STUDIES ON MICROPEROXISOMES

VII. Pigment Epithelial Cells and Other Cell Types in the Retina of Rodents

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ABSTRACT

The pigment epithelial cell of the retina actively participates in two aspects of lipid metabolism: (*a*) the fatty acid esterification of vitamin A and its storage and transport to the photoreceptors, and (*b*) the phagocytosis and degradation of the lipoprotein membrane disks shed from the photoreceptor cells. Study of the pigment epithelial cells of adult albino and pigmented rodents has revealed the abundance of an organelle, microperoxisomes, not previously known to exist in this cell type. The metabolism, transport, and storage of lipids are major functions of other cell types which possess large numbers of microperoxisomes associated with a highly developed smooth endoplasmic reticulum. Microperoxisomes were encountered, but relatively rarely, in Müller cells and vascular endothelial cells. A tubular system in photoreceptor terminals is reactive in the cytochemical procedure used to visualize microperoxisomes.

The pigment epithelial photoreceptor system of the retina system can serve as a model for analysis of genetic control of cell interactions (19). These two cell types are known to have effects upon each other during embryonic and postnatal development (9, 18), and photoreceptor recovery from injury requires the pigment epithelium (14). In the adult, the pigment epithelial cells are involved in the uptake of vitamin A, its esterification with fatty acids, and its transport to the photoreceptor cells (7–9, 17, 44, 45), most recently shown by autoradiographic studies by Bibb and Young (4). As disks are shed from photoreceptor cells they are phagocytosed and degraded by pigmented epithelial cells (2, 3, 8, 11, 14, 44).

Since lipid is prominent, morphologically and metabolically, in the interrelations between photoreceptor and pigment epithelial cell, it is of considerable interest to report the presence of an organelle not previously known to exist in the pigment epithelial cell. This organelle, termed microperoxisome (29), occurs in abundance in cell types in which the metabolism, transport, and storage of lipids are major functions (29, 31, 32, 36). Compared to the pigment epithelial cells there are far fewer microperoxisomes in the Müller cells and vascular endothelial cells.

In the course of this study a little-known tubular system in photoreceptor terminals (23) was found to be stained when tissue was incubated in the medium used to visualize microperoxisomes.

MATERIALS AND METHODS

Pigmented and nonpigmented rodents were studied. These included the C 57 black mouse and its "beige" mutant (12–30 wk old, weighing 12–21 g), albino rabbits

(4.5–6 kg), and albino guinea pigs (450–650 g). After ether or pentobarbital anesthesia, the eye was enucleated. The globe was opened along the equator and the tissue was immersed immediately in either: (*a*) freshly prepared 4% glutaraldehyde (42)-0.1 M phosphate buffer, pH 7.4 for 30 min at room temperature; or (*b*) freshly prepared cold 2.5% glutaraldehyde-2% formaldehyde (prepared from paraformaldehyde)-0.09 M cacodylate buffer, pH 7.4 with 0.025% $CaCl_2$ (26) for 90 min or for 3 h. The glutaraldehyde was diluted from a 70% solution (Ladd Research Industries, Inc., Burlington, Vt.) and the formaldehyde was prepared from paraformaldehyde (Fisher Scientific Co., Springfield, N. J.). Further dissection of the eyeball was performed in the fixative, under a dissecting microscope. Tissue from the posterior half of the globe was studied.

Tissue fixed in solution *a* was used without incubation, i.e., for morphological study only. Small blocks, less than 0.2 mm in each direction, were cut with razor blades to include retinal and choroidal layers. They were further fixed in 1% OsO_4-0.1 M phosphate buffer, pH 7.4 (27) for 1 h.

Tissue fixed in solution *b* was incubated before osmication. The tissue was rinsed in cold 7.5% sucrose; nonfrozen sections, 25–30 μm thick, were then cut with a Sorvall TC-2 tissue sectioner (DuPont Instruments, Sorvall Operations, Newtown, Conn.), according to the method of Smith and Farquhar (46). A few of these sections were first incubated for acid phosphatase activity, rinsed in sucrose, and then incubated in the alkaline 3,3'-diaminobenzidine (DAB) medium. Most sections were incubated only in the DAB medium.

The DAB medium (31) reveals the catalase in microperoxisomes. It is prepared by adding 9.3 ml of 0.05 M propanediol buffer, pH 9.0, to 20 mg DAB-tetrahydrochloride (Sigma Chemical Co., St. Louis, Mo.), adjusting to pH 9.7 with sodium hydroxide, and adding 0.5 ml 0.1 M potassium cyanide, 0.2 ml 2.5% hydrogen peroxide freshly prepared from a 30% solution, (Merck Chemical Div., Merck & Co., Inc., Rahway, N. J.), and 0.5 g sucrose. The medium is filtered before use. The incubation medium used to demonstrate sites of acid phosphatase activity is a modification (28) of that used in the procedure of Gomori (12). It is prepared by adding 10 mg 5'-cytidine monophosphate (CMP) disodium salt (Sigma Chemical Co.) to 4.8 ml distilled water, then adding 4.0 ml 0.05 M acetate buffer, pH 5.0, 1.2 ml 1% lead nitrate, and 0.5 g sucrose. The medium is filtered before use. Both DAB and CMP incubations were done at 37°C, in a shaking water bath. After incubation, the sections were rinsed in 7.5% sucrose and fixed in cold 1% osmium tetroxide-0.1 M cacodylate buffer, pH 7.2, for 60 min.

