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ULTRASTRUCTURE OF GIANT PIGMENT GRANULES IN LENTIGO SIMPLEX

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Abstract. Electron microscopic observation of lentigo simplex on the sole revealed the presence in the lesion of giant pigment granules in melanocytes and keratinocytes. The giant granules were membrane-bound bodies containing electron-dense amorphous substances and less electron-dense microvesicles. It was also revealed that compound melanosomes, similar in size and shape to giant pigment granules, were present in melanocytes. Within the compound melanosomes, melanosomes showed disintegration into electron-dense fine particles concomitant with the release of less electron-dense microvesicles. These fine particles then aggregated to the mass of electrondense amorphous substances which eventually embedded the microvesicles. This evidence strongly suggests that the giant pigment granules are formed by complete degradation of melanosomes in the large compound melanosomes which can arise within melanocytes by autophagy.

Key words: Lentigo; Melanocytes; Pigments; Autophagosomes

The occurrence of giant pigment granules in the epidermis has been observed in the café-au-lait spots of neurofibromatosis (1, 4, 10) and certain other pigmentary disturbances (2, 5, 9). Under the electron microscope, it was observed that the giant pigment granules were composed of electron-dense amorphous materials and electron-lucent microvesicles (3, 5, 11). Up to the present, however, no evidence has been forthcoming to indicate the origin and nature of the granules.

This paper reports our findings on the ultrastructure of giant pigment granules in lentigo simplex of the sole and on the process of the formation of the granules.

MATERIAL AND METHODS

A 47-year-old woman noted a small pigmented lesion on the right sole one year previously. Physical examination revealed an oval, flat, brown macule, 5×8 mm in size. No lesions consistent with neurofibromatosis or Peutz-Jeghers syndrome were present. The pigmented lesion was excised under 1% procaine anesthesia. The tissue specimen was divided into two parts. One part was fixed in 10% formaldehyde and stained with hematoxylin-eosin and ammoniated silver nitrate for light microscopy. The other part, for electron microscopy, was immediately cut into small pieces and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 hours and post-fixed in 2% osmium tetroxide in sucrose-added Veronal-acetate (pH 7.4) for 2 hours. After dehydration in a graded series of acetone, the specimen was embedded in Epon and sectioned in an LKB ultrotome III. Thin sections were stained with uranyl acetate followed by lead citrate, and examined in a Hitachi HU-12A electron microscope at 100 kV. One micron thick sections were examined by light microscopy to confirm the presence of giant pigment granules.

RESULTS

Light microscopy

There was hyperpigmentation in the lower layers of the epidermis, particularly in the epidermal ridges. Melanocytes were irregularly arranged in the basal cell layer and were sometimes crowded at the lowest pole of rete ridges without the formation of distinct clusters. These melanocytes were recognized as clear cells containing varying amounts of fine pigment granules. Sometimes they contained one or more giant pigment granules (Fig. 1). The giant pigment granules were actually spherical, varying from 1.5 to 5.5 μm in diameter. They were birefringent, appeared dark brown in hematoxylin-eosin preparations and stained black with ammoniated silver nitrate. The giant pigment granules were occasionally encountered in the keratinocytes of the lower epidermis.

Electron microscopy

Epidermal melanocytes contained varying numbers of normal melanosomes, which occurred singly

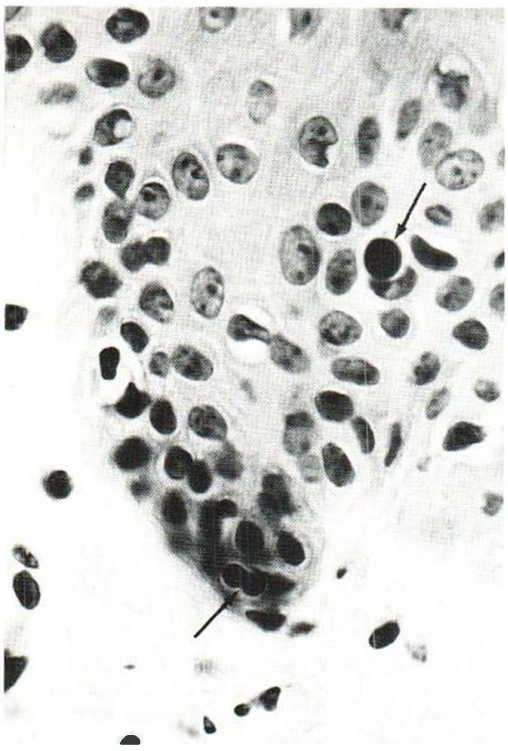

Fig. 1. Giant pigment granules (*arrows*) in a melanocyte and a keratinocyte. Hematoxylin-eosin, ×750.

