

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).

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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.

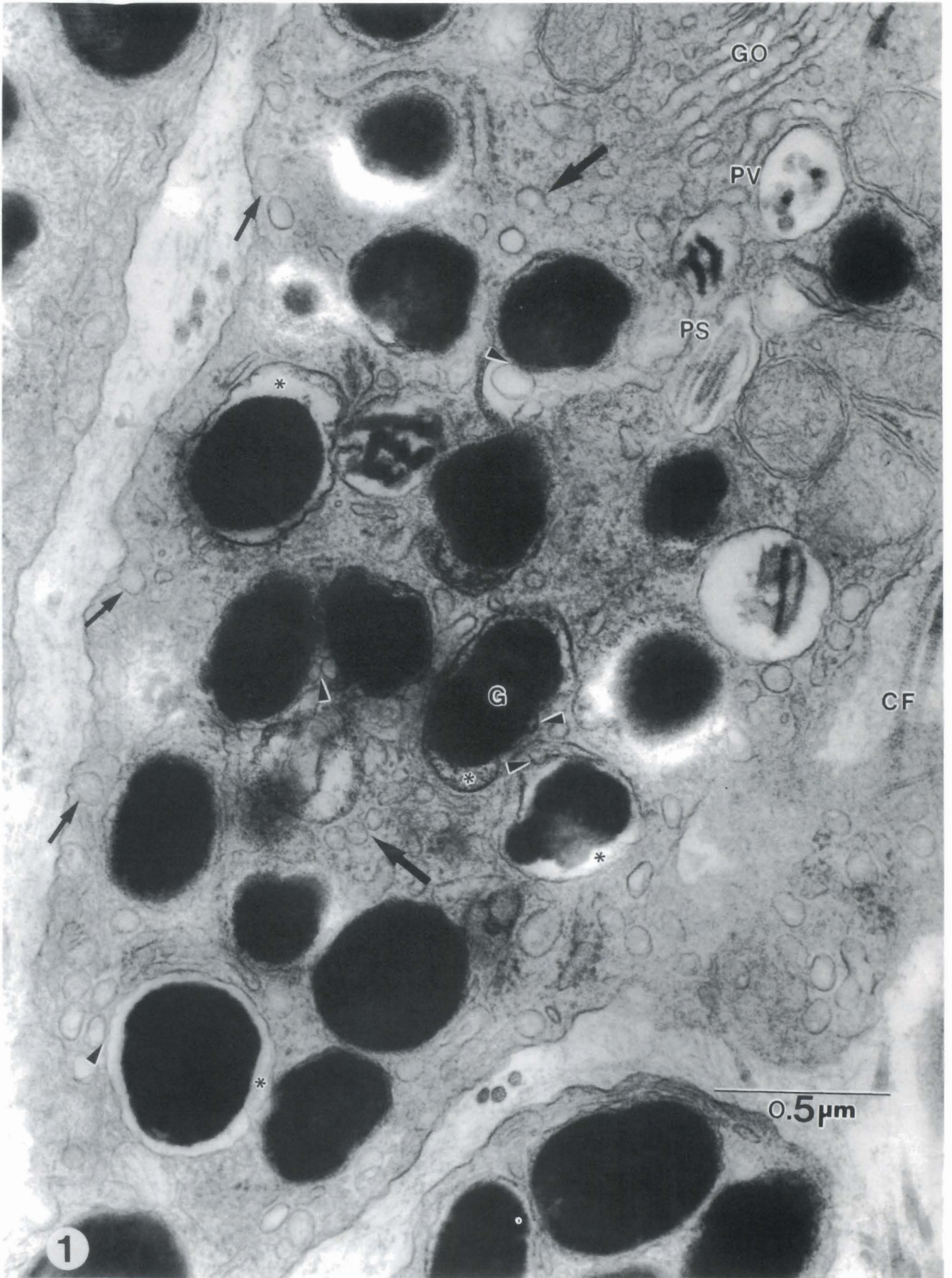
The nomenclature describing the events leading to the formation of brown (eumelanin) melanin granules in humans was also introduced by Seiji et al. (1963). The structural and chemical metamorphoses which take place in the melanin granule during melanization were described as follows.

