# STUDIES ON THE ENDOPLASMIC RETICULUM

## V. Its Form and Differentiation in Pigment Epithelial Cells of the Frog Retina

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ABSTRACT

Pigment epithelial cells of the frog's retina have been examined by methods of electron microscopy with special attention focused on the fine structure of the endoplasmic reticulum and the myeloid bodies. These cells, as reported previously, send apical prolongations into the spaces between the rod outer segments, and within these extensions, pigment migrates in response to light stimulation. The cytoplasm of these cells is filled with a compact lattice of membrane-limited tubules, the surfaces of which are smooth or particle-free. In this respect, the endoplasmic reticulum here resembles that encountered in cells which produce lipid-rich secretions. The myeloid bodies comprise paired membranes arranged in stacks shaped like biconvex lenses. At their margins the membranes are continuous with elements of the ER and in consequence of this the myeloid body is referred to as a differentiation of the reticulum. The paired membranes resemble in their thickness and spacings those which make up the outer segments; they are therefore regarded as intracellular photoreceptors of possible significance in the activation of pigment migration and other physiologic functions of these cells. The fuscin granules are enclosed in membranes which are also continuous with those of the ER. The granules seem to move independently of the prolongations in which they are contained. The report also describes the fine structure of the terminal bar apparatus, the fibrous layer intervening between the epithelium and the choroid blood vessels, and comments on the functions of the organelles depicted.

### INTRODUCTION

A few years ago an unusual development of the endoplasmic reticulum was encountered in cells of the pigment epithelium of the frog's eye. This had the form of a close lattice of tubules limited by smooth, particle-free membranes. It was observed, further, that at scattered points this reticulum showed remarkable differentiations consisting of closely stacked, paired membranes resembling the structure encountered in the outer segments of the retinal rods and cones. The disposition of the paired membranes was highly ordered and gave the impression that one was viewing a "crystalline" body (1). These structures were regarded as equivalent to the "myeloid bodies" described by Kühne (2) and by other histologists of the last century. Subsequently these observations were confirmed and extended to a variety of species, particularly among the lower vertebrates (3).

There were in these observations on the fine structure of the pigment epithelium a number of

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points of interest. The apparent sensitivity of these cells to light, which finds expression in the migration of pigment, suggested that the myeloid bodies with their special laminated structure might represent light-sensitive units. This posed the question of whether they might operate in controlling pigment migration and if so, by what means. Then, also, cells of the pigment epithelium are recognized as important in providing metabolites to the outer segments—metabolites essential for visual function. It seemed that any structure as specialized as the endoplasmic reticulum obviously is in these cells might play a role in this operation.

Since, as noted above, the ER here is of the agranular or smooth variety, its extreme expression in these pigment cells provides interesting opportunities for exploring the role of this particular type of ER in the cell. Thus far the particle-studded form of the ER has received the greater attention, and only isolated suggestions on the function of the smooth type have come from observations on its occurrence in a few types of cells and from studies of microsome fractions (12). In striated muscle, for example, it is the system associated with the myofibrils, and whether it is here for impulse conduction or carbohydrate metabolism or both are questions of some interest (4). Fawcett (5) makes note of an apparent development of the smooth ER in liver cells of fasted and refed rats and subsequent studies have linked it to glycogenesis (6). A similar conclusion derives from an intimate morphological association between the smooth ER and glycogen in the paraboloid of retinal cone cells of the turtle (7). Perhaps its most consistent development has, however, been noted in cells engaged in producing lipid-rich secretions. Thus, from observations on the sebaceous cells of the rat, Palay notes that the cells are filled with smooth ER at the time of most active fat storage (8). Muta has noted a similar development of the smooth ER in interstitial cells of the mouse ovary (9), and Christensen and Fawcett report the same in the testicular interstitial cells of the opossum (10). Reports on the fine structure of the adrenal make note of the abundance of agranular vesicles and tubules in the cytoplasm and in association with the lipid granules (11).

## MATERIALS AND METHODS

The majority of the observations reported here were made on retinas of adult frogs, *Rana pipiens* Shreber (northern Vermont). The same tissue from an aquarium fish, *Hyphessobsycon rosaceus*, a fresh water turtle, *Chrysemys picta*, and the cat, *Felis domestica*, has been examined for comparative purposes. In all cases reported the tissues were taken from light-adapted eyes.

The eyes were removed from animals under ether anesthesia, and opened by cutting with a razor blade along the equator. The retina was then flooded with fixative and the whole tissue immersed in fixative at 0°C. Caulfield's osmium-sucrose mixture at pH 7.6 to 7.8 was used in most instances for fixation (13). Fixation was continued for 1 hour. The tissue was thereafter rinsed in 50 per cent ethanol and dehydrated in increasing concentrations of ethanol. During impregnation with methacrylate the tissue was evacuated with the laboratory vacuum line (40 mm. Hg). This and the use of ultraviolet to accelerate polymerization seemed to improve the quality of the imbeddings. Following imbedding, the tissue with a small part of the surrounding plastic was removed from the original block, and after orientation fastened to a new block by wetting the contiguous surfaces with methacrylate monomer. Sections were cut, for the most part, with a glass knife on a Servall (Porter-Blum) microtome. They were mounted on either carbon- or formvar-coated grids and, in the case of the latter, were covered with a blanket of evaporated carbon. Lead hydroxide was quite generally used for staining, following the methods described by Watson (31) and by Peachey (32). Microscopy was done with an Elmiskop I (Siemens) or an EMU-2A (RCA).

## OBSERVATIONS

A few comments on the particular object of study are included here by way of orienting readers not familiar with this particular tissue.

The pigment epithelium of the vertebrate eye is a single layer of epithelial cells constituting the outermost layer of the retina. It derives in development from the outer layer of the optic cup. The free surfaces of the cells face the outer segments of rods and cones across a space which in early embryonic stages is continuous with the central canal of the neural tube. The space (interstitial) in the adult eye is filled with a fluid (14). The epithelium rests on a prominent fibrous membrane (Bruch's membrane) which is immediately adjacent to a rich vascular supply—part of the choroid. This outermost epithelial layer is continuous over the whole surface of the retina and at its peripheral margins with the epithelium of the ciliary body.

The cells of the epithelium in vertical section vary from tall cuboidal at the back of the retina to low

cuboidal at the margins. In cross-section taken near their basal surfaces they are regularly hexagonal. At a distance of about 2 microns from the basement membrane, adjacent cells are joined by terminal bars. Beyond this level the cells possess a hemispherical free surface from which slender projections or pseudopodia extend toward and between the outer segments of the rods and cones. The basal surfaces of the cells resting on a thin basement membrane are perfectly flat (Figs. 1 and 2).

The nuclei of the pigment epithelium cells are smoothly spherical and located in the basal half of the cell. The nuclear content is homogeneous in appearance except for a central nucleolus. Mitochondria are located, for the most part, in the basal half of the cells, *i.e.* below the level of the terminal bars (Fig. 2). The pigment granules, which of course are numerous in these cells, are situated almost entirely in the apical half of the cytoplasm and in the slender projections from the apical surface (Figs. 1 and 2).

It is possible at relatively low magnifications to recognize a few unusual inclusions in these cells. Some of these, a little difficult to see in light microscope images, are angular in outline and are referred to here and elsewhere as myeloid bodies. Kühne described them as "colorless, cuboidal bodies, spherical or cuboidal in shape and of wax-like luster" (2). The majority of these bodies are similar in size to mitochondria but the total size range is large and the longest dimension may reach 4 to 5 microns. The larger of these bodies are found in the basal half of the cells and the smaller ones are scattered throughout the cytoplasm except for those which extend into the finer apical projections.

More prominent than myeloid bodies are certain "lipochrin" (lipid) granules (Kühne) which occur most commonly in the apical halves of the cells. These turn black during osmium fixation and stain with common fat stains. They vary greatly in size and may reach diameters equal to that of the nucleus. These lipid granules appear dense and often perfectly homogeneous except for gross striae introduced by sectioning. In a second type, and generally smaller lipid granule, it is possible to detect, even at low magnifications, some evidence of circumferential lamellae in its structure (Fig. 2).

It is obvious from the light, and low power electron micrographs (Figs. 1 and 2) that a rich vascular bed underlies the pigment epithelium. The tissue component separating capillaries from epithelial cells is extraordinarily thin and consists in the main of a basement membrane and a special fibrous layer known as Bruch's membrane.

As noted above, the apical poles of the pigment epithelium cells face the outer segments across a space and send slender projections containing pigment granules into recesses between the outer segments of rods and cones to the level of the inner segments. In the dark-adapted eye these projections are relatively free of the pigment which now is located in the apical poles of the epithelial cells (Figs. 1 and 2).

## *Details of Fine Structure*

### *A. Interrelationship of Pigment Epithelial Cells*

Adjacent epithelial cells in this tissue are usually in contact along their margins from the level of the basement membrane to the terminal bars. This distance is relatively constant over the entire epithelium and measures about 2 $\mu$. Where in closest contact, the two adjacent plasma membranes, represented by dense lines in the EM image, are separated by a less dense space of 7 m$\mu$. Spaces of greater width are occasionally evident between the cells below the terminal bar. At the terminal bar. as is characteristic of other epithelia, the cells appear to be joined or cemented together, for at this point the space between the plasma membranes is constant (Figs. 3, 3*a*, 4, and 10). No interruptions in the continuity of this differentiated contact around the cells have been encountered.

The terminal bars in this epithelium are in certain respects unique. The condensations of dense material usually found on the cytoplasmic sides of the membranes of these structures are very extensive. In cross-section (*i.e.* vertical section of the epithelium) these condensations appear pyramidal in outline and reach a height of about 300 m$\mu$ (Fig. 3*a*). Viewed in longitudinal section (*i.e.* in horizontal section parallel to the basement membrane) the material condensed on the terminal bars is finely fibrous (Fig. 4). The fibrous units, possibly keratin in nature (called *keratin kuppe* by Angelucci (15)), run parallel to one another and in planes parallel to the basement membrane and the lateral surface of the cell in which they exist. In smaller numbers they extend deeply into the cytoplasm where they are randomly oriented. They make up a dense cortex in this region of the cell from which other cytoplasmic components are excluded (Fig. 4).

Above the terminal bars the epithelial cells may or may not be in close contact. Frequently, however, the contact is extensive, which makes the terminal bars seem extraordinarily low relative to the free surface of the epithelium. This means that above the level of the terminal bars the epithelial cells may show substantial individual shape control

and variation. The pigment epithelium is, of course, unusual among epithelia, in having so much (estimated at two-thirds) of the apical portion of the cell above the terminal bar and we suppose it possible that the prominent development of the terminal bar structure is related to this situation, in the sense of providing a more rigid frame.

### *B. Pigment Epithelium and Vascular Supply*

The cells of the pigment epithelium are separated from the endothelial cells of the underlying capillary bed by a number of "fibrous" layers (the internal limiting membrane and lamina basalis or Bruch's membrane) (Fig. 5). The first of these, and the one in closest association with the epithelium is thin ($\sim$ 30 m$\mu$ thick) and separated from the plasma membrane of the epithelial cells by a space (or a region of low density) of uniform thickness ($\sim$ 40 m$\mu$). The low density region, plus the higher density layer combined are reminiscent of basement membranes encountered in other tissues and so will together be called by that name. It is difficult to resolve any finer structure in the basement membrane, but one occasionally gains the impression that the denser layer is a feltwork of fibrous units (Figs. 3 and 5).

Just beneath the basement membrane there is a much thicker layer of loosely arranged fibers. The unit fibrils are of uniform diameter ($\sim$ 25 m$\mu$), are long and of indefinite length, and, within the limits of the layer, randomly oriented. They show an extremely fine banding with a small period of approximately 50 A. The matrix around them is of low density and without evident structure. The entire layer is about 0.2 $\mu$ thick (Figs. 3 and 5).