Subsequent processing was the same for incubated and unincubated sections. They were rinsed several times in cold 7.5% sucrose, and then treated with 0.5% uranyl acetate in Veronal-acetate buffer, pH 5.0, for 60 min at room temperature in the dark (10, 15). After dehydration in ethanols, the sections were treated with propylene oxide and flat embedded in Epon 812 according to the method of Luft (24). Thin sections were cut on a Sorvall Porter-Blum MT2 or LKB microtome with a diamond knife. They were stained in lead citrate (40) for 5–10 min and were observed in a Philips 300 electron microscope equipped with a goniometer stage, at 60, 80 or 100 kv and with a 30-μm objective aperture.

RESULTS

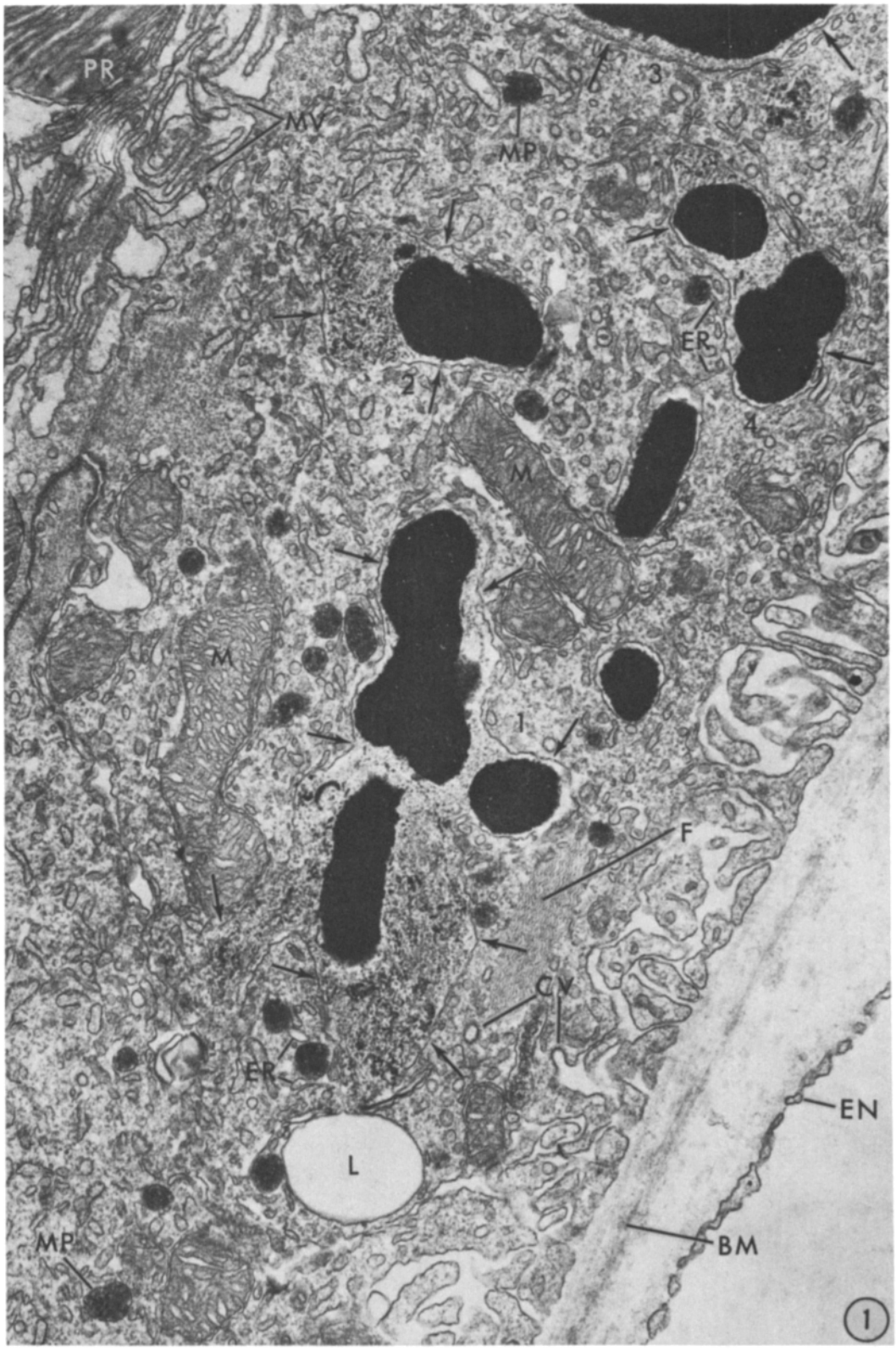
As previously described in the retina of man (2), frog (38), and rat (9), abundant smooth endoplasmic reticulum (ER) is present in the pigment epithelial cells of the beige mouse (Fig. 1), C 57 black mouse (Fig. 3), albino rabbit (Figs. 4 and 7–9) and albino guinea pig (Figs. 2, 5, 6, and 12). Related to this smooth ER, particularly in the outer portion of the cell (i.e., on the choroidal side), are numerous microperoxisomes (Figs. 2–4). Only after we had studied them in DAB-incubated tissue could we readily identify the organelles in unincubated tissue (Fig. 11).