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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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 term 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

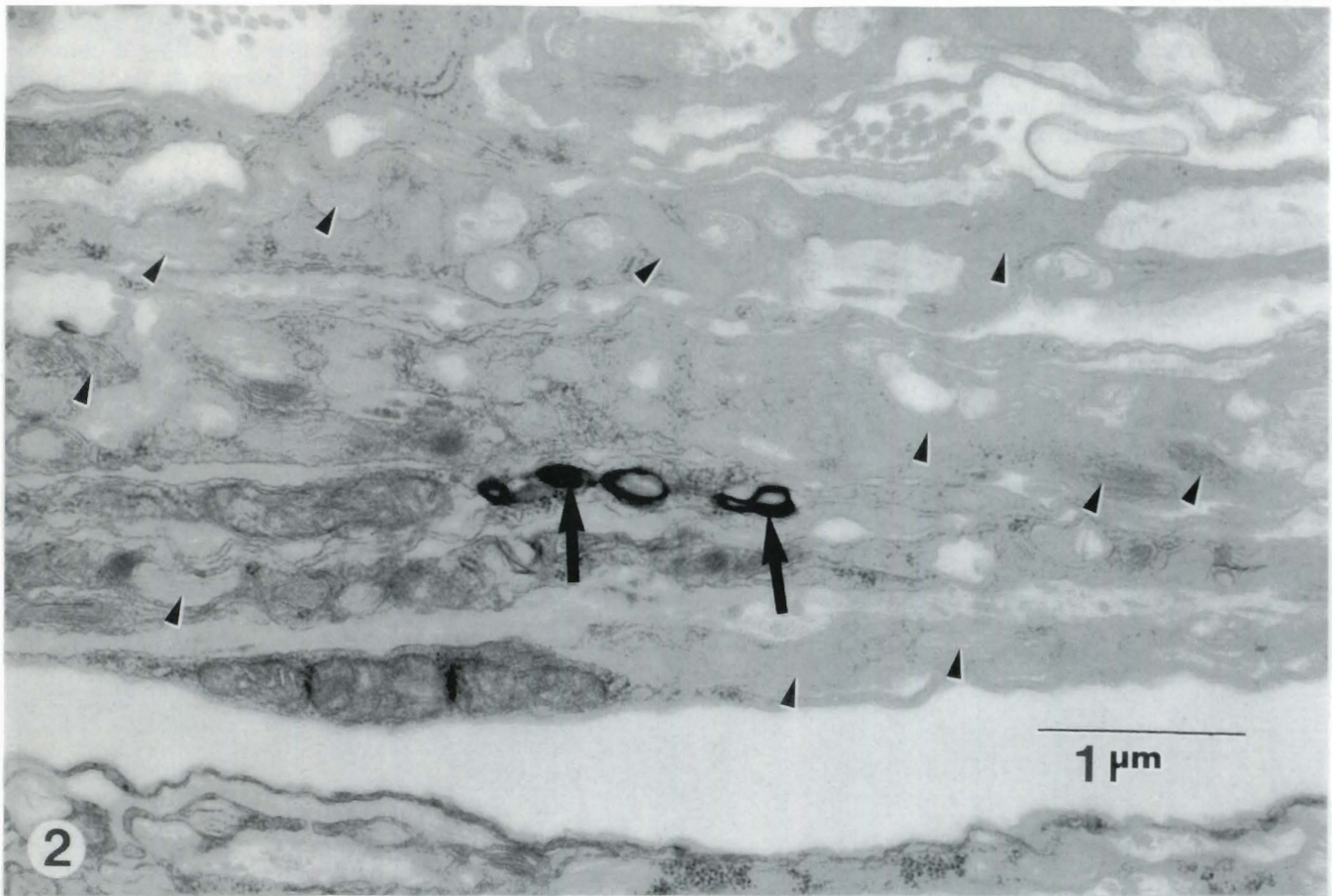


Fig. 2. Melanocytes of the choroid from the adult albino (C57B1/6J) are shown. The melanocytes contain numerous premelanosomes (arrowheads). One melanocyte contains electron dense membranous material (arrows). Nothing is known as to whether a turnover of these premelanosomes exists or not. $\times 29,500$