and showed varying degrees of melanization according to the stage of development. Fully developed melanosomes were ellipsoidal, consisting of electron-dense amorphous matrices and less electron-dense microvesicles, about 400 Å in diameter, embedded in the matrices (Fig. 2, inset). The limiting membranes of the microvesicles were usually obscure because of the high electron-density of the melanosomal matrices. In addition to the normal melanosomes, giant pigment granules were occasionally seen in the cytoplasm of the melanocytes (Fig. 2). The giant pigment granules were spherical or ovoid and reached 5 μm, or more, in diameter. They were membrane-bound bodies containing electron-dense amorphous substances and microvesicles (Fig. 3). These two components could occur in varying amounts and distribution within an individual granule. The electron-dense amorphous substances tended to aggregate to a mass in the central zone of the granules, whereas the microvesicles, similar in size and shape to those in normal melanosomes, usually appeared in the outer zone of the mass of electron-dense amorphous substances. Often the giant granules showed a peripheral zone of varying depth where electron-dense fine particles were abundant (Fig. 3). These particles seemed to aggregate to electron-dense amorphous substances of varying size.

The melanocytes containing giants pigment granules had large nuclei with some indentations (Fig. 2). There were many mitochondria in the cytoplasm. The endoplasmic reticulum was fairly abundant, consisting mostly of the rough-surfaced variety. Ribosomes, either attached to the endoplasmic reticulum or free, were often clustered. There was an extensive Golgi apparatus with associated smooth-surfaced endoplasmic reticulum and small vesicles. The giant pigment granules showed no special relationship to either the endoplasmic reticulum or the Golgi apparatus.

The following observations appear pertinent to the aim of our study: Figs. 4, 5 and 6 illustrate what may represent various stages in the formation of the giant pigment granules. Compound melanosomes, similar in size and shape to the giant pigment granules, were occasionally encountered in the melanocytes. They were membrane-bound bodies containing a large number of ellipsoidal melanosomes which were undergoing degradation. In the early stage of degradation of phagocytosed melanosomes, some of the melanosomes lost their ultrastructural characteristics and disintegrated into fragments or electron-dense fine particles which were dispersed within the confines of the limiting membranes of the compound melanosomes. As the process advanced, distinct melanosomes became less numerous, whereas electron-dense fine particles, together with less electron-dense microvesicles released from disintegrated melanosomes, became more abundant. The fine particles showed progressive aggregation, consequently forming a mass of electron-dense amorphous substances in the central zone of the compound melanosomes. In the advanced stage of degradation, almost all the melanosomes were replaced by a large mass of electron-dense amorphous substances which embedded the less electron-dense microvesicles. Here, it should be noted that the features of the large compound melanosomes in the advanced stage of degradation of phagocytosed melanosomes closely resemble those of the giant pigment granules, except for the presence of the residual fragments of melanosomes.

