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Histology and Histopathology

Retinal pigment epithelial fine structure in the Australian Galah (*Eolophus roseicapillus*) (Aves)

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Summary. As part of a comparative morphological study, the fine structure of the retinal epithelium (RPE), choriocapillaris and Bruch's membrane (complexus basalis) has been investigated by light and electron microscopy in the galah (*Eolophus roseicapillus*), an Australian cockatoo. The RPE consists of a single layer of low cuboidal cells joined basally by a series of zonulae occludentes. Basally (sclerally) the retinal epithelial cells display numerous deep infoldings while apically (vitreally) microvillar processes interdigitate with photoreceptor outer segments. Internally the RPE cells show a large vesicular nucleus, plentiful smooth endoplasmic reticulum (SER) and numerous polysomes but very little rough endoplasmic reticulum (RER). Numerous mitochondria are located basally. In the light-adapted specimens studied, the melanosomes of the RPE are almost exclusively located within the apical processes indicating that retinomotor movement of this pigment probably occurs. Phagosomes and lysosome-like bodies are present as are myeloid bodies which may show ribosomes on their outer surface. The choriocapillaris endothelium is thin but only minimally fenestrated facing Bruch's membrane. Most fenestrations of the choriocapillaris display a single-layered diaphragm while the remainder have a double-layered diaphragm. Bruch's membrane (complexus basalis) is typical of avian species in that it is pentalaminate with the central lamina densa displaced towards the choriocapillaris.

Key words: Retinal pigment epithelium (RPE), Choriocapillaris, Fine structure, Aves, Galah, *Eolophus roseicapillus*

Introduction

The retinal pigment epithelium (RPE) is the outermost (scleral) layer of the neural retina and is involved in several processes crucial to the proper functioning of the photoreceptors and ultimately to vision itself. Amongst the best understood roles of the RPE are: 1) the phagocytosis and removal of shed photoreceptor outer segment debris (Young, 1978; Bok and Young, 1979); 2) the storage and modification of vitamin A precursors of the visual pigments (Young, 1979); 3) the selective transport of materials to the photoreceptors (Kroll and Machemer, 1968; Steinberg and Miller, 1973); 4) internal adhesion within the neurosensory retina to prevent retinal detachment (Zinn and Benjamin-Henkind, 1979); 5) the architectural stabilization and proper orientation of the photoreceptor outer segments, and 6) the normal presence of pigment leads to enhanced acuity by absorbing reflected light (Moyer, 1969).

As a consequence of these several important functions, this region of the vertebrate retina has been investigated in a variety of animals and with a variety of techniques. Morphological studies in particular have shown a remarkable similarity throughout vertebrate species but with phylogenetic differences usually present (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994).

While numerous reports of the fine structure of the retinal epithelial region are available, relatively few deal with avian species (Nishida, 1964; Matsusaka, 1966; Meyer et al., 1973; Dieterich, 1975; Braekevelt, 1984, 1989; Braekevelt and Thorlakson, 1993). Consequently as part of an ongoing comparative study of the RPE region in general and with avian species in particular, this report deals with the fine structure of the retinal pigment epithelium, choriocapillaris and Bruch's membrane in the galah (*Eolophus roseicapillus*), an Australian cockatoo.

Materials and methods

For this study the eyes from two light-adapted galahs (*Eolophus roseicapillus*) were examined by light and electron microscopy. The adult birds were captured in mist nets under the Western Australian Department of Conservation and Land Management Licence # SF 000503. With the animals under deep anesthesia, the

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eyes were quickly enucleated, slit open at the equator and immersion fixed for 5 h at 4 °C in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm^2. This tissue was then postfixed for 2 h in 1% OsO_4 in the same phosphate buffer, dehydrated up through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reoriented to desired angles by means of a wax mount and thick sections (0.5 µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (60-70 nm) were then cut of selected areas and collected on copper grids. These sections were stained with aqueous uranyl acetate and lead citrate and examined and photographed in a Phillips EM201 transmission electron microscope.

Results

The RPE in the galah (*Eolophus roseicapillus*) is formed by a single layer of low cuboidal cells which average about 10 µm in height (Figs. 1, 2). Basally (sclerally) the retinal epithelial cells display numerous membranous infoldings which penetrate to a depth of about 1.5 µm (Figs. 2, 3). Apically (vitreally) these epithelial cells possess numerous finger-like processes which interdigitate with the photoreceptor outer segments (Figs. 1, 3, 7). Laterally the cell borders of the RPE cells are relatively smooth and are joined together by a series of basally-located tight junctions (Figs. 1-3, 6).

Internally the retinal epithelial cells display a single vesicular nucleus located in the mid-region of the cell in the light-adapted state (Figs. 3, 5). As has been reported in other vertebrate species, the most abundant cell organelle is smooth endoplasmic reticulum (SER) and, with the exception of the basal infoldings, is found throughout the cytoplasm of these cells (Figs. 3, 4, 6). Rough endoplasmic reticulum (RER) is noted only as small scattered profiles while polysomes are abundant and widespread (Figs. 2, 3, 6). Lysosome-like bodies were often noted and usually in a fairly homogeneous condition indicating a primary lysosome (Figs. 5, 6). Phagosomes of outer segment debris are present but uncommon within the RPE cells of the galah (Fig. 1).

Mitochondria are numerous within the retinal epithelial cells and are mainly located in the basal region of the cells adjacent to the basal infoldings (Figs. 2, 4, 5). These mitochondria are quite variable in shape, often showing a notched profile (Figs. 2, 5, 6). In addition, ring-shaped mitochondria as reported in other avian species are also present although not abundant (Figs. 2, 3, 5).

Myeloid bodies which are organelles comprised of a stacked array of smooth membranes and which are usually noted within the RPE cells of non-mammalian vertebrates are also present within the retinal epithelial cells of the galah in surprising numbers and a variety of shapes (Figs. 1, 2, 4-6). For the most part they are composed of a compact assembly of membranes which are continuous with the SER (Figs. 5, 6) but as has been noted in other avian species, the myeloid bodies in this cockatoo may display ribosomes on their outer surface, possibly indicating a continuity with the RER membranes as well (Figs. 2, 3).

In the light-adapted condition, the melanosomes within the RPE cells are almost exclusively located within the apical processes of these cells (Figs. 1, 3, 7) where they serve to surround and isolate the rod photoreceptor outer segments from each other (Fig. 7). Rod photoreceptors in this species are deeply embedded within the RPE cell body in the light-adapted condition and often reach to within 2 or 3 µm of the basal infoldings (Figs. 2, 4). Melanosomes in the galah are small and round to oval in shape (Figs. 3, 7).

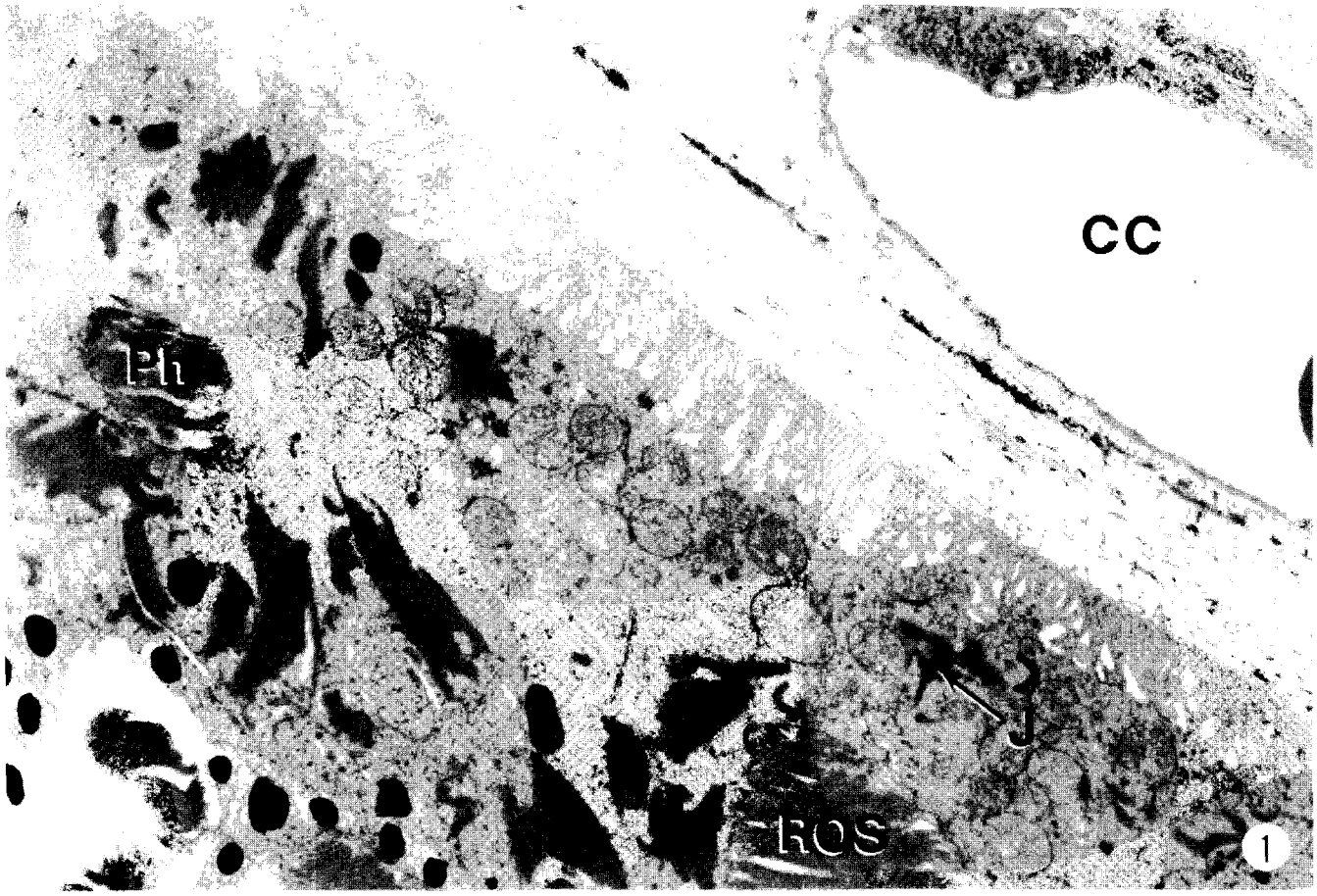
In the galah, the choriocapillaris forms a single layer of large-caliber capillaries immediately adjacent to the choroidal aspect of Bruch's membrane (Figs. 1, 2, 4, 6). The endothelium facing Bruch's membrane is normally quite attenuated but only minimally fenestrated (Figs. 2, 4, 6). Endothelial nuclei are not normally located on this aspect of the choriocapillaris. For the most part these fenestrations have a single-layered diaphragm but as is normally reported for avian species a number of them show a double-layered diaphragm (Figs. 2, 4, 6).

Bruch's membrane or complexus basalis in the galah is a pentalaminate structure consisting of: 1) the basal lamina of the RPE cells; 2) the basal lamina of the choriocapillaris enclosing; 3) a discontinuous elastic layer (lamina densa) which separates; 4) an outer, and 5) an inner collagenous layer (Figs. 2-6). As is noted in other avian species, the lamina densa is located much closer to the choriocapillaris producing an inner collagenous layer of greater width than the outer (Figs. 3, 6). Bruch's membrane averages about 1.2 µm in this species (Figs. 1-6).

