THE FINE STRUCTURE OF THE PIGMENT EPITHELIUM IN THE ALBINO RAT

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ABSTRACT

In this report, particular attention is paid to the inclusion bodies found in the apical cytoplasm of the pigment epithelial cell. These bodies are of variable size and form. The smallest (0.4 μ diameter) consist of a granular matrix enclosed by a single membrane, and are similar to the lysosomes of hepatic cells. Larger inclusion bodies contain areas of lamellated material in addition to granular matrix. The largest particles seen (2 μ diameter) are almost entirely lamellar. These different forms seem closely related, for it is possible to find all transitional stages between the smallest and largest particles. The relationship between the lamellar inclusion bodies and the rod outer segments is discussed.

For almost a century, it has been recognized that the pigment epithelium plays a vital role in the physiology of vision. Kühne in 1879 showed in a classic experiment that for visual pigment to regenerate in a frog retina in the dark the retina must be in intimate contact with pigment epithelium (14). For this to happen, Kühne noted that the pigment epithelium must be alive although the retina could be dead.

In recent years, we have begun to learn more of the intimate relationship between visual cell and pigment epithelium. For example, during light adaptation of the eye, vitamin A, formed in the rod outer segments from retinene (vitamin A aldehyde) released during the bleaching of rhodopsin, migrates to the pigment epithelium where it is stored. During dark adaptation, vitamin A flows back from the pigment epithelium into the rod outer segments as the rhodopsin is resynthesized (5). Furthermore, for the synthesis of rhodopsin, vitamin A must be both oxidized to retinene and isomerized to the 11-*cis* configuration. The only enzyme so far isolated that mediates this isomerization has been found in both the rod outer segments and pigment epithelium (10); and the pigment epithelium stores considerable amounts of 11-*cis* vitamin A (11, 13).

Several reports on the fine structure of the pigment epithelium have appeared, but little of this work has involved the pigment epithelium of mammals. Porter and Yamada's recent paper describing the pigment epithelium of the frog is particularly complete and detailed (20). In studying the pigment epithelium of the albino rat we have found that its fine structure differs somewhat from that described in lower vertebrates, and also presents certain features not before observed in pigment epithelial cells.

METHODS AND MATERIALS

The eyes of albino rats from the Harvard University colony were studied from the 7th day after birth to 12 months of age. The outer segments of the visual cells in the rat begin to differentiate at about 9 days of age and are adult in size and function by day 25 (7). The majority of the observations on adult animals were made between 2 and 6 months of age. For comparison, a few eyes of adult hooded rats of the Long-Evans strain were also examined.

Eyes from nembutal-anesthetized animals were enucleated, and the cornea and lens dissected away with a pair of fine scissors. The whole back of the eye was fixed in a 2 per cent solution of osmium tetroxide, buffered to pH 7.8 with veronal acetate solution containing 45 mg per ml sucrose and 0.002

M calcium chloride. Fixation was carried out for 20 minutes at 0°C, followed by 1 hour at room temperature. The specimens were either dehydrated through a graded series of acetone-water mixtures and embedded in Araldite (9), or were dehydrated and embedded in Aquon, a water-miscible epoxy resin (8). In some cases, the back of the eye was cut into pieces after fixation and the retina removed from the pigment epithelium. In other cases, the whole back of the eye was embedded, in an attempt to preserve the normal relation of retina to pigment epithelium. However, some separation invariably occurred, and only in occasional areas was even close approximation preserved.

Thin sections were cut with a Porter-Blum microtome and stained with saturated uranyl acetate in 50 per cent ethanol. They were examined in an RCA EMU-3D electron microscope operated at 100 kv.

OBSERVATIONS

Figs. 1 and 5 show low power views of rat pigment epithelial cells. The pigment epithelium of the rat, like that of other vertebrates, consists of a single layer of flattened cells about 6 μ in thickness and about 10 to 15 μ in diameter. They contain large, centrally placed nuclei whose fine structure is unremarkable except for occasional clumping of the chromatin about the nuclear edges (Fig. 5).

The basal surface of the pigment epithelial cell rests on a prominent basement membrane adjacent to Bruch's membrane, which separates the pigment epithelial cell from the vascular choroidal tissue. Bruch's membrane appears as an irregular band of relatively low density, about 0.5 μ thick, containing a few loosely arranged fibers exhibiting the banding of collagen (Fig. 5). A thicker, more homogeneous lamina is usually seen in the middle of Bruch's membrane (Figs. 1 and 5). This lamina probably corresponds to the *lamina elastica choroides* seen with the light microscope (21).

The plasma membrane on the basal surface of the pigment epithelial cell is very highly convoluted, sending numerous tubular invaginations a micron or so into the cell (Fig. 1). The invaginations are so extensive that they fill this basal zone to the exclusion of other cellular organelles.

At the opposite (apical) border of the cell, long villous extensions of the cytoplasm project outward toward the retina (Figs. 3 and 4). In the intact eye, these processes fit tightly between the visual cell outer segments. We have never succeeded in observing precisely how far the processes extend down the rod outer segments, but some of the isolated processes we have measured are about 6 μ long, approximately $\frac{1}{4}$ the length (20 to 25 μ) of a rat rod outer segment.

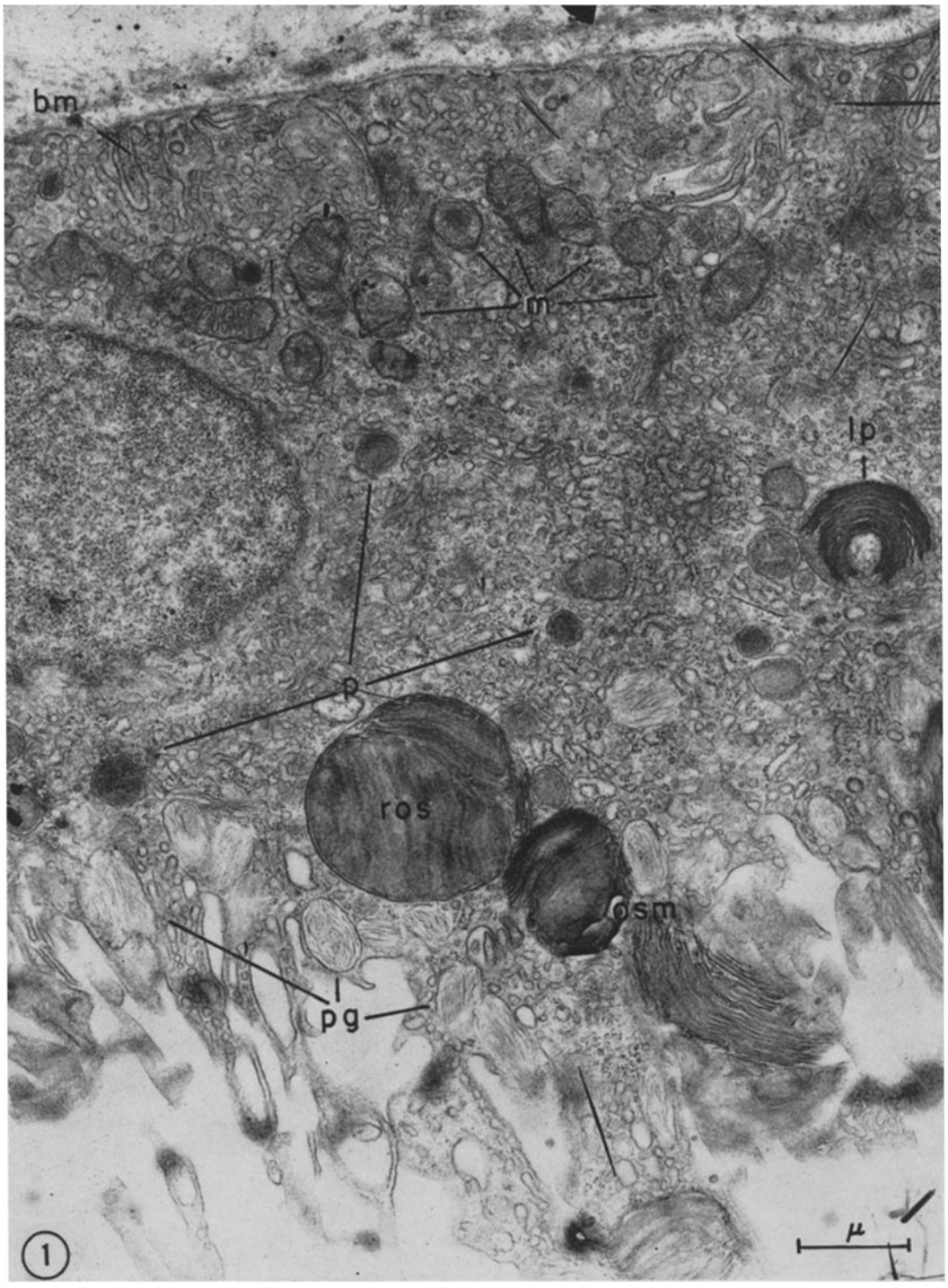
Around the apical border of the cell, between the villous processes and the main body of cytoplasm, is a thin terminal bar, which appears to be made up of a finely fibrous discontinuous net. In a few sections the terminal bar appears very prominent, but in most sections it is scarcely visible (Fig. 3).

