J. Anat. (1973), **115**, 2, pp. 289–308
With 12 *figures*
Printed in Great Britain

Ultrastructure of the receptor and epithelial layers of the bovine retina

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(*Accepted* 12 *April* 1973)

INTRODUCTION

Up to the present the tissue of most interest to the vision biochemist has been the bovine retina. The substantial yield of rhodopsin from this source suggests that it must possess a large number of rod outer segments, but the presence of cones, the morphology and ultrastructure of the outer and inner segments, and the other cellular relationships existing in the retinal structure have apparently not been studied.

Previous studies on other vertebrate retinae have revealed a number of similarities in origin and ultrastructure, as well as basic dissimilarities in the morphology and fine structure of the receptor organelles. The present paper is concerned primarily with the photoreceptor and pigment epithelial cells in the bovine retina, and the observations reported reveal for the first time the presence of cone outer segments which are similar to rod outer segments in that both receptor types are bounded by a plasma membrane distinct from the disc membranes; furthermore, the disc membranes arise, not from infoldings of the plasma membrane, but rather as the result of fusion of small disc-like membrane vesicles at the base of the outer segment.

MATERIALS AND METHODS

Whole eyes were excised from freshly slaughtered cattle and immediately fixed in 3 % glutaraldehyde in Millonig's buffer for $3\frac{1}{2}$ hours; the tissue was post-fixed in 1 % osmium tetroxide for 1 hour. Large sections of retina were blocked off together with the surrounding tissue and scleral material. The retinal tissue was then dehydrated in successive ethanol baths and infiltrated with propylene oxide for 30 minutes. It was then bathed in equal parts of propylene oxide and Epon overnight, followed by pure Epon for 2 hours. Embedding of the tissue in pure Epon followed. Thin sections were cut with an LKB ultramicrotome and stained with 1 % uranyl acetate followed by 0·25 % alkaline lead citrate in absolute methanol. Thick sections were stained with toluidine blue.

Thick sections were examined with a Reichert optical microscope and a Hitachi 650 high voltage electron microscope; thin sections were examined with a Hitachi 11-UA electron microscope.

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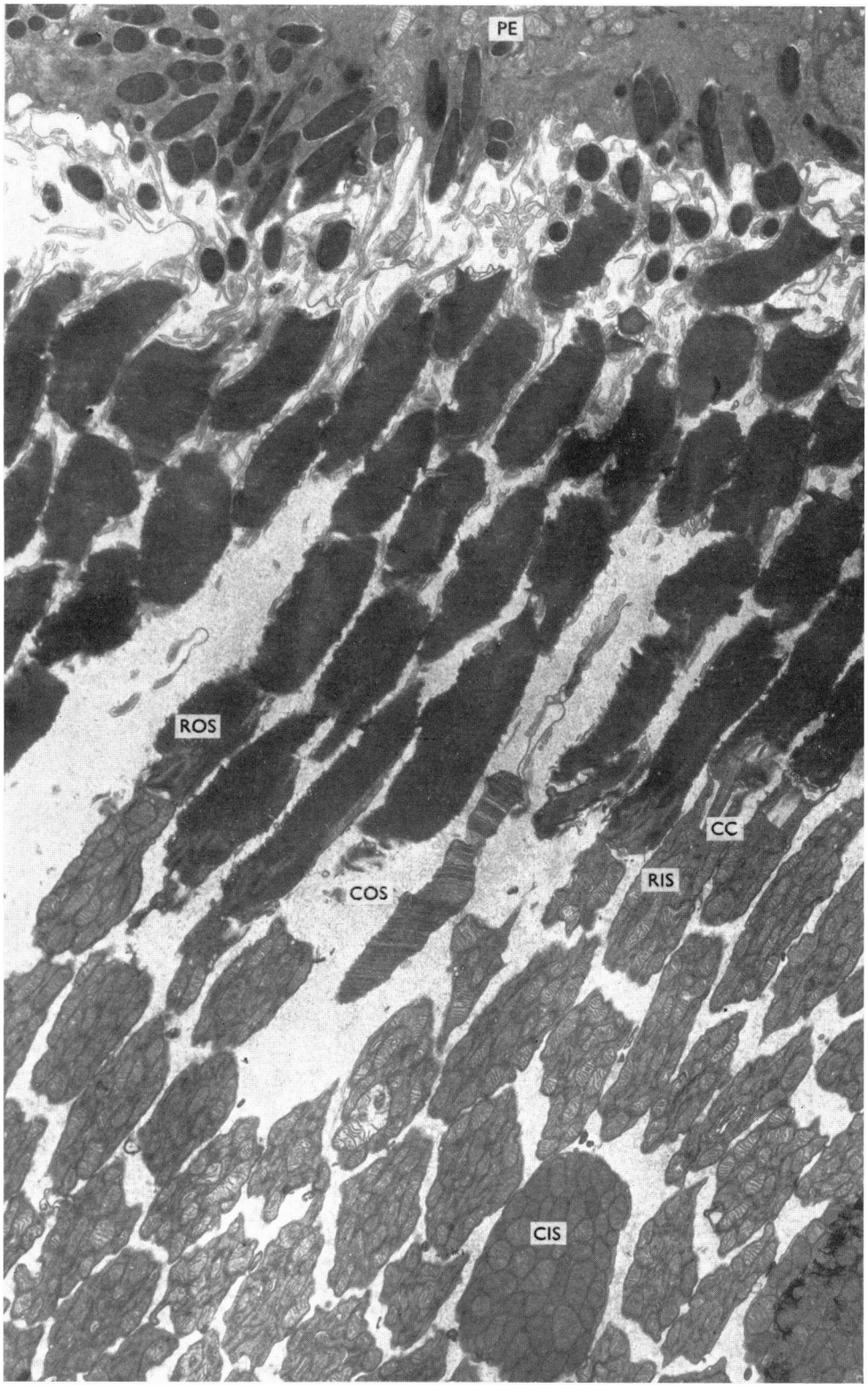
PE
ROS
COS
CC
RIS
CIS

RESULTS

The outer segments of the bovine retina are easily distinguishable as rods and cones, the rod photoreceptors clearly predominating. In all sections examined from various topographical areas of the retina there appear to be approximately 15 rods for every cone (Fig. 1).

The cone outer segments are 3–4 μm in length and 0·8 μm in average diameter. While ultrastructurally distinct from rods, the cones do not have a purely conical shape and are never in close proximity to the rod outer segments; rather, they are situated at the base of the rod outer segments and isolated by a supporting material of low electron density, so that they appear to be surrounded by an 'empty' annular region of 5–7 μm in width. Their position in the receptor layer is vitreal to the rods and intermediate between the rod outer and inner segments.

Aside from the discrete morphological placement of cone outer segments in a large intercellular space, the cones are in all cases bounded by a plasma membrane distinct from the disc membranes. The saccules in cone outer segments possess intradisc and interdisc spacings which are both larger than the corresponding spacings in the rod saccules (Fig. 2), and variation in size of the intradisc spaces of the cone segments gives the effect of banding. In Fig. 2 it is of some interest that the disc membranes of the cone are apposed at an angle of 90° to each other. Although infrequently observed, this appearance may be the result of a cone outer segment breaking off and regenerating somewhat atypically.

In the cone outer segments the overall disc thickness is 38 nm with an intradisc space of 23 nm. The saccule membrane thickness is about 7·5 nm. At the top of each cone saccule a well-defined terminal loop is observed.

The rod outer segments are 7–10 μm in length and 1–2 μm in diameter. The discs of the rod outer segment are 25 nm in thickness and exhibit a small intradisc space of 10 nm. At the lateral ends of each disc the usual loop and broadened intradisc space can be observed. As in the cone, a plasma membrane distinct and separate from the disc membrane is observed in rod outer segments. At many points along the length of the rod outer segments fissures can be seen in the disc structure parallel to the rod axis. This suggests that the disc membranes possess invaginations in their membrane structure, a point further supported by observations of cross-sectioned material. These findings are similar to those reported for the mudpuppy retina by Brown, Gibbons & Wald (1963).