Peripheral to this latter layer is yet another which is different in character. At low magnifications it appears relatively homogeneous, with only a suggestion of a fibrous component. In density it resembles the dense portion of the basement membrane. Its thickness is essentially uniform at 70 m$\mu$. As near as one can judge from comparisons with illustrations found in light microscope studies (33), this layer corresponds to the lamina elastica choroidea which stains intensely with such elastic stains as Weigert's. Occasional interruptions are

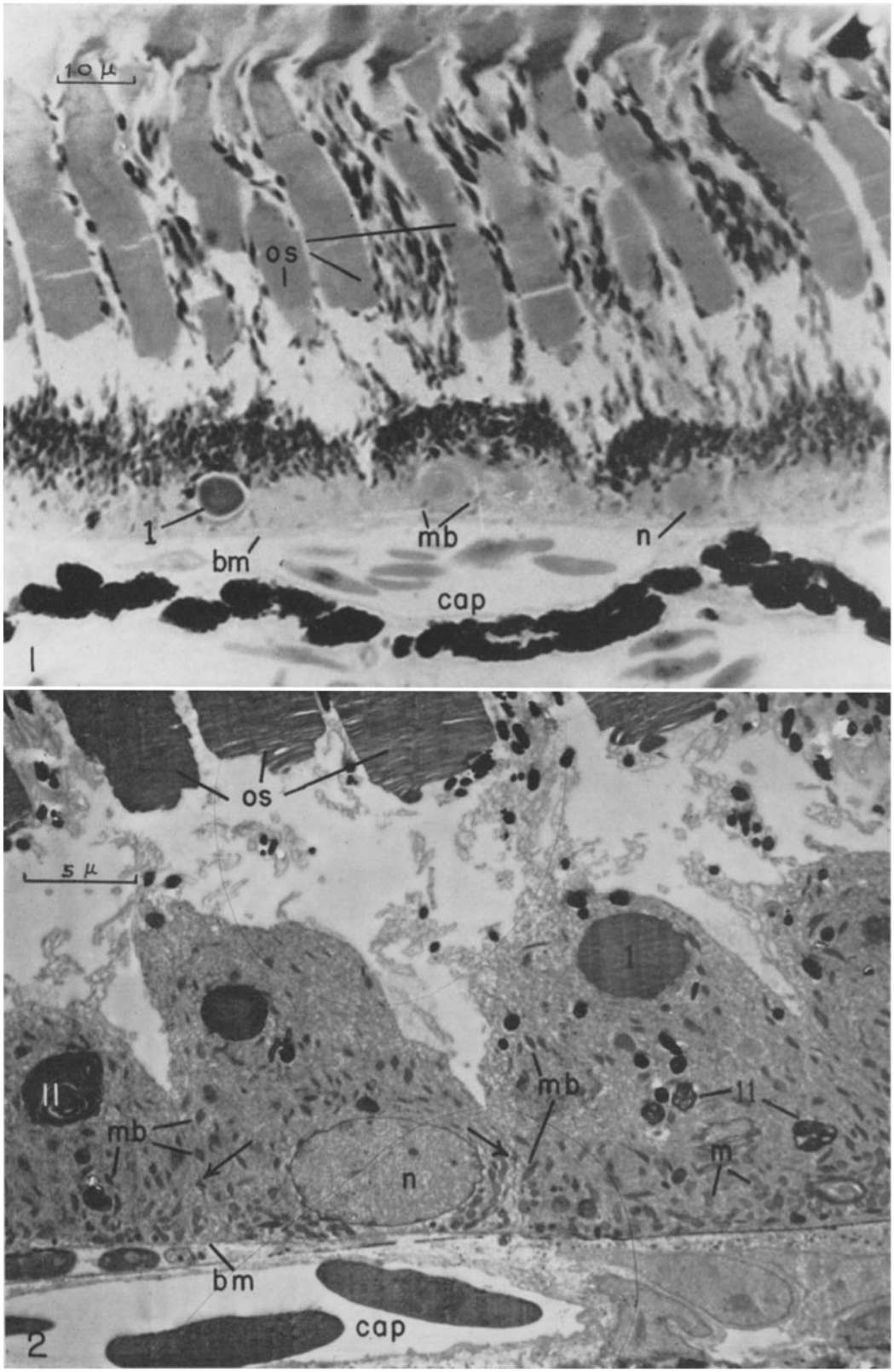
FIGURE 1

Photomicrograph of a vertical section through a frog's retina stained with PAS and fast green. The pigment epithelium lies just below the midline of the picture. Long processes can be seen to extend from the free surfaces of the epithelial cells into the spaces (possibly exaggerated by preparation procedures) between the outer segment (*os*) of the visual cells. These prolongations, like the apical halves of the epithelial cells, are filled with melanin granules. At their basal surfaces the epithelial cells rest on a basement membrane (*bm*) which in turn is underlain by the capillaries (*cap*) of the choroid. A layer of pigment cells, in the choroid, runs across the bottom of the micrograph. Besides pigment granules it is possible to identify within the cytoplasm of the epithelial cells: lipid droplets (*l*), a more faintly evident nucleus (*n*), and a few myeloid bodies (*mb*) or clusters of same. Magnification $\times$ 1200.

FIGURE 2

Low power electron micrograph of pigment epithelium. The epithelial cells occupy the center of the micrograph and their free surfaces are close to the distal ends of the rod outer segments (*os*). Sections through processes from apical surfaces of the epithelial cells are seen in the intervening space. Because of the thinness of the section only a relatively few jet black pigment granules are present. The basal surfaces of the cells rest on a thin basement membrane (*bm*), which in turn rests on the capillary (*cap*) bed of the choroid.

Cell inclusions are identified as follows: mitochondria (*m*), mostly in the basal half of the epithelial cells; nucleus (*n*); numerous myeloid bodies (*mb*) in basal and, to a lesser extent, in apical halves of cells; lipid droplets (*l*), fairly large in this instance; and laminated lipid bodies (*ll*). The terminal bars (and desmosomes) between the epithelial cells are located at a constant distance from the basement membrane and are indicated by arrows. Magnification $\times$ 3500.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evident in the continuity of this membrane. Its appearance in the EM image resembles closely that of elastic tissue components encountered elsewhere (Fig. 5).

The layer intervening between the lamina elastica and the endothelial cells is filled with fibers having the characteristics of those on the inner side of the elastica. According to Wislocki and Sidman (33) these two layers are strongly PAS-positive. The plasma membrane limiting the endothelial cells is exposed directly to this outer layer without the presence of the usual basement membrane. It is assumed that this construction, as well as the presence of perforations in the elastica, is designed to facilitate the diffusion of metabolites between capillary and pigment epithelium. Fenestrations in the endothelial cells have been seen only infrequently.

### *C. The Endoplasmic Reticulum*

The cytoplasm of the pigment epithelial cells consists in very large part of a complex system of membrane-limited tubules. These are associated in a manner to form a compact three-dimensional reticular structure (Figs. 3, 4, 7, and 10). The tubules have a mean diameter of approximately 75 mμ and the variation from this (except in such local differentiations as the myeloid body) is remarkably slight. The compactness of tubules—or membrane-enclosed elements—is such that the volume of spaces between them seems less than the volume of the system itself. Only in a few other instances in recorded studies of cell fine structure have similar condensations of the ER been observed. These include sustentacular cells of the olfactory epithelium (1), and several types previously mentioned, *e.g.* adrenal cells of human foetus (11), meibomian gland cells (8), interstitial cells of ovary (9), and testis (10).

This form of the endoplasmic reticulum extends into almost all parts of the cytoplasm and seems to be characteristic of pigment epithelial cells in all parts of the retina and in all retinas studied. There are, however, at least two variations of this general morphology. One is not particularly remarkable. It is found in those parts of the reticulum extending into the projections from the apical poles which "interdigitate" with the outer segments. In these projections and around their bases the ER is less compacted and the individual elements (tubules) may be slightly more slender (Figs. 12 and 13). Possibly this is a reflection of a greater mobility of the system in those parts of the epithelial cells. Within the projections elements of the ER achieve a close association with the plasma membrane (Figs. 12*a* and 13). The other variation or local differentiation of the ER is much more striking

FIGURE 3

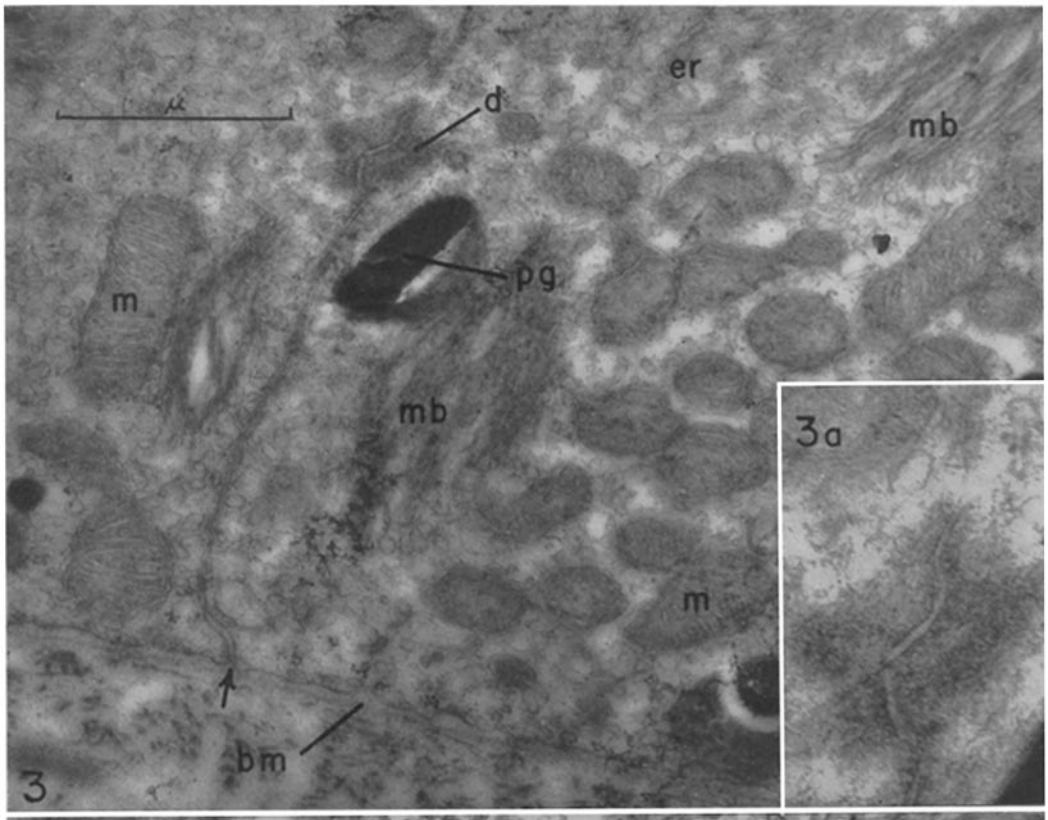
Micrograph of basal portion of two adjacent cells of pigment epithelium. The location of the facing membranes of the two cells and the intervening space are indicated by an arrow. The terminal bar and desmosomes are shown at *d*. The basement membrane is at *bm* and is continuous below with the more coarsely fibrous layer of Bruch's membrane. Myeloid bodies (*mb*) are evident along with numerous mitochondria (*m*) and a few pigment granules (*pg*). The cytoplasm is filled otherwise with profiles of vesicles and tubules—elements of a smooth surfaced endoplasmic reticulum (*er*). Magnification × 30,000.

FIGURE 3*a*

Enlargement of desmosomes to show detail.