Their abundance is best appreciated in Figs. 1 and 10. As in the microperoxisomes of most cell types (29, 31–36), the features of pigment epithelial cell peroxisomes are: (*a*) the presence of catalase, as revealed by the staining of the organelles in tissue incubated in the alkaline DAB medium (Figs. 1–10 and 12) (and the absence of acid phosphatase activity [Fig. 13]); (*b*) the absence of nucleoids which characterize the peroxisomes of most mammalian hepatocytes and epithelial cells of the proximal convoluted tubule portion of the renal tubule; (*c*) the presence of a tripartite delimiting membrane (Figs. 5, 6, 9, 11, and 12); and (*d*) numerous slender continuities with smooth ER (Figs. 2–4 and 6–8), these being often evident only when the specimen is viewed at specific tilt angles (Figs. 5–9).

The microperoxisomes and the smooth ER with which they are continuous are often seen on the surface of lipid droplets (Figs. 1, 10, and 11). Mitochondria may also be seen near the microperoxisome-smooth ER aggregates (Figs. 3 and 4).

In the beige mouse, the microperoxisome-smooth ER complexes are also found close to the "giant melanin granules" (25). These often contain two or more melanin granules, and they demonstrate dense accumulations of reaction product when incubated for acid phosphatase (20).[1] They

[1] This has been confirmed recently by Robison et al. (41).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may, therefore be referred to as compound melanosomes (30) or, as we suggest, melanolysosomes. The complexity of melanolysosome structure is well illustrated in Fig. 1. Within a tripartite membrane may be found large, irregular melanin granules, scattered melanin and unidentified materials of lower electron opacity.

In the beige mouse, bundles of parallel wavy filaments, apparently not membrane enclosed, are encountered (Fig. 1). Their nature and function were not investigated.

Many microperoxisomes in the pigment epithelial cells appear circular or only slightly elongate (Figs. 1–10). However, more elongate forms are encountered (Figs. 10 and 12) and it is likely that most, if not all, microperoxisomes are elongate, about 0.3 μm in length.

We did not search for microperoxisomes in the other cell types of the retina, but occasionally they were encountered in vascular endothelial cells (Fig. 14) and Müller cells (Fig. 15).

In albino guinea pigs (Figs. 16 and 17) and in rabbits and rats (P.M. Leuenberger, unpublished observations), an extensive DAB-positive tubular system was seen in the synaptic terminals of the photoreceptor cells in tissues incubated in the DAB medium, pH 9.7. The system is composed of twisted anastomosing tubules, approximately 13 nm in inner diameter.