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. $\times 65,000$

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-

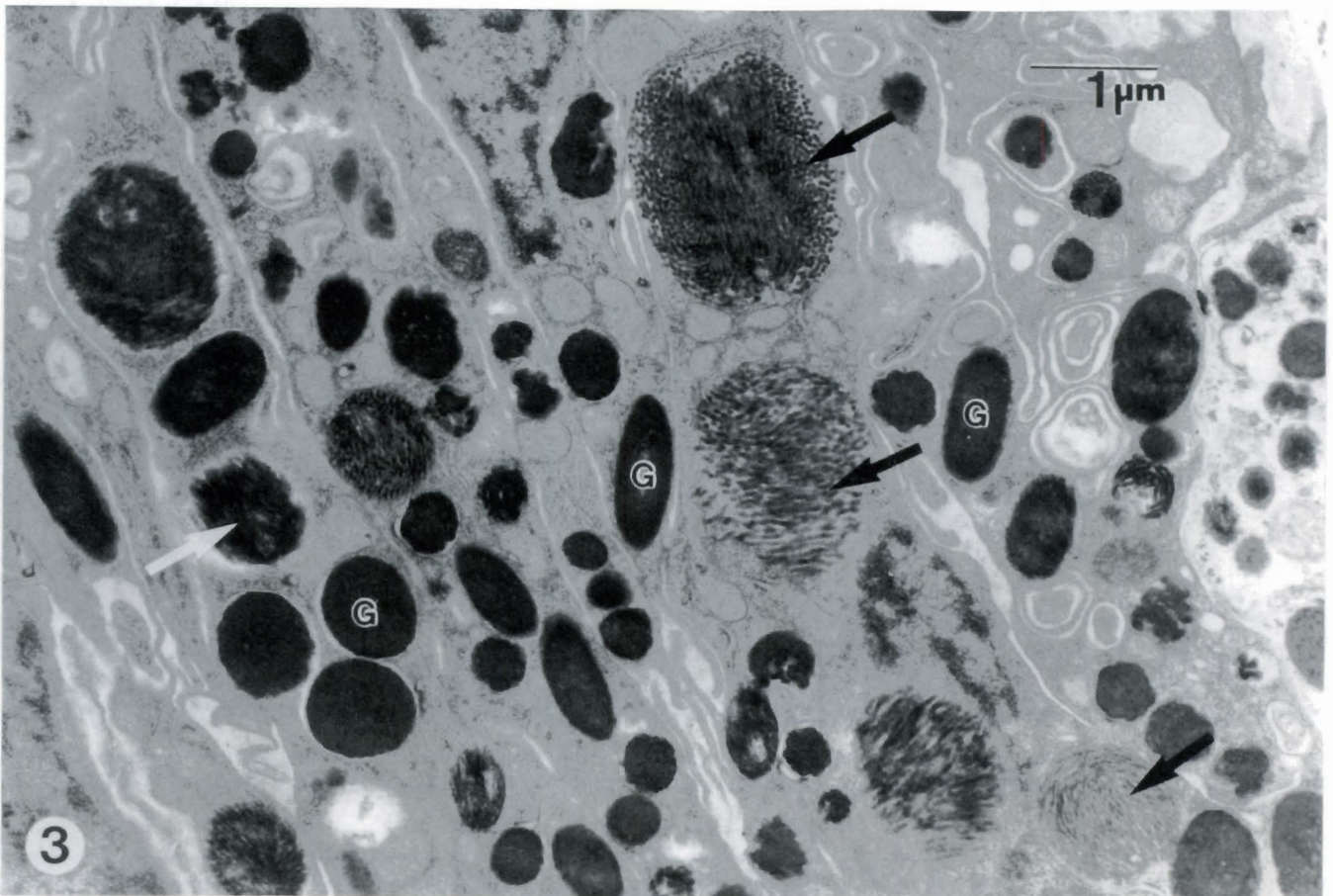


Fig. 3. A tangential section through the retinal pigment epithelium of the adult golden hamster demonstrates the size difference between the mature melanin granules (G) and the roundish large type of melanosome (black arrows). The large type melanosome (white arrow) may condense and change into mature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

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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 approximately 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; Zimmermann 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 1.3×0.6 to $0.3 \times 0.1 \mu\text{m}$ (for review see Robins, 1991). The length of melanosomes from 14 different sources ranged from $3 \mu\text{m}$ (black poodle hair) to 0.18 mm (*Loligo* ink) (Hach et al., 1993).