FIGURE 4

Micrograph of a horizontal section through pigment epithelium at level of terminal bar, which is shown along the bottom margin of the picture. The thin and uniform space between the epithelial cells bisects the image of the bar. The denser portions (*d*) of the image represent the desmosomes; the lighter, finely fibrous parts are associated thin layers of fibrous cytoplasm. Extremely fine fibrous elements extend out into the cortical cytoplasm from the region of the bar and intermingle with profiles of the ER (*er*). Mitochondria (*m*) and myeloid bodies (*mb*) are represented. Magnification × 38,000.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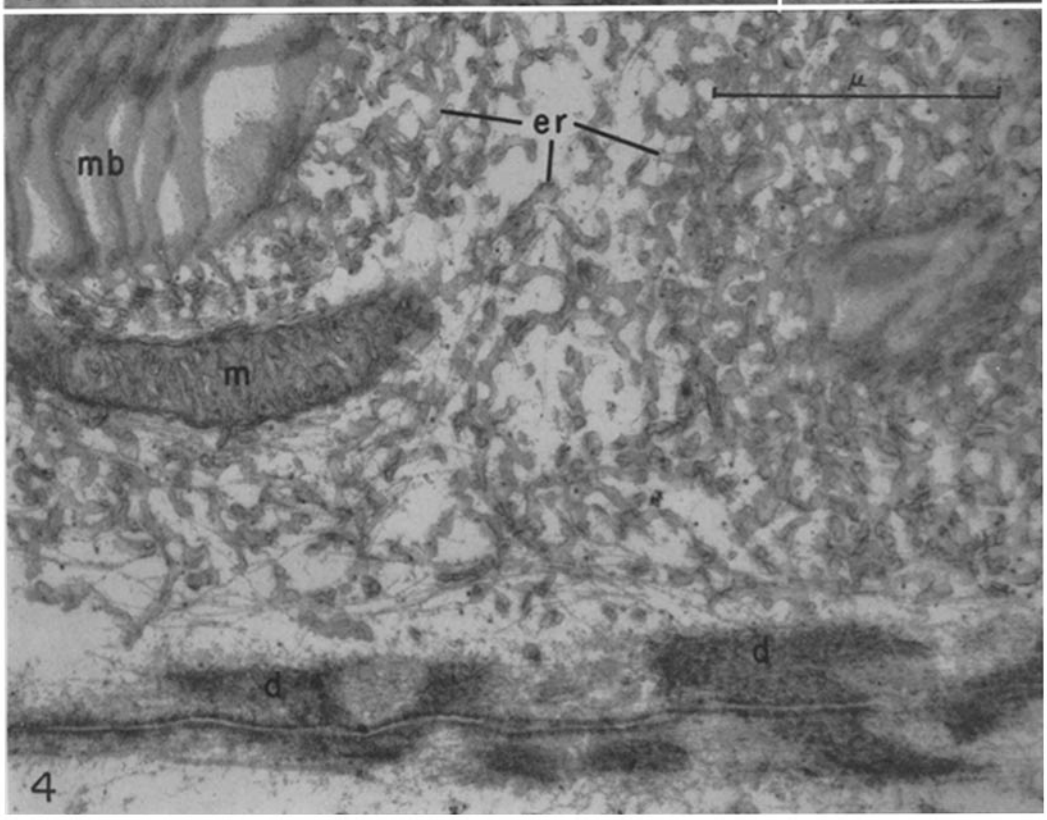
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and possibly of greater functional interest. It constitutes what has been known since the observation of Kühne (2) and of Angelucci (15) as the myeloid body.

### *D. Myeloid Bodies*

Light microscopy of stained and even unstained preparations of the pigment cells defines in these cells cytoplasmic bodies of small (mitochondrial) size, with angular outlines as noted above. These are PAS-positive, and are stained with haematoxylin. In polarized light they appear birefringent (16). Their staining properties and physical characteristics are identical with the rod and cone outer segments.

This similarity is borne out by the electron microscope image (Figs. 6 to 10). In vertical sec-

FIGURE 5

This shows the basal halves of two epithelial cells and the underlying basement membrane, plus various fibrous layers of the lamina basalis, or Bruch's membrane. The section was stained with $Pb(OH)_2$. Myeloid bodies (*mb*) are prominent in the cytoplasms of the two cells. Mitochondria, pigment granules, and densely developed ER are readily identified.

The micrograph is of greatest interest for the details it shows of the basement membrane etc. The plasma membrane (*pm*) can be seen limiting the basal cytoplasm. Just beneath it are the light and dense layer of the basement membrane (*bm*). From here, in order peripherally, there is a loose fibrous layer, a dense layer, which is identified with the lamina elastica choroidea (*le*), and a second loose fiber layer. Beneath this latter lies the endothelial cell (*ec*) of a capillary and the capillary lumen (*c*). Magnification × 32,000.

FIGURE 6

High power of myeloid body (*mb*) to show regular spacings (average repeating unit, 155A) within fine structure. The double-line units represent vertical profiles of thin discs. The space between them (7 mμ) is uniform here and represents the inter disc space. It is continuous with the cytoplasmic matrix. The line, $X$–$X_1$, marks the location from which was taken the densitometric tracing shown in Fig. 6*a*. A large lipid body (*l*) intrudes into upper half of figure, Magnification × 80,000.

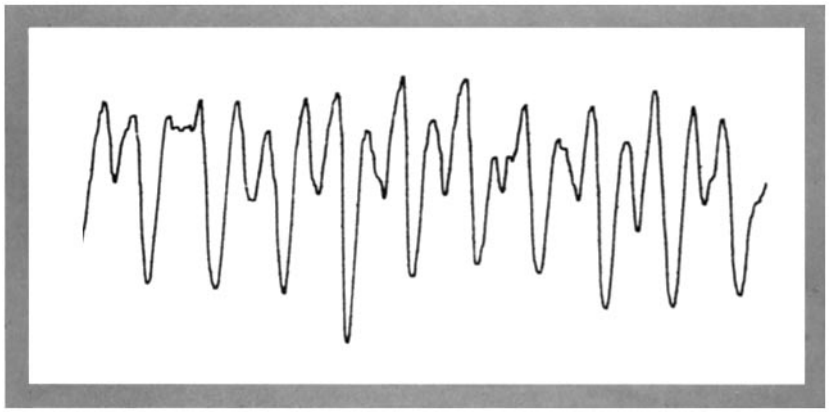
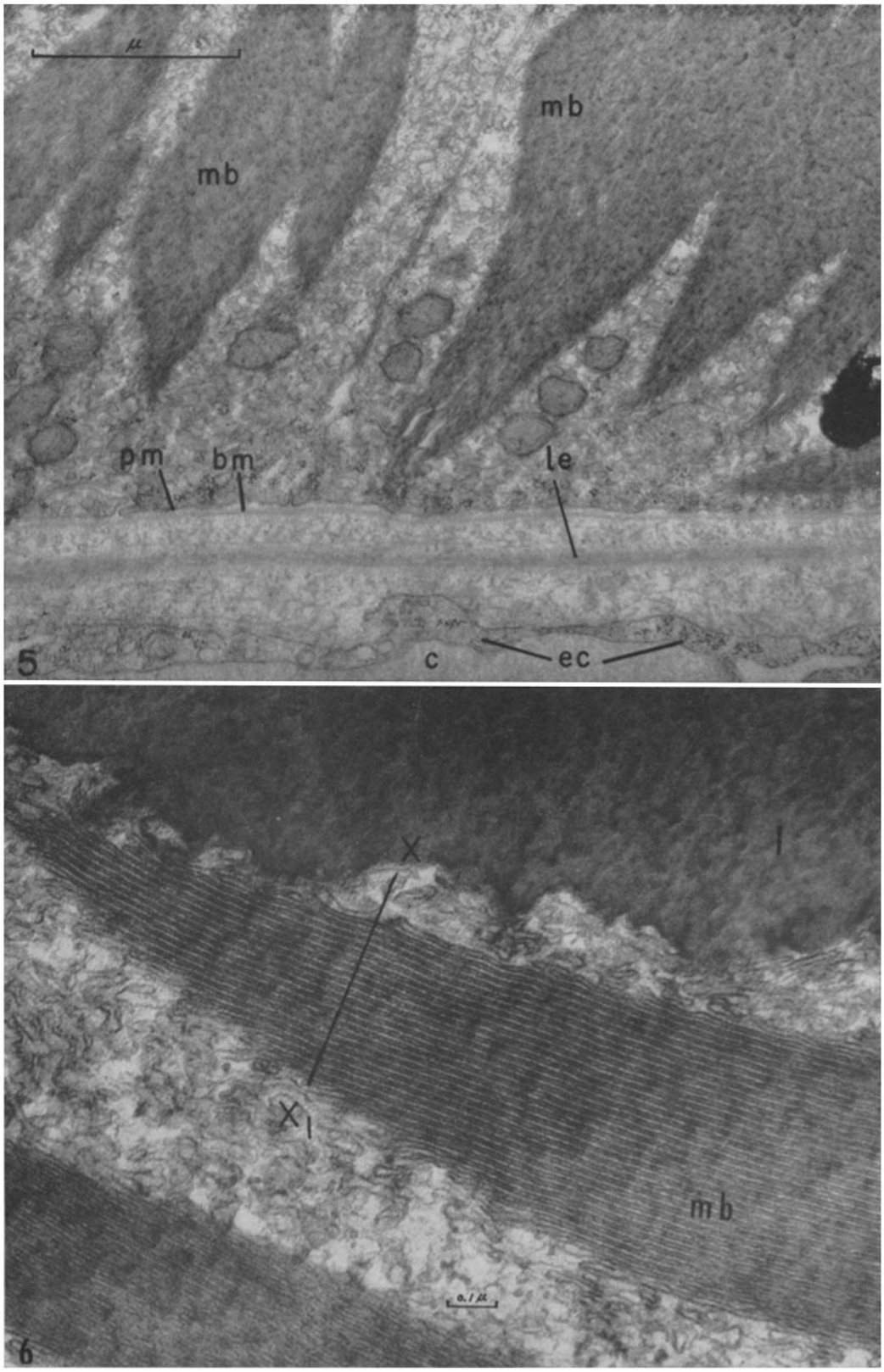


FIGURE 6*a*

Densitometric tracing made from negative along line ($X$–$X_1$) indicated on Fig. 6. The upper peaks in the tracings represent the centers of the membranes; the troughs between them represent alternately the intra- and (greater) inter-disc spaces. The magnification is 50 times that on negative (× 11,300). It is obvious that the spacing is regular, and that the space between the pairs of membranes (inter-disc) represented by the deep trough is slightly greater than that between the membranes.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tion they appear as stacks of membranes which are obviously paired as in the outer segments. The membranes are slightly more dense and slightly thicker than those that limit the tubules and vesicles of the general ER. The space between the pairs of membranes (center to center of line) is constant and of the order of 70 A. The alternate space, *i.e.* between the membranes, is more variable and is sometimes less and sometimes more than that between the pairs, especially in instances where the organization of the body is obviously disturbed (Fig. 7). If followed carefully to the margin of the myeloid body, this thin space limited by the paired membranes is found to be continuous with the cavity of the connecting elements of the ER (Figs. 8 to 10). Any one lamellar unit within a myeloid body may therefore be thought of as a flat disc, the peripheral limits of which are determined by the presence of marginal fenestrae. The extensions of the disc (trabeculae) between the fenestrae are parts of and continuous with the general reticulum of the adjacent ER (Fig. 11). The discs, so defined, differ in diameter within any one stack and the diameter difference between any one disc and that adjacent to it, tends to be uniform over the whole stack. Thus the diameter of the central disc in a stack is greater by a measurable and uniform amount than the adjacent disc above and below it. It therefore develops that as one measures the discs in order away from the central one, the diameters are found to decrease by uniform decrements (Figs. 5 to 7 and 10). This results in the stack having a double, flat-conical form, roughly the shape of a biconvex lens (Fig. 11). In its regularity this subdivision of the ER has the character of a crystal, though obviously a liquid one, for its form varies somewhat and occasionally departs from the precise regularity here described.

The evidence for this description comes from an examination of many sections passing horizontally and obliquely as well as vertically through the body. In horizontal section, *e.g.*, the myeloid body is circular in outline and the ring of fenestrations marking its limits is readily seen (Fig. 9). These same images, as well as those of sections passing "vertically" through the myeloid bodies (Figs 8 and 10), illustrate clearly the continuity of the individual discs with elements of the surrounding ER.