Fig. 1. Low power electron micrograph of the RPE layer to indicate the height and width of the cells. A cell junction (J) is indicated as is a phagosome (Ph), a rod outer segment (ROS) and the choriocapillaris (CC). x 10,600

Fig. 2. Electron micrograph to illustrate numerous basally-located pleomorphic mitochondria (Mi) and plentiful basal infoldings (BI). For orientation the choriocapillaris (CC) and Bruch's membrane (B) are also indicated. x 10,700

Fig. 3. Electron micrograph to illustrate apical processes (AP), a deeply embedded rod outer segment (ROS) and an RPE nucleus (N). Bruch's membrane (B) is indicated. x 9,500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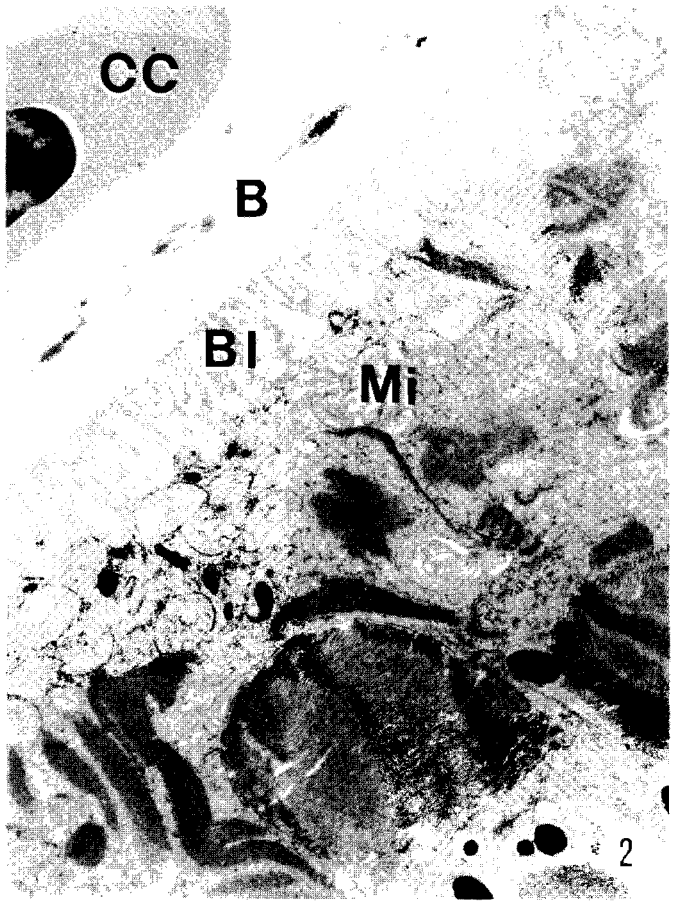
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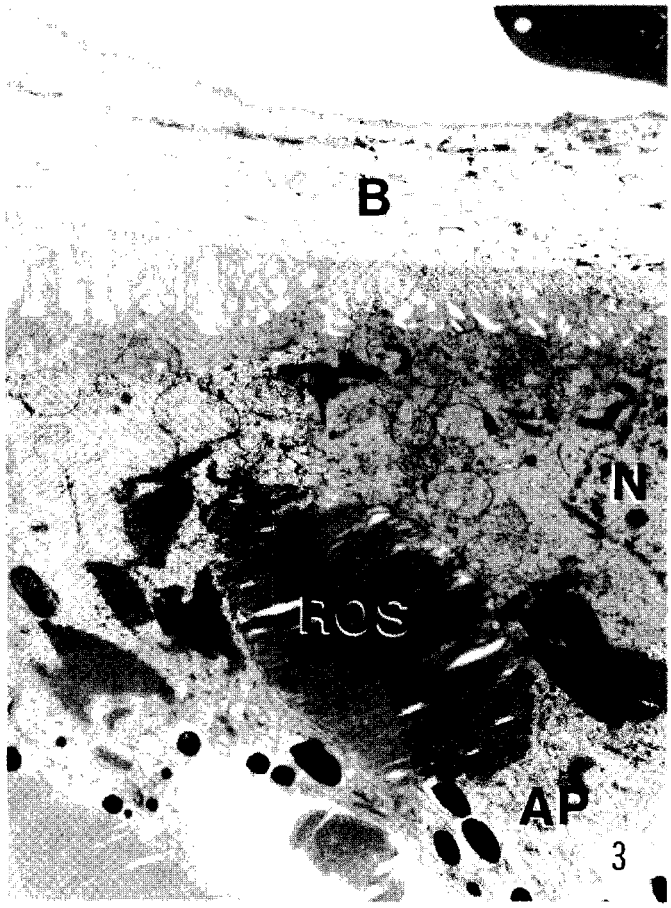
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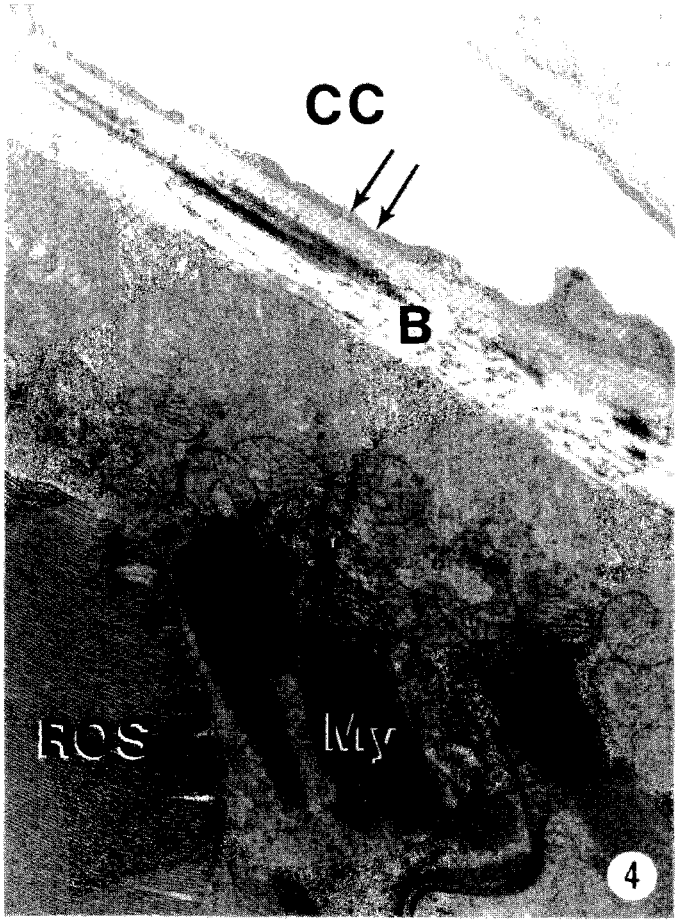
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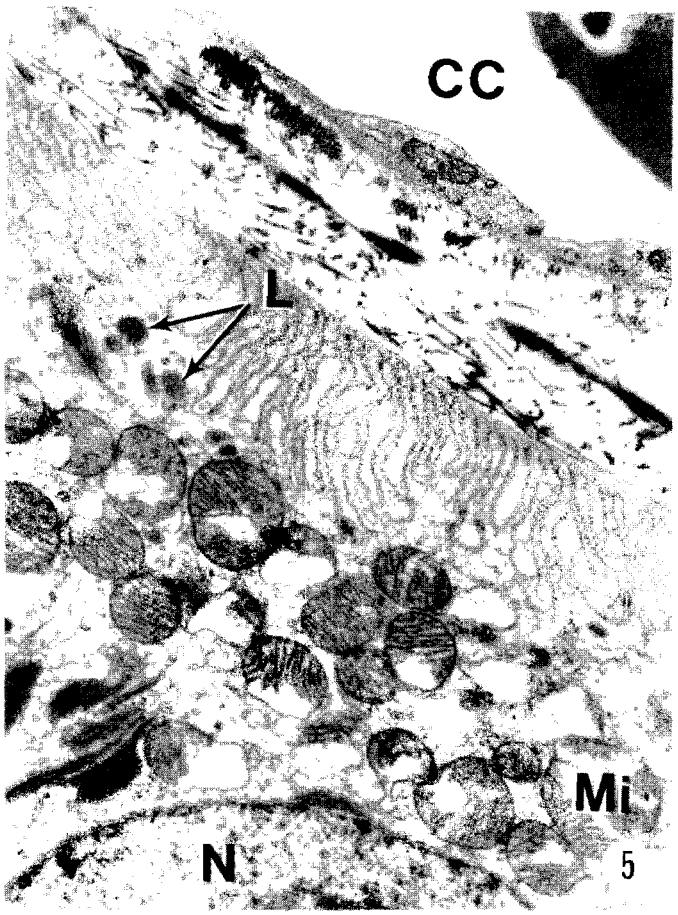
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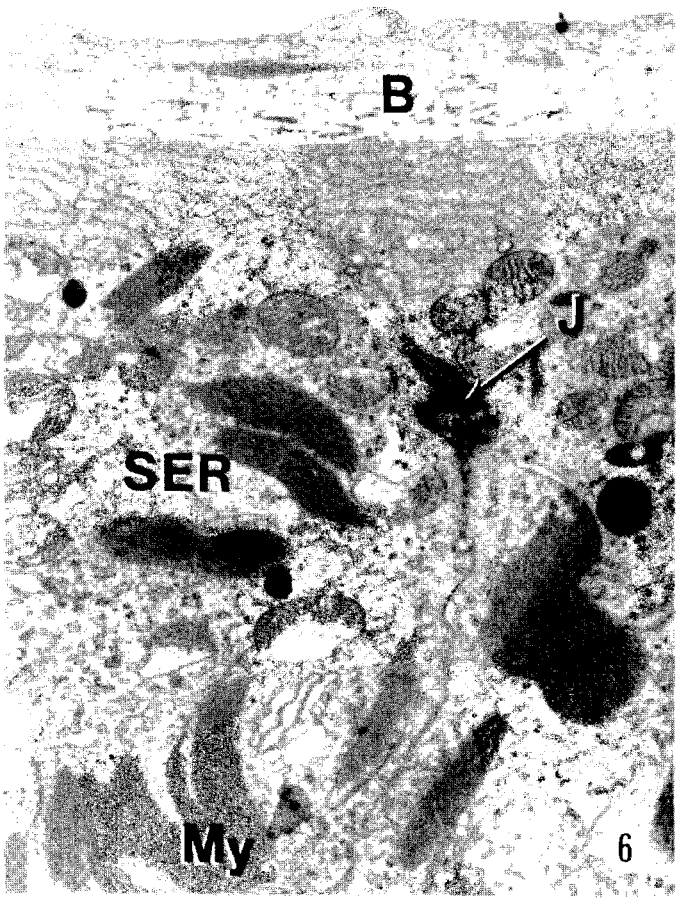
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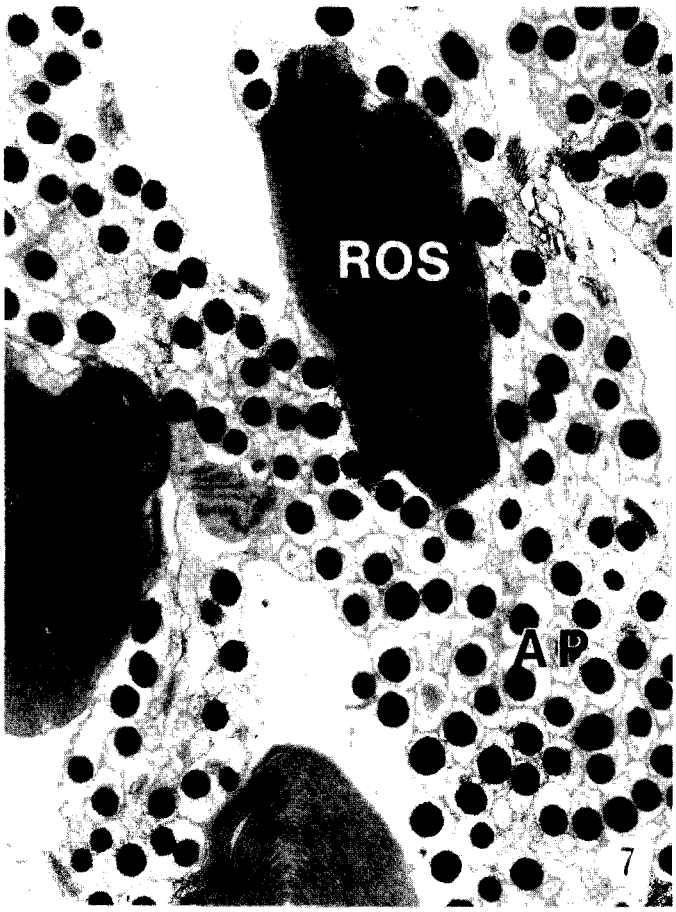
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Discussion

The RPE, Bruch's membrane (complexus basalis) and choriocapillaris of the galah (*Eolophus roseicapillus*) is essentially similar to that described for other vertebrates with characteristics that appear to be specific to avian species (Rodieck, 1973; Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994).

As in all vertebrate species the retinal epithelium of the galah is composed of a single layer of cells which display extensive basal infoldings as well as numerous apical processes which interdigitate with photoreceptor outer segments. The presence of highly infolded basal membranes in epithelial cells along with plentiful mitochondria is known to be indicative of a heavy involvement in transport, a function which is well established for the retinal epithelium (Steinberg and Miller, 1973). The basal infoldings in birds are normally quite deep which may indicate an enhanced transport role for the RPE in avian species (Braekevelt, 1990, 1992; Braekevelt and Thorlakson, 1993).

The numerous apical processes of RPE cells are involved in a variety of activities. They are certainly required for the structural support and efficient orientation to the incoming light of the elongated photoreceptor outer segments (Bernstein, 1961; Enoch, 1979). They are also important in maintaining the internal adhesion required within the neural retina to prevent retinal detachment (Zinn and Benjamin-Henkind, 1979). Finally the apical processes are involved in the phagocytosis and removal of shed outer segment disc material from the subretinal space, so as not to physically interfere with the transport of materials between the RPE and the photoreceptors (Bok and Young, 1979).

The series of tight junctions located at the lateral cell borders of the retinal epithelial cells is a constant feature of all retinal epithelial cells. They constitute an effective barrier to the intercellular movements of materials and hence form part of the blood ocular barrier (Zinn and Benjamin-Henkind, 1979). As noted for non-mammalian species, these cell junctions in the galah are located in the mid to basal region of the epithelial cells (Kuwabara, 1979; Braekevelt, 1984, 1989).