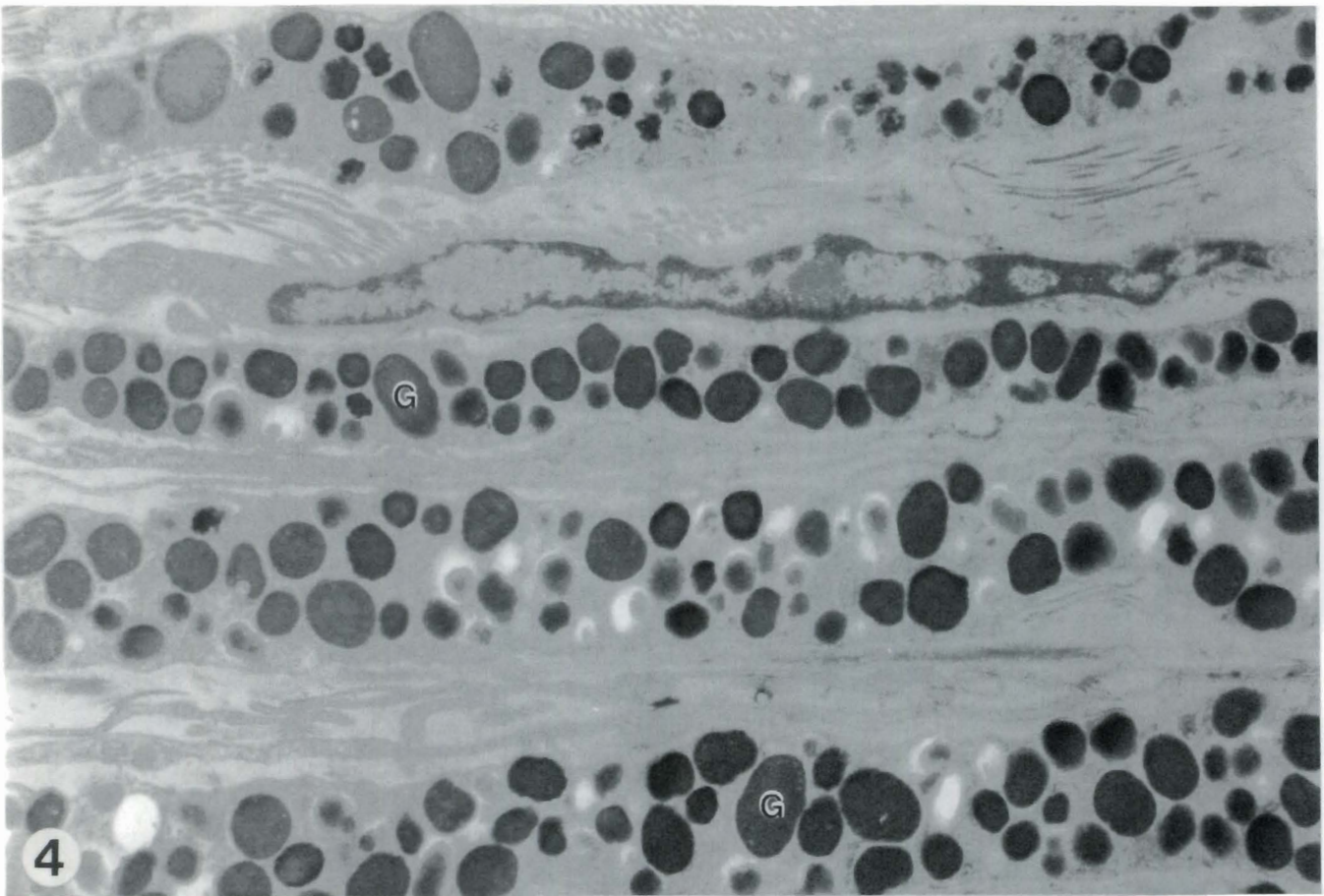


Fig. 4. In hamsters that had been kept under the normal light conditions of a laboratory, the dendritic melanocytes of the choroid contain mature melanin granules (G) and appear metabolically inactive judging by the poor development of cytoplasmic organelles. Premelanosomes are not observed. $\times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

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 melanosomes 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 melanofilaments, 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).