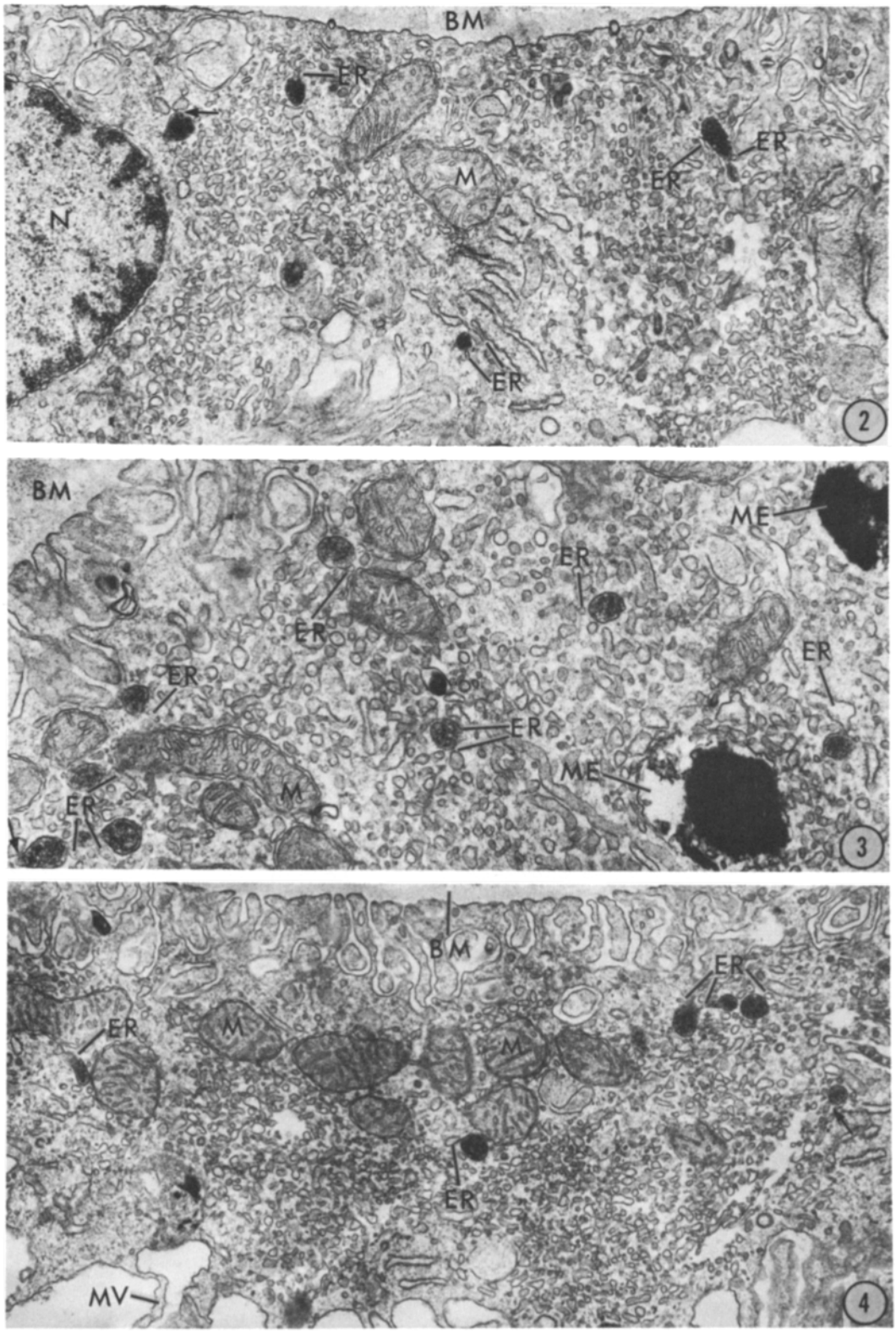
DISCUSSION

An earlier publication (32) from this laboratory described microperoxisomes in 31 mammalian cell types in tissue incubated in the alkaline DAB procedure used in the present investigation. The cells were classified roughly on the basis of the relative number of microperoxisomes. The pigment epithelial cells were placed in the second most numerous class, on the basis of our preliminary observations. The more extended observations reported in this communication would place these cells either at the top of this class or in the first class, i.e., that with a "large number" of these organelles. In any event, in all four rodents studied, pigmented and albino, microperoxisomes are numerous in pigment epithelial cells. This feature and an abundance of smooth ER with which the microperoxisomes are closely related characterize cell types in which the metabolism, transport, and storage of lipids are prominent. Recently, specific functional roles for microperoxisomes have been proposed for specific cell types of animals (1, 33).