As has been reported in most other species, SER is abundant within retinal epithelial cells while RER is not (Nguyen-Legros, 1978; Kuwabara, 1979; Braekevelt, 1983, 1986, 1990, 1992, 1994). This preponderance of SER is due almost certainly to the heavy involvement of this epithelium in the production, storage, transport and esterification of the lipid precursors of the visual pigments (Zinn and Benjamin-Henkind, 1979). In the adult birds, the much smaller amount of RER present would indicate that little protein for export is being produced by these cells, whereas the abundance of polysomes would reflect the ongoing production of protein for the internal use of these epithelial cells (Alberts et al., 1989). The large vesicular nucleus coupled with much SER and numerous mitochondria are all indicative of metabolically very active cells.

The wealth of basally located mitochondria within RPE cells is noted in many species but the ring-shaped mitochondria noted in the galah appear to be unique to avian species (Lauber, 1983a; Braekevelt, 1989, 1990, 1992; Braekevelt and Thorlakson, 1993). Lauber (1983a,b) has shown that this ring-shape effectively doubles the surface area of the mitochondrion. While pleomorphic mitochondria have been reported in other species, avian mitochondria are normally quite irregular in shape as noted in the galah. Reports of mitochondrial shape in owls indicates an extreme pleomorphism that may be unique to that order of birds (Braekevelt and Thorlakson, 1993; Braekevelt et al., 1996).

Lauber (1983a,b) has also shown a variation in the number of ring-shaped mitochondria associated with the photoperiod, with a peak in the early dark period. This may explain their relative scarcity in the galah, as only light-adapted specimens were available for study. Perhaps the degree of mitochondrial pleomorphism will also vary with the photoperiod. In like manner the phagosomes of outer segment material noted within the RPE cells of the galah are presumably the remains of the burst of rod outer segment shedding which is known to occur soon after the onset of light. Their numbers would be lower by the mid to late portion of the light component of the circadian cycle (Young, 1978, 1979).

Judging by their almost exclusive location within the apical processes during light-adaptation, it is felt that the melanosomes of the RPE of the galah undergo photomechanical or retinomotor movements. Meyer (1977) states that photomechanical movement of the pigment within RPE cells of all birds is both rapid and extensive but examination of the RPE of a fully dark-adapted galah would be required to absolutely confirm this in the galah.

Fig. 4. Electron micrograph to indicate the pentalaminate Bruch's membrane (B), a myeloid body (My) and a rod outer segment (ROS). Note that some of the fenestrations in the choriocapillaris (CC) have a double diaphragm (arrows). x 19,900

Fig. 5. Electron micrograph to indicate the abundant mitochondria (Mi), lysosome-like bodies (L) and a vesicular nucleus (N). The choriocapillaris (CC) is also indicated. x 15,500

Fig. 6. Electron micrograph to illustrate the abundance of smooth endoplasmic reticulum (SER) and myeloid bodies (My) and the paucity of RER. A cell junction (J) and Bruch's membrane (B) are also labelled. x 16,700

Fig. 7. Electron micrograph to indicate the pigment-laden apical processes (AP) surrounding rod outer segments (ROS). x 16,700

Myeloid bodies which are usually present as a stack of smooth-surfaced membranes are a common feature within the retinal epithelial cells of non-mammalian species (Kuwabara, 1979; Braekevelt, 1982, 1984, 1988). While they have been implicated as sites of storage of lipids prior to esterification (Yorke and Dickson, 1984, 1985) and as the organelle that triggers photomechanical movements (Porter and Yamada, 1960), their function remains uncertain. Only in avian species however have ribosomes been reported on the surface of myeloid bodies and this may indicate another or secondary function for these organelles (Meyer et al., 1973, Braekevelt, 1989, 1990, 1992; Braekevelt and Thorlakson, 1993). The number, location and size of myeloid bodies has been reported to change dependent on the circadian cycle in the chick (Dickson and Morrison, 1993) but as only light-adapted specimens were examined in this study it is uncertain as to whether the myeloid bodies of the galah will undergo such changes.

Bruch's membrane or complexus basalis in mammalian species is invariably a pentalaminate structure with the five layers as described in the results portion of this report being quite obvious (Nakaizumi, 1964; Braekevelt, 1986, 1988, 1990). In contrast teleosts characteristically display a trilaminate Bruch's membrane with the central elastic layer (lamina densa) being absent (Braekevelt, 1982, 1985). In avian species, Bruch's membrane is pentalaminate and while the central lamina densa is often poorly represented, it is always shifted towards the choriocapillary side of Bruch's membrane (Braekevelt, 1984, 1989, 1990). The significance (if any) of these variations in the structure of the complexus basalis between species is obscure.

The choriocapillaris in all vertebrate species is composed of a single layer of large caliber anastomosing capillaries (Rodieck, 1973; Kuwabara, 1979). The endothelium facing Bruch's membrane is typically very attenuated and highly fenestrated indicative of the movement of large quantities of material across these capillaries to the RPE (Bernstein and Hollenberg, 1965). Teleost species normally show only a minimally fenestrated choriocapillaris which is felt to be due to the presence of a choroid gland critical for maintenance of a high oxygen pressure due to its counter current arrangement (Wittenberg and Wittenberg, 1974). Avian species including the galah also normally show a reduction in the number of fenestrations in the choriocapillary endothelium which in this case may be due to the presence of a pecten oculi (Braekevelt, 1989, 1990). Finally a number of the fenestrations that are present in the choriocapillary endothelium of birds and snakes show a double-layered diaphragm rather than the more conventional single-layered diaphragm (Kuwabara, 1979; Braekevelt, 1990, 1992, 1994). Such double diaphragm fenestrations are also seen in the galah but again their significance is unknown.

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Histology and Histopathology

Invited Review

The intracellular origin of the melanosome in pigment cells. A review of ultrastructural data

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Summary. This paper is a review about the ultrastructural data dealing with the origin of the melanin granules in retinal pigment epithelial cells, in melanocytes, in the ink gland of cuttle fish, in Kupffer cells of the liver, in neuronal tissues, in cultured pigment cells. The role and structure of lysosomes in melanogenesis are discussed in a separate chapter. The early steps of melanogenesis are ultrastructurally very heterogeneous, even in the same cell types. With respect to this heterogeneity and the considerably different views on melanosome origin in the literature, the author hypothesizes that pigment cells may use protein matrices originated from different cellular pathways.

1) They may either produce a specific protein matrix and be converted into melanin in the classical way, or 2) alternatively, a matrix resulting from lysosomal protein degradation or endocytotic pathways may be used and converted into melanin, as found in fibroblasts transfected with the tyrosinase gen or in Kupffer cells.

The very heterogeneous ultrastructure of the polymerizing melanin may be influenced by the amount and sterical availability of tyrosine residues in the protein moieties and the activity of tyrosinase.

Key words: Lysosome, Melanocyte, Retinal pigment epithelium, Melanin

Introduction

Whereas it is generally accepted that the key enzyme for mammalian melanogenesis is tyrosinase (Lerner et al., 1950; Körner and Pawelek, 1982; Hearing and Tsukamoto, 1991), the role and the origin of the matrix component of melanogenesis, the premelanosome, has remained obscure. However, it seems that pigment abnormalities may also result from the melanosomal matrix (Jimbow et al., 1982; Yanai and Takeuchi, 1987). This even seems to be the case in the tyrosinase deficient albino mouse (Granholm et al., 1990).

There are two types of melanin in humans and the animal kingdom: *eumelanin*; and *pheomelanin* (for review see Robins, 1991; Hearing and King, 1993; Prota, 1992). Eumelanin is the black-brown compound found in the skin, hair and all the other melanocyte-bearing tissues. Pheomelanin is the yellow to reddish-brown pigment that has been identified in mammalian hair (including human red hair) and the feathers of chicken. Pheomelanin and eumelanin differ in certain chemical and physical properties; for example, pheomelanins contain sulphur from the incorporation of cyteinyldopa and are soluble in dilute alkali.

The melanosome concept was developed by Seiji et al. (1961) by isolation of the subcellular fractions of the melanocyte by sucrose gradient ultracentrifugation combined with histochemical and electron microscopic analysis.

The nomenclature describing the events leading to the formation of brown (eumelanin) melanin granules in humans was also introduced by Seiji et al. (1963). The structural and chemical metamorphoses which take place in the melanin granule during melanization were described as follows.

1. At first stage, in which polypeptides are synthesized and condensed into the secondary and tertiary structures of the protein molecules-subunits containing tyrosinase molecule should be formed. In contrast to this original concept it is now clear that tyrosinase is not incorporated into the first stage organelles but later transported into premelanosomes by Golgi-derived coated vesicles (Maul, 1969).

2. An intermediate stage, in which the subunits are arranged in structural form, is called the premelanosome. The subunits are aligned in an ordered pattern, but melanin synthesis does not begin (Figs. 1, 2).

3. In a third stage, melanin biosynthesis begins and melanin accumulates inside the organelle. During this stage the granule contains tyrosinase activity and is called a melanosome (Fig. 3).

4. The final product, in which melanization is

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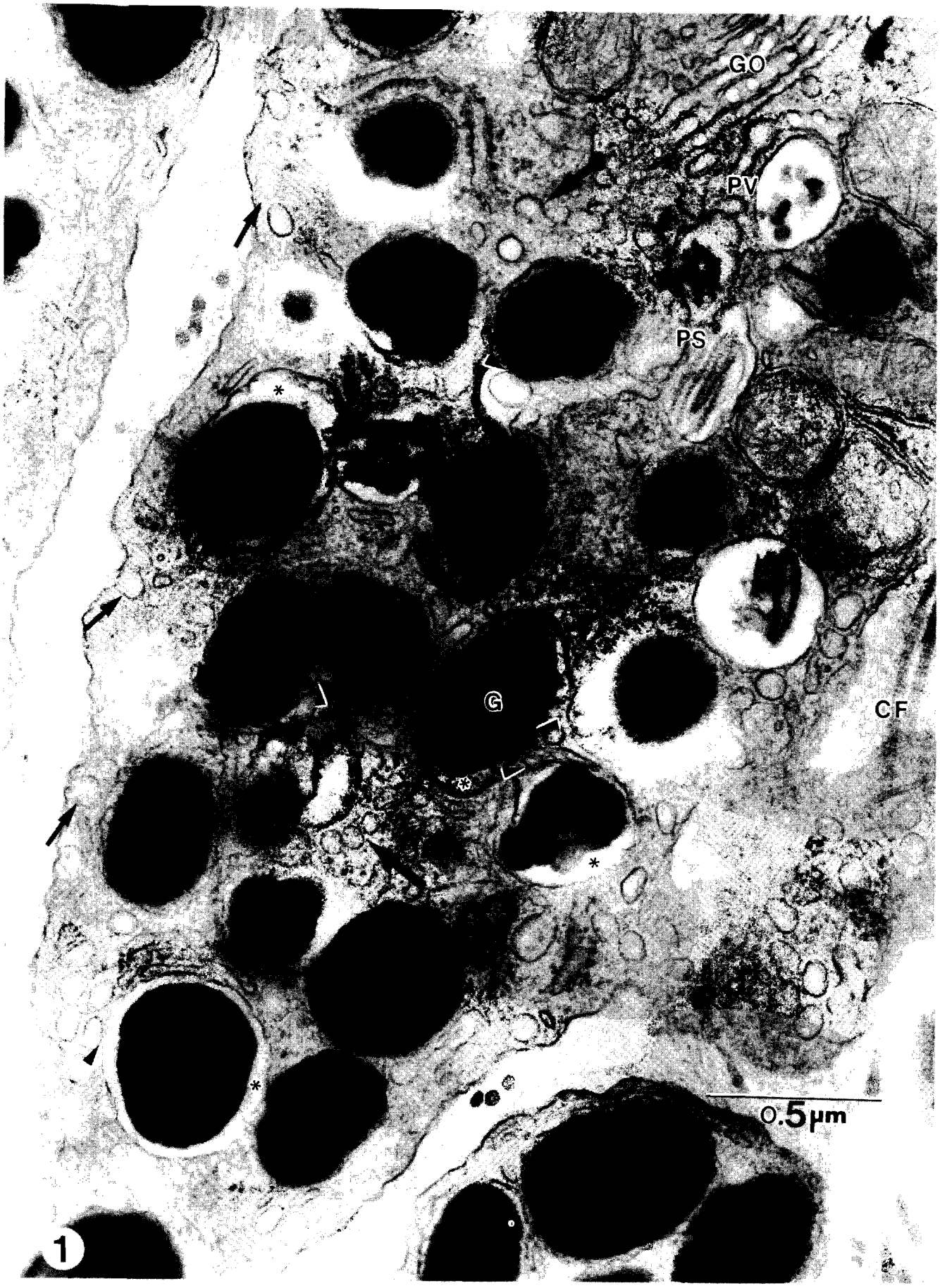
GO
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completed and in which no tyrosinase activity can be detected, is called a melanin granule (Fig. 3).

Later, these stages were termed stage I-IV melanosome (Toda and Fitzpatrick, 1971). In the recent literature the tem melanosome is also often used if melanin granules or premelanosomes are meant.