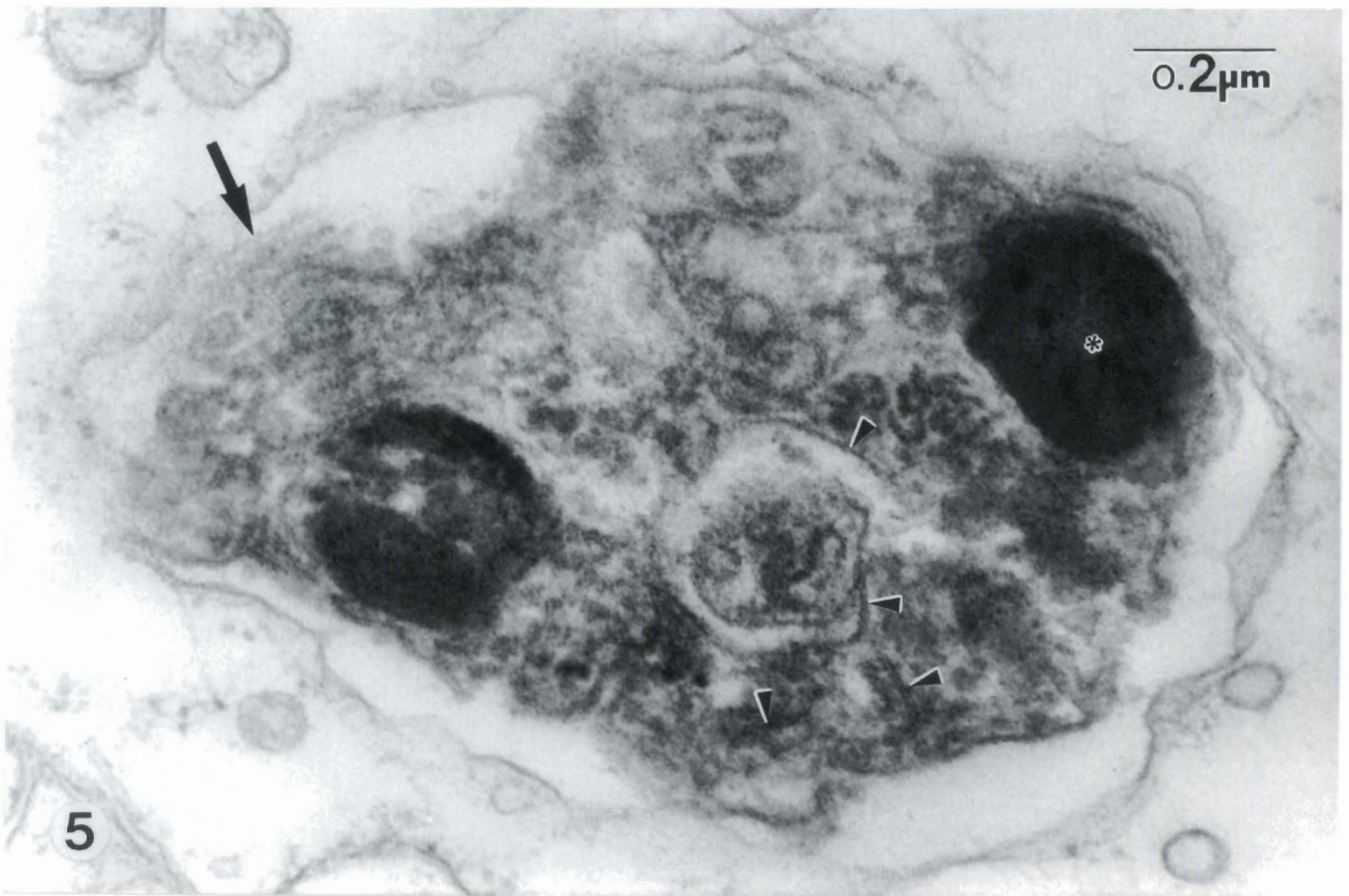


Fig. 5. In embryonic chicken (7 days old), besides the more classical vesicular-globular or lamellar premelanosomes, atypical large melanosome complexes containing melanofilaments (arrow), membranous material (arrowheads) and melanin (asterisk) can be seen. This organelle may also represent an autophagic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

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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 shedded 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).

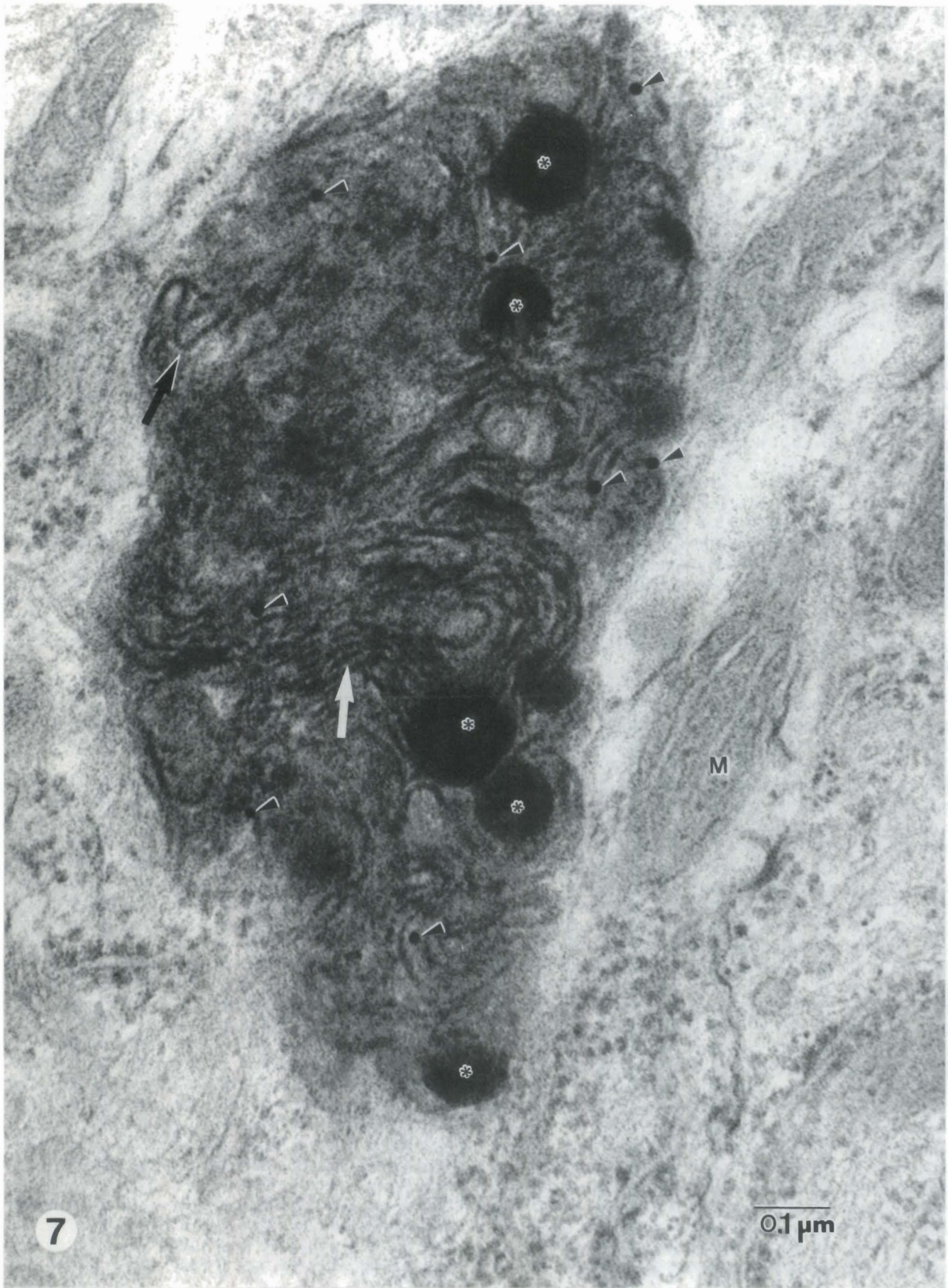
Multivesicular bodies were also reported as precursors of pheomelanin-containing melanosomes in the agouti mouse (Sakurai et al., 1975). Abnormally-differentiated lamellar and granular melanosomes were detected in the melanocytes of rats after copper deficiency (Miranda et al., 1992).