At the lateral margins of the apical border, the pigment epithelial cells show regularly shaped interdigitations (Figs. 2 and 3). These are circular in cross-section, and may extend 1 to 2 μ into the neighboring cell. In Fig. 2, two interdigitations can be seen, one running at right angles to the other. The terminal bar runs into the interdigitating extensions and forms a desmosome-like structure between one cell and the next.

The cytoplasm of the rat pigment epithelium is filled with a dense vesicular and tubular endoplasmic reticulum which extends into the apical processes. In the thicker processes, the vesicles characteristically lie in quite regular rows parallel to the long axis of the process (Fig. 4). The vesicles often appear to fuse, forming long tubules which run down the process. Occasionally the

FIGURE 1

Low power electron micrograph showing pigment epithelial cell from 15-day-old animal. At the top of the micrograph, mitochondria (*m*) are seen just below the infolded basement membrane. A prominent lamellar inclusion particle (*lp*) is seen in the right center of the micrograph, along with some granular particles (*p*). Precursor pigment granules (*pg*) are scattered in the apical cytoplasm. A rod outer segment (*ros*), seen in cross-section, appears enclosed within pigment cell cytoplasm along the lower border of the cell. In young eyes, outer segment material (*osm*) is frequently seen, as here, in close association with the apical processes. Bruch's membrane, *bm*. Magnification, 16,000.


bm
m
lp
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ros
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1

tubules appear collapsed, giving the appearance of parallel membranes running down the process. At the distal end of the processes, the tubules may be open and in direct contact with the extracellular space (Fig. 4).

The rod outer segments, especially when seen in cross-section, are so completely enveloped by the apical processes as sometimes to give the appearance of being entirely enclosed within the pigment epithelial cell (Fig. 1). This is seen particularly frequently in young retinas (10 to 20 days after birth), when the outer segments are still irregular and lengthening. In this condition, the distal ends of the rod outer segments seem to adhere much more firmly to the pigment epithelium. Frequently in young retinas, outer segment membranes are seen closely applied to the pigment epithelial processes, and oriented parallel to the long axis of the outer segment, instead of transversely, as in the adult rod (Fig. 1).

Throughout the bulk of the pigment epithelial cytoplasm, many particles having the density and dimensions of ribosomes are seen (Fig. 1). These particles are usually associated with the endoplasmic reticulum, but are also in unattached clusters. There are very few ribosome-like particles, however, in either the apical processes or the convoluted basal zone.

The mitochondria of these cells are typical, their cristae often extending completely across their width. The mitochondria have a very characteristic distribution, being found primarily just below the edge of the convoluted basal margin and along the lateral boundaries of the cell (Figs. 1 and 3). Along the lateral borders of the cell, there is a single row of mitochondria, parallel with the cell margin and closely applied to the membrane (Fig. 3). Relatively few mitochondria occur elsewhere in the cell.

One of the most striking features of the pigment epithelial cell in rats is the presence in the apical cytoplasm of a large number of inclusion bodies (Figs. 5 and 6). The morphology of these particles is extremely variable, but close inspection reveals features common to most of them. All related particles are characteristically surrounded by a single membrane. We occasionally see a different type of inclusion body, not surrounded by a membrane, but these are seen only rarely.

The membrane-limited particles vary greatly in size and structure. The smallest and most numerous are small round bodies about 0.4 μ in diameter enclosed in the single limiting membrane, and containing a granular matrix. Separating the matrix from the surrounding membrane is a fairly regular space (100 to 120 A thick) of relatively low density (Fig. 10). Next in order of frequency of occurrence are slightly larger particles 0.5 to 0.7 μ in diameter. These have exactly the same fine structure as the smaller particles but they characteristically contain one or more areas of darkly staining material within their granular matrix (Figs. 6 and 7). In many instances, presumably when the orientation is favorable, the darkly stained areas consist of evenly spaced lamellae (Figs. 7, 8). In other cases the darkly stained substance has a polygonal profile that suggests a crystalline nature (Fig. 8).

Quite frequently, larger particles (about 1 μ) are seen which are almost entirely lamellated, yet also contain some granular matrix, and are surrounded by a membrane (Fig. 9). Entirely membranous bodies of about the same size or larger (up to 3 μ) are also seen (Figs. 10, 11, 12, 14). In some cases the internal membranes in the bodies are parallel, so that the particle appears highly organized (Figs. 9 to 11).