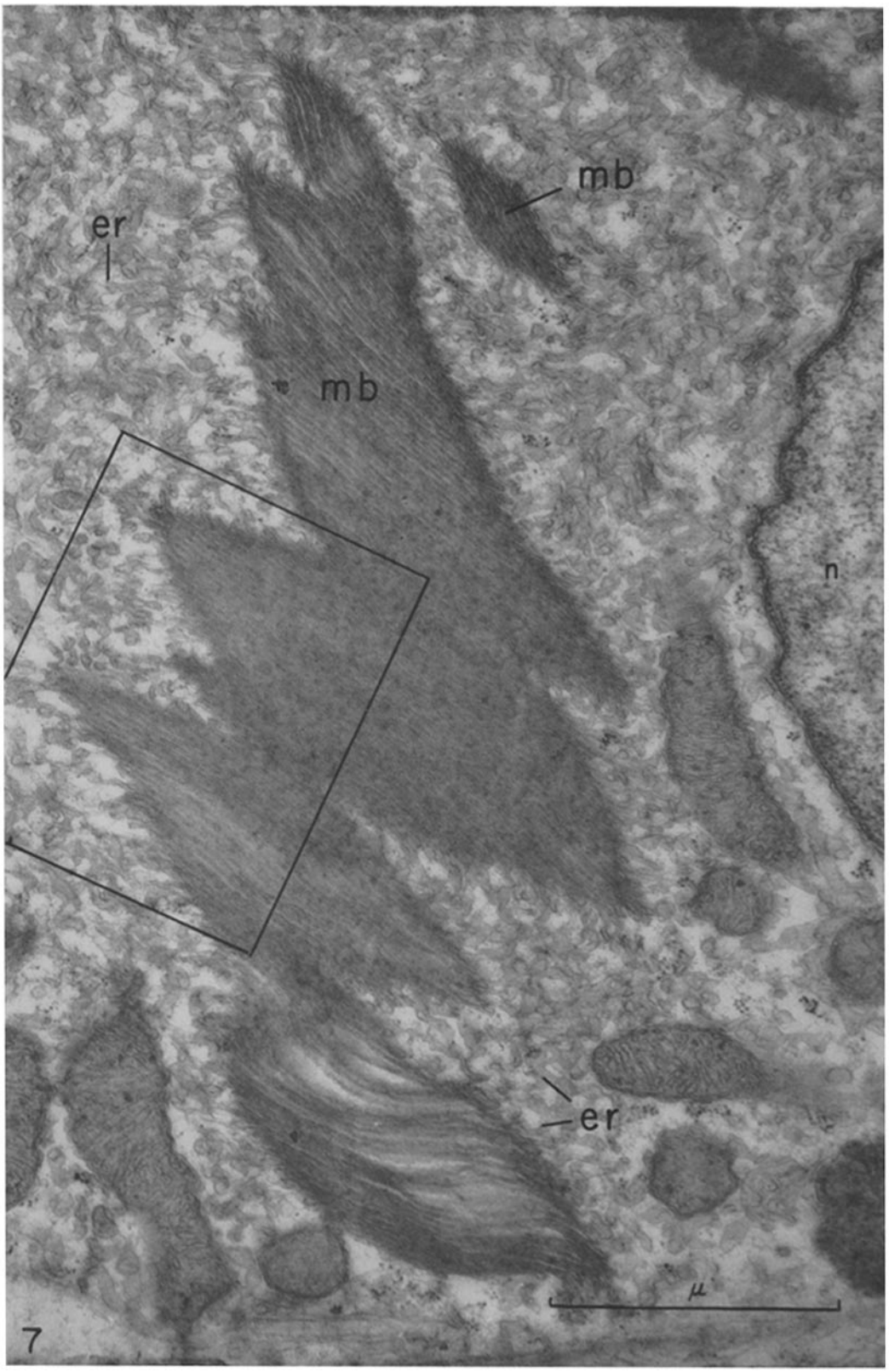
The membranes limiting the units of the general ER and also those of the myeloid bodies are of the smooth or agranular variety encountered, as already noted, in several cell types. Granules similar in size to RNP particles of other cells occur in small numbers only. These, in part, are free in the matrix between the trabeculae of the ER and infrequently are attached to the membranes (Figs. 7 and 17 and others).

The size variations in myeloid bodies depicted in any one section is substantial. It is reasonable to question, however, whether the actual variation is as great as that depicted because much of what is seen could be referred to the variable relation of section plane to medial axis of the bodies. In other words, it is difficult with bodies of this shape to dis-

FIGURE 7

Basal portion of pigment epithelial cell showing a characteristic array of myeloid bodies. The section was stained with $Pb(OH)_2$. The edge of the nucleus (*n*) is at the right. Mitochondria are evident in the basal regions of the cytoplasm. The endoplasmic reticulum (*er*), as before, is represented by a dense, irregular lattice work of tubular elements. Dense particles scattered in clusters throughout the cytoplasm are thought to represent RNP particles encountered elsewhere.

The myeloid bodies, occupying the center of the image, appear to consist of closely stacked, paired membranes. These are evenly spaced at *mb*, at which point the plane of sections is normal to the lamellar units of the body. The long dimensions of these units, in any one group, diminish progressively and uniformly at positions further removed from the center of the body, so that the entire structure has a fairly regular rhomboidal profile. The solid form of the whole body is that of a biconvex lens (see Fig. 11). The excuse for the continuity between myeloid bodies is not known. At their edges the paired membranes come together or are continuous with membranes limiting tubules of the ER. The region enclosed in the rectangle is shown at a higher magnification in Fig. 8. Magnification × 45,000.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tinguish between a medial section of a small body and a marginal section of a large body, although in actual fact a difference should be apparent. What can be said with certainty is that large profiles of myeloid bodies, 4 to 5 microns in longest dimension, are frequently observed in the basal halves of the cells whereas only smaller units (never larger than 1 $\mu$) are found in the apical poles. This extent of variation is therefore established.

Another feature of the myeloid bodies deserving mention is their associations. It is common, *e.g.*, to find them connected to one another by their convex faces (but never by their margins) to form groups of 5 or 6. The character of the association varies greatly (see Figs. 5 and 7). Myeloid bodies also are found attached to the surface of the nucleus as though they had included in their structure a part of the nuclear envelope (Fig. 17). This association is further descriptive of the common properties of myeloid bodies and the endoplasmic reticulum.

Finally, and possibly of some functional importance, is their association with large lipid bodies. Here several myeloid bodies may be arrayed on both sides of the lipid body and in such a manner that those in closest proximity to the granule are crescentic in form and essentially envelope part of the surface of the granule.

### *E. Fuscin Granules*

Pigment granules in electron micrographs appear as dense as in equivalent light micrographs (compare Fig. 1 with Figs. 2, 12, and 13). They can therefore be readily identified in these preparations of pigment epithelial cells.

They are found to be confined to the apical half of the cell and also in the finger-like prolongations which extend from the apical poles into the spaces between the outer segments. The granules in the prolongations are uniformly oval in profile when the plane of section is parallel to the long axes of the prolongations. In cross-sections they appear round. This means that the individual granule is cylindrical, with spherical ends (Figs. 12 and 13). Their size in this part of the cell is relatively uniform. The long dimension is about 2 $\mu$ and the cross-diameter about 0.4 $\mu$. They tend, as might be expected, to orient with their long axes parallel to the long dimension of the prolongations.

The pigment granules within the cell body are less uniform. Many appear similar in shape and size to those in the cell extensions, but many others are spherical and, in some instances, substantially smaller (or larger). These latter forms seem never to find their way into the prolongations. It appears, therefore, that there is in these cells more than one species of pigment granule. It should be noted that the ER extends up into the prolongations in very much the same form as that found in the apical portions of the cell body where the prolongations arise (Fig. 12). This appears as a loose reticulum of small tubular elements. The content of these is more dense than that of similar elements more deeply located in the cell, and they achieve a very close contact with the inner surface of the plasma membrane.

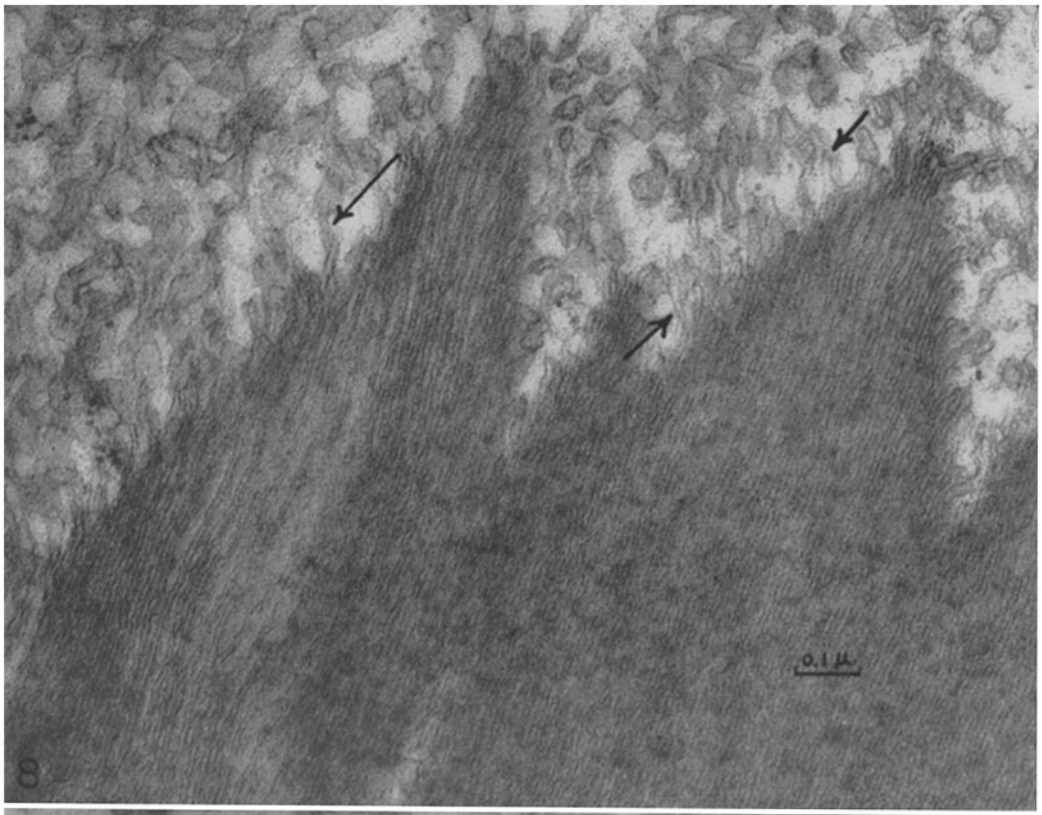
The relation of the fuscin granules to the endoplasmic reticulum is of special interest for a number of reasons and the two structures have therefore been examined with care, especially in the apical prolongations where changes in granule disposition take place. Thus it has been observed

FIGURE 8

An enlargement of the outlined area in Fig. 7, designed to show to better advantage the membranous structure of the myeloid bodies, and the continuity between these membranes and those limiting the tubules of the ER. Such continuities are evident at arrows. Magnification × 86,000.