In both rod and cone outer segments, a plasma membrane 10 nm thick can be observed (Figs. 2–4). In longitudinal sections the plasma membrane is distinct from

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EXPLANATION OF FIGURES

Plates are representative electron micrographs of the bovine retina. All samples are glutaraldehyde-OsO_4 fixed, uranium-lead stained.

Fig. 1. Section through the central region of the bovine retina. Note the large cytoplasmic region surrounding the cone outer segments (COS). Note also the large cone inner segments (CIS), the smaller rod inner segments (RIS), numerous oblique cilia (CC), rod outer segments (ROS) and the pigment epithelium (PE). × 8100.

Fig. 2. Detail of a section through a cone outer segment showing the extensive plasma membrane surrounding the cone as well as the pigmentation from which macroscopic banding arises; note alternate discs exhibit differential stain affinity in the intradisc area. × 60900.

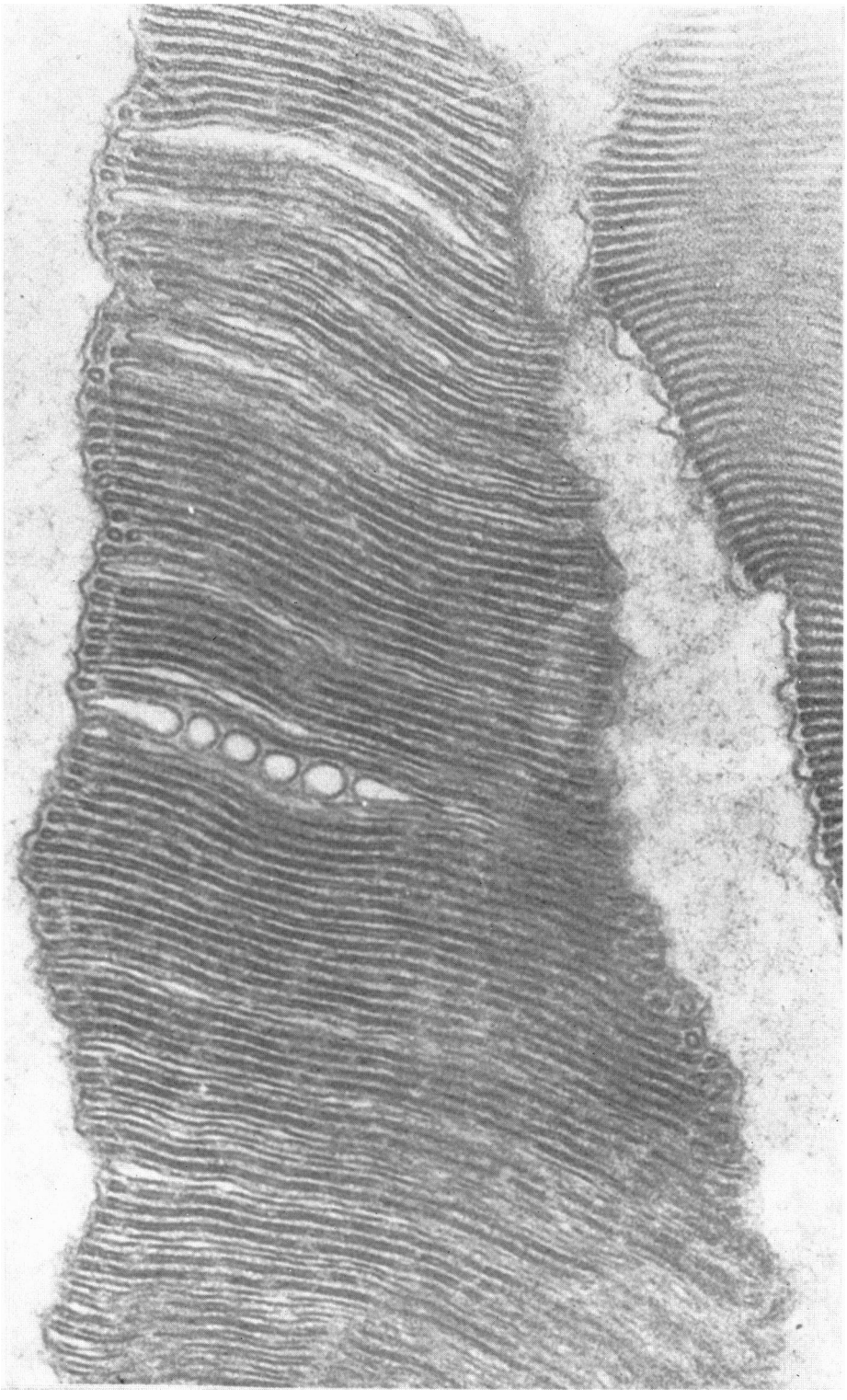


Fig. 3. Section through a cone outer segment; note vesiculation intermediate along segment length. × 66000.

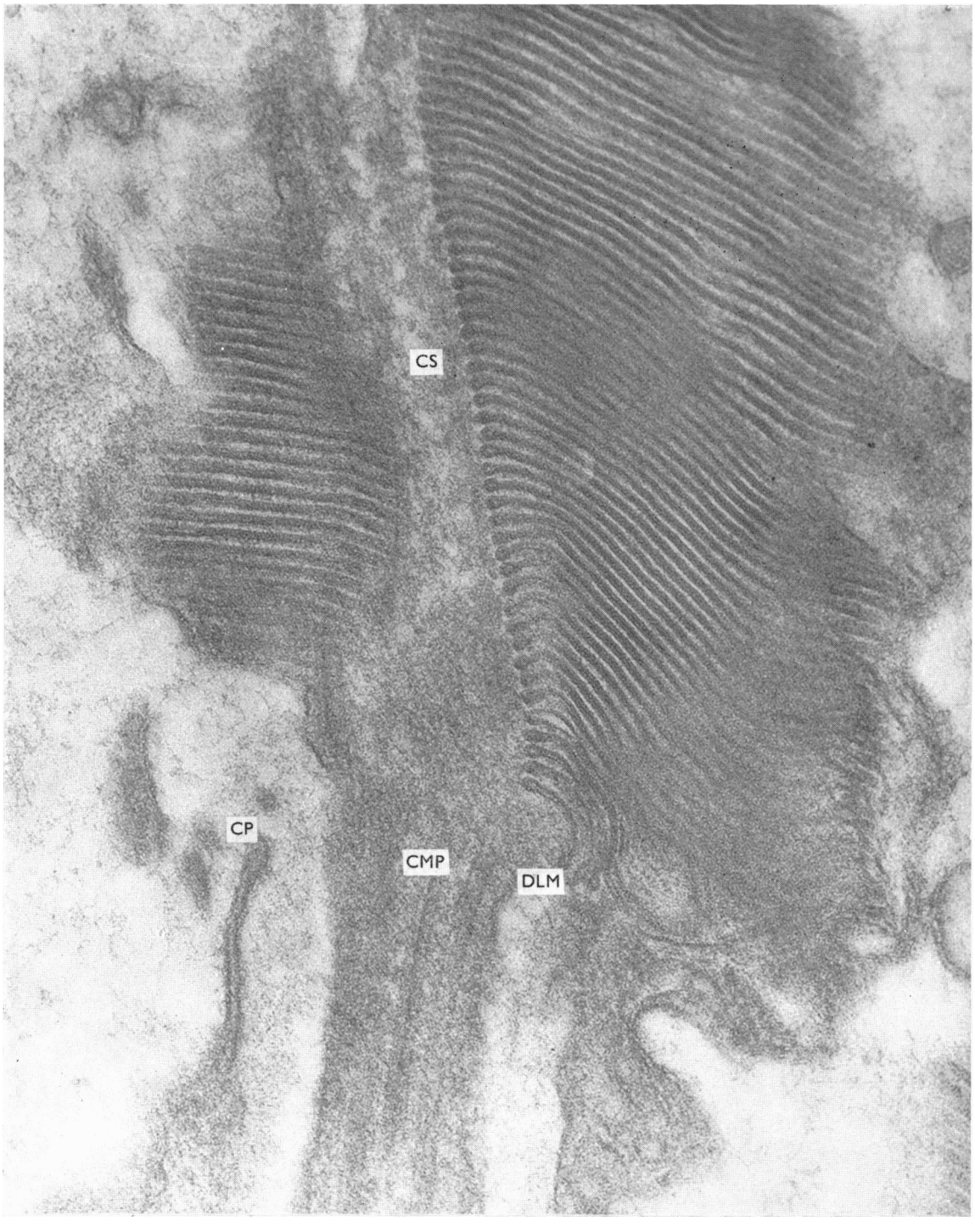


Fig. 4. Section through the base of a rod outer segment. Note the extension of the ciliary stalk (CS) along the length of the outer segment and the organizational capacity of the stalk with respect to the terminal loop; note also the ending of the calycal process (CP), the small disc-like vesicles (DLM), and the central microtubule process (CMP). × 131 800.