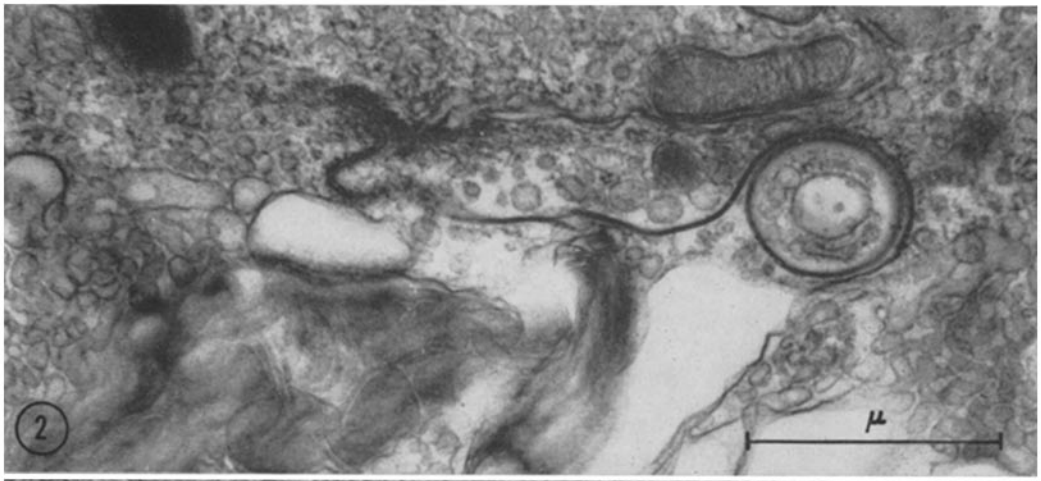
In the smaller particles each dense lamella is about 75 to 80 A thick, and close inspection shows it to consist of a pair of closely applied membranes

FIGURE 2

Micrograph of apical border of pigment epithelial cells showing finger-like interdigitations. One interdigitation is in the plane of the section; the other runs at right angles to the micrograph. Aquon-embedded specimen. Magnification, 32,000.

FIGURE 3

Apical border of two pigment epithelial cells showing detail of finger-like interdigitation and apical processes. Mitochondria (*m*) are seen, closely applied to the lateral borders of the cells. The terminal bar (*tb*) runs between the apical processes and the rest of the cell cytoplasm. Aquon-embedded specimen. Magnification, 35,000.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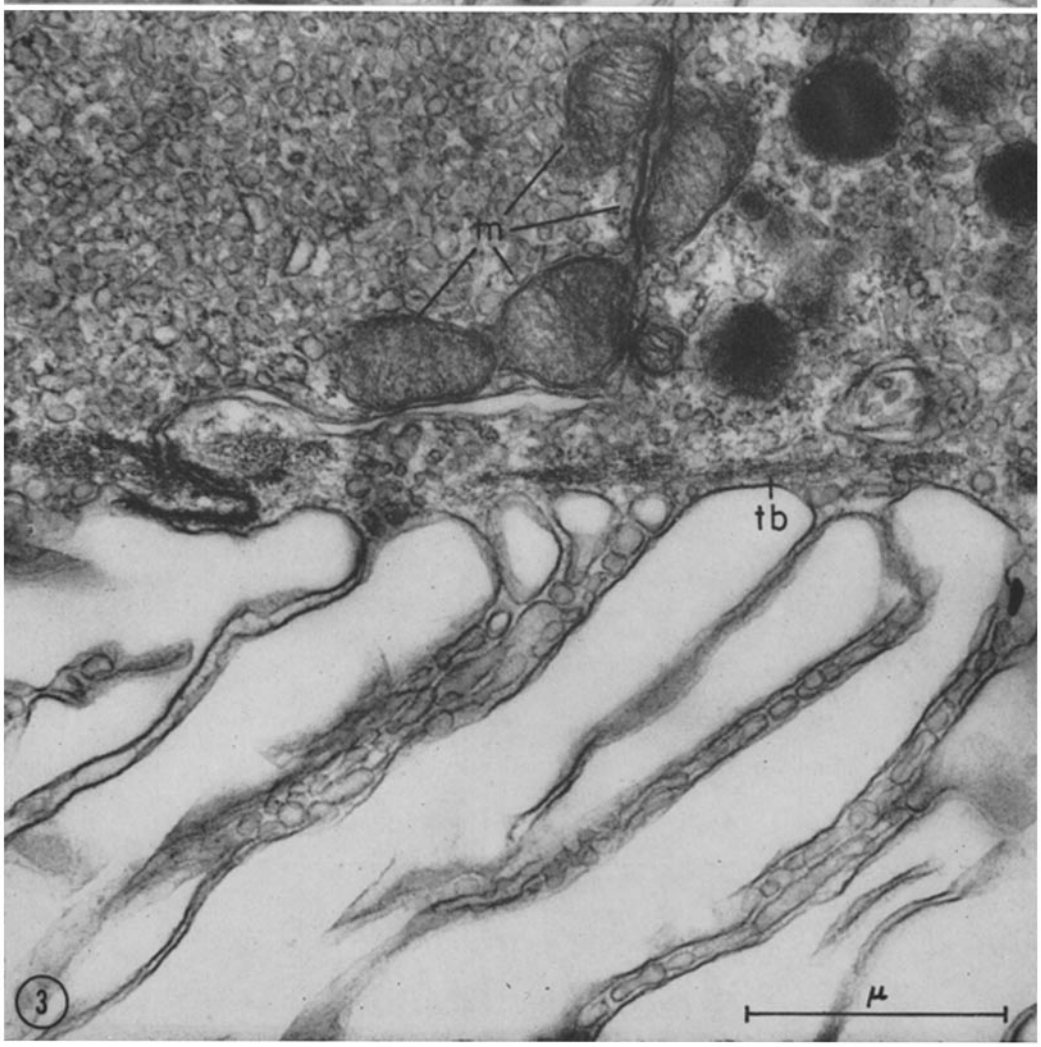
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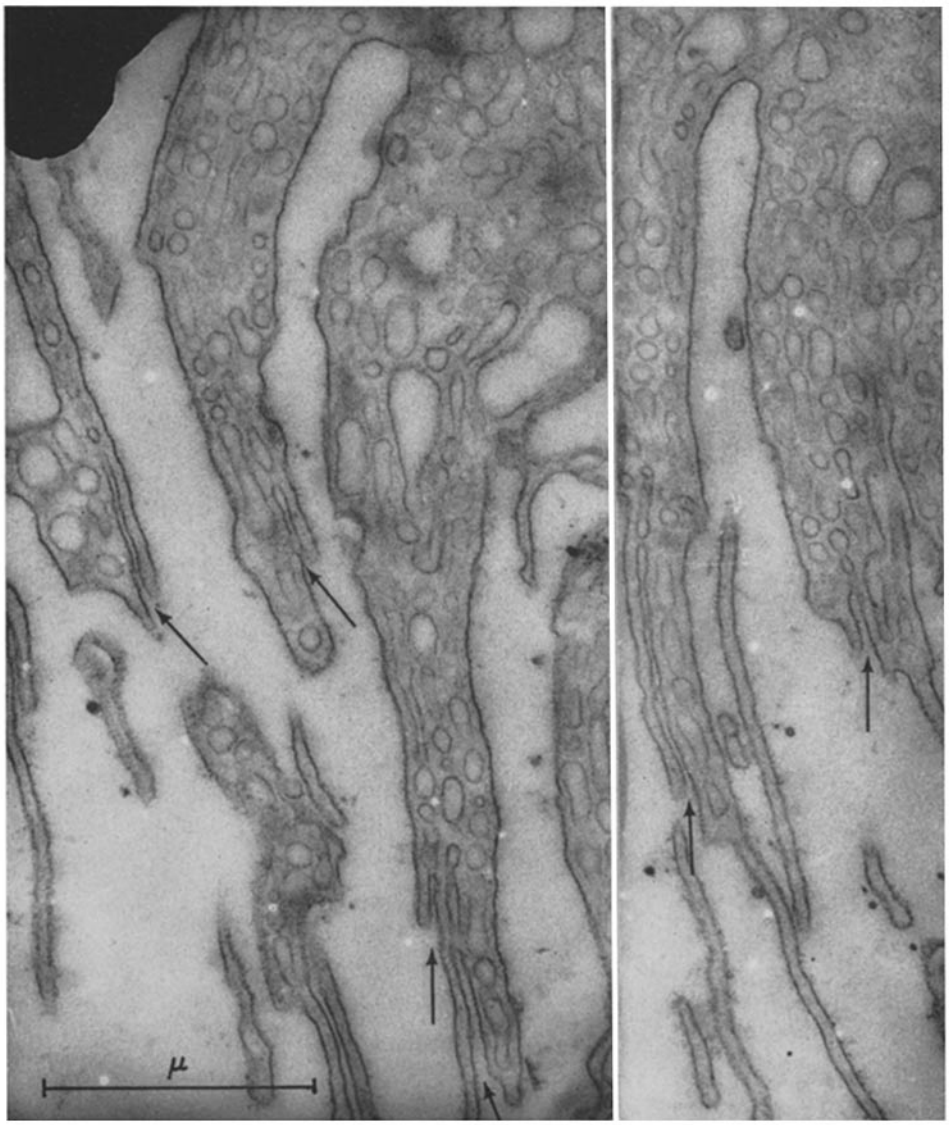
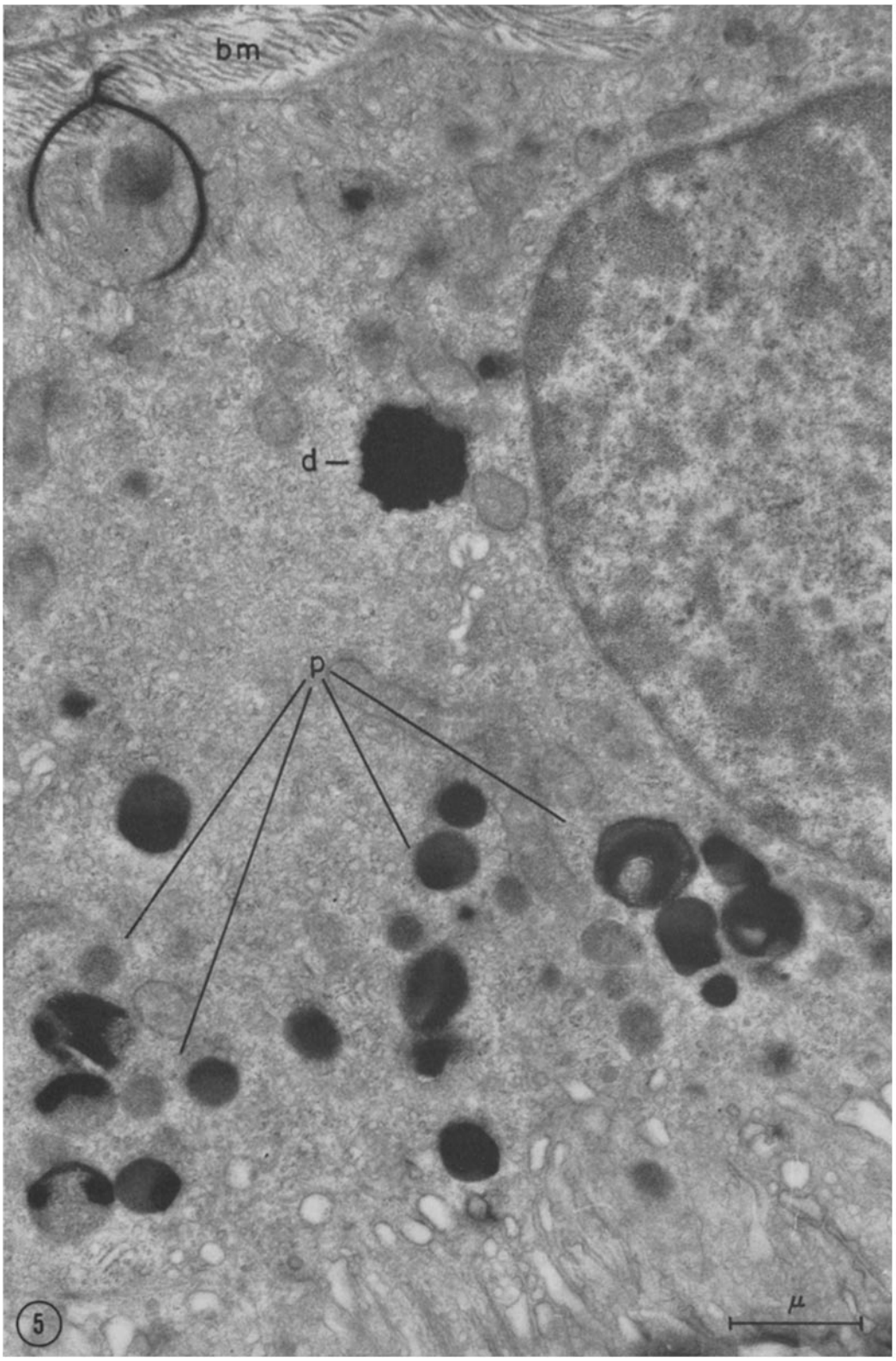