Whereas in the concept of Seiji et al. (1963), it was assumed that all matrix proteins were already present in the premelanosome it has been shown that during stimulated melanogenesis by PUVA therapy an unknown mechanism is activated, causing increased amounts of structural proteins to be incorporated into stage II melanosomes (Ree, 1983). Later, it was detected that melanosomes of human black hair characteristically contained so-called vesiculo-globular bodies; membrane delimited vesicles with a diameter of 40 nm (Jimbow and Kukita, 1971). These vesicles maintain the electron lucent areas during eumelanogenesis whereas they become amorphous and embedded in electron dense matrix during pheomelanogenesis (Jimbow et al., 1979). Whereas in the original concept tyrosinase should already be incorporated into the premelanosome in an inactive form (protyrosinase) it is now clear tyrosinase is transported in coated vesicles from the Golgi apparatus to the melanosome (Maul, 1969; Maul and Brumbaugh, 1971; Tomita et al., 1983; Imokawa, 1989; Granholm et al., 1990).

Electron microscopic histochemical studies have shown that tyrosinase is catalytically competent in these coated vesicles, but it is not completely clear how melanogenesis is delayed until its arrival at the melanosome *in vivo*. New evidence (Hatta et al., 1988; Mishima, 1990) was presented suggesting that coated vesicles play more than a passive role of carriers for the

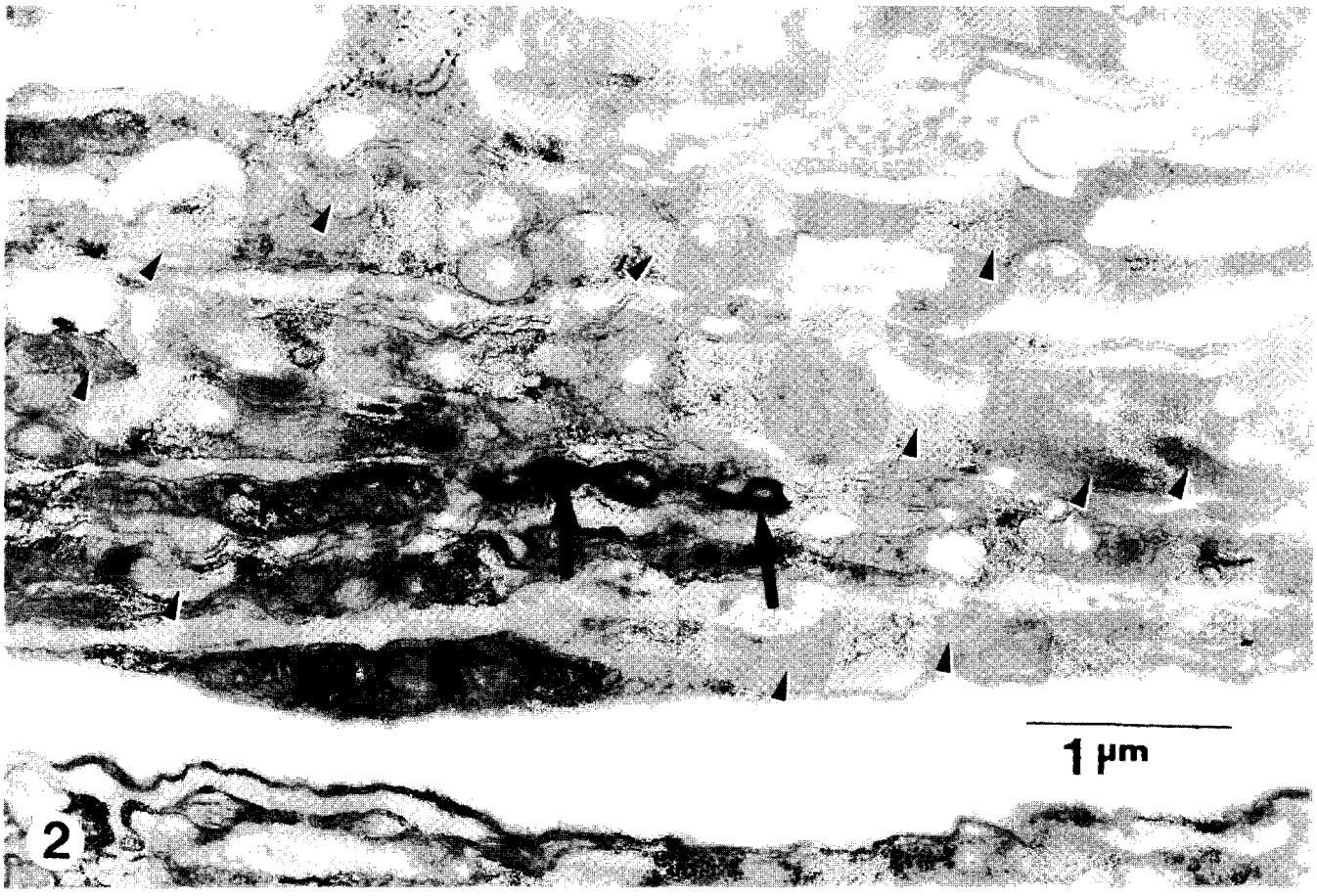


Fig. 2. Melanocytes of the choroid from the adult albino (C57B1/6J) are shown. The melanocytes contain numerous premelanosomes (arrowheads). One melanocyte contains electron dense membranous material (arrows). Nothing is known as to whether a turnover of these premelanosomes exists or not. x 29,500

Fig. 1. After intense illumination, melanocytes of the choroid of the adult golden hamster appear metabolically highly active. This is indicated by Golgi stacks (GO) and large amounts of internal (large arrows) and endocytotic (small arrows) vesicles becoming visible. Vesicles seem to fuse (arrowheads) with the membranes of the melanin granules (G), and the lumen (asterisk) between limiting membrane and melanin matrix appears enlarged in some melanin granules. Premelanosomes containing either melanofilaments with a striated periodicity (PS) or vesiculo-globular bodies (PV) are present. In the extracellular space collagen fibers (CF) with their characteristic periodicity are shown. x 65,000

translocation of tyrosinase into the premelanosome, but their functional significance in the melanization process is not yet clear. A lot of attempts have been made to relate the ultrastructure of melanogenesis to specific types of melanin or pigment cells.

Recently, the ultrastructure of melanosomes has been divided into two basic groups (Hach et al., 1993): 1) elliptical (ovoid) lamellar or fibrillar melanosomes with a protein matrix arranged into coiled lamellae or fibrils or rolled up sheets arranged parallel to the long axis of the organelle; 2) spherical globular melanosomes characterized by a granular appearance possibly due to a sponge-like architecture of their structural proteins with melanin stuffing the empty spaces.

A comparison of eu- and pheomelanogenesis further revealed that the earliest form of melanosomes are identical (Jimbow et al., 1979). The vesiculo-globular bodies are involved in melanosomal constituents (Jimbow and Kukita, 1971; Maul and Brumbaugh, 1971; Ide, 1972; Jimbow and Fitzpatrick, 1973; Sugiyama, 1979; Ito et al., 1982). In pheomelanogenesis lamellae are not formed, but these bodies fuse with each other to form an amorphous matrix on complete differentiation of melanosomes. Mature melanosomes in eumelanogenesis are ellipsoidal, whereas those melanosomes in pheomelanogenesis are spherical (Parakkal, 1967; Jimbow et al., 1979).

In the agouti mouse (Sakurai et al., 1975) and in one rare case in human (Inazu and Mishima, 1993), both eu- and pheomelanogenesis can take place in the same melanocyte or even the same melanosome, which has been termed mosaic-type melanosome (Sato et al., 1985). Therefore, the ultrastructure of the melanosome is not determined by the type of melanin produced as was already suggested by Moyer (1966).

In the agouti mouse, melanocyte-stimulating hormone induces a switch of synthesis from yellow to black melanin (Geschwind et al., 1972; Granholm et al., 1990; Granholm and van Amerongen, 1991). Such a switch was also observed after chemical modification in hairless mouse skin (Nishimura et al., 1982).

Eumelanogenesis in the retinal pigment epithelium (RPE) and melanocytes seems to follow a common pathway (Feeney et al., 1965; Hearing et al., 1973; Hu and Mah, 1983; Sarna, 1992) and a lot of detailed descriptions have been presented. Fine vertically-

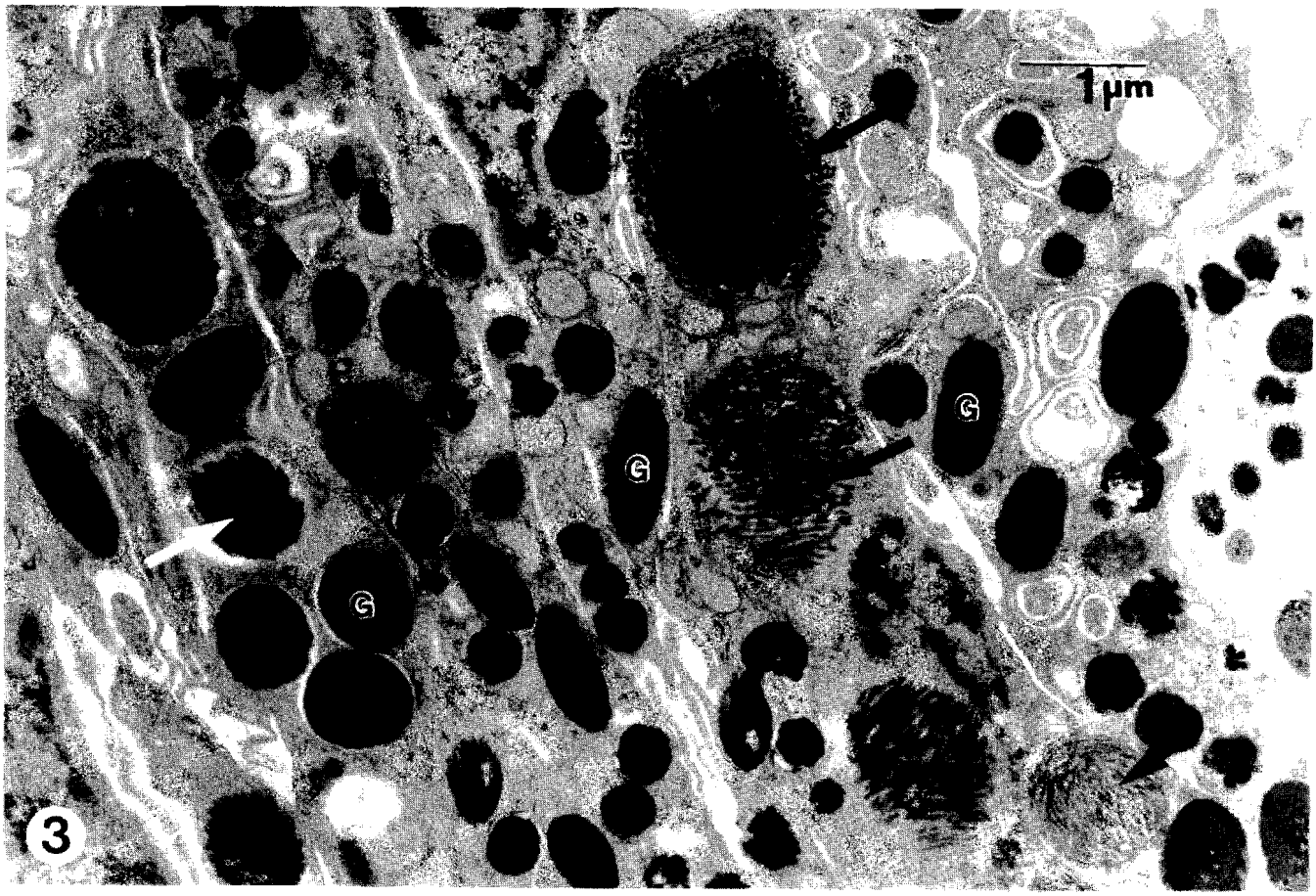


Fig. 3. A tangential section through the retinal pigment epithelium of the adult golden hamster demonstrates the size difference between the mature melanin granules (G) and the roundish large type of melanosome (black arrows). The large type melanosome (white arrow) may condense and change into mature melanin granules. x 17,000

running filaments of 3-5 nm have been reported to be the basic developmental structure of the premelanosome (Moyer, 1961, 1966; Tousimis, 1963; Mottaz and Zelickson, 1969; Jimbow and Kukita, 1971; Stanka, 1971; Lutzner and Lowrie, 1972; Hearing et al., 1973). These filaments, described as either spiral or zigzag in nature, are thought to run the length of the melanosome. As melanization proceeds, the fine filaments thicken, either through melanin deposition or by compounding the number of filaments (Moyer, 1961; Toshima et al., 1968; Rittenhouse, 1968; Hearing et al., 1973). This results in fibers which are aproximately 20 nm thick (Moyer 1961, 1966; Wellings and Siegel, 1963; Toshima et al., 1968; Rittenhouse, 1968; Hearing et al., 1973), 10 nm apart (Moyer, 1966; Mottaz and Zelickson, 1969) and which have a periodicity ranging from 6 to 13 nm. A second structural characteristic of the premelanosome is a striated periodicity in the order of about 5 nm in the filaments that are about 4 nm in diameter (Hu et al., 1967; Zimmerman et al., 1981). In the thicker filaments, probably in later stages, the periodicity is in the range of 10-15 nm (Hearing et al., 1973; Granholm et al., 1990). The nature of the material causing the striated periodicity that resembles that of collagen is not yet known (Fig. 1). When the striated periodicity is seen, many of the filaments within a single melanosome are often aligned in phase, thus imparting an overall periodicity to the granule, which is oriented perpendicular to the long axis of the granule (Birbeck, 1963; Jimbow and Kukita, 1971; Hearing et al., 1973). By solubilization of mature eumelanosomes it was shown that 19 nm fibers arranged in a zigzag fashion were surrounding 24 nm diameter electron-lucent spaces and an internal gridwork enclosing 4-5 nm diameter spaces (Zimmermann et al., 1981). In contrast, in freeze-fractured mammalian epidermal melanosomes, a very fine particulate matter was found without evidence of an internal structure (Garcia and Szabo, 1981).