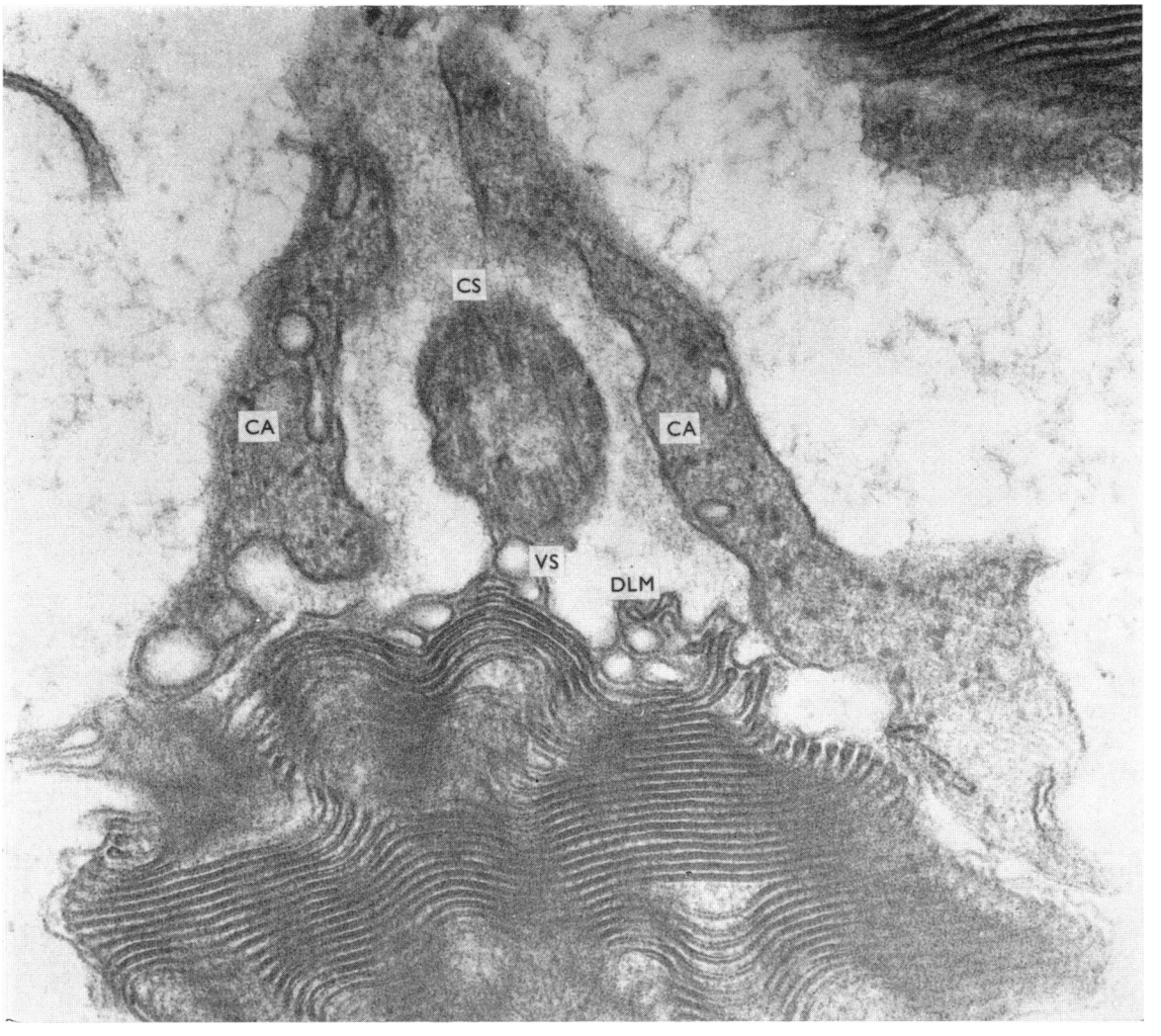


Fig. 5. Oblique section through the inner segment/outer segment interface at the cilium level. Note the calycal process (CA) surrounding the segment at this level, the central ciliary stalk (CS) and the numerous vesicles (VS) at the base of the outer segment. Note also the small disc-like material (DLM) which appears to be analogous to the larger disc material. × 102900.

the saccules of the rod, both at the base and along the length of the rod outer segment. However, numerous small disc-like vesicles evident at the base of both segment types suggest a possible morphogenetic origin of the saccules (Fig. 4). In the first 4–5 layers of these vesicles successive fusion of the membranes is noted, until eventually discs of 1–2 μm diameter become apparent. In all cases, both the vesicles and the disc structure are morphologically distinct from the plasma membrane (Figs. 1, 4) and the plasma membrane is intact. These observations suggest that the rod discs may not arise from the plasma membrane, as shown by Young (1967) in the