FIGURE 9

Here the plane of section is parallel to the equatorial plane of one myeloid body (*mb*) and so provides a top view of a portion of the body. It is seen to be circular in outline. The circular lamellar units at the edges show fenestrations or reticulate and continue on outward as elements of the general ER. A pigment granule appears at the far right. RNP particles are present in two large clusters and vertical sections through edges of two myeloid bodies are shown at upper center and upper left. Magnification × 75,000.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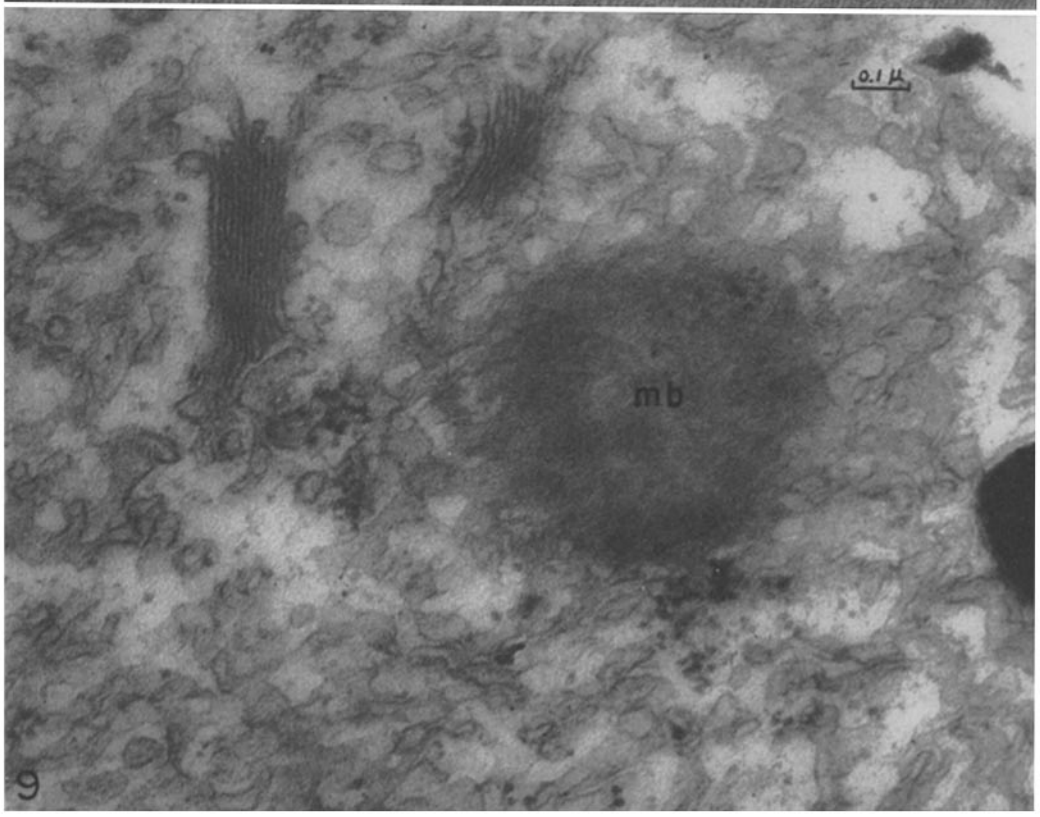
0.1 μ
mb
9

that each fuscin granule is surrounded by a membrane which is loosely associated with its surface (Fig. 13). That is to say that there is in these fixed specimens a narrow space between the surface of the granules and the membrane. This membrane is not apparently different from that which limits the tubules of the ER *and in certain instances has been noted to be continuous with the latter*. It would therefore seem that the melanin granules reside in special cisternae (or their morphological equivalents) of the endoplasmic reticulum. Presumably migration of the pigment is accompanied by shifts in the disposition of the ER. However, from preliminary observations on dark-adapted retinas two points of interest emerge: The apical projections of the pigment epithelial cell are not withdrawn nor are they completely depleted of profiles of the ER. It seems, therefore, that the shift in fuscin granules is not accompanied by a corresponding shift in the entire ER content of the pseudopodia.

### *F. Lipid Inclusions*

The existence of prominent lipid inclusions in these pigmented epithelial cells has been known since the original observations of Boll (1877) (17), Angelucci (1878) (15), and Kühne (1878) (2). In the unstained preparations studied in the time of these investigators it was possible to distinguish between several kinds of fat globules. The distinction was based chiefly on the color of the globules, recognized now as a distinction of doubtful significance. It was noted by these early observers that the color bleached in the light.

In the material that we have examined, the lipid granules are of two kinds. Each varies in size from submicroscopic units to bodies 10 microns in diameter. The large globules are located, as a rule, in the apical portions of the cells while the smaller units of each kind tend to occupy the basal regions. In one kind, the osmiophilic substance of the granule is laminated in a way that resembles the fine structure of the myelin sheath (18) (Fig. 15). In its finer details, as well, the lamination resembles that of myelin. It consists of parallel dense lines separated by a space of 12 m$\mu$, in which there is a less obvious, interrupted line (Fig. 15*a*). This organization of the material in these lipid, or lipoprotein, granules is most striking in the cortical region of the granules and less so in the central. In the fixed material, massive breaks in the continuity of structure are common and probably artificial.

The other type of lipid body is also osmiophilic, though possibly less so per unit volume than the first kind. The content is not laminated and appears homogeneous (Fig. 14). The majority of these lipid granules are large (*i.e.* 5 to 10 $\mu$). The margins are usually scalloped in profile and it is common for vesicular elements of the ER to be closely applied to the surface. This surface is further peculiar in that it appears more dense than the deeper parts of the body, as though some metabolic activity at the surface were rendering the material there more osmiophilic.

The relationship between the lipid bodies and ER seems an intimate one but otherwise difficult to define with certainty. In the case of the homogeneous type, the smooth elements of the ER are, as noted above, closely applied to its surface. The suggestions is that some transfer of metabolites is in progress. This same association is not apparent in the case of the laminated form and here, if anything, the same elements of the ER are repelled from the surface, for a space is commonly present.

### *G. Other Components of the Cytoplasm*

*Mitochondria:* The mitochondria are readily identified by their structure which is, in general, characteristic for these organelles wherever they occur. They are spherical to cylindrical in form.

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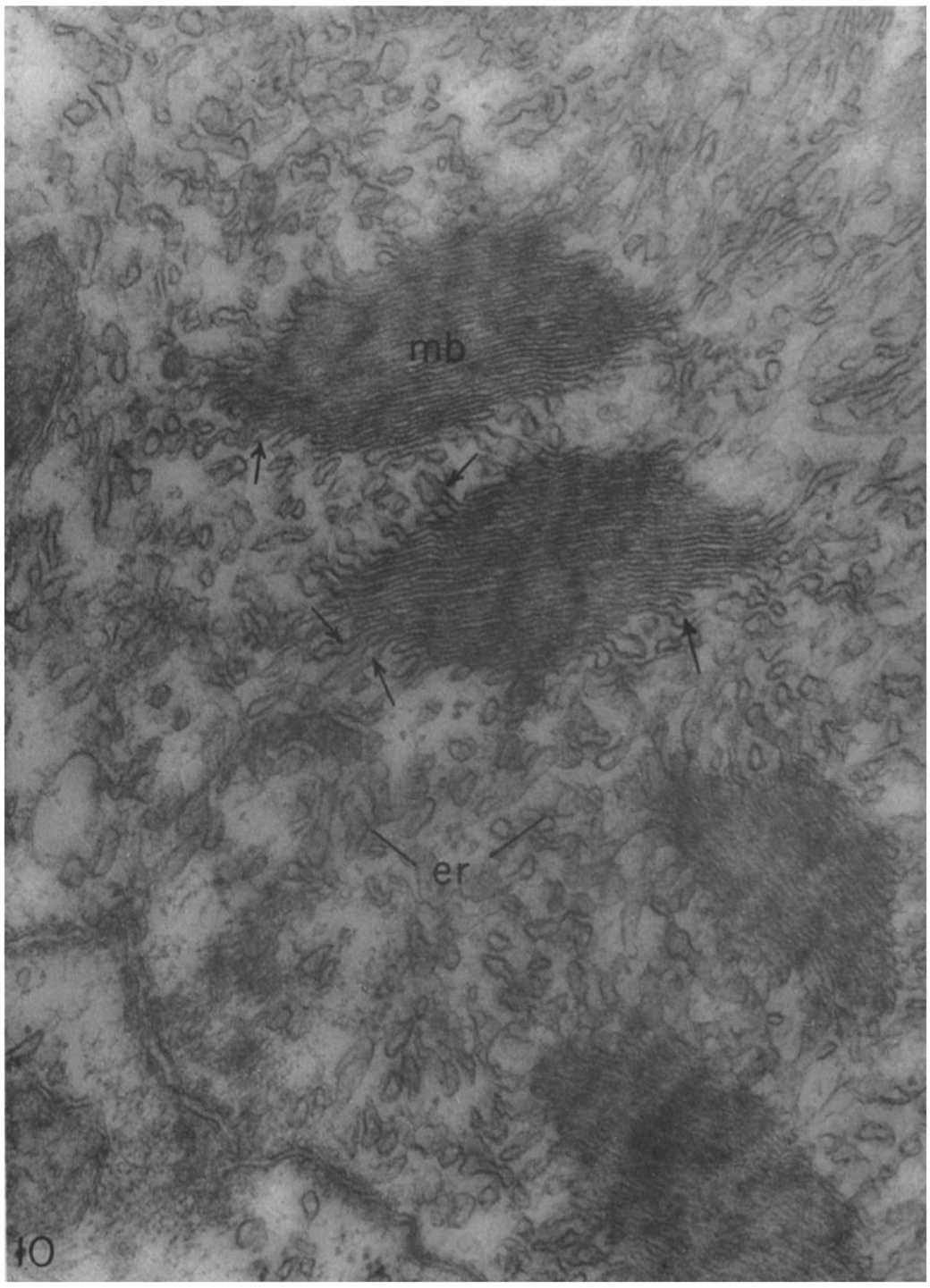
FIGURE 10

Myeloid bodies (*mb*) and endoplasmic reticulum (*er*) at level of terminal bar. Micrograph illustrates more clearly then elsewhere the continuity between paired membranes of the myeloid body and those of the surrounding ER (see arrows).

The plasma membranes of two adjacent cells run parallel to one another across the lower left corner of the micrograph. At one region in this association there can be seen the finely fibrous material making up the density of the terminal bar. Magnification × 80,000.


mb
er
40

They are limited by the usual double membrane and contain a large number of cristae. These latter are long and frequently extend to the wall opposite their origin. The mitochondria are essentially confined to the basal half of the cell, they show no noticeable tendency to associate with any other inclusions or organelles of the cell, except in so far as these are also concentrated in the basal half of the cell.

*Golgi Complex:* In a cytoplasm that is so rich in membrane-limited elements and includes numerous lamellar structures it can be difficult to identify a structure which itself is made up of vesicles and stacks of lamellar units. That a Golgi complex exists among all the other vesicular elements seems, however, certain.

It is common to find in the supranuclear portion of the cytoplasm a region that is different in that many of the vesicular components are spherical, seem unconnected, and surround a series of 4 to 5 parallel lamellar sacs having the form characteristic of the Golgi component. The entire structure cannot be called obvious but it is identifiable. A few of the vesicles are marked by a content and cortex of greater density (see enclosed space, Fig. 16).

*RNP Particles:* Particles having the density and size expected of RNP particles are distributed in small clusters throughout the cytoplasm of the main body of the cell. They are occasionally attached to the matrix surface of a tubule or vesicle, and in such instances appear arranged in patterns (rows, rosettes, whorls), as in pancreas and other types of cells engaged in protein synthesis. There seems not, however, to be any major section or part of the endoplasmic reticulum associated with these particles and they most frequently appear in clusters of 8 to 12 particles lying free in the cytoplasmic matrix.

*The Nucleus:* The nuclei are located in the basal half of these pigment cells. They are of homogeneous density except for small marginal condensations (presumably chromatin) and a single, centrally-located nucleolus. The nuclear envelope is not unusual in most respects; it shows occasional pores (Fig. 17). Connections between the outer membrane and those of the adjacent ER can be found. Where a myeloid body is in contact with the nuclear surface the lamellae of the body lie parallel to the outer membrane and as close to it as to the adjacent lamellae of the body. In other words, the association between the outer nuclear membrane and the membranes of the myeloid body may be as intimate as between the membranes in the body itself.

## DISCUSSION

*Form and Function of Endoplasmic Reticulum:* The endoplasmic reticulum, including the nuclear envelope, is now regarded as a unit system of the cell which appears in a great variety of forms or patterns. Some of these patterns repeat in cells performing similar functions and have therefore been correlated with these functions. This is especially true of that form or differentiation of the system associated with RNP particles, the so called rough ER or ergastoplasm, which is apparently involved in protein synthesis. Less, however, is known about other variants of the system, and especially about the agranular or smooth form. For this reason it is important to continue to study cell types where this is prominently developed.

One such cell is found in the pigment epithelium of the retina. Here the ER shows a number of unusual features which initially attracted our attention and stimulated this more detailed study. In these cells, as noted above, it is made up in large part of smooth (or agranular) tubular elements associated in a close tridimensional lattice. In the basal half of the cell this is extraordinarily compact and constitutes more than 50 per cent (estimated) of the total volume of the cytoplasm. RNP particles occur in small numbers but show no special affinity for the surfaces of the ER. This is one of the purest expressions of the agranular form of the ER thus far encountered. In a terminology used by Grassé, among others, this would represent a reticular form of the ergastoplasm, though it would have none of the staining properties traditionally used to characterize this cell component.

In these cells of the pigment epithelium the general reticular pattern of the smooth ER is interrupted at a number of points by striking differentiations of the system referred to as myeloid bodies. These are essentially highly ordered stacks of thin lamellar units of the reticulum. As is easily recognized, this gives these structures precisely the form characteristic of the rod outer segments and other photoreceptor units.