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



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 β -hexosaminidase, β -galactosidase, β -glucuronidase and cathepsin B and L have all been shown to cosediment with tyrosinase-rich densely sedimenting melanosomes (Diment et al., 1995). Also,

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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

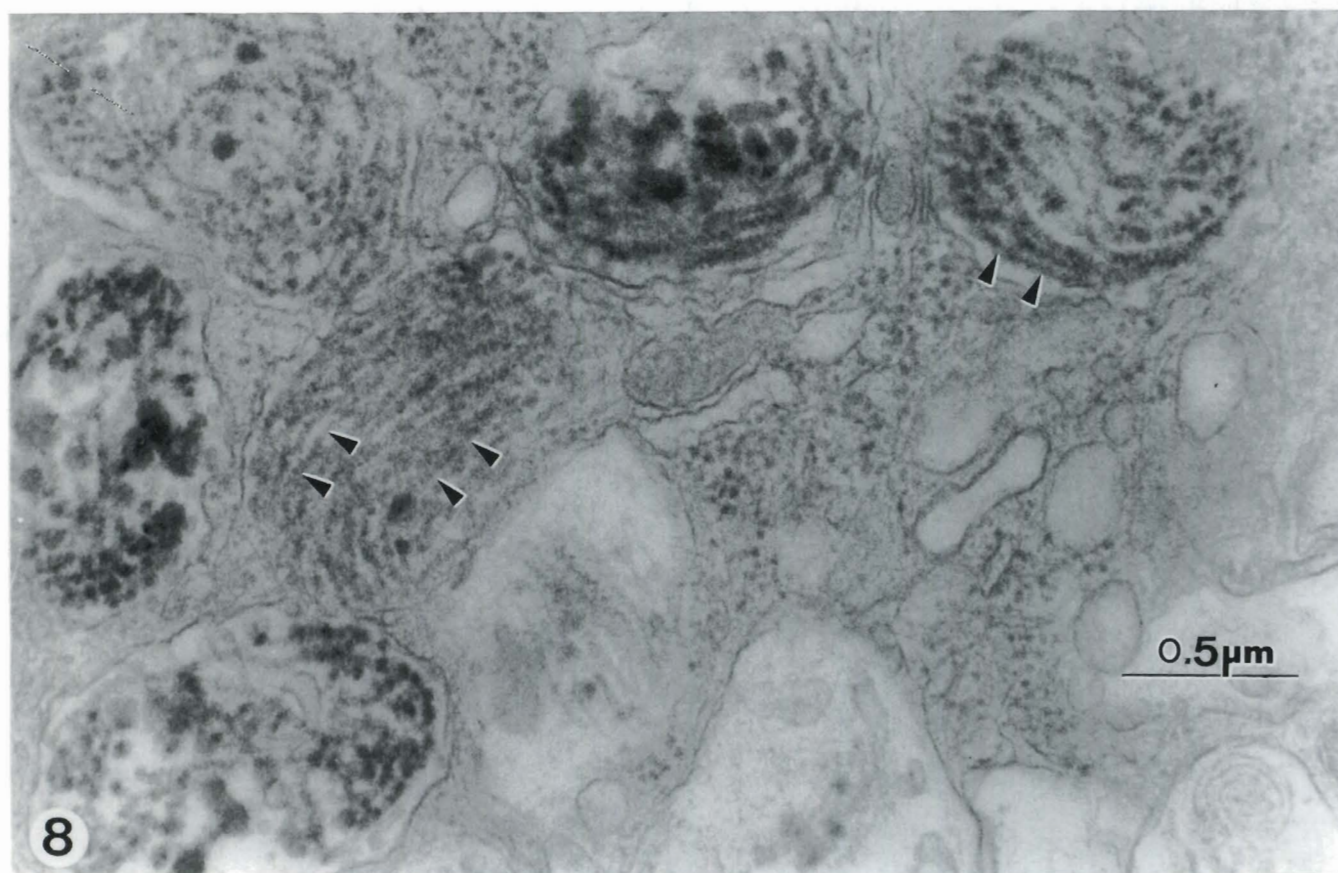