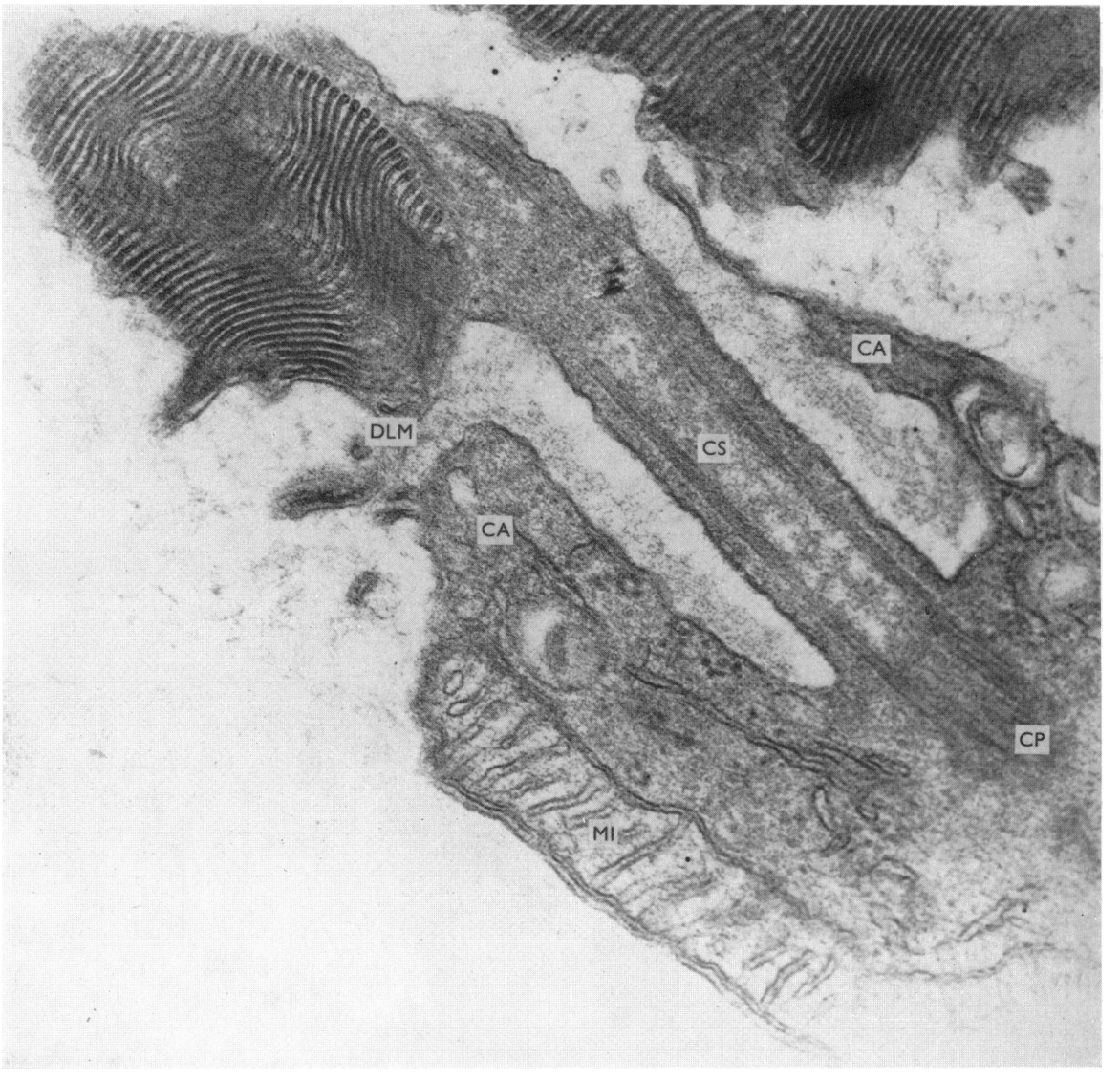


Fig. 6. Section through area similar to 6. Note the appearance of the calycal process (CA), the elongated mitochondria (MI), the ciliary stalk (CS), centriole pair (CP) and the newly forming disc-like material (DLM). ×128300.

frog, but rather as the result of a coalescence of material from the connecting cilium region (Figs. 4, 5).

The outer and inner segments are connected by a cilium structure similar to that observed in other vertebrates (Cohen, 1960, 1961; Brown *et al.* 1963; Cohen, 1965; Holmberg, 1970). A calycal process of the inner segment is also observed adjacent to the cilium at the apex of the inner segment and surrounding the outer segment base (Fig. 5). In longitudinal section the cilium structure is seen to be composed of a

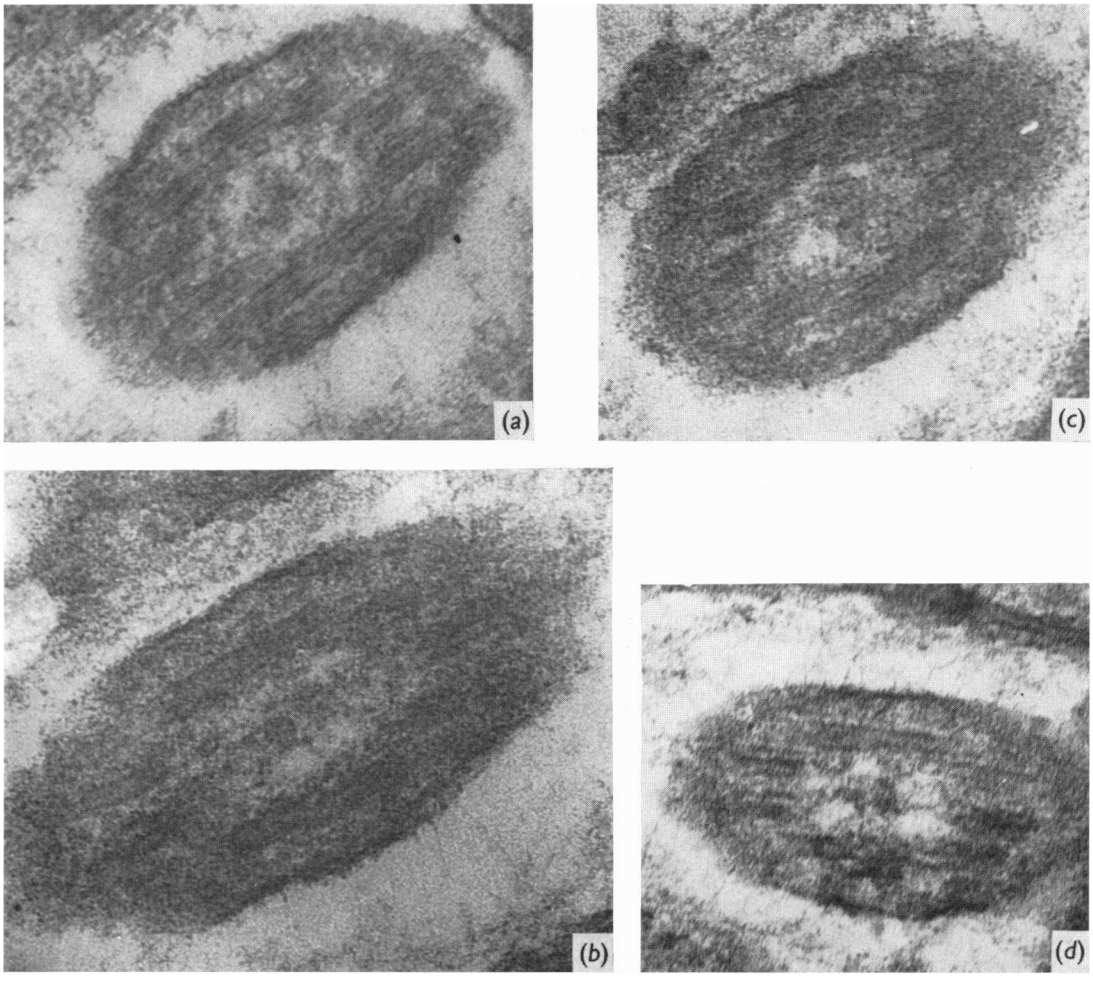


Fig. 7. Sections through the ciliary stalk at the level of the inner/outer segment. Note the consistent appearance of a central microtubule pair. (*a*) × 117600; (*b*) 131000; (*c*) 108900, (*d*) 96502.

connecting cilium and basal body (axial centriole) (Fig. 6). An oblique centriole immediately posterior to the basal body is also observed in the distal region of the inner segment. The connecting cilium is consistently observed to make contact with disc membranes along the length of the outer segment, and may exist in an organizational capacity at the base of the outer segment (Fig. 4).

In cross-section the connecting cilium clearly consists of nine peripheral microtubular processes oriented axially to the cilium. A central structure of intermediate electron density can also be observed in most cilia and appears to be more diffuse than the peripheral microtubules (Fig. 7).

The mitochondria of the receptor cell are concentrated at the apex of the inner segment close to the outer segment layer. Other supporting material surrounds the mitochondria, namely small osmiophilic vesicles and granules, numerous strands of