FIGURE 4

Higher magnification micrographs of the apical processes. The vesicles in the processes typically lie in regular rows, parallel to the axis of the process. The vesicles are often fused, forming tubules which run down the processes. At the end of the process, the tubules may be open and in direct contact with the extracellular space (arrows). Magnification, 40,000.

FIGURE 5

Micrograph of a typical adult pigment epithelial cell. Numerous inclusion particles (*p*) are seen in the apical cytoplasm. A small lipid droplet (*d*) in the top half of the cell is identified by its scalloped outline. Many fibers exhibiting the banding of collagen are seen in Bruch's membrane (*bm*) at the top of the micrograph. Magnification, 20,000.


bm
d
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5
μ

each about 40 A thick. This doubleness is clearest near the ends of the lamellae where the membranes, often loop around or fray apart (Figs. 9, 13). The largest of the particles, on the other hand, contain principally single membranes (40 A thick) which are only occasionally paired in close approximation (Fig. 14). Particles of intermediate size, such as that shown in Fig. 13, contain both paired (Fig. 13 *a*) and single membranes (Fig. 13 *b*) in different regions. It is notable that the membranes are usually more tortuous and their arrangement less regular when they are single (*e.g.* Fig. 14) than when they are paired (*e.g.* Fig. 11).

Although all these membrane-limited particles vary considerably in appearance, they seem to be related, and one can construct from the micrographs what appears to be a continuous transition of stages from the smallest, entirely granular particle to the largest of the membranous bodies. We shall have more to say of this below.

The second type of inclusion body, which has no limiting membrane, is observed much less frequently. Bodies of this type usually have rather irregular scalloped contours and may measure from 0.5 to 2 μ in width (Fig. 5). Their interior is homogeneous and of moderate density; no evidence of internal structure has been found. These bodies appear similar to the non-laminated lipid bodies that Porter and Yamada found prominent in pigment epithelial cells of frog (20).

Development of the Pigment Epithelial Cell

In the course of studying the development of the retina, we have had opportunity to observe the pigment of epithelium before, during, and after the development of the rod outer segments. Here we will not describe the entire development of this cell, but only aspects that are pertinent.