The melanocytes containing large compound

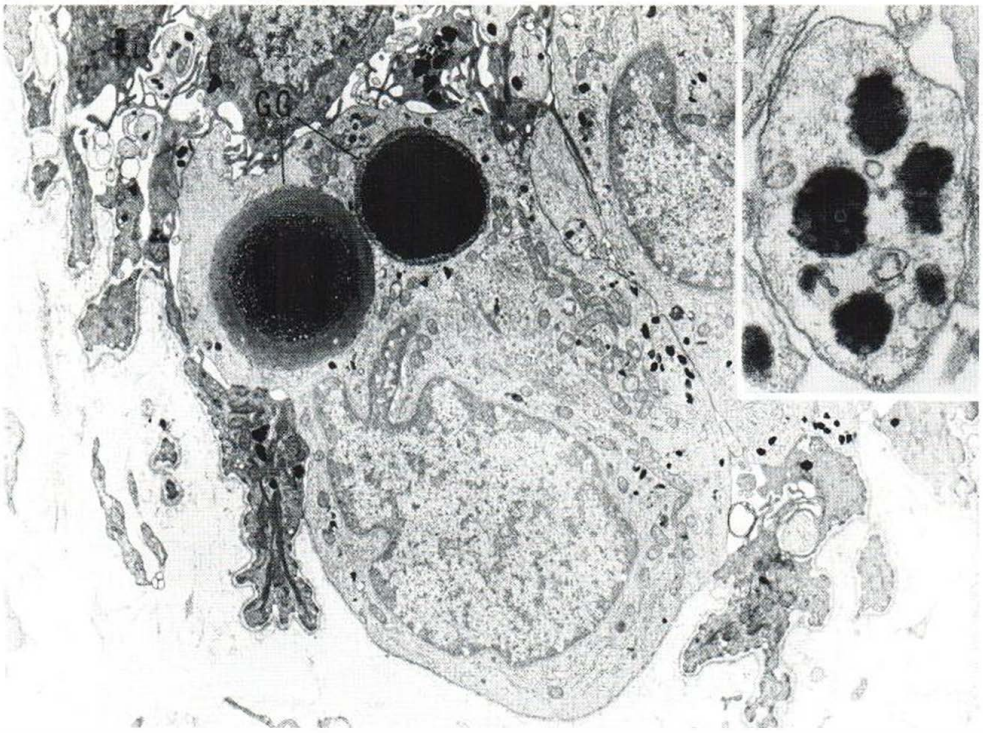

Fig. 2. A melanocyte containing giant pigment granules (GG). ×6 000. Inset shows fully developed melanosomes, consisting of dense amorphous matrices and less dense microvesicles, in a dendritic process. ×45 000.

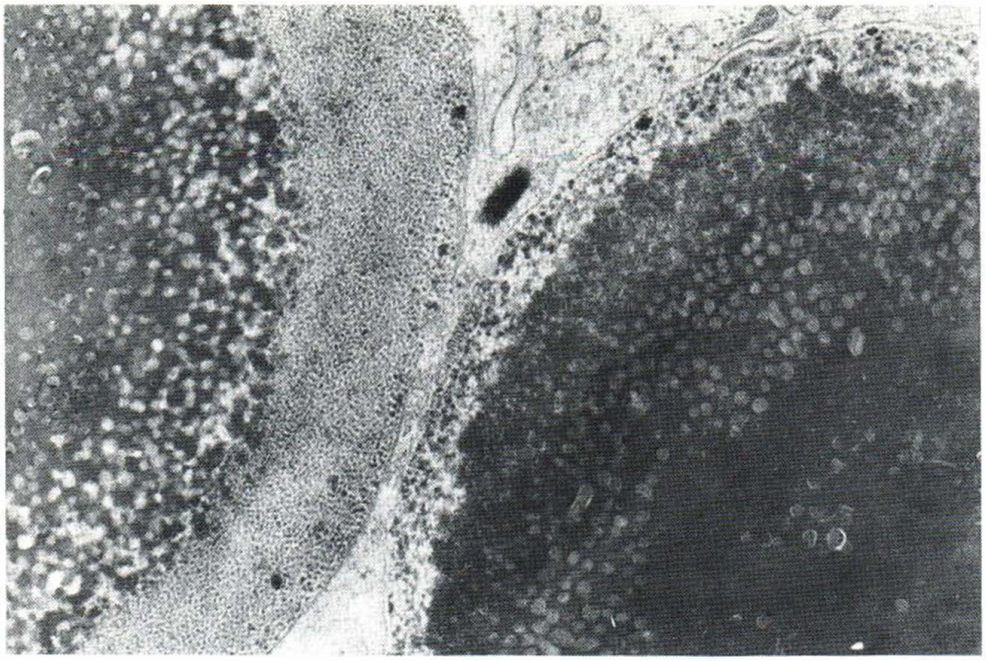

Fig. 3. Enlargement of parts of giant granules shown in Fig. 2. A central mass of dense amorphous substances embedding microvesicles and a peripheral zone containing fine particles or amorphous substances of various sizes are seen in each granule. ×45 000.