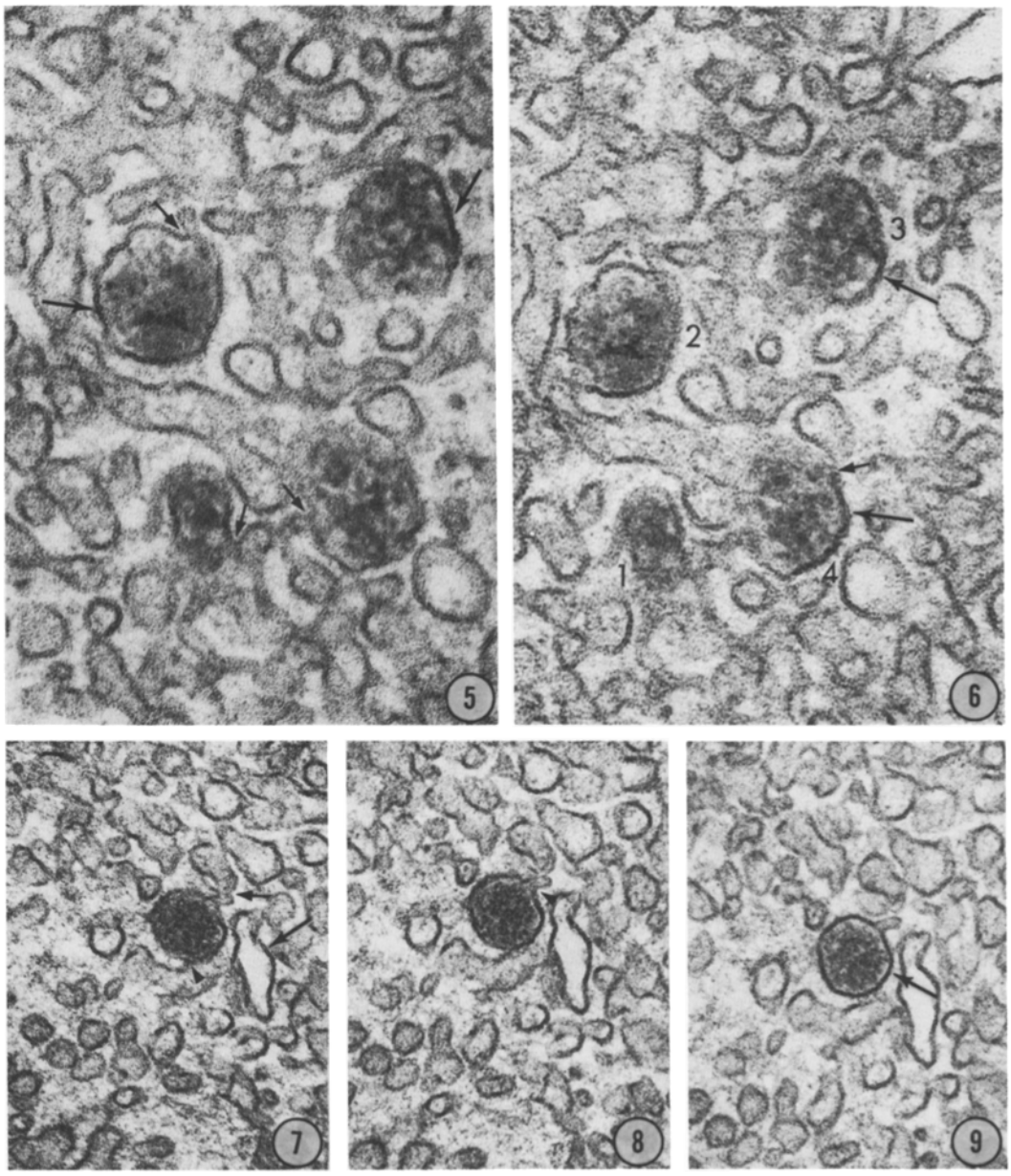
A recent autoradiographic analysis of frog retina (4) showed that fatty acids were incorporated very rapidly (in less than 5 min) in the pigment epithelial cells; then there was a progressive shift of label into the photoreceptor cells (cf. references 8 and 44). It has long been realized that the pigment epithelial cells phagocytose and degrade the lipoprotein disks discarded by the photoreceptor cells (2, 3, 14, 48–50). Also long known is the involvement of the pigment epithelial cells in vitamin A turnover (4, 8, 9, 16, 45) (also see references 22 and 39). There are high levels of vitamin A esters in the pigment epithelial cells. It is conceivable that the large number of microperoxisomes is related to reactions which remove vitamin A from the plasma β-lipoproteins and esterify it.

Two morphological findings in pigment epithelial cells are similar to those in other cell types. The first is the presence of microperoxisomes and smooth ER on the surface of lipid droplets. This is seen in hepatocytes (Fig. 4 of reference 29) and other cell types. The other is the presence of the two organelles, smooth ER and microperoxisomes, adjacent to complex lysosomes, the melanolysosomes, in the pigment epithelial cells of the beige mouse. This is similar to their relation to

FIGURE 1 A portion of the outer retina of a beige mouse. Incubation: DAB, pH 9.7, 90 min. Most of the field is occupied by a pigment epithelial cell. The "giant melanin granules" (25) or melanolysosomes (see text) are numbered *1–4;* the delimiting membrane of each is indicated by arrows. Large electron-opaque irregular melanin granules, scattered melanin, and less electron-opaque materials are seen within the melanolysosomes. There are about 20 microperoxisomes in the field, two of which are labeled (*MP*); they are DAB positive. All microperoxisomes show proximities to the endoplasmic reticulum (*ER*). Most of the ER in this cell type is smooth. One microperoxisome abuts a lipid droplet (*L*). Several microperoxisomes are seen adjacent to melanolysosome *1*. At the upper left of the figure a small portion of a photoreceptor cell (*PR*) is seen. Microvilli of the adjacent pigment epithelial cell are seen at *MV*. Also labeled are the endothelium of a choriocapillary (*EN*), Bruck's membrane (*BM*), coated vesicles (*CV*), mitochondria (*M*), and an area of parallel wavy filaments (*F*). × 28,000.