The rod outer segments in the rat do not begin to form until the 9th or 10th day after birth (Fig. 15). At day 9, the inner segments have extended beyond the external limiting membrane and are beginning to lengthen. An occasional cilium or basal body is seen at the distal end of the inner segment, but little outer segment material is present. The apical border of the pigment epithelial cell, at this stage of development, is very different from that of an adult cell, in that there are no processes extending outward (Fig. 15). In the apical part of the cell there are many prominent vesicles similar to those found later in the processes, but they are randomly scattered through the cytoplasm.

As soon as the outer segments begin to form, however, the apical processes are seen. By day 11 or 12 they are very prominent, and by day 15 are adult in appearance (Fig. 1). Our micrographs suggest that the processes arise from the vesicles in the apical cytoplasm which line up in parallel rows and then fuse to form long tubular invaginations (see Fig. 4). Such a mechanism for forming an elaborately infolded system of plasma membranes has been suggested from observations on the aqueous-humor-secreting ciliary epithelium (17). The appearance of the membranous inclusion bodies in the pigment epithelial cytoplasm also occurs at the same time as the formation of the outer segments. Only granular particles are seen before the rods begin to form, but by day 15 (when the rod outer segments are all present but only about half their mature length) both granular and membranous particles are present (Fig. 1), though much less numerous then in the adult.

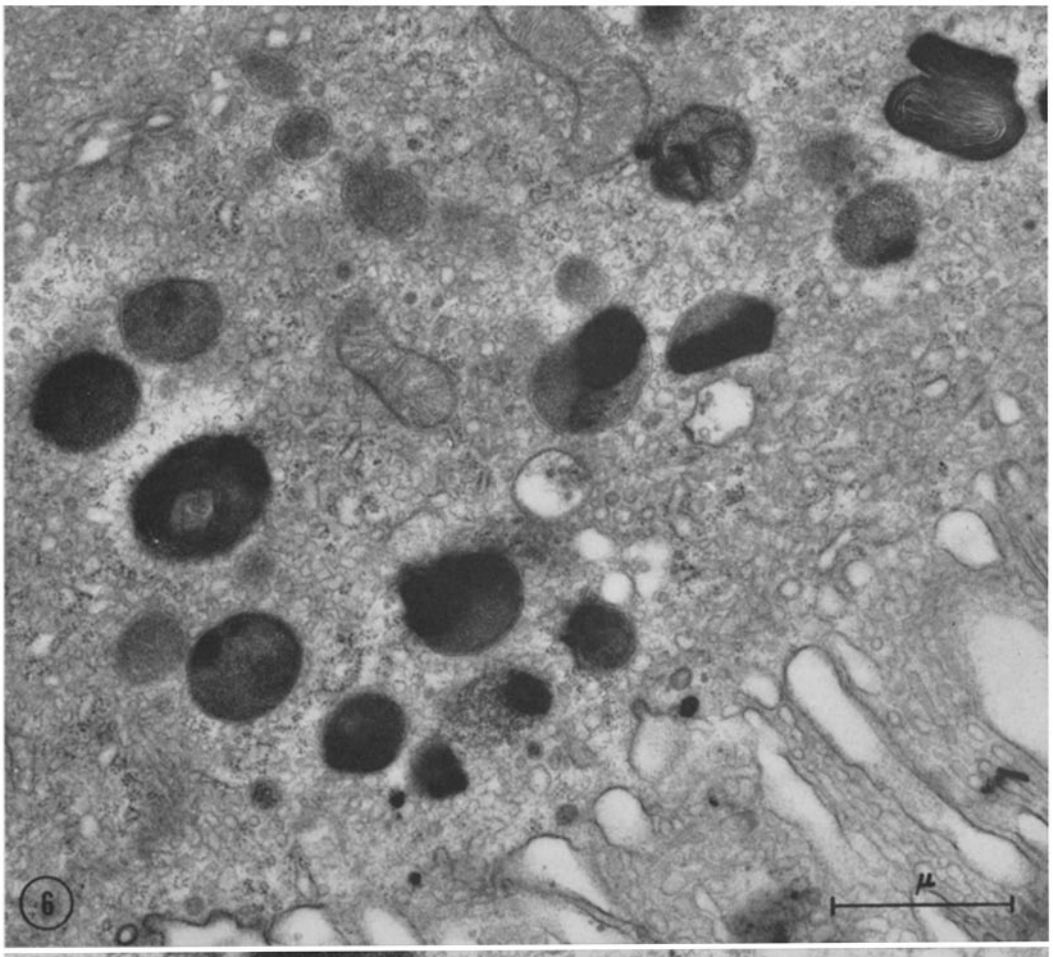
In the pigment epithelium of an adult albino rat, there are no pigment granules (Fig. 5). However, in the young animal, one sees precursor or pigmentless pigment granules (15) (Figs. 1 and 15). The pigment granules develop apparently normally up to the point of pigment formation. At this stage, the genetic block is expressed, pre-

FIGURE 6

Micrograph showing a variety of small inclusion particles in the apical cytoplasm of a pigment epithelial cell. The particles are all surrounded by a single membrane and contain varying amounts of dense material. Magnification, 20,000.

FIGURE 7

Higher magnification of several of the particles shown in Fig. 5. When the orientation is favorable, the densely stained material in the particles often appears lamellated. Magnification, 50,000.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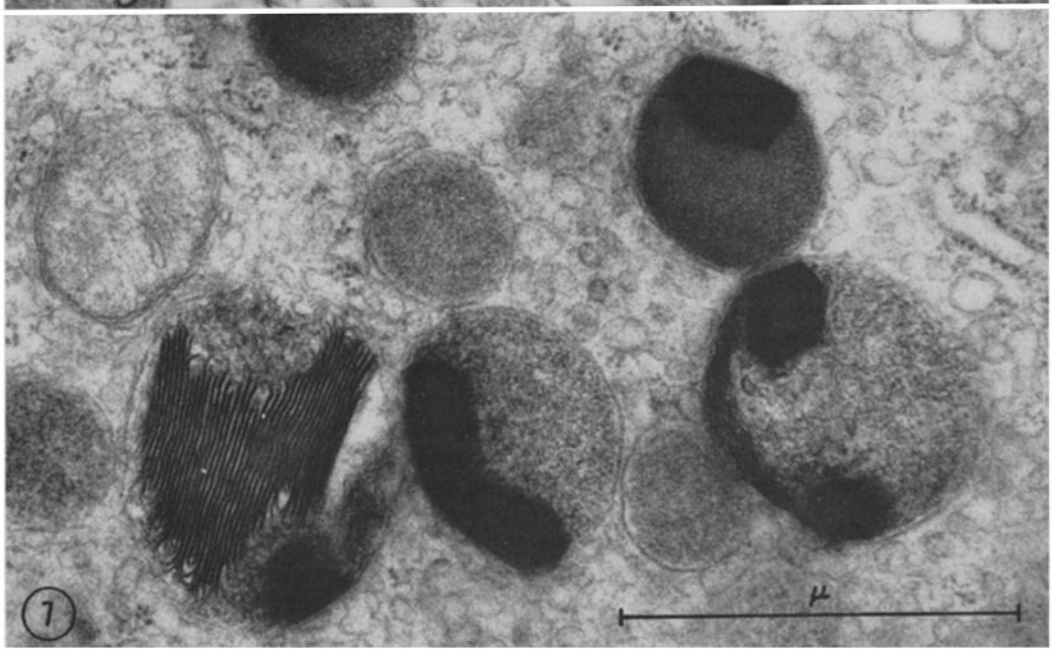
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venting melanization, and subsequently the granule disappears. The pigmentless pigment granules are most numerous at about 12 days of age, and are completely gone by the time the animal is 25 to 30 days old. Figs. 15 *a* and *b* show two pigmentless pigment granules at high magnification, one cut longitudinally, the other in cross-section. They are usually oval in shape, about 0.7 μ long and 0.4 μ wide, and are surrounded by a prominent membrane which occasionally, however, appears incomplete (Fig. 15 *b*). Fine parallel lamellae inside the granule, when seen in cross-section, are arranged in a spiral. The internal structure of the granule therefore seems to be made up of a single sheet of material curled in jelly-roll fashion.