In human races the size of individual melanosomes in the skin can vary between 1.3 x 0.6 to 0.3 x 0.1 µm (for review see Robins, 1991). The length of melanosomes from 14 different sources ranged from 3 µm (black poodle hair) to 0.18 mm (*Loligo* ink) (Hach et al., 1993).

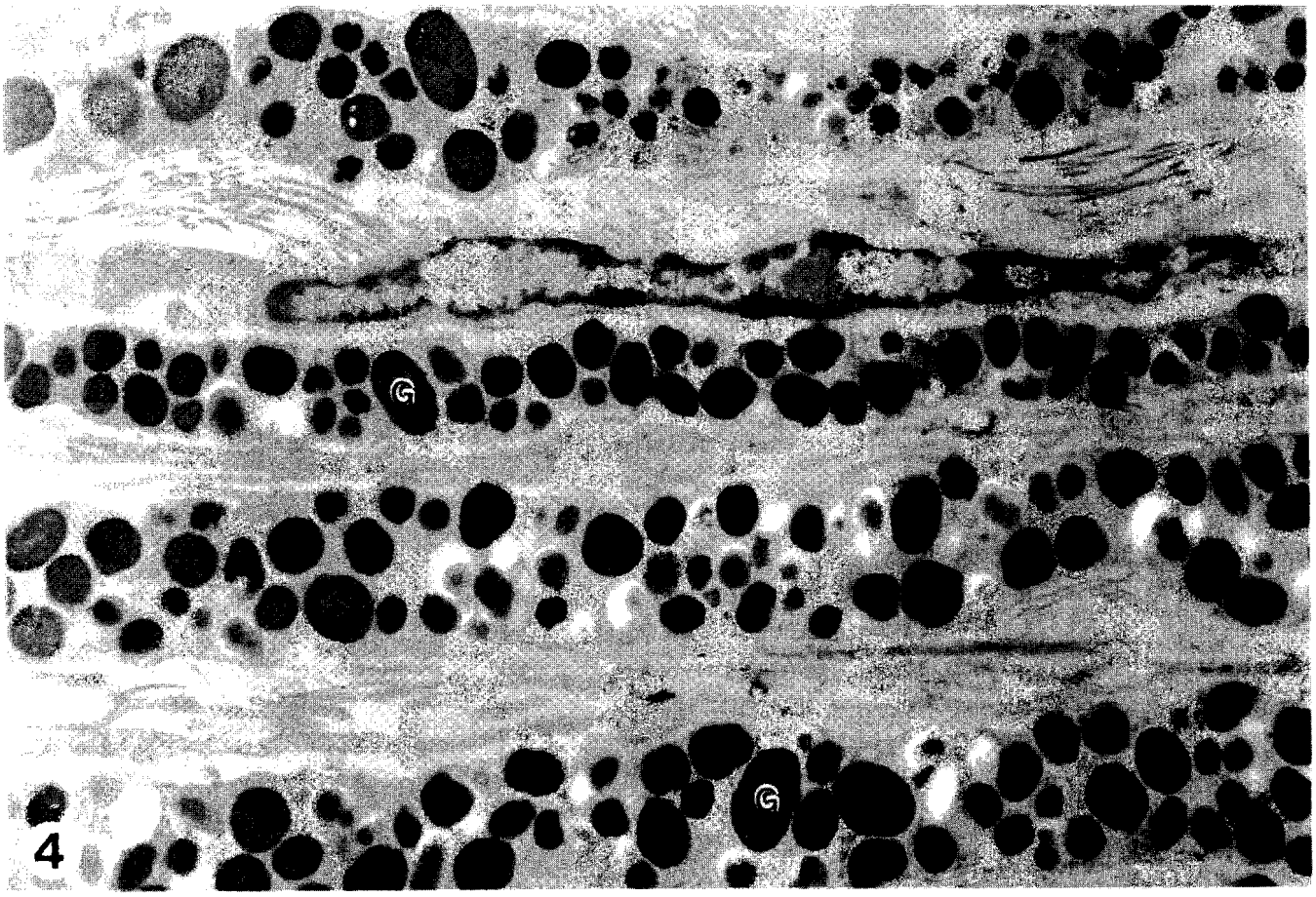


Fig. 4. In hamsters that had been kept under the normal light conditions of a laboratory, the dendritic melanocytes of the choroid contain mature melanin granules (G) and appear metabolically inactive judging by the poor development of cytoplasmic organelles. Premelanosomes are not observed. x 17,000

This article reviews the ultrastructural data on the intracellular origin of the melanosome.

Melanosomes in pigment cells of vertebrates

In vertebrates two types of pigment cells belonging to different cell lines are present. The RPE originates from the outer neuroectodermal sheath of the eyecup, whereas the melanocytes of skin, hair-bulbs, internal ear (Rawles, 1953) and the uvea (choroid, ciliar body and iris) (Heimann, 1972; Ozanicks et al., 1978; Torczynski, 1982) immigrate from the neural crest.

The ultrastructure of melanosomes in retinal pigment epithelial (RPE) cells

Turnover of melanin was believed to be completely absent in the RPE of adult mammals for a long time (for review see Schraermeyer, 1993) and was believed to be restricted to the prenatal period (Carr and Siegel, 1979; Dorey et al., 1990; Sarna, 1992) although age-related changes in the morphology of melanosomes have been reported (Boulton et al., 1990). However, all classical stages of melanin granule formation are present in the RPE of adult hamsters (Schraermeyer, 1993) which indicates that synthesis of melanin can occur in the RPE of adult mammals. However, in this study the formation of classical premelanosomes may have been induced by intense illumination. Stage II-IV melano-somes have been found in the RPE of adult tree squirrels (Tabor et al., 1980). In contrast to melanocytes, the RPE cells are specialized in degrading large amounts of proteins and lipids shed from the rod outer segments of the photoreceptor cells (Bok and Young, 1979; Young, 1977, 1978) which is important for the normal function of the retina. Although melanogenesis in the RPE and choroid seems to follow a common pathway (Feeney et al., 1965; Hearing et al., 1973; Hu and Mah, 1983; Sarna, 1992) the RPE of adult hamsters and probably all vertebrates contains unique melanosomes (Fig. 3) never present in melanocytes of the choroid (fig. 4). These are large, spherical melanosomes with loosely-packed melano-filaments, the origin of which is as yet unknown. Whereas a high number of melanin granules are spindle-shaped in the RPE (Fig. 3), they are smaller and more spherical in the choroid (Fig. 4) (Schraermeyer, 1993).

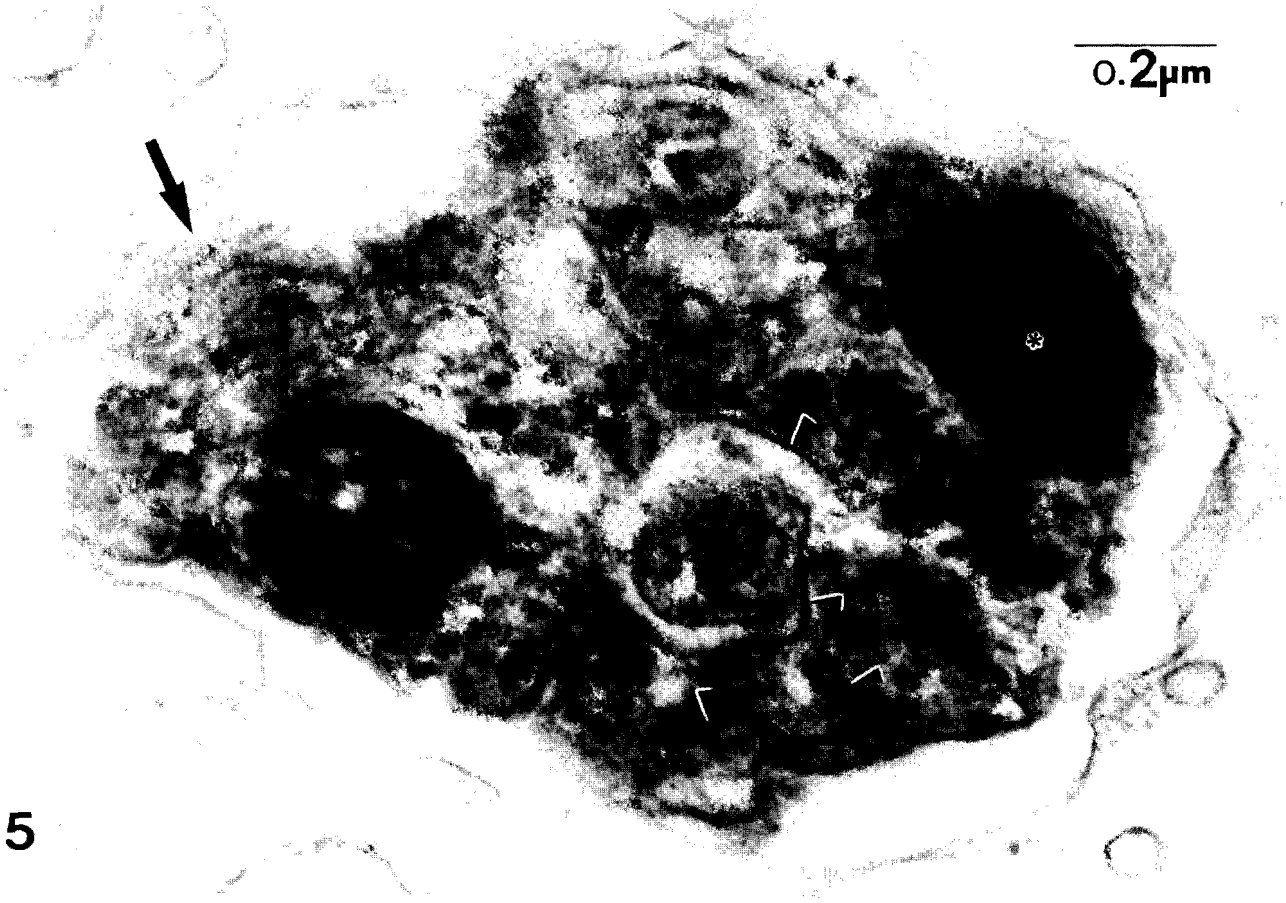


Fig. 5. In embryonic chicken (7 days old), besides the more classical vesicular-globular or lamellar premelanosomes, atypical large melanosome complexes containing melanofilaments (arrow), membranous material (arrowheads) and melanin (asterisk) can be seen. This organelle may also represent an autophagic vacuole degrading intracellular proteins including melanin. x 94,500

The large type melanosomes often fuse with earlier-stage melanosomes (Schraermeyer, 1993) and have been described as late immature melanosomes in the prenatal RPE of humans (Mund and Rodrigues, 1979). Similar organelles have been shown in the RPE of different coat color mutants of the mouse (Hearing et al., 1973). In the RPE melanosomes contain melanofilaments ordered concentrically, as are the membranes in phagosomes (Schraermeyer, 1992).

Only a small amount of melanin granules corresponding in size and shape to choroideal melanin granules is also present in the RPE. The cause of these differences is not known. Also unique to the RPE, an association of residual bodies and premelanosomes has been repeatedly observed. Young (1977) found such associations in RPE of the lizard. Additionally, some of the sheedded tips of the rod outer segments, still located in the extracellular space, resembled premelanosomes, which is shown by Young (1977) in his Fig. 5. Premelanosomes also contained relatively large, pale, spherical areas typical of lysosomes, shown in Fig. 20 of the same study. Moreover, in the RPE of chicken parallel filaments which appeared to have the same structure as those forming the framework of melanosomes were frequently found in phagosomes (Young, 1978).

Goldman-Herman and Steinberg (1982) concluded that in the *Opossum* newly-formed melanosomes fused with phagosomes derived from outer segments of the photoreceptors. Fused phagosomes and melanosomes were also observed in the RPE of adult hamsters (Schraermeyer, 1993).