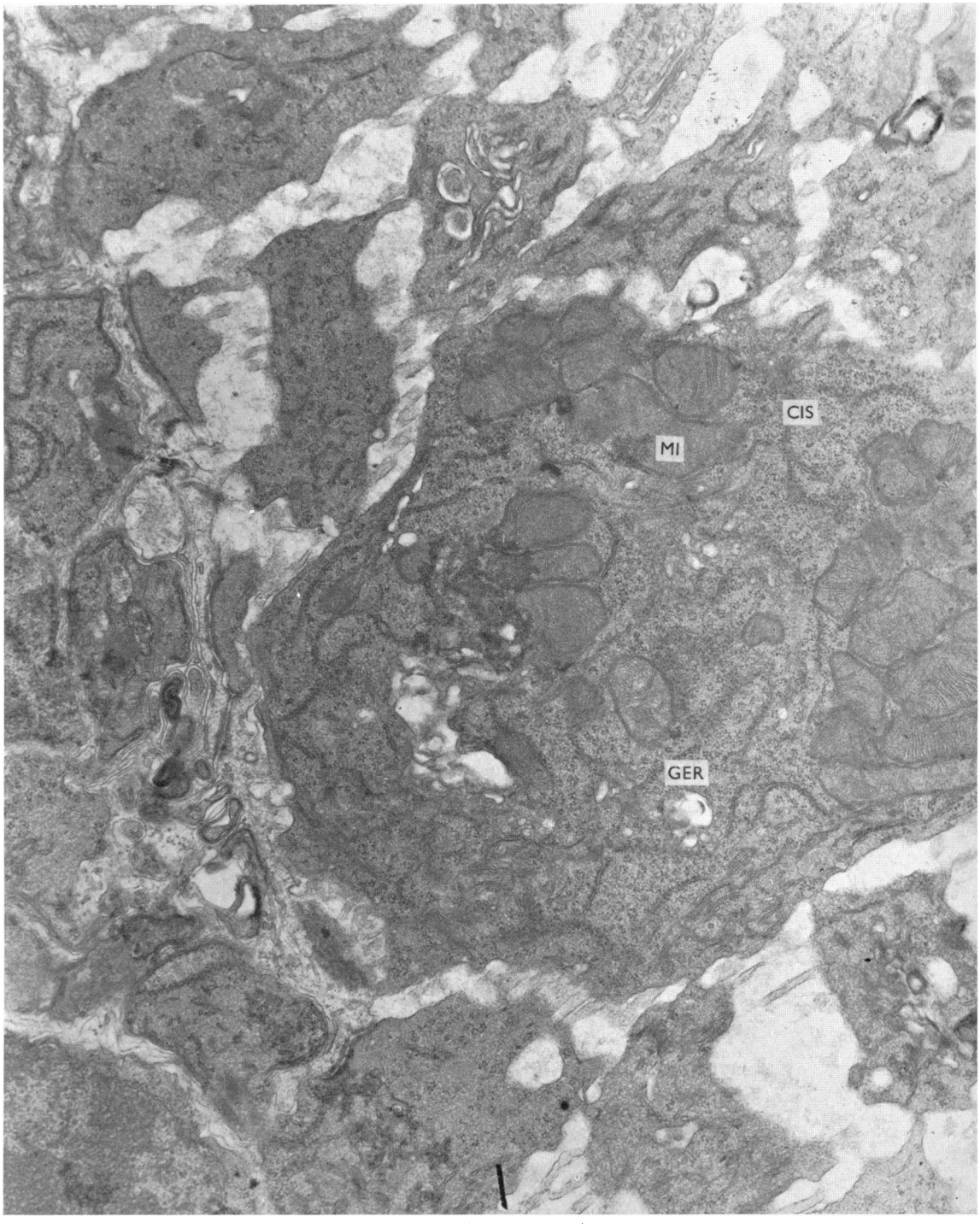


Fig. 8. Section through the supranuclear region of the retina; note high density of mitochondria (MI) at the apex of cone inner segments (CIS) and density of granular endoplasmic reticulum (GER) at the base of the segment. ×35000.

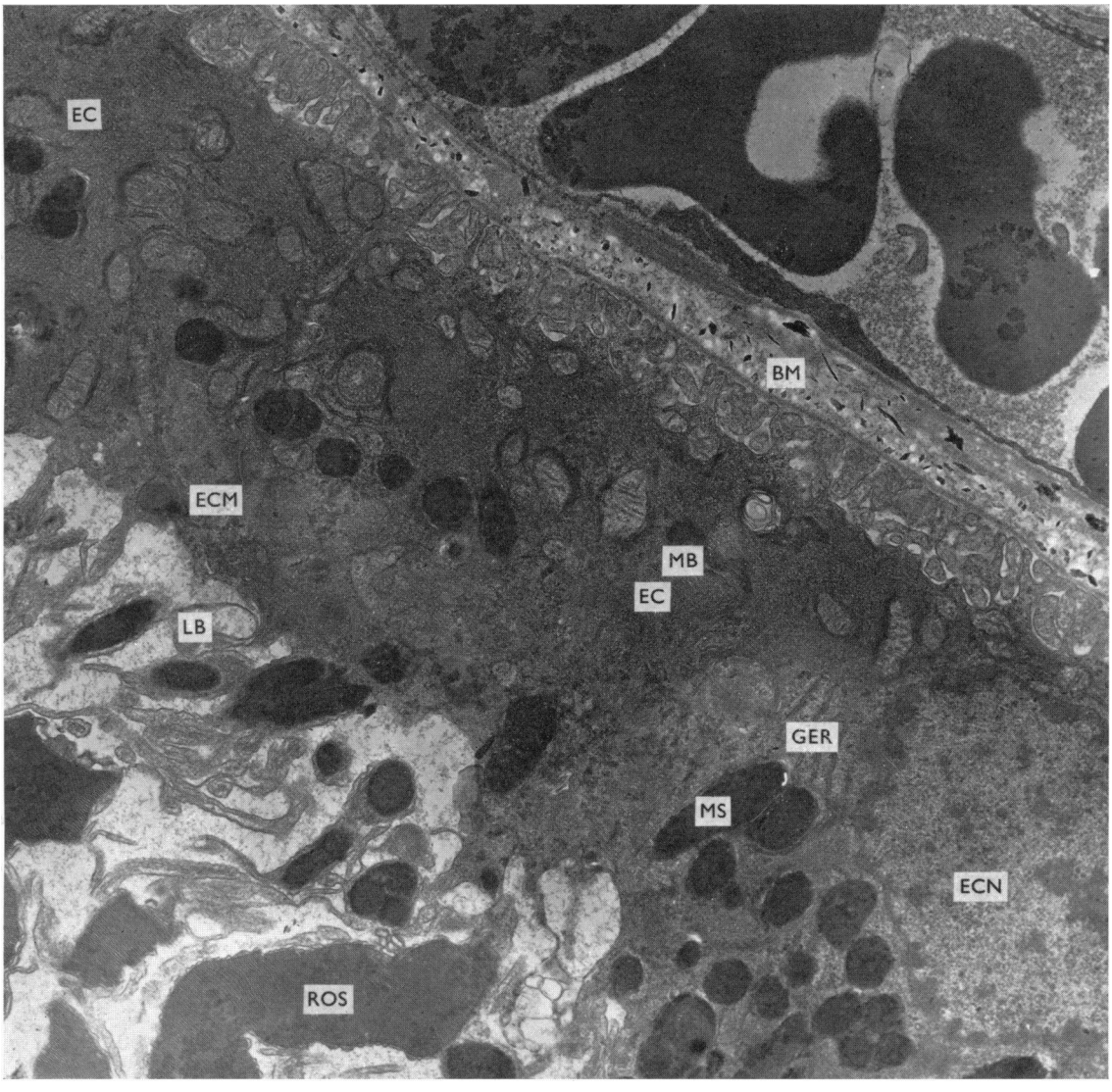


Fig. 9. Section through the pigment epithelium; note epithelial cell (EC), epithelial cell membrane (ECM), Bruch's membrane (BM), choroid endothelial cells (EC), nucleus of epithelial cells (ECN), melanosomes (MS), myeloid bodies (MB), lysosomal bodies (LB), granular endoplasmic reticulum (GER) and rod outer segment (ROS). × 21 800.

granular endoplasmic reticulum, and some free ribosomes and polyribosomes (Fig. 8).

In the vitreal end of the inner segment, mitochondria are sparse, an extensive Golgi complex is evident, and long fibrils of granular endoplasmic reticulum predominate, in most cases oriented parallel to the long axis of the inner segment. This endoplasmic reticulum is as long as 400 nm in some instances, and is densely lined with ribosomal material.