DISCUSSION

There are several reasons for believing that the pigment epithelium plays a key role in maintaining the nutrition and metabolism of the rod outer segments. The rod outer segments, made up of flattened disks piled one on top of another, contain none of the cytoplasmic organelles we associate with protein synthesis (ribosomes) or oxidative phosphorylation (mitochondria), and, therefore, probably have to depend on assistance from neighboring structures, *i.e.* the pigment epithelium or inner segments. The contact between the pigment epithelium and the outer segment is more intimate and of much greater area than the contact between the inner and outer segments, which in the rat consists solely of the tenuous connecting cilium. Indeed, the entire distal end of the outer segment may be in direct contact with the body of a pigment epithelial cell, in addition to being surrounded by the apical processes extending along the length of the rod. When retina and pigment epithelium become separated, vision is quickly lost, but can be restored if contact can be reestablished, even if the separation has continued for many months.

Furthermore, the rat, like other animals, has only a minor retinal circulation, and it appears that at least the outer segments and perhaps the whole visual cells receive nutriment from the choroidal blood supply (19). For substances to get to the outer segments, therefore, they must, of course, pass through the pigment epithelium. Thirdly, as we have already noted, exchange of vitamin A between outer segments and pigment epithelium takes place during the course of light and dark adaptation (5).

The fine structure of the pigment epithelial cells supports the notion of their having a nutritive role. The extensive infoldings of their basal membrane and the close presence of numerous mitochondria are features reminiscent of the cells which line the convoluted tubules in the kidney (18).

This condition suggests that this surface of the pigment epithelial cell is actively absorbing substances from the vascular choroidal layer. The characteristic lining up of a single row of mitochondria along the lateral membranes of the cell perhaps indicates an energy-requiring process in

FIGURE 8

A high magnification micrograph showing both lamellar and polyhedric nature of dense substance in the inclusion particles. Magnification, 62,000.

FIGURE 9

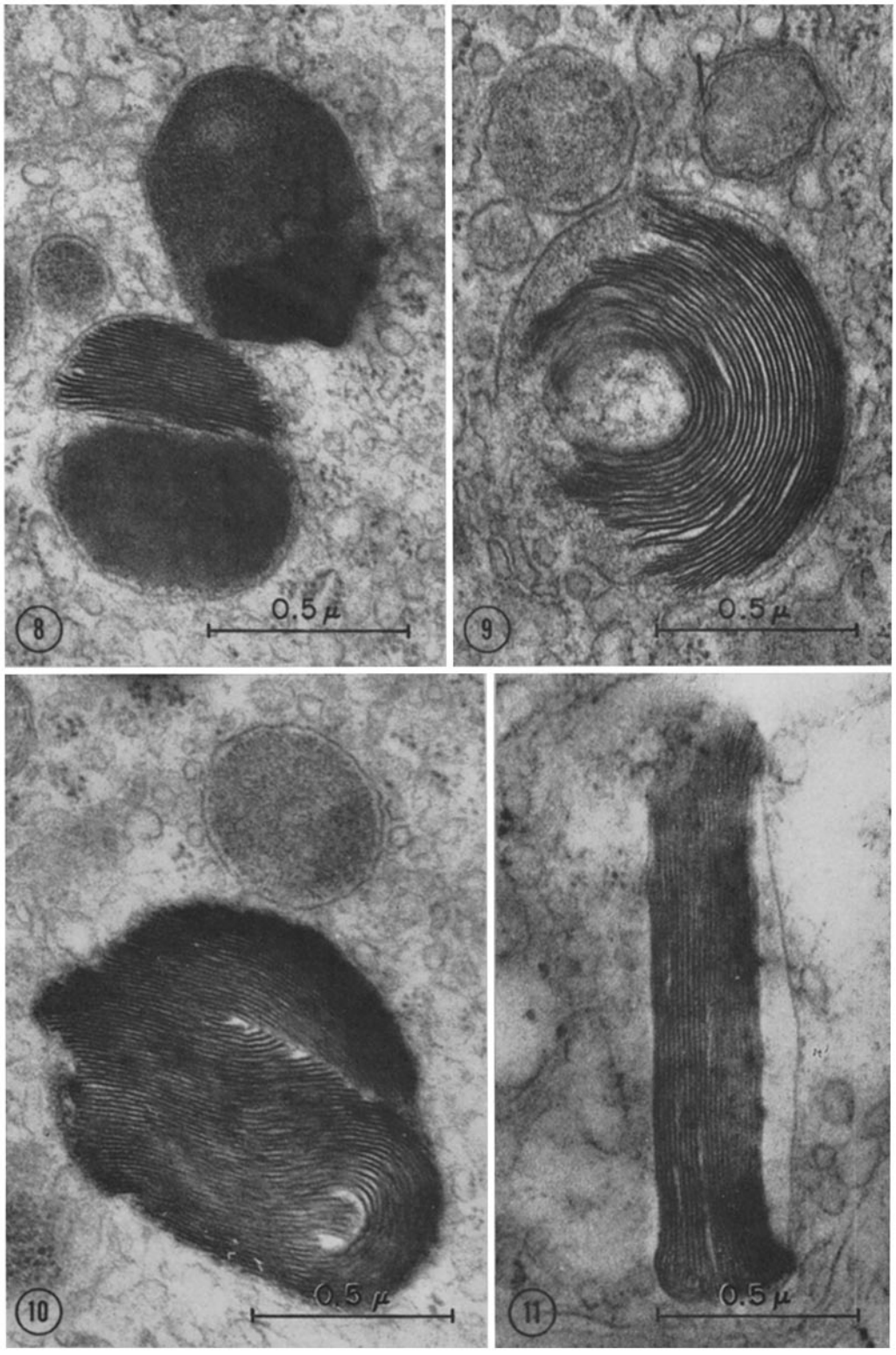
Enlargement of lamellar inclusion particle seen in Fig. 1. The lamellae are seen to be double at the ends where they have frayed apart. Magnification, 62,000.

FIGURE 10

Micrograph showing granular and lamellar particles side by side. The regular electron-transparent zone between the enclosing membrane and granular matrix in the granular particle is seen in many of the particles (See Fig. 6). Magnification, 62,000.

FIGURE 11

A very regular lamellar particle. The lamellae are seen clearly as double in this micrograph. Magnification, 62,000.


0.5 μ
8
0.5 μ
9
0.5 μ
10
0.5 μ
11

these membranes or in the narrow intercellular space.