In the RPE of embryonic chicken (7 days old) besides the already described vesicular-globular or lamellar premelanosomes (Jimbow et al., 1979) atypical large melanosome complexes (Fig. 5) resembling lysosomes containing irregularly-ordered melanofilaments and membranous material can be seen.

Ultrastructural similarities between phagosomes and melanosomes have also been reported. In the RPE of cattle, phagosomes were found that contained an electron dense melanin-like material that was not autofluorescent and therefore not lipofuscin. Additionally, electron dense vesiculo-globular bodies (10-100 nm) were found in phagosomes during disk membrane

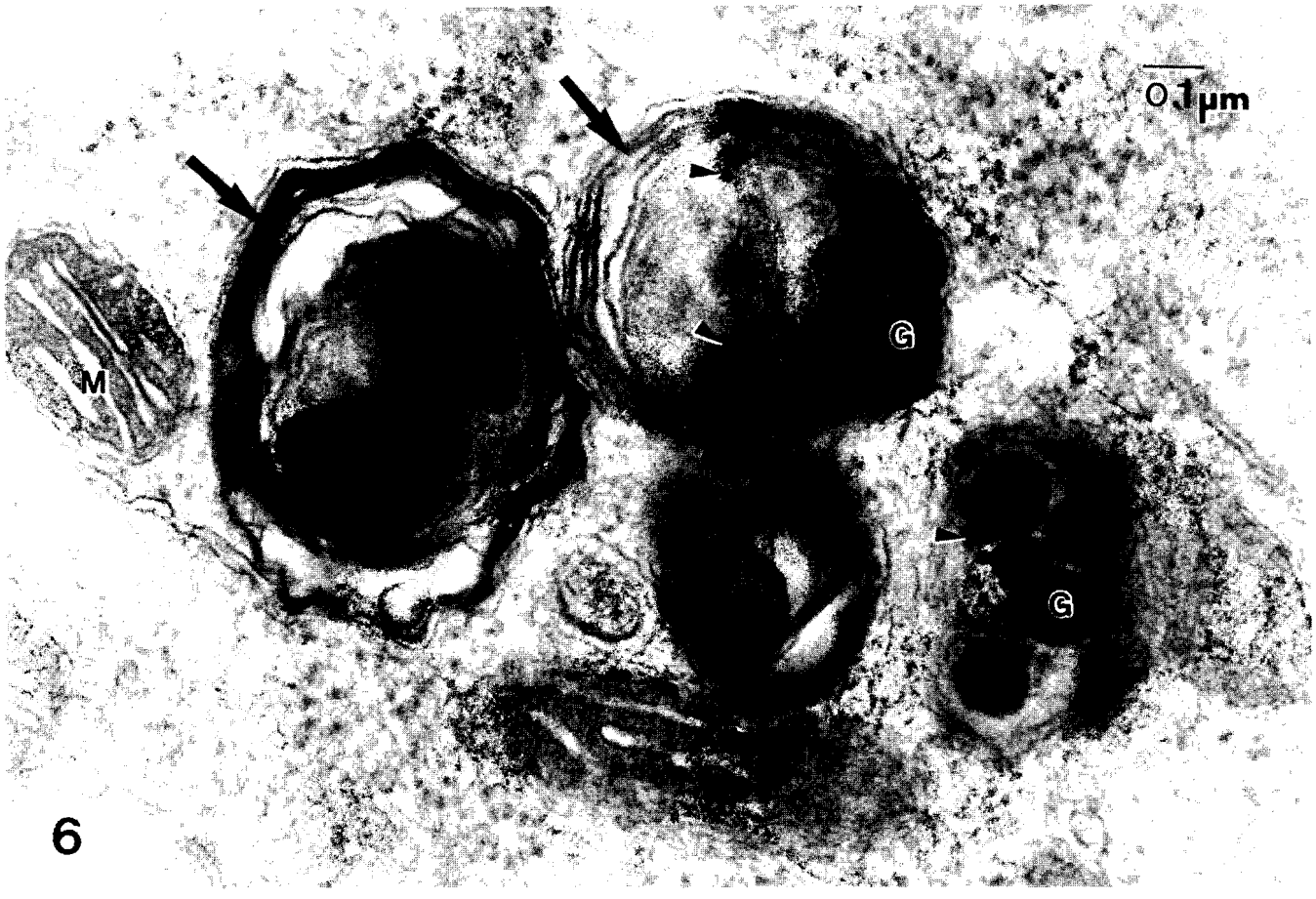


Fig. 6. Melanosomes in the ink gland from *Sepia officinalis* contain some electron dense spots (arrowheads) that probably indicate the beginning of melanogenesis and, additionally, mature melanin granules (G). The melanosomes are completely or partially surrounded by several layers of membranes (arrows). Mitochondria (M) are also seen. x 82,500

degradation as well as within mature melanin granules (Schraermeyer, 1992).

The presence of early-stage melanosomes inside phagosomes degrading photosensory membrane and the structural similarities between lysosomes and phagosomes have not yet been understood. However, the existence of these structures could be explained by a potential transformation of phagosomal tyrosine residues into melanin, as suggested by Schraermeyer and Stieve (1994).

The ultrastructure of melanosomes in melanocytes

In mammals and other vertebrates, besides hair bulbs, skin and choroid, internal organs not directly exposed to light, such as leptomeninges, mucous membranes, mesenteric and internal ear are also immigrated into by melanocytes. A specific influence on the structural events leading to melanin formation depending on the tissue location site of the melanocytes has only occasionally been reported. A recent review describes the melanogenesis processes in melanocytes of epidermis and hair follicles of the mouse (Hirobe, 1995).

Uveal melanocytes in the iris stroma of monkey eyes differ from those in the choroid: in the time of appearance during fetal development (Hu and Montagna, 1971) and in the fine structure of their melanosomes (Endo and Hu, 1973; Hu et al., 1973). In the iris of leaf frogs a melanosome has been described which is composed of a fibrous cortex containing pteridines surrounding a core of eumelanin (Bagnara and Ferris, 1974). Nevertheless, in general melanogenesis in melanocytes seems to correspond to the common scheme outlined in the introduction. However, in aberrant melanocytes, i.e. melanoma cells (for review see Borovansky et al., 1991; Bhuta, 1993), pigmented nevi and other pigmentary disorders (for review see Levine, 1993), the classic forms were frequently difficult to identify and abnormal variant forms often predominated (Mishima, 1965; Curran and McCann, 1976; Mintis and Silvers, 1978; Mazur and Katzenstein, 1980; Jimbow et al., 1989). Also, in melanocytes of clinically non involved skin of patients with malignant melanoma unusual melanosomes were found (Szekeres and Orfanos, 1978). The ultrastructure of melanosomes in disorders is only randomly striped and is not subject of this article.

Some special features, however, were observed in melanosome formation induced by osmotic stress in the skin of goldfish (Turner et al., 1975). It was found that multi-vesicular bodies (MVB) were the main type of premelanosome (Turner et al., 1975) but multilamellar bodies and granules having a homogeneous matrix also function as premelanosomes (Schraermeyer et al., 1996). According to Turner et al. (1975) Golgi-derived tyrosinase containing vesicles fuse with larger rough endoplasmic reticulum-derived vesicles. Then small vesicles invert and are incorporated into the larger ones now exposing tyrosinase at their outer side. Melanin is synthesized around the periphery of the inverted vesicles and fills the intervesicular spaces and finally the internal vesicles (Turner et al., 1975). This mechanism, however, includes one important aspect. During invagination of vesicles into the lumen of MVB, cytoplasm is also incorporated into the internal vesicles. Because the content of the internal vesicles is also converted into melanin, which can be affirmed by its ultrastructure, cytoplasmic material consequently also seems to serve as substrate for melanogenesis (see Fig. 12 in Turner et al, 1975). MVB as premelanosomes have also been observed in *Salamandra atra atra* (Trevisan et al., 1991). In xanthic sailfin mollies melanosome formation has been described from MVB and alternatively from lamellar framework-type premelanosome (Blanchard et al., 1991)

Lamellar premelanosomes were additionally observed in the eyes of an albino goldfish (Abramowitz et al., 1977). A remarkable polymorphism in the ultrastructure of melanosomes covering lamellar, multivesicular, fibrillar, and macroglobular types was found in the goldfish (Matsumoto et al., 1989).

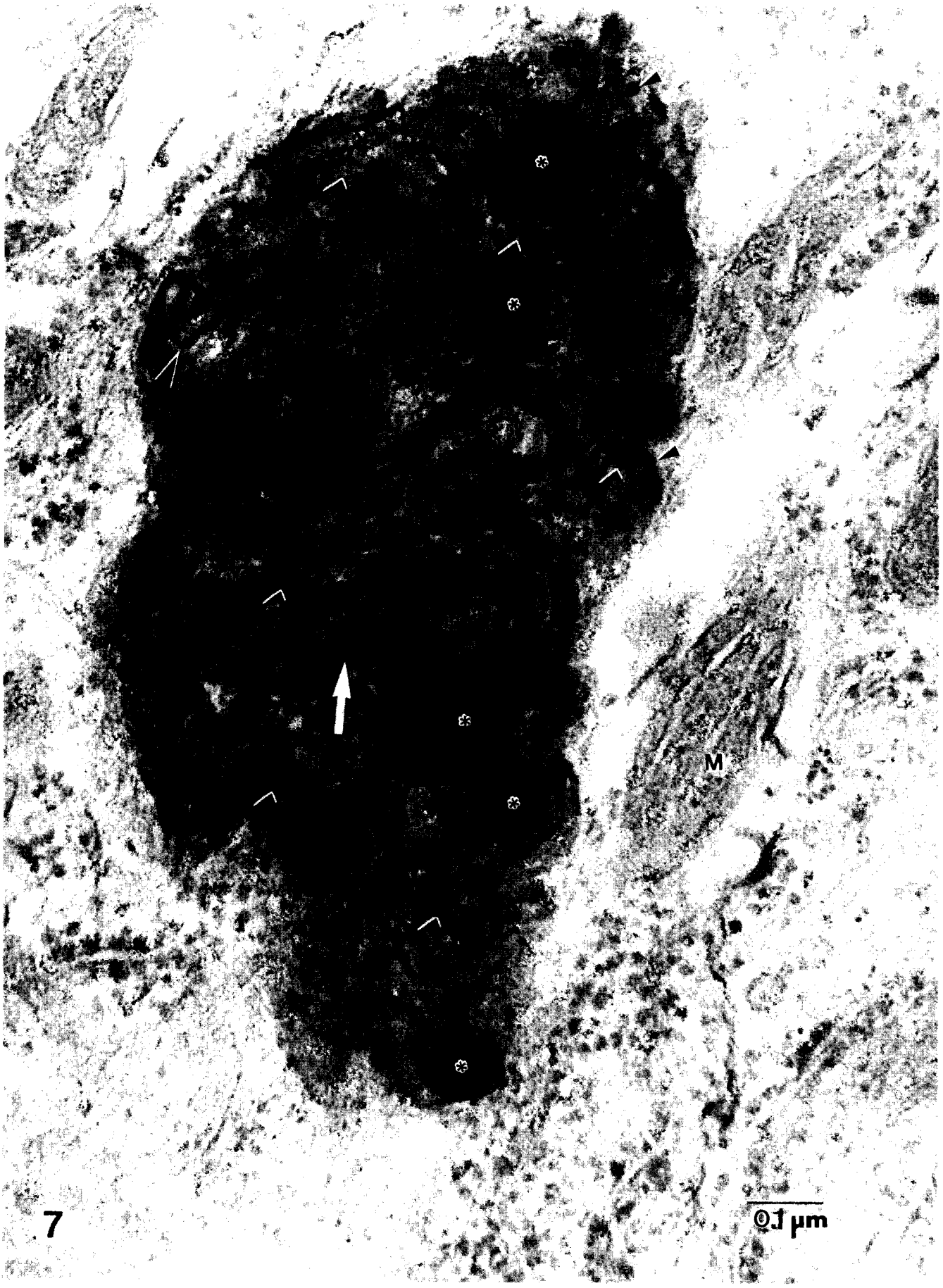
Multivesicular bodies were also reported as precursors of pheomelanin-containing melanosomes in the agouti mouse (Sakurai et al., 1975). Abnormally-differentiated lamellar and granular melanosomes were detected in the melanocytes of rats after copper deficiency (Miranda et al., 1992).

Taken together, the structure of the matrix component in melanogenesis of normal melanocytes is extremely heterogeneous.