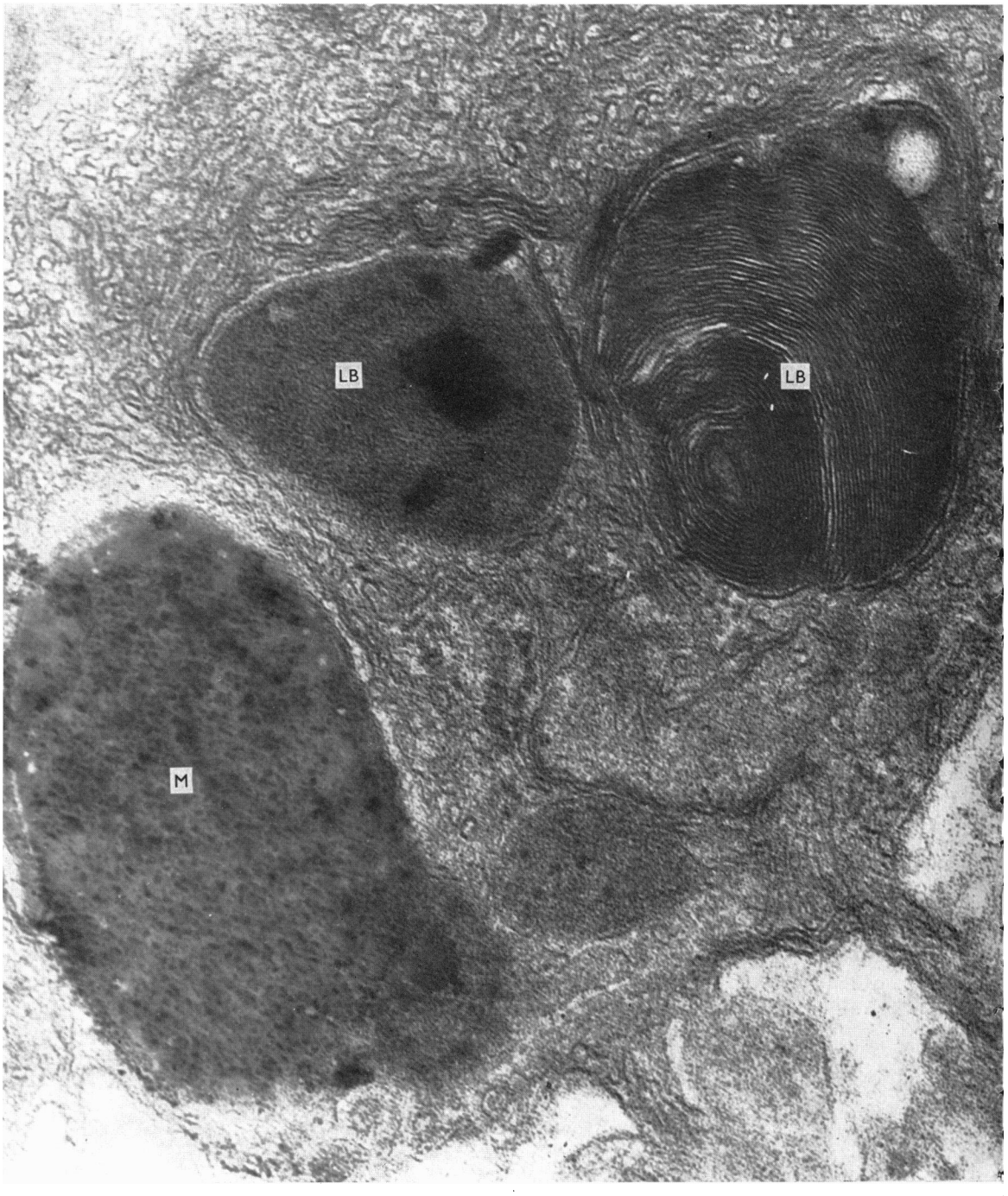


Fig. 10. Section through the pigment epithelium; note lysosome body (LB) containing whorled disc material, melanosome (M), and additional lysosomal body exhibiting dense pigmentation (LB). ×119000.

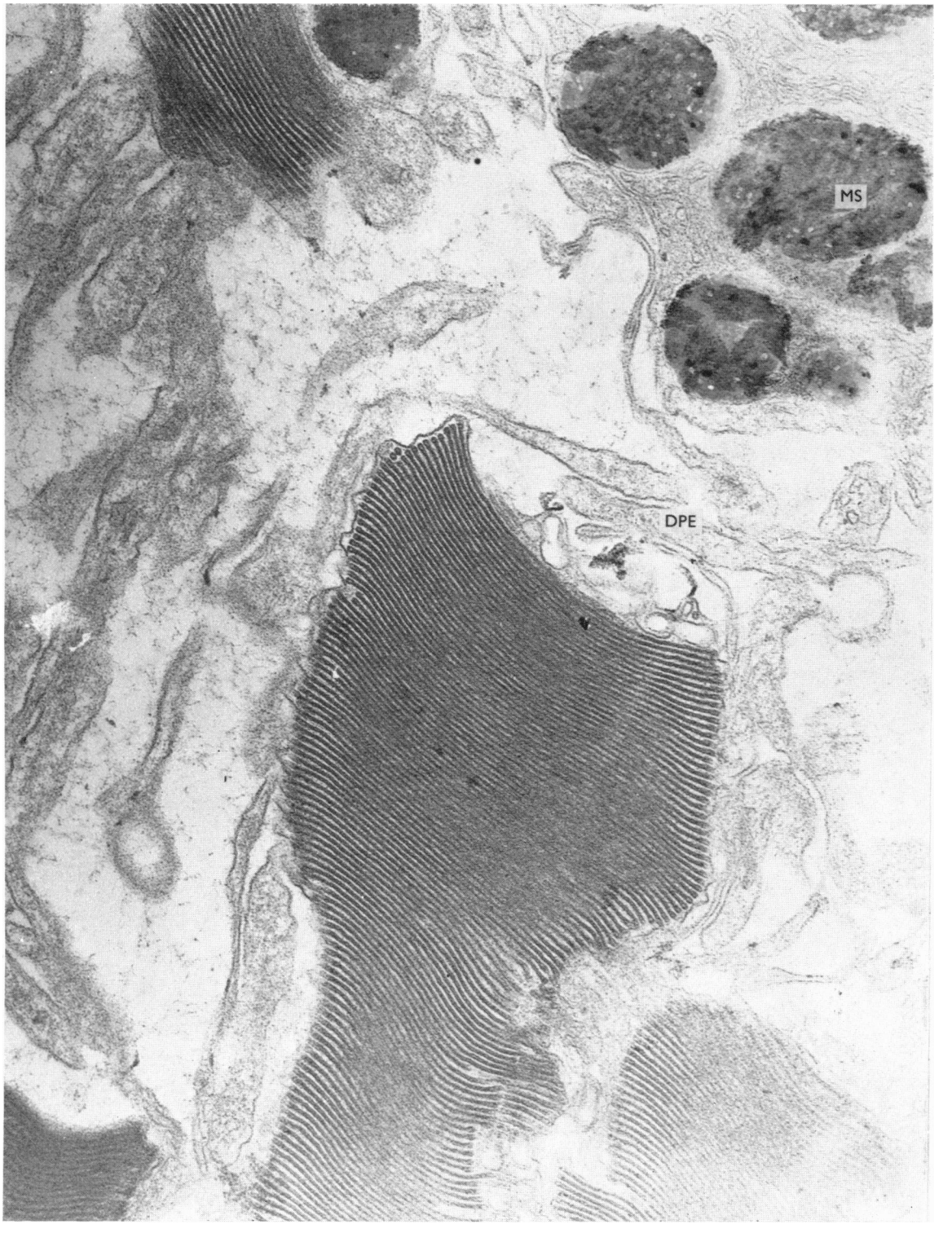
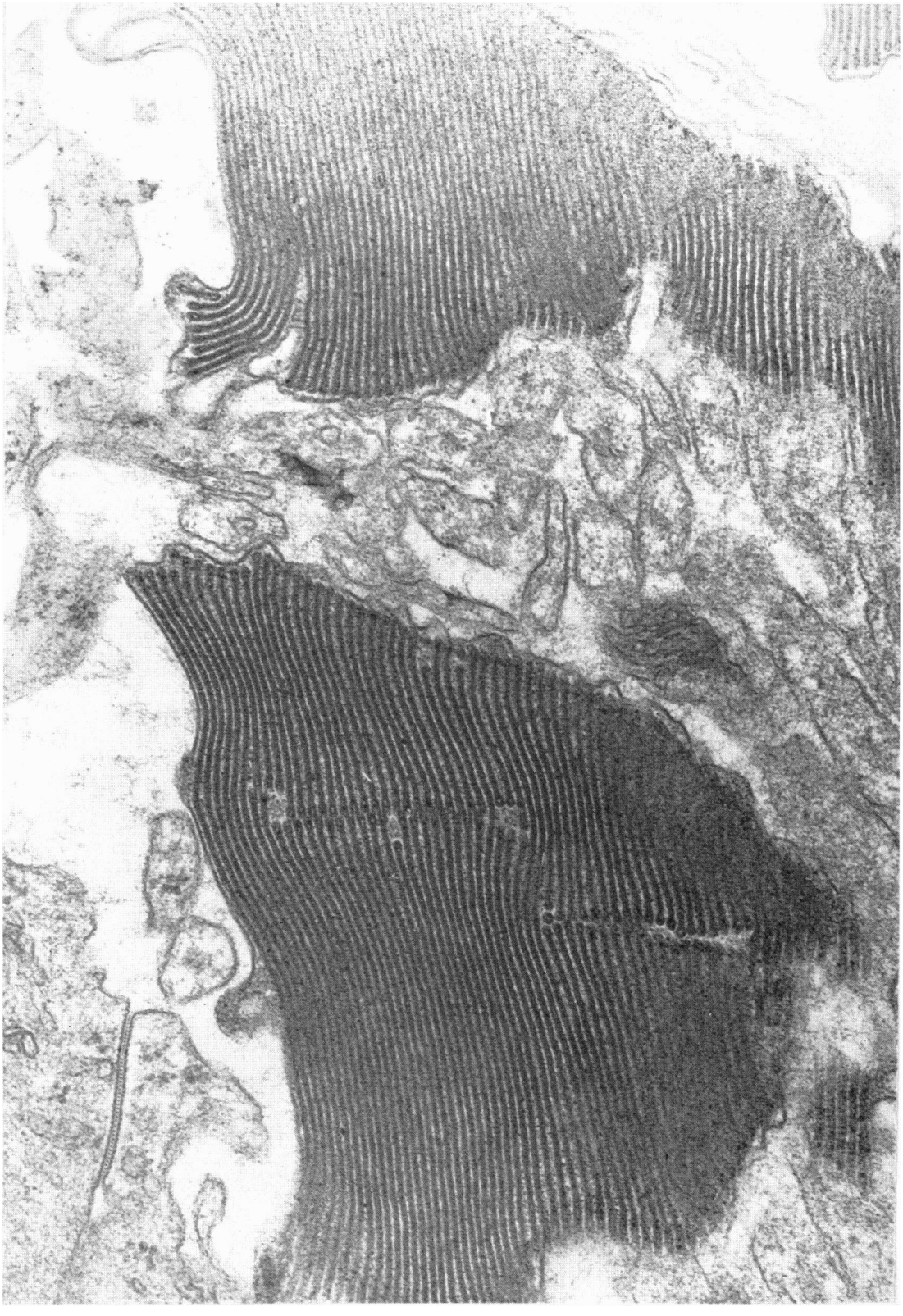