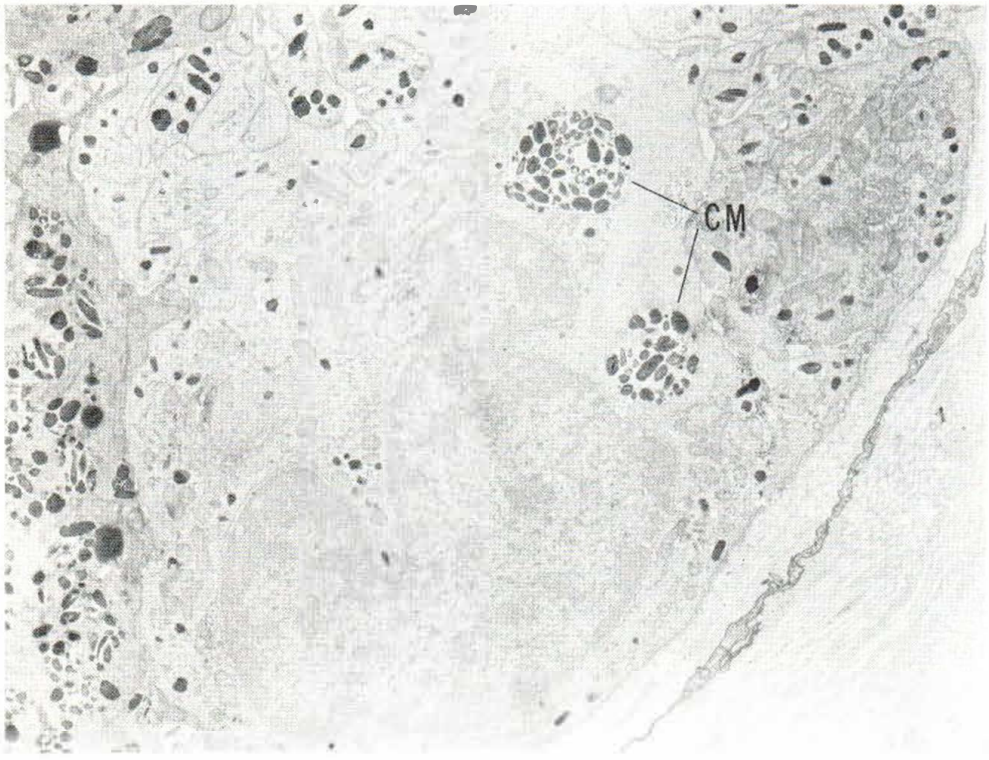

Fig. 4. A melanocyte containing large compound melanosomes (CM). ×10 500.

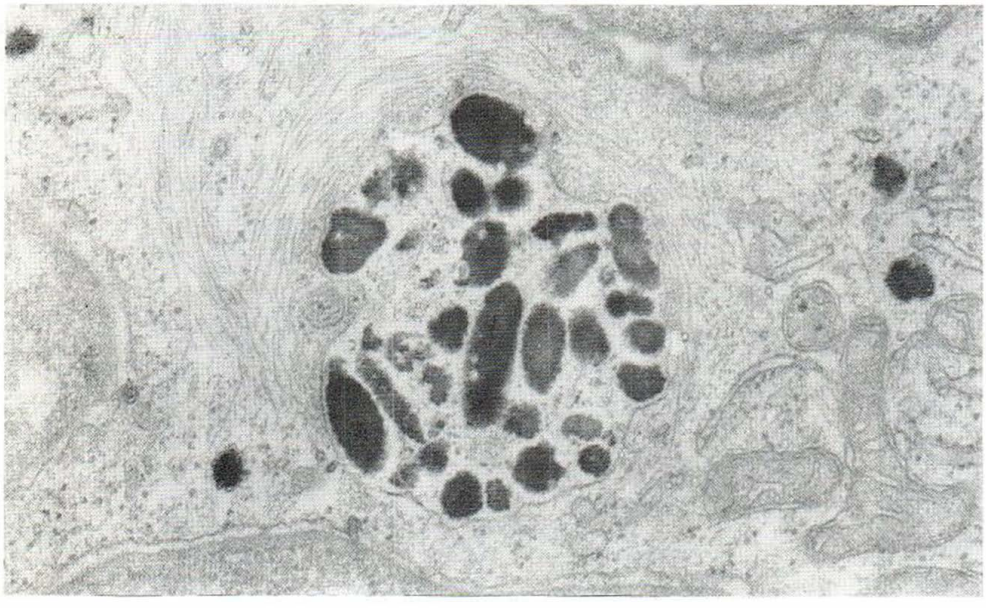

Fig. 5. A compound melanosome showing an early stage in the degradiation of autophagocytosed melanosomes. ×45 000.