Melanosomes in Kupffer cells of vertebrates

In poikilotherm vertebrates Kupffer cells are also able to synthesize melanin (Sichel, 1988; Cicero et al., 1989). Melanin was also occasionally detected in mouse spleen (Weissman, 1967; Sundberg, 1989). The steps of melanin formation in these cells has not yet been documented ultrastructurally. An ultrastructural investigation of pigmented cells from the liver of frogs revealed that these cells shared properties with liver macrophages, such as phagocytic activity (Guida and Cicero, 1993). Because Kupffer cells are professional phagocytes and play an important role in removing

Fig. 7. A cultured retinal pigment epithelial cells of cattle is shown partially after feeding with gold-labeled rod outer segments. Here one can see the partially degraded disk membranes in a lysosome that has some properties of a melanosome. The membranes have become thicker than in the original disks (white arrow). Some of the cross-sectioned former photoreceptor membrane profiles resemble melanofilaments with a striated periodicity of about 5 nm (black arrow). The gold-granules are indicated by arrowheads. Additionally, melanin-like material is present in these lysosomes (asterisk). Tyrosinase, the key enzyme in melanogenesis, is additionally localized inside such lysosomes (Schraermeyer and Stieve, 1994). Mitochondria (M) are also seen. x 137,700



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blood cells it seems likely, but remains to be checked, that melanogenesis occurs in their lysosomes.

Ultrastructure of neuromelanin

Whereas it is generally believed that melanogenesis in RPE and melanocytes follows a common pathway (Feeney et al., 1965; Hearing et al., 1973; Hu and Mah, 1983; Sarna, 1992) melanogenesis took place without tyrosinase or formation of premelanosomes in the nervous system (Graham, 1979; Carstam et al., 1991). The ultrastructure of neuromelanin from the brain of frogs and tadpoles was described by Kemali and Gloffré (1985). In the substantia nigra of humans and monkeys ultrastructurally the neuromelanin pigment granule was composed of three distinct components (Moses et al., 1966). The first was a finely granular medium-dense matrix that occasionally had linear configurations. The second component was a very dense, coarsely granular material that appeared to be deposited on the finely granular matrix. The third component of the neuromelanin granule was a lipid globule that was not preserved in glutaraldehyde-fixed tissue. The structural description of the first and second component resembled some melanosome found in the cultured RPE of cattle (Fig. 10).

Ultrastructure of melanosomes in invertebrate animals

In arthropods melanin is involved in body coloration. However, the precise steps in melanin formation have not yet been described ultrastructurally (for review see Ghidalia, 1985; Kayser, 1985). Nevertheless, premelanosomes-like material with a granular not lamellar structure was observed within epidermal processes that probably transport melanin in lepidopteran cuticles (Kayser-Wegmann, 1976).

One of the richest sources of eumelanin in the animal kingdom is the ink gland of cephalopods, which has recently been investigated ultrastructurally (Schraermeyer, 1994a). Melanogenesis in a simplified view seemed to follow the general scheme of melanin formation in vertebrates. Firstly, a membrane-bound protein matrix was formed, called early-stage melanosome. The early-stage melanosomes were more or less irregular in shape with a size of up to 1.5 µm and contained membranous, granular or vesicular material (Fig. 6). They seemed to originate from Golgi bodies and/or endoplasmic reticulum. Membranes that were frequently present in the early-stage melanosomes seemed to originate from fusion of vesicles or from incorporation of Golgi membranes into early-stage melanosomes. Free cytoplasmic material or mitochondria were also probably incorporated into the melanosomes or early-stage melanosomes. Therefore, the origin of the early-stage melanosomes was suggested to be similar to that of autophagosomes. The early-stage melanosomes mature to melanosomes in which several dozens of melanin granules are formed. These melanosomes at last release the melanin granules together with other cellular material, including early-stage melanosomes, into the lumen of the ink gland. This finding confirmed the earlier postulated holocrine character of the release (Prota et al., 1981). Moreover, the material of broken down cells inside the lumen of the ink sac seemed to be converted into melanin.

Melanosomes and lysosomes

Structural and histochemical evidence for conversion of proteins into melanin in lysosomes was presented in the cultured retinal pigment epithelium or melanocytes of cattle (Schraermeyer and Stieve, 1994; Schraermeyer, 1995). In these studies, gold-labeled proteins were digested by the pigment cells. After degradation of the proteins the gold granules appeared inside the melanin granules. Fig. 7 shows an engulfed fragment of a rod outer segment within a cultured pigment epithelial cell of cattle. In these lysosomes tyrosinase was also present (Schraermeyer and Stieve, 1994). Some of the cross-sectioned former photoreceptor membrane profiles resemble melanofilaments with a striated periodicity of about 5 nm (Fig. 7). Additionally, melanin-like material was present in these lysosomes. A recent review brought together the growing body of evidence in the literature which shows that melanosomes are specialized lysosomes (Orlow, 1995).

Besides reports describing degradation of melanosomes in lysosomes of keratinocytes (Hori et al., 1968) evidence for a common functional role and origin of melanosomes and lysosomes has been presented by the study of certain disorders.

An association of lysosomes and melanosomes is particularly prominent in diseases like the Chediak-Higashi syndrome (Robison et al., 1975; Collier et al., 1984) or retinitis pigmentosa (Kroll and Kuwabara, 1964; Kolb and Gouras, 1974; Szamier and Berson, 1977). The morphological hallmark of the former disorder, which is associated with albinism, is the presence of large myeloperoxidase-positive cytoplasmic granules in all granule-containing cells. This is postulated to result from the fusion of primary lysosomes (Jones et al., 1992). Association of melanosomes and lysosomes was also observed in the RPE of the adult pallid mouse (Ito et al., 1982) and in avian albinos (Boissy et al., 1987, 1988). In the pallid mouse (Novak and Swank, 1979) and in the Chediak-Higashi syndrome lysosomal dysfunction is associated with hypopigmentation. Moreover, in a vitiligo mouse model the ability to form phagosomes, which become lysosomes, in the RPE is reduced (Smith et al., 1994). Biochemical data also indicate the relationship between lysosomes and melanosomes. Thus, lysosomal hydrolases, such as ß-hexosaminidase, ß-galactosidase, ß-glucoronidase and cathepsin B and L have all been shown to cosediment with tyrosinase-rich densely sedimenting melanosomes (Diment et al., 1995). Also,

melanosomal and lysosomal proteins have been colocalized by immuno-electron microscopy (Smit et al., 1993). Furthermore, it has been shown that the melanosomal glycoprotein (gp 75), which is related to the B (brown) locus of the mouse, can be glycosylated into two different forms, as was also observed in the lysosomal-associated membrane protein 1 (LAMP-1) and other proteins (Vijayasaradhi et al., 1991). As in lysosomes, the pH in melanosomes is acidic and the lysosomal enzymes work at a pH lower than 4.6 (Bhatnagar et al., 1993). This is in accordance with old and new findings reporting that most melanosomes in the RPE (Toda and Fitzpatrick, 1972; Hollyfield and Ward, 1974; Novikoff et al., 1979) or melanoma cells (Seiji and Iwashita, 1965) contain the lysosomal marker enzyme acid phosphatase. A recent study has confirmed the long-suspected relationship between melanosomes and the lysosomal lineage of organelles (Zhou et al., 1993). In the later study it was shown that there is a common step in the genesis of both types of organelles (Zhou et al., 1993). Furthermore, molecular biological results support the relationship between melanosomes and lysosomes. It was observed in fibroblasts transfected with the tyrosinase gene (Winder et al., 1993) that, surprisingly, the enzyme tyrosinase was transported into the lysosomes in which melanogenesis then took place. Ultrastructural data from this artificial system of melanogenesis are not yet available. However, it is clear that in transgenic cells a protein matrix having a specific sequence is not necessary for melanin formation.

Structure of melanosomes in cultured pigment cells

Under culture conditions structural changes of the melanosomes are often observed. Cultured embryonic chick retinal pigment cells synthesize small, spherical melanosomes containing an irregular internal matrix in contrast to those made in ovo and in vivo. However, the same cells in embryo extract-free and serum-free defined media are found to synthesize melanosomes of normal size and shape (Garcia and Szabo, 1983). Therefore, unknown factors in the culture environment have produced a shift to a different type of melanogenesis. It cannot be excluded that endocytosed serum proteins have been used as matrix for melanin formation and thereby influence the structure. Interestingly, formation of melanin granules without any (classical) premelanosomes has been observed in multilamellar bodies of

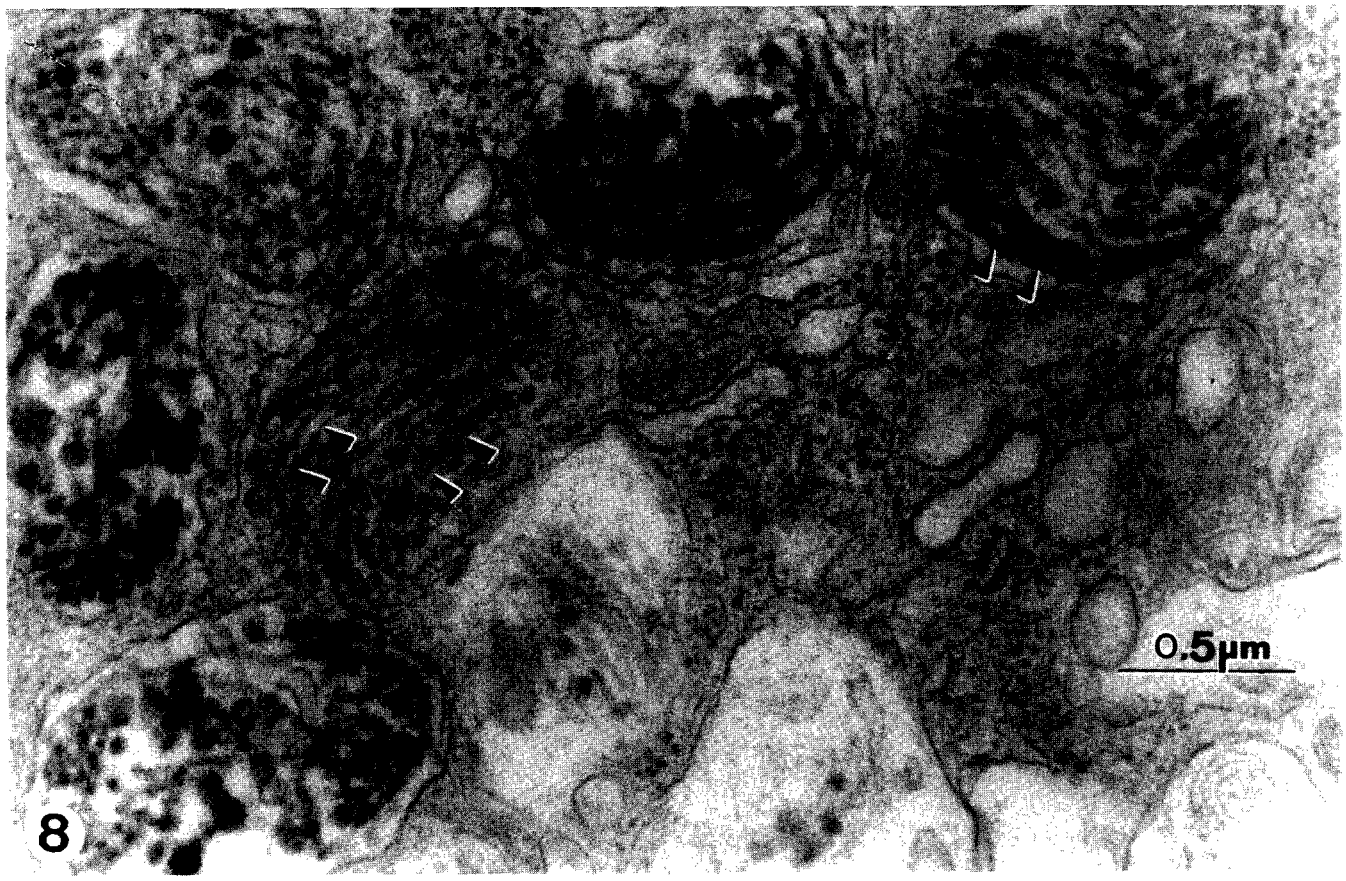

Fig. 8. Two weeks after culture under conditions described by Schraermeyer and Stieve (1994). Early-stage melanosomes of the lamellar type containing melanofilaments (arrowheads) are occasionally present in the RPE of cattle. x 72,600

cultured RPE of adult pigs (Dorey et al., 1990). Three types of electron dense granules were described: simple spheres 0.3-0.5 µm in diameter, large spheres 1-2 µm in diameter and lysosomal aggregations of the smaller spheres. The matrix of both spheres was composed of 40 nm microvesicles that were also found free in the cytoplasm and aggregated within vacuolar structures.

After long-term culture of bovine RPE under conditions as described by Schraermeyer and Stieve (1994) lamellar (Fig. 8) as well as granular (Fig. 9) melanosomes from those types described by Dorey et al. (1990) were found, and additionally a panel of a different pattern of internal melanin formation (Fig. 10).

The role of the protein matrix

Most investigators favor the view that natural eumelanins are melanoproteins (see Prota, 1992). This concept was first introduced by Görtner (1911) who described the pigment isolated from black wool by extraction with NaOH followed by precipitation with acid as melanoprotein. It was suggested that chemical differences, i.e., quantitative and qualitative, of the matrix proteins were responsible for the ultrastructural differentiation of melanosomes (Jimbow et al., 1982). The amount of protein from 14 different tissues ranged from 5% in melanin from the ink sac of Sepia to 63% in melanin from Harding-Passey melanoma (Hach et al., 1977).