It is not, however, quite so easy to point to equivalents in other cell types of the general reticular form of the ER. The sarcoplasmic reticulum described earlier in this series of papers (4) is

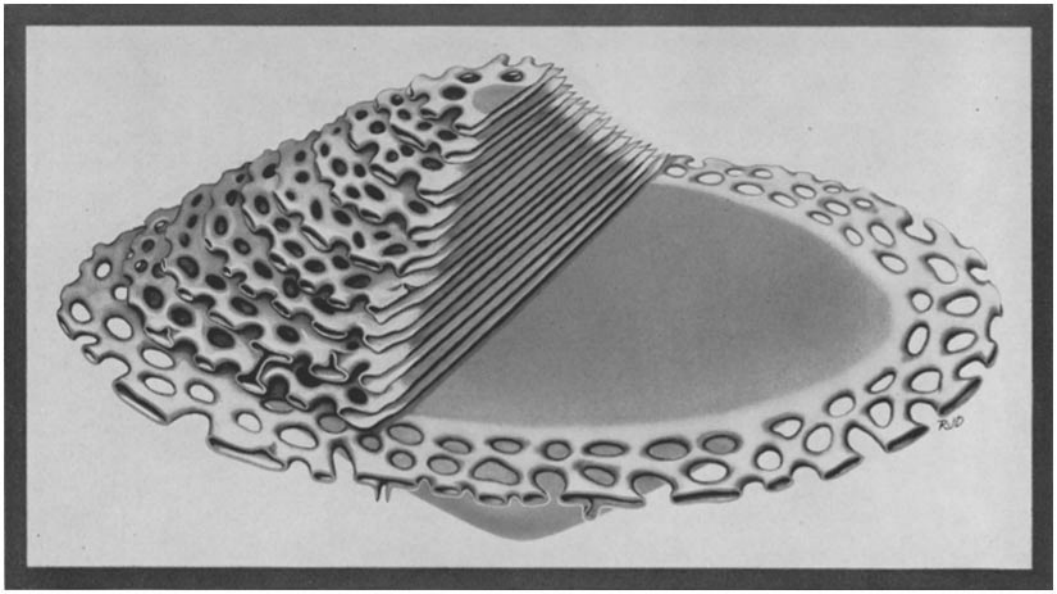


FIGURE 11

Drawing representing in three dimensions, structure of myeloid body. The paired membranes of each lamellar unit are represented as thicker than the membranes limiting the ER. The development of fenestrae in the margins of the lamellar units leads to a reticular form continuous with the general ER. The double line used to indicate the fine edges of membranes is an artist's device and has no structural significance.

similar in being made up very largely of agranular and tubular elements, but there the similarity largely ends. The smooth type of the ER encountered in glycogen areas of liver cells of fasted rats is more nearly equivalent in form (6, 19). The same may be said for the smooth ER associated with the glycogen-rich paraboloid of the turtle retina (2). In these two instances there is evidence suggesting that the smooth ER is involved in carbohydrate metabolism and for reasons mentioned below, this structural similarity may have some significance here. On the other hand, in cells of the sebaceous glands, Palay (8) has noted networks of fine tubular elements which, through a close morphological relation to lipoid droplets, would appear to function in fat metabolism. Similar developments of the smooth ER have been observed by Muta (9) in the interstitial cells of the mouse ovary and by Christensen and Fawcett (10) in the interstitial cells of the opossum testis. It should perhaps also be mentioned that reports on the adrenal cortex make note of an abundance of agranular vesicles and tubules in association with lipid granules (11). There seems, therefore, in these morphological observations some excuse to associate the smooth ER with lipid as well as with carbohydrate metabolism. The close affinity noted above in these pigment cells between the ER and the surface of the fat globule would bear out this assumption. This is recognized as a feeble form of evidence, but there is at least some biochemical evidence on agranular microsomes which supports the general idea (12).

It is regrettable in this regard that more is not known about the functions of the pigment epithelium for then one might look to this information for clues to the role of this unusual and extreme expression of the agranular form of the ER. Though the information is meager it is, however, worth summarizing.

There is some evidence that the pigment epithelium plays an important role in the physiology of vision. Existing as the sole cell barrier between the richly vascular choroid (which supplies the retina with the bulk of its metabolites) and the photoreceptors of the retina, it has the responsibility for transmitting metabolites in unaltered form to these receptors, or for transforming such

metabolites into materials physiologically important in vision. The intactness of this cell barrier, as evidenced by the close and complete apposition of the cells at the terminal bar level, strengthens the conclusion that the bulk of metabolites essential for visual perception must go through the epithelial cells rather than between them. Quite apart, then, from the phenomenon surrounding pigment migration the epithelium is an important tissue. Eichner (21) has suggested from his consideration of the problem that the epithelium provides for the functional metabolism of the outer segments as well as for the more general cellular metabolism of the inner segments and cell bodies. The glucose and oxygen requirements of the visual epithelium are most certainly satisfied by transport through the pigment cells. In cases of detached retina, where the visual layer separates from the pigment layer, not only is there a loss of vision but there is a shift in metabolism from highly glycolytic to highly oxidative (22).

The gross structural relationship of the two cell layers is well suited to an effective transfer of these metabolites from one to the other. In the first place, the barrier between the vascular supply and the epithelial layer is extremely thin and should offer slight impediment to the movement of diffusible metabolites. Then it is to be noted that the epithelial cell essentially enfolds the outer segments. Not only does the whole body of the pigment cell project into the interstitial space but it sends long processes into the space between the outer segments and even as far as the outer limiting membrane. These processes correspond to the fibrils noted by Sidman (14). This morphology obviously increases greatly the total cell surface in contact with the interstitial matrix and so provides for more rapid exchanges. The fine structural elements of possible significance in this exchange are not so readily defined. The form of the ER along the basal half of the pigment cell, as mentioned above, is reminescent of the form in the liver cell which is apparently involved in glucose storage. Since there is no demonstrable glycogen in the pigment epithelium it is reasonable only to consider the involvement of the smooth ER in glucose uptake and transport to the interstitial space. Whether it is phosphorylated is unknown, but the relatively large population of mitochondria along this surface of the cell suggests that some ATP-requiring reaction is in progress. It is impossible to say at the moment what is involved here, but one is encouraged to assume that the pronounced development of the smooth ER is, in part, for the purpose of carbohydrate transport.

A number of chemical units in the visual cycle could conceivably be associated with the large membrane surfaces provided by the smooth ER and its local differentiation—the myeloid body. There is, for example, as part of the pigment epithelial cell, the "pigment layer factor" of Bliss (23) which promotes the reconstitution of rhodopsin from retinene and opsin—the equivalent of retinene isomerase (30). Since the isomerization

FIGURE 12

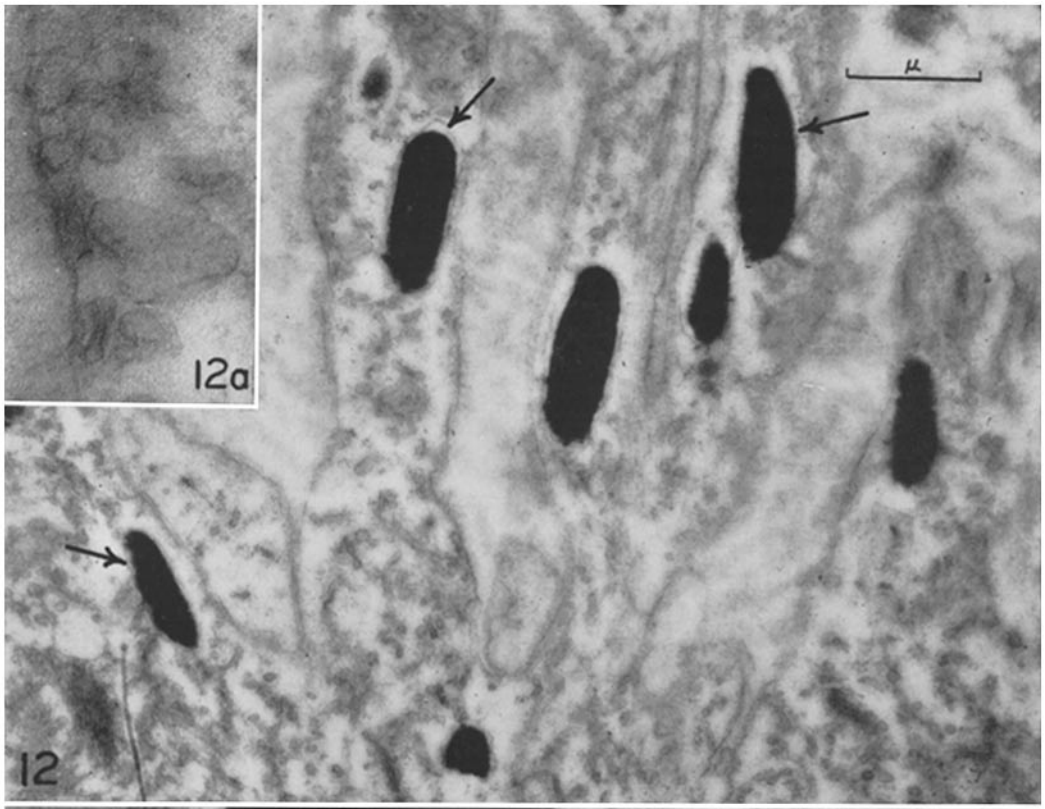
Micrograph taken to show apical portion of pigment epithelial cell at point where several processes leave the cell body. Elliptically shaped pigment granules are present in each process as are also many profiles of small tubules and vesicles of ER. These latter are identical with ER elements in the apical pole of the cell. A thin membrane around the pigment granules can be discerned at a few points (see arrows). Magnification × 18,000.

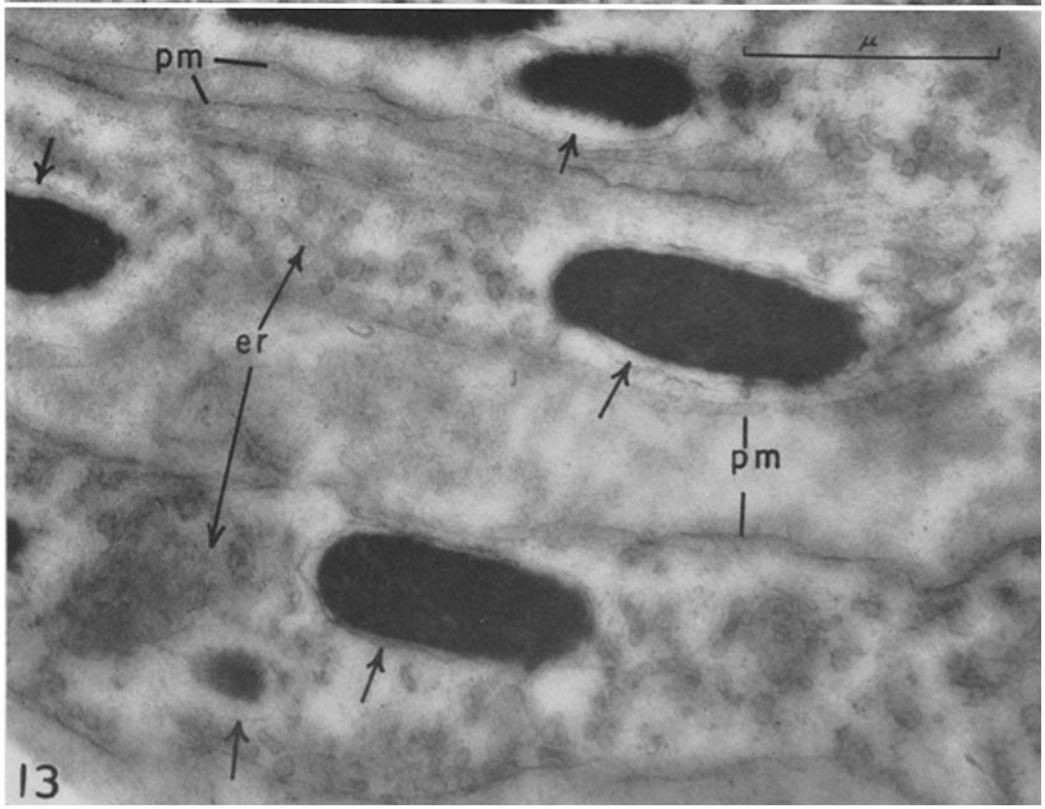
FIGURE 12*a*

Greater enlargement of surface of adjacent cell process designed to show intimate relation between cytoplasmic vesicles and plasma membrane. Magnification × 70,000.