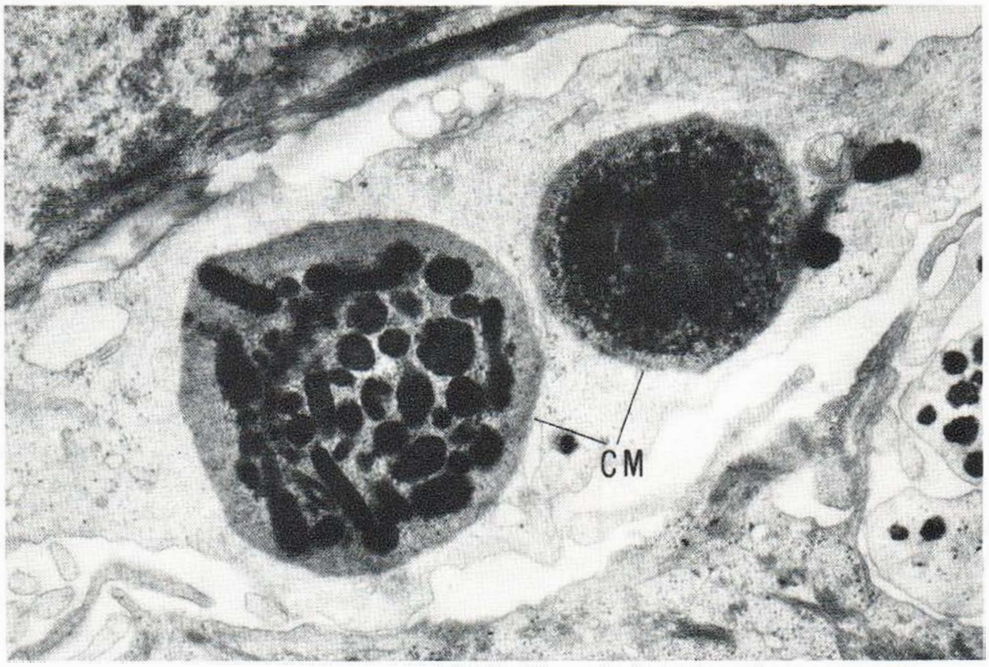

Fig. 6. Compound melanosomes (CM) in the dendritic process of a melanocyte. They show more advanced stages in the degradation of autophagocytosed melanosomes. ×27 000.

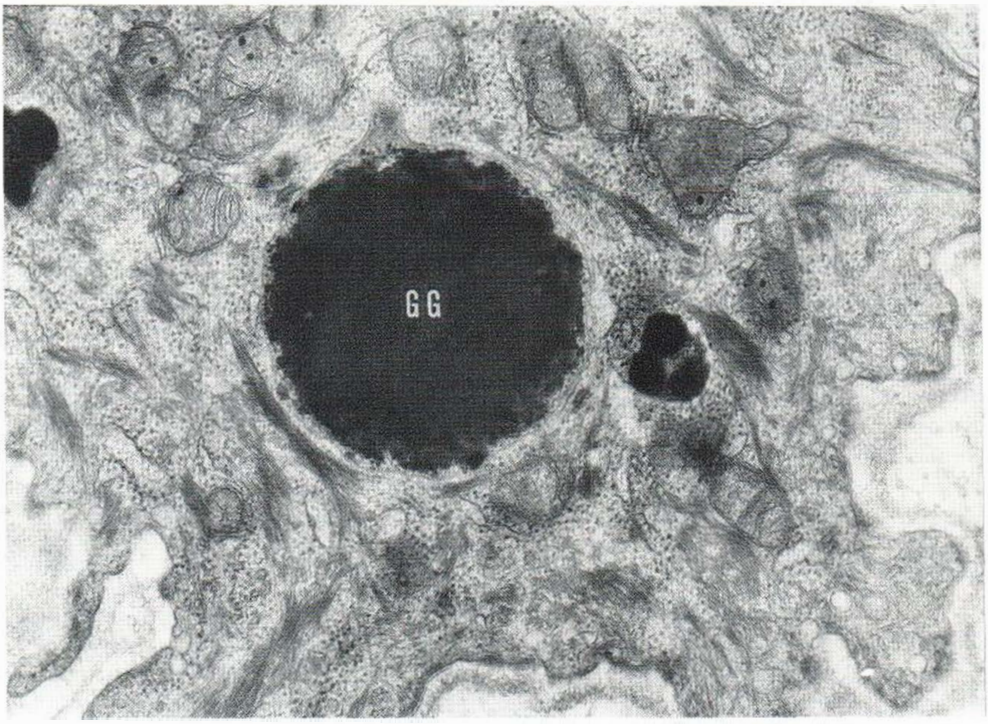

Fig. 7. A giant pigment granule (GG) in a basal keratinocyte. ×30 000.