The structure of the apical processes suggests their involvement also in transport. Since they cover the whole apical surface of the pigment epithelium they must be involved in some way in the migration of vitamin A between the visual cells and pigment epithelium that occurs in the course of light and dark adaptation. The detailed mechanism by which this migration occurs is not established, but the numerous parallel rows of vesicles and tubules in the processes suggest a process of micro-vesiculation, somewhat similar to that by which fat is absorbed from the jejunum (16). However, we have not as yet been successful in following the migration of vitamin A directly in our electron micrographs. A preliminary autoradiographic study with C^{14}-labeled vitamin A (Sidman and Dowling, unpublished results) has shown that vitamin A is completely extracted from the retina and pigment epithelium in the course of the usual preparation of specimens for electron microscopy.

As we have noted, perhaps the most interesting feature of the pigment epithelial cells is the presence of numerous inclusion bodies in the apical cytoplasm. The smallest and most numerous of these particles have a very regular morphology and are strikingly similar in appearance to the lysosomes (4) of the liver and other cells. Both lysosomes and the particles described here are spherical, about 0.4 μ in diameter, and consist of a granular matrix, surrounded by a single membrane with a characteristic electron-transparent zone 100 A wide between the matrix and the membrane. The liver lysosome particles are known to contain large amounts of enzymes (4). The great majority of enzymes so far demonstrated in lysosomes are hydrolytic in nature, but it appears that the particles are very heterogeneous and may contain many different enzymes.

The other inclusion particles we see have many characteristics similar to the lysosome-like particles. They also are surrounded by a single membrane, and usually contain granular matrix material. However, they typically contain densely staining material which is frequently seen to be lamellar in nature. The larger particles are usually almost entirely lamellar.

Lamellar particles similar to these in the rat

FIGURE 12

A lower power micrograph showing a large membranous inclusion particle in the center of a pigment epithelial cell. Other inclusion particles (*p*) are seen in the cytoplasm, and apical processes. Magnification, 14,000.

FIGURE 13

An intermediate-sized lamellar particle containing both single and double lamellae. Magnification, 37,000.

FIGURE 13*a*

An enlargement of the double lamellae from the particle shown in Fig. 13. Magnification, 148,000.

FIGURE 13*b*

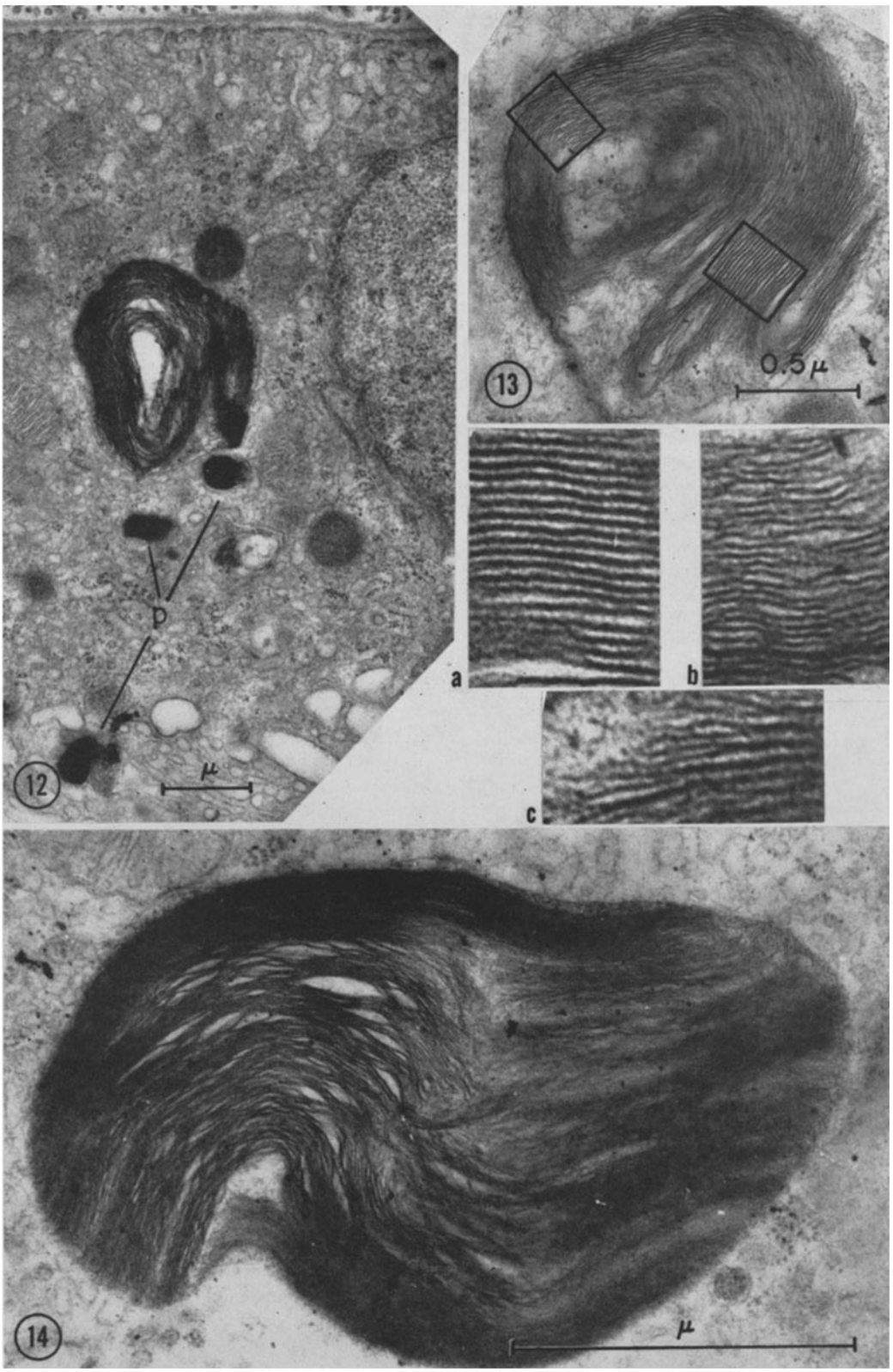
An enlargement of the single lamellae from Fig. 13. Magnification, 148,000.

FIGURE 13*c*

An enlargement from Figure 13 showing separation of double lamellae into single lamellae. Magnification, 180,000.

FIGURE 14

A large lamellar particle containing mostly single membranes. The lamellae in these large particles characteristically are less regular than those in the smaller particles. Magnification, 50,000.


12
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c
14
μ

have been seen and noted in pigment epithelial cells of other mammals (1, 2, 23). In lower vertebrates, such as the frog and turtle, the pigment epithelial cells also contain prominent lamellar bodies (20, 22), but these structures, called myeloid bodies, appear fundamentally different from the lamellar body in the pigment epithelial cell of the rat. The myeloid bodies of the frog are apparently derived from and continuous with the endoplasmic reticulum and are not surrounded by a membrane. Furthermore, the myeloid bodies of the frog and turtle have a very regular and characteristic lamellar structure similar to that of a rod outer segment and do not show the variability of lamellar orientation and spacing seen in the inclusion bodies of the rat.