FIGURE 13

This micrograph shows portions of three pigment-containing processes. The plasma membranes limiting the processes are indicated by *pm*. Each pigment granule is surrounded by a membrane as though contained within a vesicle (see arrows). These membranes are continuous at certain points with the membranes limiting elements of the ER (*er*). Magnification × 34,000.


12a
μ
12


pm
μ
er
pm
13

catalyzed by this enzyme is accelerated by light, one is encouraged to relate the reaction to the myeloid body by reason of the structural similarity between it and known photoreceptors. It is attractive to speculate further that the smooth ER may be significant (in supporting requisite dehydrogenases) in the interconversion between retinene and vitamin A and the transport of these compounds. It is known that vitamin A, like the smooth ER, is abundant in the pigment epithelial cells and is distributed rather widely in the cytoplasm (20 and 23). The yellow color of the lipid droplets probably reflects a high content of carotinoids here also, and here again we find morphological evidence of ER involvement. The close relation between the ER and the surface of the fat globule has been indicated and contrasted with the relative indifference shown by the ER towards the laminated (phospholipid) inclusions.

Obviously these are only primitive suggestions of functional relationships between pigment cell fine structure and the photoreceptive elements of the retina. It is, however, not reasonable to expect more, for, as Wald has recently emphasized (24),

FIGURE 14

Micrograph selected to show homogeneous type of fat globule (or granule) and the close relation between its surface and the surrounding elements of the endoplasmic reticulum. The fat globule with a dense margin is at the upper right. Myeloid bodies (*mb*) and the densely developed ER occupy the rest of the cytoplasmic field. Magnification × 45,000.

FIGURE 15

This shows a part of the laminated type of lipid granule also encountered in pigment epithelial cells. The spacing and lines in the lamination repeat approximately those observed in the myelin of the nerve sheath. Details of spacings etc. are displayed within the circular enlargement, and in the densitometric tracing along line $X$ to $X_1$ shown in Fig. 15*a*. Profiles of the ER are present in the surrounding cytoplasm. Magnification × 85,000. Magnification of overlay × 170,000.

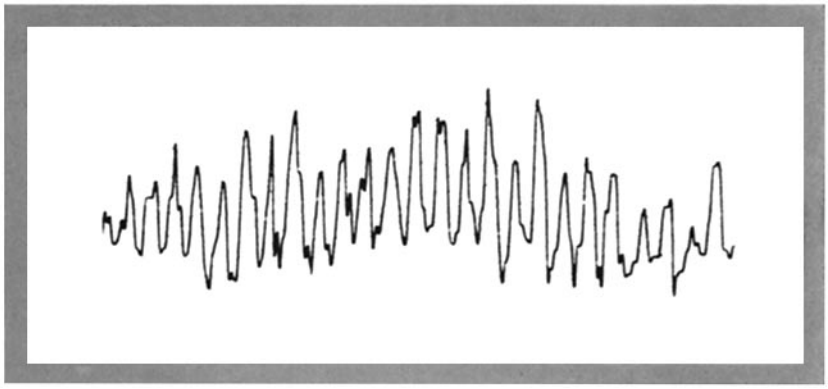
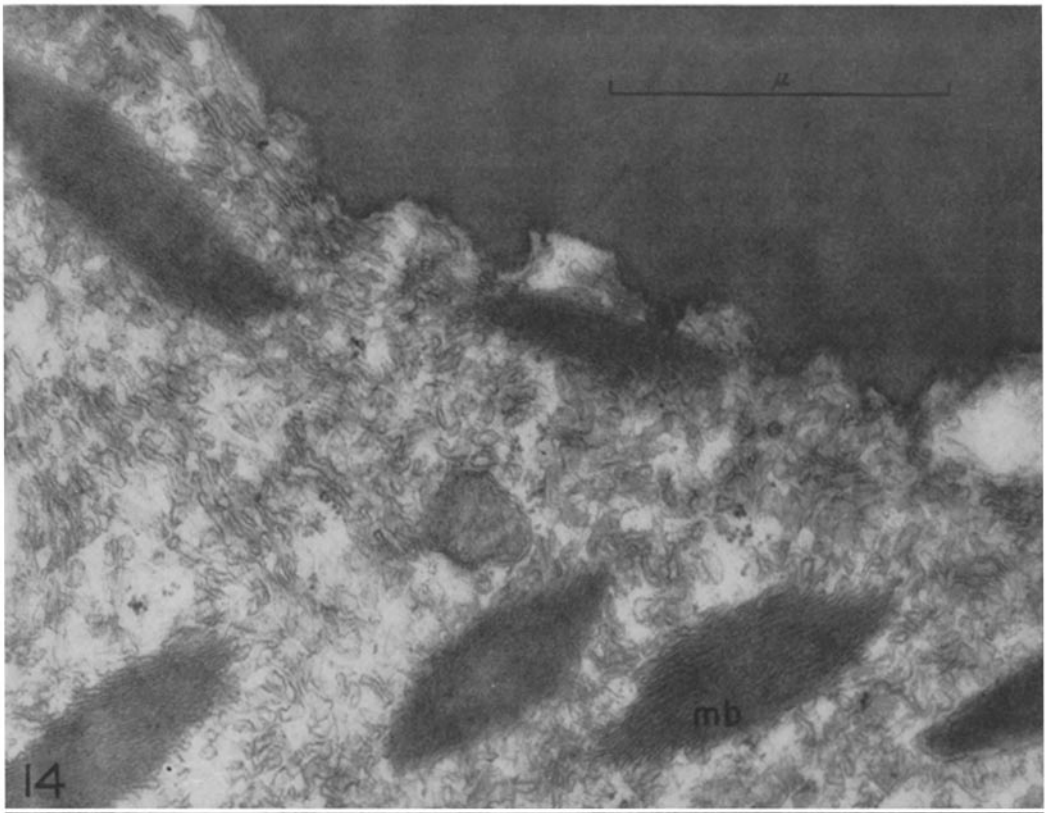
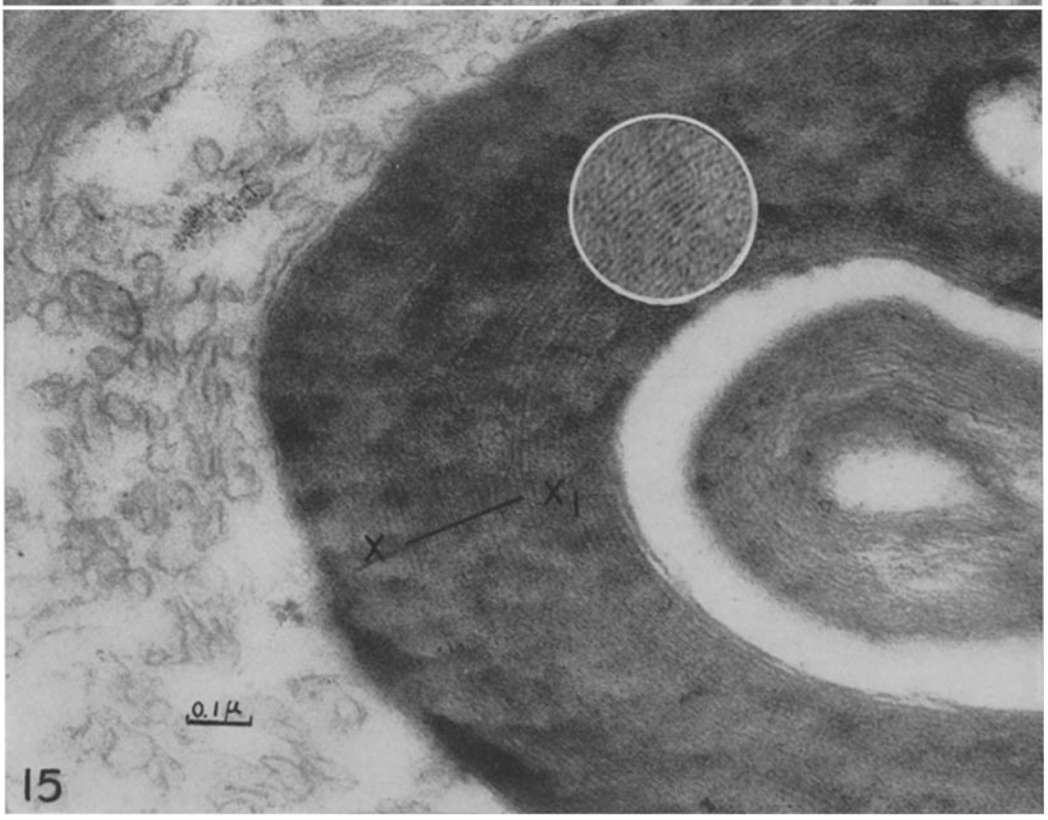


FIGURE 15*a*

Densitometric tracing taken from negative for Fig. 15 along line $X$–$X_1$ in that figure. The upper peaks represent the major lines and the troughs, the separating spaces. Intermediate peaks of lesser magnitude reflect points where densitometer recorded density of intervening minor line. The magnification is 50 times the original negative or × 567,000 the original material.


μ
mb
14


X1
X
0.1μ
15

we have only a fragmentary knowledge of the physiological relationships between the whole tissues, to say nothing of their fine structure.

The concept of the pigment epithelial cell as a secretory cell was proposed first by Kolmer (25), later denied, and more recently revived again. The latest suggestion from Sidman (14) is that these cells secrete substances into the interstitial matrix whence they might diffuse to the outer segments and limiting membrane of the visual epithelium. We cannot report that we have observed any evidence of secretory granules, and would suggest that the PAS-positive granules observed by Sidman may be the phospholipid granules (laminated) evident in these preparations. (See Lillie (26) on staining properties of galactolipids.)

*The Myeloid Body:* The single other known activity of the pigment epithelium is to bring about the migration of the pigment. In the amphibia, birds and fishes, the pigment is largely withdrawn into the apical pole of the cell in the dark-adapted eye, whereas under light stimulation it moves out again between the outer segments. This is said to cut down on scatter of light between the rods. Observations made here suggest that fucsin granules are contained within membranes and that these are continuous with those of the ER. In migration, however, the pigment alone seems to move; *i.e.* cell projections and some elements of the ER stay in place.

The factors involved in pigment migration are not understood. The presence of the myeloid body with a structure like that of other photoreceptors suggested that here was a unit capable of receiving a light signal and possibly translating it into pigment migration. The myeloid body may indeed be thus involved but if so, it seems more likely that it activates the pigment epithelial cell to perform other functions required by the visual receptor cells because pigment migration itself seems to be influenced by general humoral substances such as adrenalin (27). Even though its function is debatable, the myeloid body remains an interesting differentiation of the endoplasmic reticulum.

Myeloid bodies were described fairly early in light microscopy by Kühne (2) and Angelucci (15). They are birefringent and can be stained by the PAS procedure as can also the outer segments.

Their fine structure and relation to the ER deserve some comment. There is no question of the continuity of the individual discs at their margins with the tubules of the reticulum. The structural transition from the solid to the reticulate form at the margins of the discs is illustrated in Fig. 11, and requires only that the solid disc become fenestrated at its margins. This transition is similar to that reported earlier by Hodge *et al.* (28) for plant dictyosomes and more recently by Manton (29).