melanosomes resembled those containing giant pigment granules, showing evidence of pronounced melanogenic activity (Fig. 4). They contained a large number of normal ellipsoidal melanosomes which were disposed singly in the cytoplasm. Both the rough-surfaced endoplasmic reticulum and the Golgi apparatus were well developed.

The keratinocytes in the lower epidermis contained a large number of normal ellipsoidal melanosomes, most of which were aggregated within small vacuoles. These keratinocytes occasionally contained giant pigment granules, which were membrane-bound bodies filled with electron-dense amorphous substances (Fig. 7).

DISCUSSION

The ultrastructure of giant pigment granules has been studied previously in café-au-lait spots by Jimbow et al. (3) who demonstrated that giant pigment granules are large membrane-bound bodies containing electron-dense amorphous materials and electron-lucent microvesicles, and that several subtypes of the granules are distinguishable according to the amounts and distribution of these components. They suggested that these subtypes may represent the different stages in the developmental sequence of the granules in melanocytes. Konrad et al. (5) showed that giant pigment granules in a nevoid pigmented lesion were similar in ultrastructure to those in café-au-lait spots. On the basis of their findings they proposed that giant pigment granules are extraordinarily large melanosomes which contain numerous microvesicles participating in melanization and are formed by asynchronous melanization from the central core to the peripheral zone. These authors, however, provided no evidence to indicate the presence of the precursors of giant pigment granules and the relation between normal melanosomes and giant pigment granules.

In this study, it has been revealed that there are giant pigment granules in the melanocytes and keratinocytes in lentigo simplex of the sole. Our findings confirm the basic similarity in the ultrastructure of giant pigment granules in these three kinds of pigmentary disturbances. In addition, we observed that large compound melanosomes occur within melanocytes as the precursors of giant pigment granules. The evidence indicates that the melanosomes show disintegration into electron-dense fine particles, which are dispersed and accumulated within the confines of the limiting membranes of compound melanosomes, concomitant with the release of less electron-dense microvesicles. It is also indicated that the fine particles aggregate to the mass of electron-dense amorphous substances and which eventually embeds the microvesicles. These observations strongly suggest that the giant pigment granules in melanocytes originate from the large compound melanosomes where melanosomes undergo degradation. Some biochemical support for these observations may be found in the report by Saito & Seiji (7). In their digestion experiments on melanosomes, they demonstrated that melanosomes were degraded by lysosomes not at the melanin moiety but at the protein moiety and suggested that the same transformation of melanosomes could occur in compound melanosomes.

It is generally accepted that compound melanosomes in melanin-producing cells are some kind of lysosomes and arise within the cells by autophagy (6, 8). Our observation has demonstrated that the melanocytes containing large compound melanosomes show evidence of pronounced melanogenic activity. Consequently it seems likely that, when melanosomes are produced in excess of the amount to be transferred to keratinocytes, this excess is enclosed in autophagic vacuoles within the melanocytes. If this is the case, it follows that the giant pigment granules in melanocytes are large autophagosomes containing completely disintegrated melanosomes.

Concerning the origin of giant pigment granules of keratinocytes, Jimbow et al. (3) proposed that the giant granules were transferred from melanocytes to keratinocytes as a single unit after their maturation (i.e., after completion of their formation process). In Fig. 13 of their paper, however, these authors showed an image suggesting fusions of a giant pigment granule with some aggregates of ellipsoidal melanosomes in the keratinocyte. This observation and those in our study make it possible to suggest that in keratinocytes giant granules may increase in size by the accumulation of additional amounts of the electron-dense amorphous substances resulting from the degradation of the engulfed melanosomes. Whether giant pigment granules arise *de novo* within keratinocytes is left for further study.

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