Fig. 11. Section of bovine retina at outer segment/pigment epithelium interface; note dendritic processes of pigment epithelium (DPE) surrounding rod outer segment and melanosomes (MS): also note continuation of plasma membrane at apex of outer segment. × 66000.



While not unique among vertebrate receptors, the most interesting feature of the inner segment region is the large difference in size of rod and cone inner segments. In longitudinal sections of bovine retina, cone inner segments are invariably 3–4 times the diameter of rod inner segments and 10 times the total volume of rod inner segments. Additionally, a cone inner segment is estimated to contain 600–700 mitochondria whereas a rod inner segment contains only 50 mitochondria. The cone inner segments are concentrated in the intermediate and lower regions of the inner segment area, corresponding to the placement of cone outer segments vitreal to rod outer segments.

The pigmented epithelial cells of the bovine retina lie on the scleral side of the outer segment region and are separated from the choroid endothelial cells by an osmiophilic region of diverse composition, namely basal laminae of the pigment and epithelial capillary cells, lateral processes of the endothelial cells, darkly staining inclusions and fibrillated collagenous-like material. This assemblage is roughly 0·5–0·7 μm in width and constitutes Bruch's membrane (Fig. 9).

On the vitreal side of Bruch's membrane is the pigment epithelium. The apical surface of the outer membrane of the epithelial cells frequently produces evaginations which project approximately 500 nm into the intracellular space of the epithelial cell. Lateral infoldings of the plasma membrane can also be noted, so that adjacent plasma membranes of epithelial cells interdigitate into the extracellular space of adjacent epithelial cells. This membrane organization continues down to the receptor level of the retina.

The retinal epithelial cells are characterized by a large nucleus with a diffuse nucleolus (Fig. 9). Extensive systems of granular endoplasmic reticulum and mitochondria are located in the dense cytoplasm of the pigment epithelium. These systems are highly organized and are usually observed in laminar stacks of 5–15 reticule units.

The epithelial cells contain a variety of intensely osmiophilic and pigmented inclusions, as shown in Fig. 10. These lysosomal and melanosomal bodies bear an intimate relationship with the outer segments (Fig. 11), and often appear to be in the process of digesting several disc membranes (Fig. 12). Most lysosomal bodies are spherical and composed of disc membrane in the internal compartment of the body. Other melanosomal bodies are packed with a dense pigment and reveal no internal fine structure. Some inclusions are spherical or oval in shape, and are occasionally composed of a densely staining core and a less tightly packed cytoplasm which is usually made up of disc-like material and appears to be undergoing compression into more densely packed material (Fig. 10).

The dynamic digestive character of the pigment epithelium is very marked. In many cases digestive vesicles are present in the tip of the outer segments, where they appear to be in the process of separating 6–8 disc membranes from the other segment. The discs are presumably taken up into one of the epithelial inclusions and subsequently digested (Fig. 12).

Fig. 12. Section through pigment epithelium/outer segment interface showing eight disc membranes being sloughed off from the rod outer segment; note appearance of vesicles in area of disc separation; note also continuity of plasma membrane around the periphery of the outer segment. × 194 600.

DISCUSSION

The visual receptor cells of the bovine retina appear to have many points of similarity as well as dissimilarity to other vertebrate visual receptor systems. Because the retina was preserved *in situ* in the whole eye and fixed with both glutaraldehyde and osmium, fixation artifacts appear, by the accepted criteria of good fixation (Fawcett, 1966), to be minimal.

The central and peripheral regions of the bovine retina differ in cone frequency, the central region possessing the higher density of cones. Even so, the cone density in the central region is relatively low in comparison with other vertebrates. The lightly staining 'pocket' surrounding the cone outer segments is upwards of 5–6 μm in diameter, and this, taken together with the location of the cone outer segments vitreal to the rod outer segments but scleral to the rod inner segments, virtually precludes the possibility of artifact. The additional fact that cone inner segments are displaced to the vitreal side of the inner segment region would seem to confirm their marked isolation, although the functional necessity for this isolation is obscure.

Cones of the central retinal region are surrounded by a plasma membrane for their entire length. Previous investigations in other vertebrates have suggested that the disc membranes of the cone outer segment are primarily invaginations of the plasma membrane, and have further demonstrated numerous discontinuities in the surrounding membrane system. This characteristic is not observed in the bovine retina. Some of these earlier studies were performed with retinal material fixed only with osmium, and the rapid oxidizing nature of osmium reagents may have generated cellular artifacts which could account for this discrepancy (Tormey, 1964; Eakin, 1965; Röhlich, 1966).