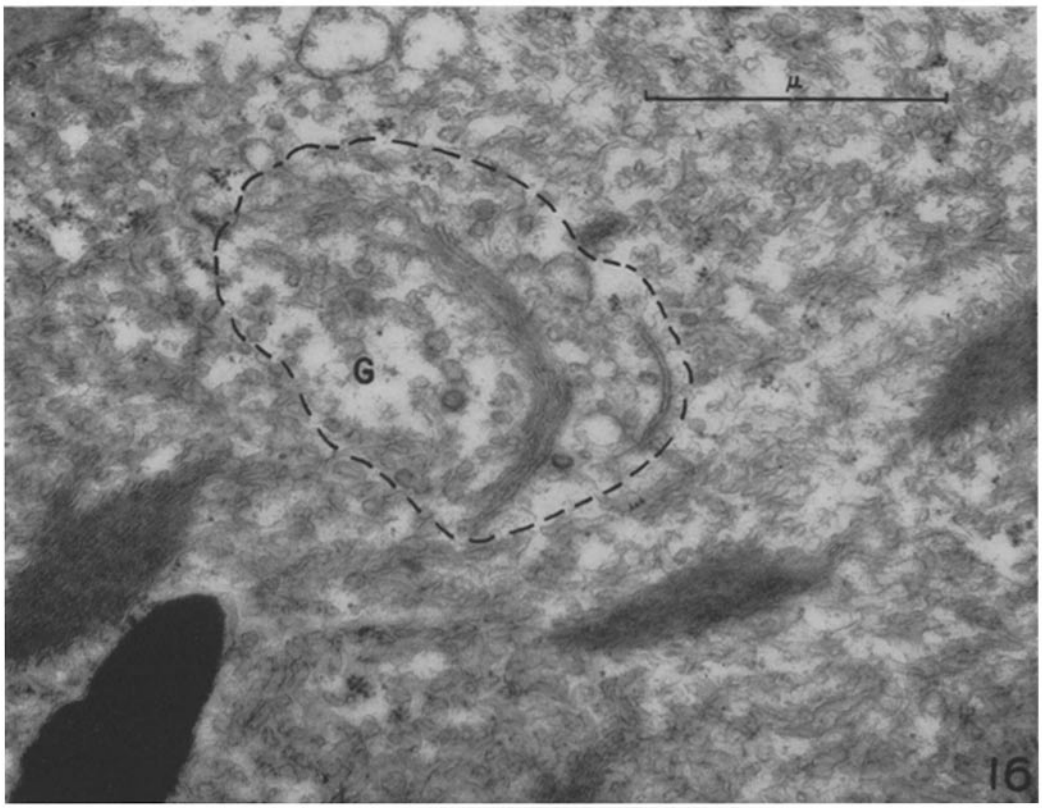
The membranes constituting the discs and the myeloid body are thicker than those limiting the ER, but similar to those in the discs making up the photoreceptor outer segments. This is probably descriptive of a content of photosensitive pigments, though, thus far, the presence of any in the pigment epithelium has not been demonstrated. The pairing of these membranes is explained by the fact that they limit a space that is continuous with the cavity of the ER. The myeloid body is therefore a stack of discs, as indicated earlier. The thickness of these is similar to that of those in the outer segments, as is also the space between them. Besides this evidence of order along the vertical

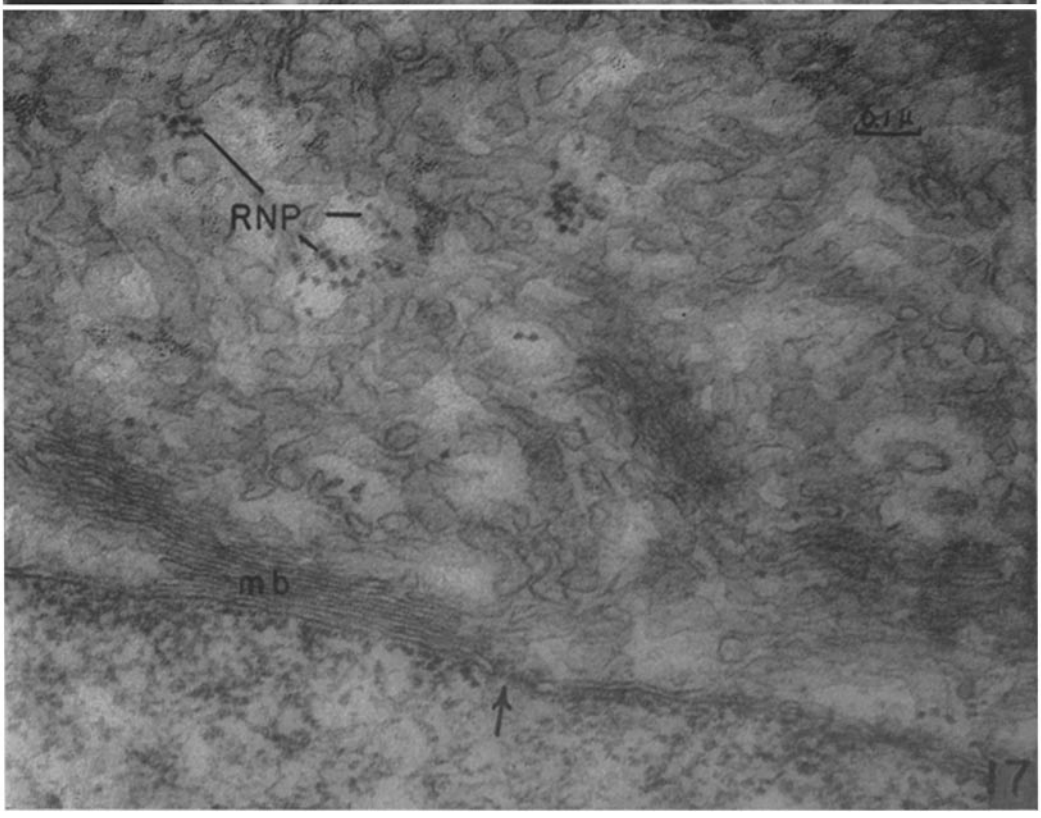
FIGURE 16

This depicts a part of the supranuclear cytoplasm in which a portion of the Golgi complex (*G*) can be identified; it is enclosed by a dotted line. The cytoplasm otherwise shows familiar structures. Magnification × 40,000.

FIGURE 17

Micrograph showing small portion of margin of nucleus, and adjacent cytoplasm. The nuclear envelope has the characteristic thin vesicular structure (double membrane) and is interrupted by a pore at the arrow. A myeloid body, closely applied to the envelope, is evident at *mb* and is separated from the envelope by a distance which duplicates that between lamellar units of the body itself. RNP particles are so indicated. Magnification × 86,000.


μ
G
16


0.1 μ
RNP
m b
17

axis of the myeloid body, there is also apparently some order radially in the discs. The gross expression of this is the uniform cut-back, stepwise, in the diameters of successive discs in the stack as they are taken in either direction from the equator of the body. This is true, at any rate, in most of the larger, possibly more fully differentiated bodies. The tendency, in other words, is toward an essentially crystalline form. This orderly arrangement would seem to be the necessary expression of a pattern in the molecular design of the membranes and the suggestion is that the pattern repeats radially every 65 mμ, which is the dimension of the cut-back. This describes as well the existence of a pattern in a derivative of the ER and reminds one of the probable general presence of patterns in membranes of the ER which could account for the distribution of RNP particles in whorls and rosettes, but more significantly, in the over-all form the system adopts under normal intracellular conditions.

The functional significance of the myeloid body must remain for future investigations to solve. We have thus far seen no evidence, in terms of orientation or number, of any response to light or dark. That they must be designed to function as photoreceptive units seems almost certain, but proof is lacking. As mentioned earlier, even if not involved in the initiation and control of pigment migration they could stimulate the cell to perform other activities important in retinal photoreception.

It is a pleasure for the authors to acknowledge the assistance of Mr. George Schidlovsky in the early phases of the microscopy of this material.

## REFERENCES

1. PORTER, K. R., The submicroscopic morphology of protoplasm, *Harvey Lectures*, 1957, **51**, 175.
2. KÜHNE, W., Chemische Vorgänge in der Netzhaut, *in* Hermann, L., Handbuch der Physiologie, Leipsic, F. C. W. Vogel, 1879, **3**, 235.
3. YAMADA, E., TOKUYASU, K., and IWAKI, S., The fine structure of retina studied with electron microscope. II. Pigment epithelium and capillary of the choriocapillary layer, *J. Electronmicr.*, 1958, **6**, 42.
4. PORTER, K. R., and PALADE, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
5. FAWCETT, D. W., Observations on the cytology and electron microscopy of hepatic cells, *J. Nat. Cancer Inst.*, 1955, **15**, 1475.
6. PORTER, K. R., and BRUNI, C., Fine structural changes in rat liver cells associated with glycogenesis and glycogenolysis, *Anat. Rec.*, 1960, **136**, 260.
7. YAMADA, E., The fine structure of the paraboloid in the turtle retina as revealed by electron microscopy, *Anat. Rec.*, 1960, **136**, 352.
8. PALAY, S. L., The morphology of secretion, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958.
9. MUTA, T., The fine structure of the interstitial cell in the mouse ovary studied with electron microscope, *Kurume Med. J.*, 1958, **5**, 167.
10. CHRISTENSEN, A. K., and FAWCETT, D. W., The fine structure of testicular interstitial cells in the opossum, *Anat. Rec.*, 1960, **136**, 333.
11. ROSS, M. H., PAPPAS, G., and LANMAN, J. T., Electron microscope observations on the endoplasmic reticulum in the human fetal adrenal, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 659.
12. LYNN, W. S., and BROWN, R. H., The conversion of progesterone to androgens by testes, *J. Biol. Chem.*, 1958, **232**, 1015.
13. CAULFIELD, J. B., Effects of varying the vehicle for $OsO_4$ in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
14. SIDMAN, R. L., Histochemical studies on photoreceptor cells, *Ann. New York Acad. Sc.*, 1958, **74**, 182.
15. ANGELUCCI, A., Histologische Untersuchungen über das retinale Pigmentepithel der Wirbelthiere, *Arch. Anat. u. Physiol., Physiol. Abt.*, 1878, 353.
16. MAJIMA, K., Studien über die Struktur der Sehzellen und der Pigmentepithelzellen der Froschnetzhaut, *Arch. Ophth.*, 1925, **115**, 286.
17. BOLL, F., Zur Anatomie und Physiologie der Retina, *Arch. Anct. u. Physiol., Physiol. Abt.*, 1877, 4.
18. FERNANDEZ-MORAN, H., and FINEAN, J. B., Electron microscope and low angle X-ray diffraction studies of the nerve myelin sheath, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 725.
19. PORTER, K. R., and BRUNI, C., An electron microscope study ot the early effects of 3′-Me-DAB on rat liver cells, *Cancer Research*, 1960, **19**, 997.
20. WALD, G., Vitamin A in eye tissues, *J. Gen. Physiol.*, 1934–35, **18**, 905.

21. EICHNER, D., Zur Histologie und Topochemie der Netzhaut des Menschen, *Z. Zellforsch.*, 1958, **48,** 137.
22. WEVE, H. J. M., and FISCHER, F. P., Le metabolisme de la rétine décollée, *Ann. ocul.*, 1938, **175,** 817.
23. BLISS, A. F., Properties of pigment layer factor in the regeneration of rhodopsin, *J. Biol. Chem.*, 1951, **193,** 525.
24. WALD, G., Photochemical aspects of visual excitation, *Exp. Cell Research*, 1958, suppl. 5, 389.
25. KOLMER, W., Über einen sekretartigen Bestandteil der Stäbchenzapfenschicht der Wirbeltierretina, *Arch. ges. Physiol.*, 1909, **129,** 35.
26. LILLIE, R. D., Histochemical studies on the retina, *Anat. Rec.*, 1952, **112,** 477.
27. DETWILER, S. R., On the role of chemical factors in retinal photomechanical responses, *Am. J. Anat.*, 1945, **77,** 117.
28. HODGE, A. J., MCLEAN, J. D., and MERCER, F. V., A possible mechanism for the morphogenesis of lamellar systems in plant cells, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 597.
29. MANTON, I., On a reticular derivative from Golgi bodies in the meristem of *Anthoceros*, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 221.
30. HUBBARD, R., Retinene isomerase, *J. Gen. Physiol.*, 1956, **39,** 935.
31. WATSON, M., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 727.
32. PEACHEY, L., A device for staining tissue sections for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 511.
33. WISLOCKI, G. B., and SIDMAN, R. L., The chemical morphology of the retina, *J. Comp. Neurol.*, 1954, **101,** 53.