From the association of protein with melanin arises a principal question about its origin. Is a specific protein matrix necessary (Mochii et al., 1991) or can any cellular proteins be converted into melanoprotein? The melanosomal matrix protein found by Mochii et al. (1991) contains 66 tyrosine residues among 761 amino acids and its role in the melanization process remains unclear. The substrate for melanin formation is tyrosine. Therefore, a premelanosomal matrix should be rich in tyrosine.

At present, it is unknown whether and how tyrosine is concentrated in melanosomes. It has been repeatedly shown that peptides and protein can be converted into melano-protein by tyrosinase using biochemical experiments (Ito et al., 1984; Marumo and Waite, 1986; Rosei et al., 1991). Bovine serum albumin contains 19 tyrosine and 1 cysteine residues per molecule (Ito et al.,

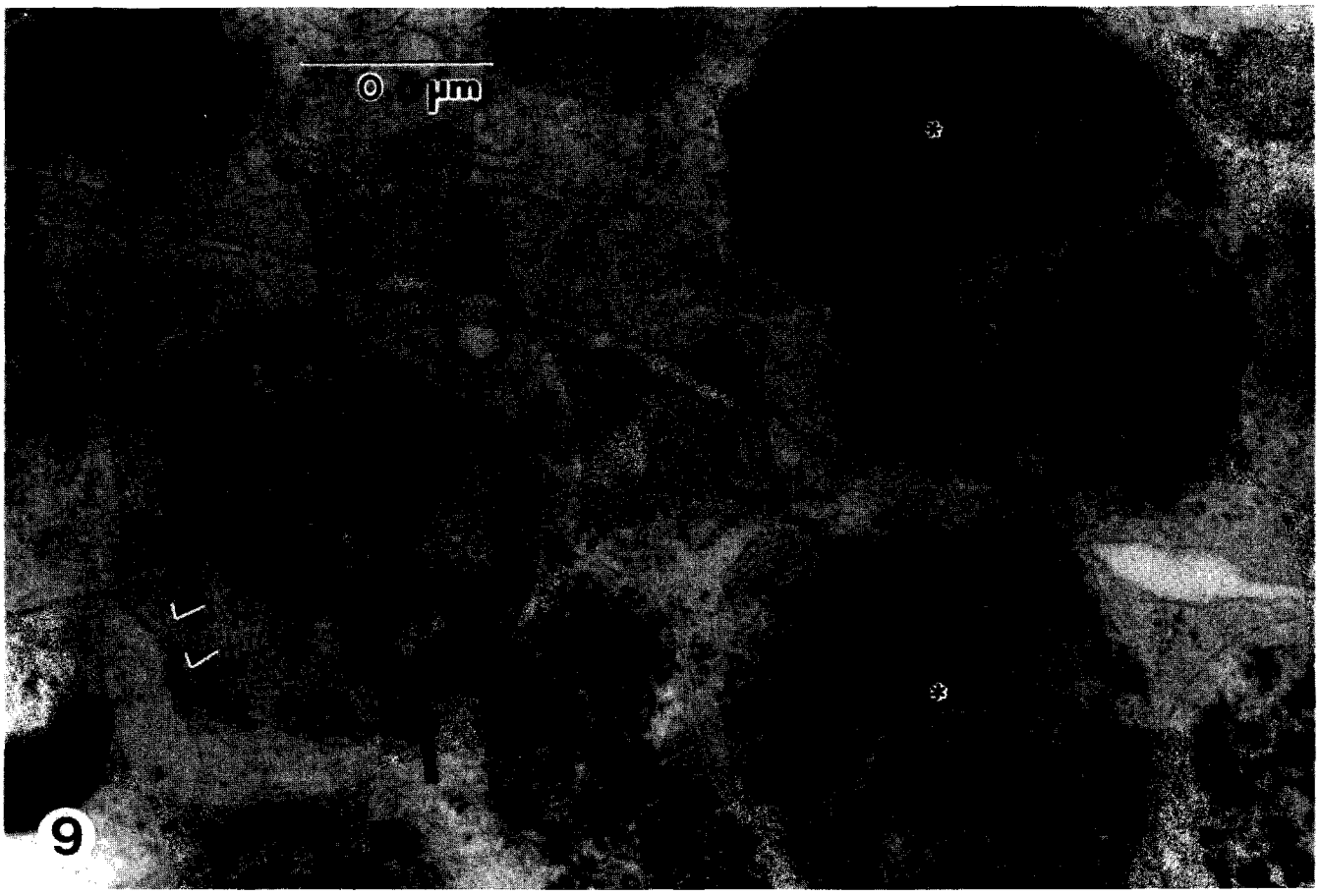

Fig. 9. After culture of bovine RPE under conditions described by Schraermeyer and Stieve (1994) for 6 months. Granular melanosomes (asterisks) are shown. One melanosome additionally contains melanofilaments (arrowheads) and probably melanin (arrow). x 52,500

1984). The latter workers showed that tyrosinase could catalyze the hydroxylation of tyrosine residues in bovine serum albumin and the subsequent oxidation to the dopaquinone form. Also, degradation of albumin by hydroxyl radicals *in vitro* resulted in the formation of melanin (Schraermeyer, 1994b).

The hypothesis as to whether the continuous synthesis of proteins is necessary for the continuous synthesis of melanin has been elegantly tested by Whittaker (1979) by incorporation experiments during early differentiation of chick retinal pigment cells, which have a very high rate of melanin synthesis. Using radiolabeled tyrosine he found that short-term formation of labeled melanin was not affected by inhibitors of protein synthesis, such as puromycin and cycloheximide. Moreover, melanin from tissues that had been incubated with radiolabeled valin had no associated radioactivity when compared with the radioactivity of melanin prepared from tissues in the same series incubated with labeled tyrosine. These results strongly suggest that coordinated synthesis of protein is not necessary for melanogenesis to occur and that no significant among of new protein is bound to the pigment. However, these results are not in contradiction according to a hypothesis that aged proteins, for example resulting from lysosomal protein degradation, would be incorporated into newly-formed melanin.

What is the origin of melanosome?

The origin of the premelanosome is not clear at present. Premelanosomes should be derived either from the rough (Stanka, 1971; Stanka et al., 1981; Hirobe, 1982), smooth (Mishima et al., 1978; Jimbow et al., 1988) or not specified (Sakurai et al., 1975) endoplasmic reticulum, Golgi vacuoles (Hirobe, 1982) or from polysomes (Moyer, 1961).

The Golgi-Endoplasmic Reticulum-Lysosome Complex (GERL) was found to be the origin of melanosomes in melanoma cells (Novikoff et al., 1968). Even mitochondria should be involved in the process of melanosome formation in cultured HPM-73 melanoma cells (Schjeide et al., 1976). Other workers were not able to determine the origin of melanofilaments in the eyes of

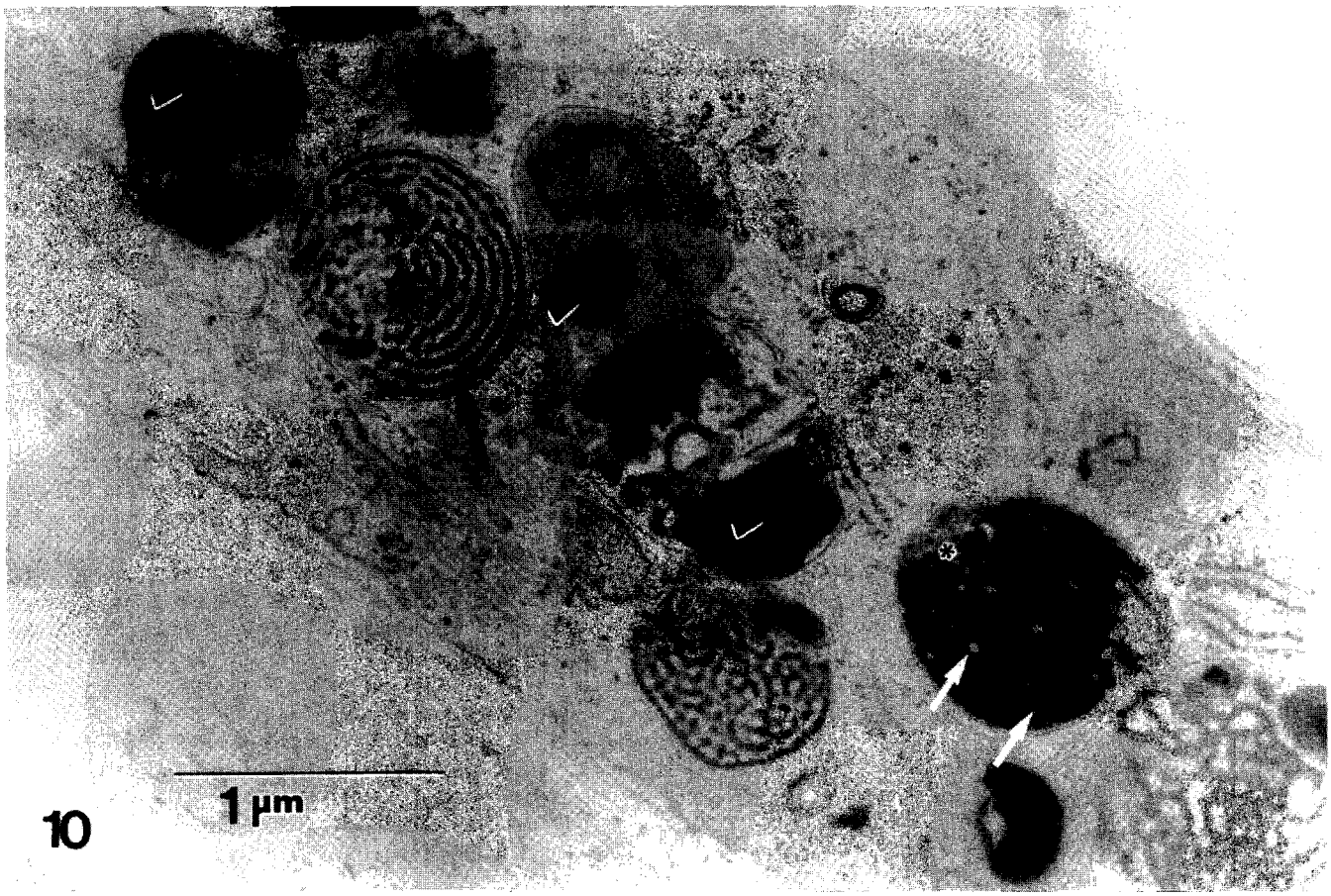

Fig. 10. Different patterns of polymerizing melanin can be seen after 6 months of culture of the RPE of cattle under conditions described by Schraermeyer and Stieve (1994). In one melanosome melanin seems to polymerize in highly ordered concentric shells (black arrow) whereas in other it seems to polymerize more solid (arrowheads). In one melanosome (asterisk) the electron lucent spaces (white arrows), probably corresponding to those of the melanosome containing the concentric shells also seem to be filled with melanin. x 36,000

black and beige mice (Lutzner and Lowrie, 1972). Alternatively, autophagosomes (Novikoff et al., 1979; Schraermeyer, 1994a) or phagosomes (Schraermeyer, 1992; Schraermeyer and Stieve, 1994) have been taken into consideration as matrix components for melanin granule formation. According to Moellman and Halaban (1989) the melanosome is a modified peroxisome.

With respect to these considerably different views on the melanosome origin it seems possible that pigment cells may use protein matrixes originated from different cellular pathways, dependent on their functional state. 1) They may produce a specific protein matrix to be converted into melanin. This classical protein matrix may be rich in tyrosine residues and may be composed of regular repetitions of subunits causing a striated periodicity. This type of premelanosome may be predominant in pigment cells that do not have to degrade high amounts of proteins, i.e. in embryonic RPE and in unstimulated melanocytes. 2) Alternatively, a matrix resulting from lysosomal protein degradation may be used and converted into melanin. It is possible that these lysosomes concentrate tyrosine residues, whereas other amino acids may be recycled. The ultrastructure of the polymerizing melanin may be influenced by the amount and sterical availability of tyrosine residues in the protein moieties. It has to be borne in mind that in fibroblasts transfected with the tyrosinase gen, and probably in Kupffer cells, proteins can be converted into melanin within melano-lysosomes. In other pigments, lipofuscin, is also known to originate from lysosomal degradation (for review see Porta, 1991). A possible relationship of these two pigments cannot be excluded, because melanolipofuscin develops in the RPE with the progression of age and is thought to be a complex aggregate of both melanin and lipofuscin (Feeney-Burns, 1980).

Therefore, it might be useful to investigate whether in normal pigment cells damaged proteins that may contain preexisting DOPA-residues (Simpson et al., 1993) can be used as substrate for melanogenesis. This may be the case in cultured pigment cells degrading serum or other proteins, in melanocytes of skin that may degrade UV light-damaged proteins, in the RPE cells degrading rod outer segments or in melanophores of fish in which melanogenesis could be induced by a panel of different protein damaging factors (Chavin, 1956).

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