The morphogenesis of bovine rod and cone saccules differs, the cone saccules being disorganized and highly vesiculated at the base, leading to a loosely packed array of saccules and numerous vesicles in the scleral region of the receptor. On the other hand, the rods show a high degree of organization at the outer segment base, with little vesiculation. This is reflected in a highly organized disc membrane system in the distal portion of the outer segment.

The developmental origin of receptor membranes in the vertebrate retina has been studied predominantly in the rod outer segment of the developing frog. It has been suggested that the disc membrane derives from invaginations of the basal plasma membrane (Sjöstrand, 1953; Tokuyasu & Yamada, 1959; Moody & Robertson, 1960; Young, 1967). A similar situation is not observed in the bovine retina. The vesicles seen in Figs. 3 and 4 appear to undergo a fusion process to form the disc membranes observed along the length of the outer segment. These vesicles are not observed to associate in any way with the receptor cell plasma membrane. Such a fusion process is quite likely considering the highly fluid nature of the bovine receptor membrane, as suggested by its high degree of lipid fatty acid unsaturation (Anderson, Feldman & Feldman, 1970; Borggreven, Daemen & Bonting, 1970; Poincelot & Abrahamson, 1970), and the thermal broadening of the X-ray diffraction maxima arising from the membrane (Blasie & Worthington, 1967). The highly organized nature of the disc material observed in the bovine retina, and the lack of disruption in the plasma membrane proximal to the discs, make it appear likely that

these observations are valid. A further point which bears on this discussion of the morphogenetic origin of disc material is that the membrane thickness of the plasma (10 nm) and disc (7·5 nm) membranes is markedly different, implying that the former is not a precursor of the latter. This conclusion is further supported by Hagins' observations that Procian yellow, a charged dye, does not penetrate the rod at the outer segment base. If invaginations of the plasma membrane of the rod did exist, one would expect to see diffusion of the dye into the rod (Hagins, personal communication). Conversely, lack of dye penetration does imply the existence of an intact plasma membrane consistent with the observation herein.

In these studies we observed vesiculations intermediate along the outer segment only in cones and never in rods. As vesicles intermediate along the cone outer segment resemble the newly formed vesiculated material at the outer segment base in size, shape and staining characteristics, it seems likely that they represent the normal course of disc membrane assembly or degeneration. Dowling & Gibbons (1961) have demonstrated a similar phenomenon in the pigeon retina.

The appearance of cone outer segments in longitudinal section is unique because they are so osmiophilic relative to rods. This may result either from differential uptake of stain because of basic differences in the intradisc fluid, or from variations in the size of the intradisc space.

In cross-sections and longitudinal sections of bovine retina at the nuclear level, several interesting structures are noted. A system of agranular vesicles predominates in the nuclear region, while mitochondria predominate at the receptor end of the inner segment. In close association with the inner segment at the nuclear level are numerous granules of high electron density. Such structures are found in both the surrounding cytoplasm and inner segment, and are suspected to be either glycogen or mucopolysaccharide (Lillie, 1952; Zimmerman, 1958; Ocumpaugh & Young, 1966; Freeman & Wortman, 1966) and/or glycogen (Lillie, 1952; Wislocki & Sidman, 1954; Eichner & Themann, 1962). The presence of mucopolysaccharides in the intercellular space between the outer segments has been well documented by Sidman (1958), Young & Bok (1969) and others. Holmberg (1970) has detected glycogen in the hagfish retina and has suggested that the glycogen granules may support the metabolic activity of mitochondria in the inner segment. The positive identification of glycogen granules in the bovine retina, however, must await further study by specific staining techniques.

The inner segments of the retinal photoreceptors vary widely in size, the rod inner segments possessing 10 % of the number of mitochondria contained in cone inner segments, although the mitochondria found in the two cell types are bascially identical. This difference may reflect the greater need of cones for metabolic energy. The abundance of mitochondria in the inner segments is also of interest from a different standpoint. The cone outer segments contain less than 20 % of the disc material found in rod outer segments; nevertheless the cones require a greater metabolic supply than do rods. Further, the ratio of mitochondrial volume to outer segment volume is approximately 100 times greater in cones than in rods. From this we may reasonably infer that cone receptor segments possess a vastly more active metabolic system than do rod receptor segments.

The presence of large concentrations of mitochondria in the rod inner segment

has been shown by Hagins (1973) to be associated with an active sodium pump. The mitochondria in the cone in the same region seem likely to have a similar function, but it is not clear why there is such a vast difference in the proportion of photopigment to mitochondria. It is possible also that some of the energy production is associated with the rapid pigment turnover at high light intensities.

The pigmented epithelial cells of the bovine retina resemble those found in other vertebrate retinae (Sjöstrand, 1953; De Robertis, 1956; Moody & Robertson, 1960; Cohen, 1960; Cohen, 1961). In addition to an extensive system of granular endoplasmic reticulum, mitochondria and densely pigmented inclusions, the bovine pigment epithelium cells contain numerous myeloid bodies similar to those identified in the frog (Porter, 1956), the rat (Dowling & Gibbons, 1961) and the turtle (Yamada, 1961). These myeloid bodies may exist in a free form in the cytoplasm, or, more frequently, surround some of the spherical vesicles and mitochondria in the epithelial cells.

The phagocytic digestive function of lysosomal bodies on the vitreous side of the epithelial cells has been well documented in vertebrates by Young (1967). This degradative function appears to exist in cattle, and several different types of osmiophilic bodies have been identified here as well. It is admittedly difficult to differentiate one inclusion from the next, but it appears that in the bovine retina six to eight discs are peeled away from the outer segment by the digestive vesicles (Fig. 12); these discs are then enclosed by a large vesicle, presumably containing digestive enzymes. The vesicles degrade the disc material and compress it into a dense, pigmented core. These pigmented bodies are always observed in the vitreous half of the pigment epithelium, and never approach the scleral side of the epithelium or Bruch's membrane (Fig. 1). Autoradiographic studies have failed to elucidate the fate of this material (Hall, personal communication).

From the examination of the bovine retina at the level of the outer nuclear/inner segment interface it appears possible that the large amount of granular endoplasmic reticulum present in the vitreal end of the inner segment may be the origin of newly synthesized material for the outer nuclear layer as well as for outer segment material. It has been shown that the inner segment is the probable source of membrane protein for the disc membranes of the outer segment (Young, 1967), and it seems likely that inner segments could function as an additional source for outer nuclear material.

SUMMARY

Thin sections of bovine retina have been examined by electron microscopy and found to contain morphologically distinct rods and cones. The cone outer segments were surrounded by a plasma membrane discrete from the cone saccules, and in no case were the rod or cone saccules observed to be invaginations of the receptor cell membrane.

The origin of rod photoreceptor material in the bovine retina appears to be a system of disc-like vesicles 50–75 nm in width and located at the base of the segment. A similar system was observed in cones; the vesicles in the cone, however, are not localized in the base of the outer segment but are distributed along the length of the segment. This observation is in conflict with other reports on the vertebrate retina.

The cone outer segments are 3–4 μm in length and 1–2 μm in diameter. Cone saccules are 35 nm thick with a 20 nm intradisc spacing. Rod outer segments are 7–10 μm in length and 1–2 μm in diameter and rod discs are 25 nm thick with a 100 nm intradisc space. Both receptor cells possess a highly developed connecting cilium. This cilium consists of a central electron-dense structure surrounded by nine peripheral doublets, in contrast to other vertebrate retinae which do not contain a central structure.

The inner segment system of cones is also discrete from the rod inner segments. The cone inner segments were observed to contain 10–15 times the number of mitochondria found in a rod inner segment and were correspondingly larger.

Retinal function is discussed from the standpoint of inner segment activity in the supranuclear region and a general study of pigmented epithelial cells is reported.

We wish to acknowledge the assistance of Dr John Gabrovsek of the Department of Anatomy, and Dr Ida Kaiserman-Abramoff for her critical reading of the manuscript and helpful comments. This work was supported by grants EY-00471 and EY-00209 from the National Eye Institute, National Institute of Health.

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