Like other workers, we have in the past termed the large membranous particles we observed myeloid bodies, also in the rat; yet in light of the fundamental differences between the two types of structure, it might be wise to drop this term for the particles in mammalian pigment epithelium.

Although the particles in the rat pigment epithelium are extremely varied in size, shape, and content, they all do seem related because of the structural characteristics they share. The many transitional stages suggest that the different forms of the particles might represent a sequence in which the larger membranous particles are formed from the smaller granular ones (or *vice versa*). However, in the absence of other evidence, static micrographs are insufficient to decide whether or not this represents an actively evolving sequence in the cell.

The presence of membranous structures in the pigment epithelium tempts one to consider whether these particles are related to the rod outer segments. We know little of the mechanism by which rod lamellae are formed in the eye. A recent study of inherited retinal dystrophy in the rat showed that, early in the disease process, there is an over-production of rhodopsin and rod-like lamellae which accumulates between the apparently normal visual cells and the pigment epithelium (7). This extracellular lamellar material appeared more closely linked to the pigment epithelium than to the rod cell, and it was suggested that the pigment epithelium might be a source of lamellae in the eye. The lamellated particles seen in the pigment epithelium may represent this activity of the cell.

On the other hand, there is some evidence that pigment epithelial cells are capable of ingesting trypan blue particles and are phagocytic (12). In the inclusion bodies we might be observing rod outer segment fragments engulfed in such a phagocytic process. The relation of the pigment epithelium particles to lysosome-like particles might be held to strengthen this view. However, electron microscopic examination of pigment epithelial cells during the active degeneration of visual cells in inherited retinal dystrophy in the rat gave no indication of phagocytic activity (7).

A more direct proof of the relation of the lamellated bodies in the pigment epithelium to the rod outer segments would be the detection of rhodopsin or photosensitive pigments in the particles. The pigment epithelial cells of the rat eye do not contain measurable quantities of vitamin A aldehyde (retinene), the prosthetic group of rhodopsin (5), and we have been discouraged so far from making a more exhaustive search for photosensitive pigment in pigment epithelial cells because of the difficulty of obtaining cells completely free from contaminating rod fragments. Recent refinements in the techniques of microspectrophotometry,

FIGURE 15

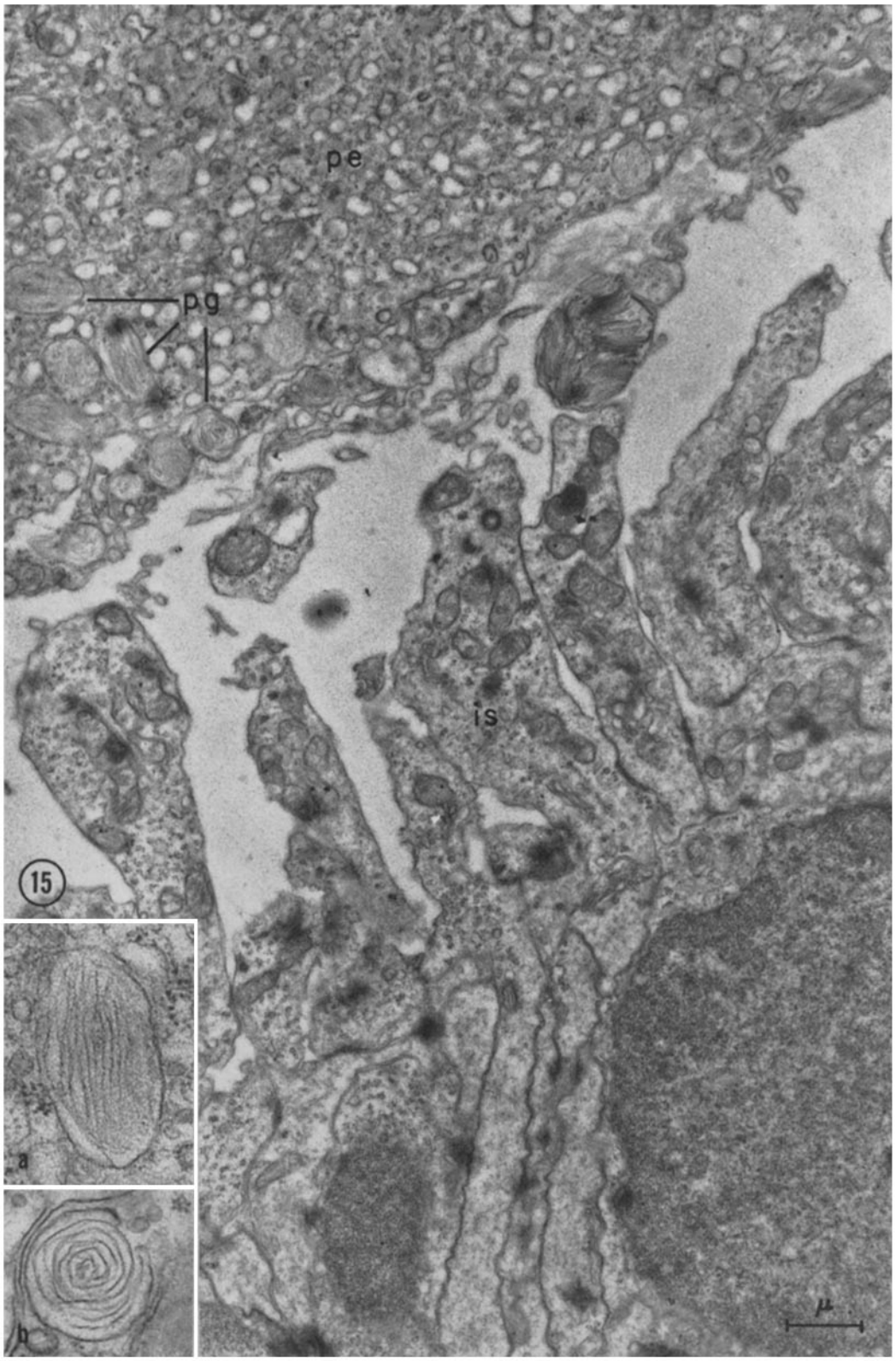
A low power micrograph showing lower border of pigment epithelial cell (*pe*) and inner segments (*is*) from 9-day-old animal. As yet, little outer segment material has formed. The lower border of the pigment epithelium has no processes, but its cytoplasm contains many small vesicles. Precursor pigment granules (*pg*) are present. Magnification, 10,000.

FIGURE 15*a*

Enlargement of precursor pigment granules. A number of fine lamellae are seen inside the granule. Magnification, 50,000.

FIGURE 15*b*

A cross-section of a precursor pigment granule. Magnification, 50,000.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however, may make it feasible to identify such pigments in small intracellular bodies *in situ* (3).

We have examined the pigment epithelium from vitamin A-deficient animals (6) and find that the larger laminated bodies disappear from the pigment epithelium at about the same time the rod outer segments disappear. The small granular particles do not disappear; indeed, the deficient cells appear to contain about the same number of them as normal cells. However, few of these particles contain any dark staining material. If vitamin A is re-fed to the deficient animal, the particles in the pigment epithelium assume a variety of forms, and within a few days large lamellar bodies are seen again. In summary, it appears that as the outer segment is dependent on vitamin A for its structural integrity; so also are the formation and maintenance of the membranous particles in the pigment epithelial cell. Beyond this we can say little that is definite about the function and significance of these particles.

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