Fig. 8. Two weeks after culture under conditions described by Schraermeyer and Stieve (1994). Early-stage melanosomes of the lamellar type containing melanofilaments (arrowheads) are occasionally present in the RPE of cattle. $\times 72,600$

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.,

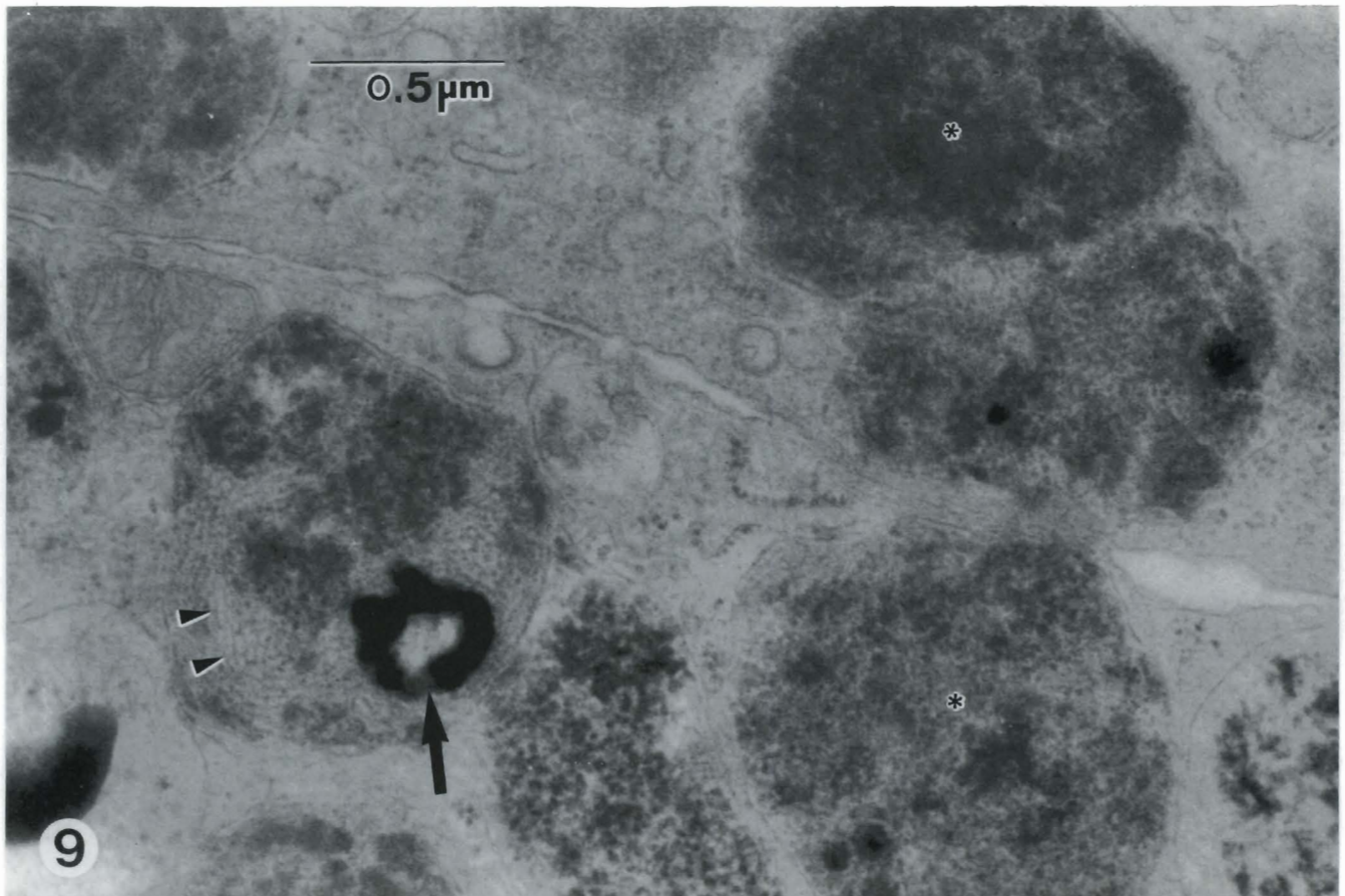


Fig. 9. After culture of bovine RPE under conditions described by Schraermeyer and Stieve (1994) for 6 months. Granular melanosomes (asterisks) are shown. One melanosome additionally contains melanofilaments (arrowheads) and probably melanin (arrow). $\times 52,500$