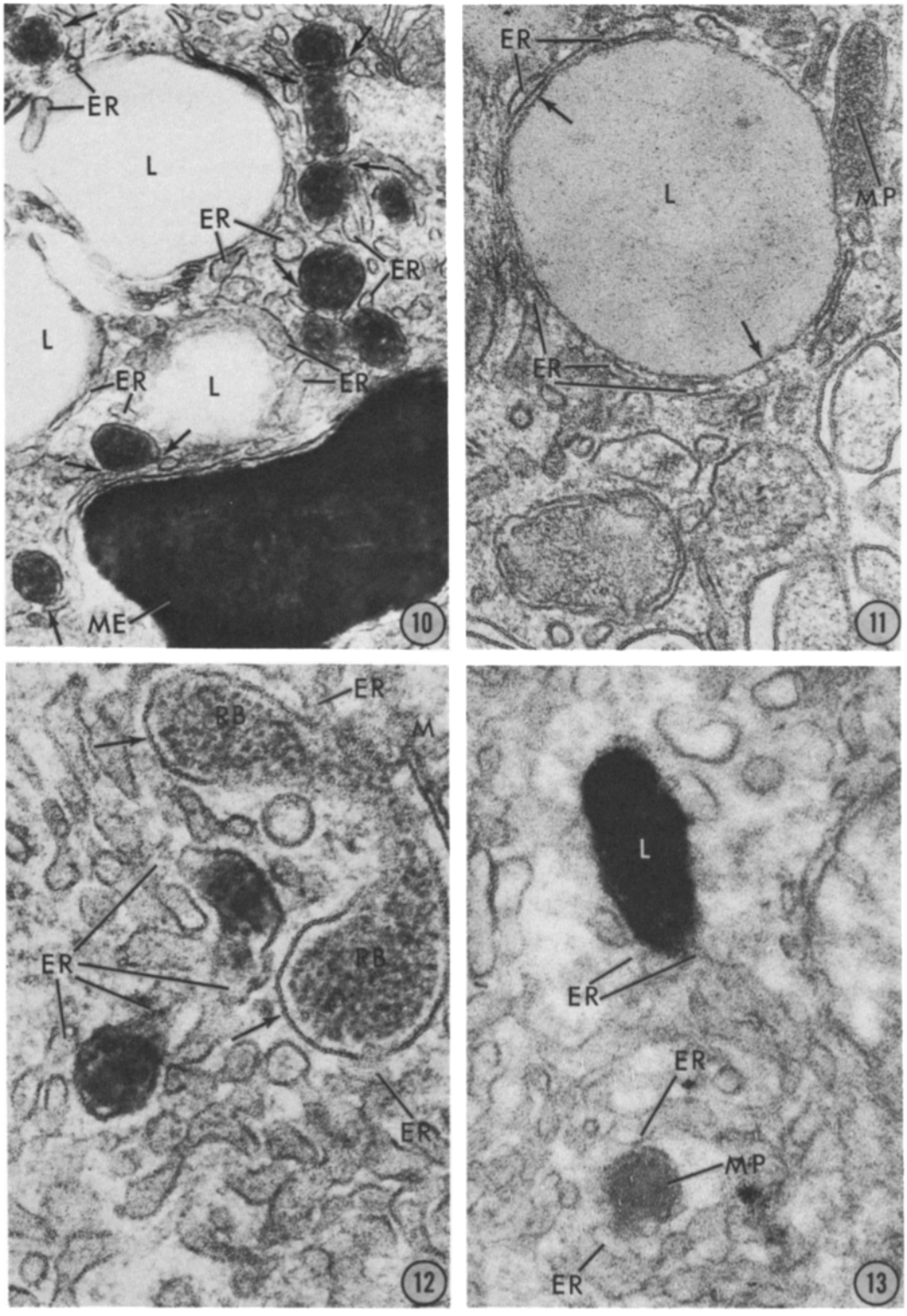


FIGURES 2–4 Portions of pigment epithelial cells of albino guinea pig (Fig. 2), C 57 black mouse (Fig. 3), and albino rabbit (Fig. 4). Incubation: DAB, pH 9.7, 80 min (Fig. 2), 60 min (Fig. 3), and 120 min (Fig. 4). Note the abundance of smooth ER. The microperoxisomes are DAB positive. They show their proximity to endoplasmic reticulum (*ER*) or continuity with (one arrow in each figure) the smooth ER. Also labeled are Bruch's membrane (*BM*), mitochondria (*M*), melanin granules (*ME*), microvilli (*MV*), and a nucleus (*N*). Fig. 2, × 19,000; Fig. 3, × 27,000; Fig. 4, × 19,000.



FIGURES 5 and 6 A portion of a pigment epithelial cell of an albino guinea pig. Incubation: DAB, pH 9.7, 60 min. Fig. 5, untilted specimen; Fig. 6, specimen tilted to $-30°$. The microperoxisomes are numbered *1–4* in Fig. 6. Different regions of the membrane delimiting the microperoxisomes show their tripartite nature (longer arrows) in the two views. The same is true of the smooth ER. The shorter arrows indicate the proximity of smooth ER and the delimiting membranes of the microperoxisomes. × 131,000.

FIGURES 7–9 A portion of a pigment epithelial cell of an albino rabbit. Incubation: DAB, pH 9.7, 80 min. Fig. 9, untilted specimen; Fig. 8, specimen tilted to $+36°$; Fig. 7, specimen tilted to $+42°$. Longer arrows in Figs. 7 and 9 indicate regions where the tripartite nature of the smooth delimiting membrane of the microperoxisome and of the ER are evident. The short arrow in Fig. 7 shows a continuity between two regions of smooth ER that is not evident in Fig. 9. Arrowheads in Figs. 7 and 8 show different continuities between the delimiting membrane and smooth ER. × 71,000.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lipofuscin granules, also complex lysosomes, in human liver (29, 34). The biochemical significance of these spatial relations is presently unknown.

It is of interest that the number and structure of the microperoxisomes are the same in the albino and pigmented eyes. These organelles appear to be dilated areas of, or derivatives from, smooth ER widely distributed in the cytoplasm. In contrast, premelanosomes are probably derived from the specialized region of ER found at the inner (trans) surface of the Golgi apparatus (GERL) (30).

Connock et al. (5) have recently isolated microperoxisomes from homogenates of the absorptive cells of the small intestine. The isolated fraction was rich in D-amino acid oxidase activity as well as catalase activity. Cytoplasmic particles rich in D-amino acid oxidase activity have been separated from rabbit retina (13) but their localization in pigment epithelial cells was not tested.

The first publication on microperoxisomes from this laboratory dealt with absorptive cells of the small intestine (35). Although these cells contain huge numbers of microperoxisomes their existence was unrecognized by the many electron microscopists who had studied these cells. However, they became unmistakable when sections were incubated in the alkaline DAB medium for catalase. Similarly, in previous studies of pigment epithelial cells in unincubated tissue by one of us (P.M. Leuenberger), and by others, these organelles were overlooked.

Incubation in the alkaline DAB medium also revealed an extensive DAB-positive tubular system in the terminals of photoreceptors. These tubules, approximately 13 nm wide, have some resemblance to smooth ER. Connections with ER or synaptic vesicles were not encountered. The basis of its DAB reactivity was not studied. This tubular system appears to be the same as that first described by Yamada (47), then by others (6, 23, 37, 43), most extensively by Lovas (23).

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The work has been presented in preliminary form (21).

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FIGURE 10 A portion of a pigment epithelial cell of a beige mouse. Incubation: DAB, pH 9.7, 60 min. Three lipid droplets (*L*) are seen. As in liver (29, 36) smooth ER is found at the surface of the lipid droplets (*ER*). Related to the ER on the lipid surface are microperoxisomes (DAB positive) (cf. Fig. 21 in reference 36 and Fig. 4 in reference 29). At *ME* a melanolysosome (cf. Fig. 1) is seen; smooth ER and microperoxisomes are present at its periphery. Arrows, there and elsewhere, indicate proximities of microperoxisomes to smooth ER. × 50,000.

FIGURE 11 A portion of a pigment epithelial cell of a C 57 black mouse. The tissue was *not* incubated in DAB medium. Much of the field is occupied by a lipid droplet (*L*). The limit of the lipid is indicated by two arrows. Covering portions of the lipid surface are smooth ER (*ER*) and a microperoxisome (*MP*). × 73,000.

FIGURE 12 A portion of a pigment epithelial cell of an albino guinea pig. Incubation: DAB, pH 9.7, 60 min. A small part of a mitochondrion is seen at *M*. As in guinea pig duodenum (see Figs. 31 and 32 in reference 35), residual bodies (*RB*) (arrows indicate thick tripartite membrane with "halo" underneath) and microperoxisomes (DAB positive) are related to adjacent regions of smooth ER (*ER*) (cf. Fig. 13). × 111,000.

FIGURE 13 A portion of pigment epithelial cell of an albino guinea pig. Incubation: CMP, 13 min, followed by DAB, pH 9.7, 30 min. Reaction product covers the lysosome (*L*), probably a residual body. The DAB reaction product in the microperoxisome (*MP*) is light, partly because the organelle is sectioned tangentially and partly because of the relatively short incubation time. Both organelles are related to adjacent smooth ER (*ER*) (cf. Fig. 12). × 90,000.