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 amount 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 (Schjerve et al., 1976). Other workers were not able to determine the origin of melanofilaments in the eyes of

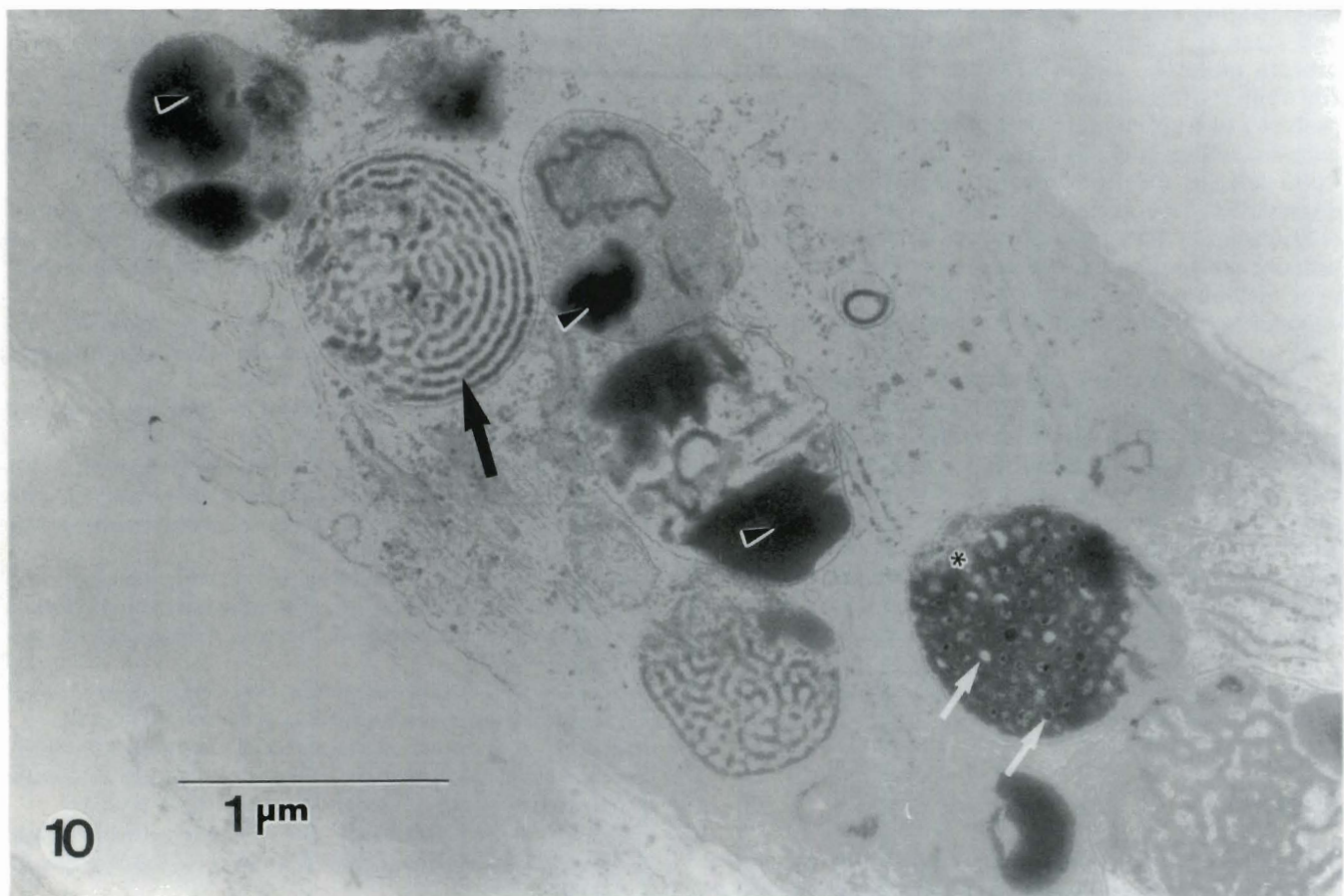


Fig. 10. Different patterns of polymerizing melanin can be seen after 6 months of culture of the RPE of cattle under conditions described by Schraermeyer and Stieve (1994). In one melanosome melanin seems to polymerize in highly ordered concentric shells (black arrow) whereas in other it seems to polymerize 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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