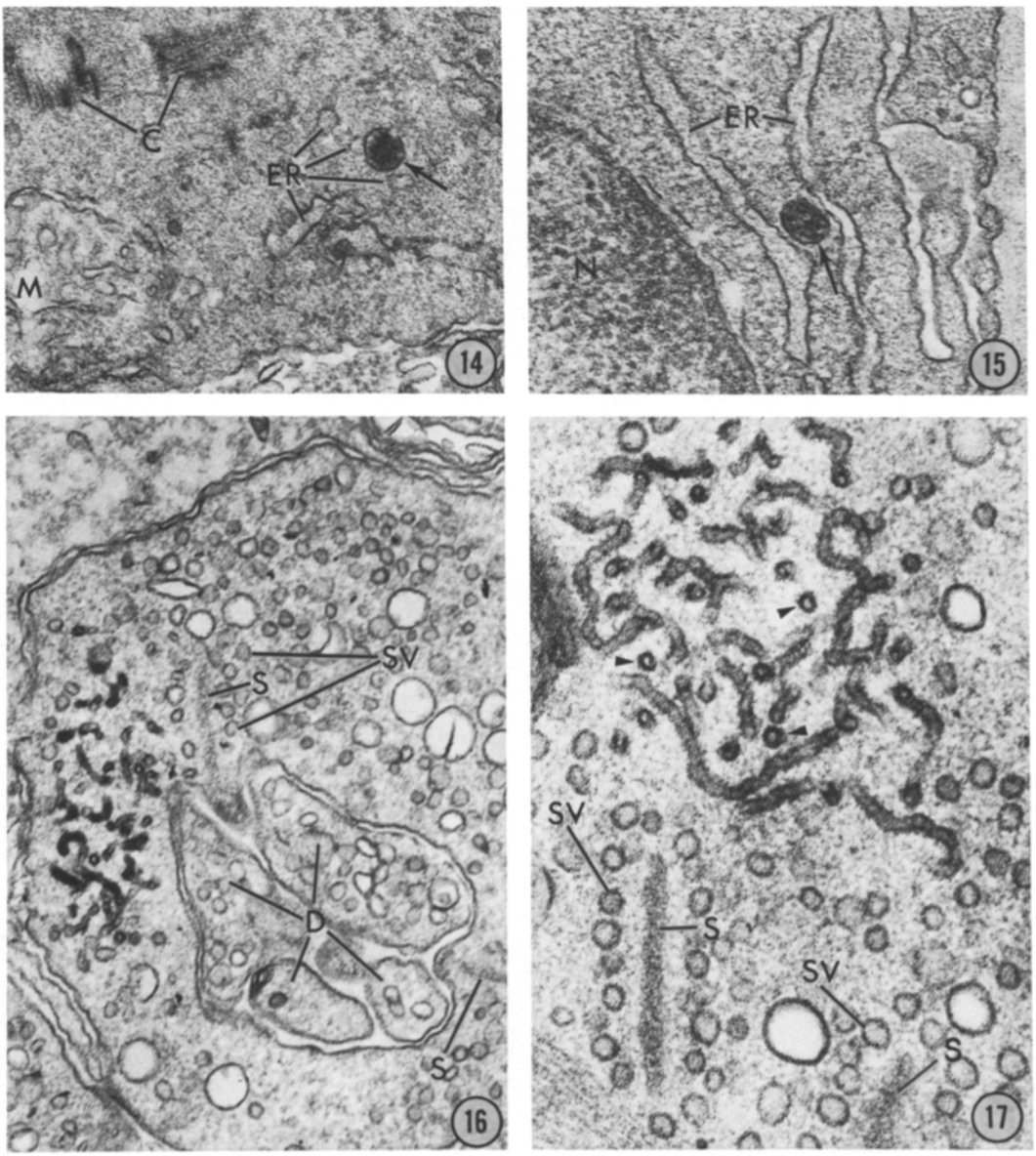

FIGURE 14 A portion of an endothelial cell in the choriocapillary of an albino guinea pig. Incubation: DAB, pH 9.7, 60 min. A microperoxisome is seen at the arrow; it is DAB positive. Smooth ER is indicated (*ER*). Also labeled are a mitochondrion (*M*) and a pair of centrioles (*C*). × 57,000.

FIGURE 15 A portion of a Müller cell in the beige mouse. Incubation: DAB, pH 9.7, 90 min. A microperoxisome is seen at the arrow; it is DAB positive. Note adjacent endoplasmic reticulum (*ER*). A portion of the nucleus is seen (*N*). × 55,000.

FIGURE 16 A portion of a photoreceptor cell terminal in the albino guinea pig. Incubation: DAB, pH 9.7, 60 min. Dendrites present in a deep invagination into the terminal are indicated (*D*). Within the terminal may be seen synaptic ribbons (*S*) surrounded by synaptic vesicles (*SV*). At the left of the field is a system of narrow tubules that are DAB positive. Both transverse and longitudinal sections of the tubules are evident. × 50,000.

FIGURE 17 A portion of a photoreceptor cell terminal in an albino guinea pig. Incubation: DAB, pH 9.7, 60 min. Two synaptic ribbons are seen (*S*) and many synaptic vesicles (*SV*). The DAB-positive system is composed of tortuous branching tubules. The tubule cavity is seen most clearly in transverse sections (arrowheads). × 80,000.

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