MELANOSOMES IN PHAGOCYTIC VACUOLES IN LANGERHANS CELLS

Electron Microscopy of Keratin-Stripped Human Epidermis

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The nature of Langerhans cells is disputed. They have been considered effete melanocytes (1, 2), postmelanin-synthetic cells (3), melanocyte precursors (4), intraepidermal nerve endings (5, 6), or postdivisional stages of previously melanogenic melanocytes (7). Under the electron microscope, melanocytes and Langerhans cells are distinguished by highly characteristic cytoplasmic organelles: melanosomes in the melanocytes, and disc-shaped bodies in the Langerhans cells. Any postulated relationship between the two types of cells must remain hypothetical as long as there is no evidence that a cell can turn from synthesis of one type of organelle to that of the other.

Since removal of the stratum corneum of the epidermis by Scotch tape stripping has been shown to increase epidermal cell turnover and to cause characteristic subcellular changes (8), and since melanocytes and keratinocytes form a symbiosis (9), it seemed possible that the events after stripping might provide a better opportunity to observe the proposed melanocyte-Langerhans cell conversion.

MATERIALS AND METHODS

Specimens of normal adult Negro and Caucasian skin were obtained from the flexor surface of the forearm at various intervals after Scotch tape stripping (10). Control sections were taken from a corresponding unstripped area. Specimens were immediately fixed in OsO_4 for 120 min and embedded in Maraglas 655. Staining was carried out with 1% phosphotungstic acid at the absolute ethanol stage of dehydration and with lead citrate (11) after ultrathin sectioning. Sections were examined with an RCA EMU-3F or EMU-3G microscope.

RESULTS

Cells containing both melanosomes and Langerhans cell granules have not been found previously in normal integument, and the present study did not reveal such cells in control sections. However, the number of typical Langerhans cells seen in the epidermis of human skin obtained 18 and 24 hr after keratin layer stripping appears to be significantly increased as compared to the number found in normal control skin.

Fig. 1 shows two Langerhans cells in juxtaposition to a middle level prickle cell 24 hr after stripping. These Langerhans cells characteristically exhibit absence of tonofilaments and desmosomes, in contrast to surrounding keratinocytes. They have an indented or convoluted nucleus in addition to their distinctive organelles. Melanosomes are present in both Langerhans cells, although the upper cell contains fewer granules. Higher magnification of one of these Langerhans cells (Fig. 2) reveals typical Langerhans cell granules. They appear rod-shaped with rounded ends and often have vesicles continuous with their outer limiting membrane. The rod-shaped structure contains an array of relatively electron-opaque particles lying in the midlongitudinal axis of the rod. Perpendicular to this longitudinal structure are periodically occurring particle bands, approximately 50 to 90 A apart, which form planes parallel to the short axis. Although these organelles

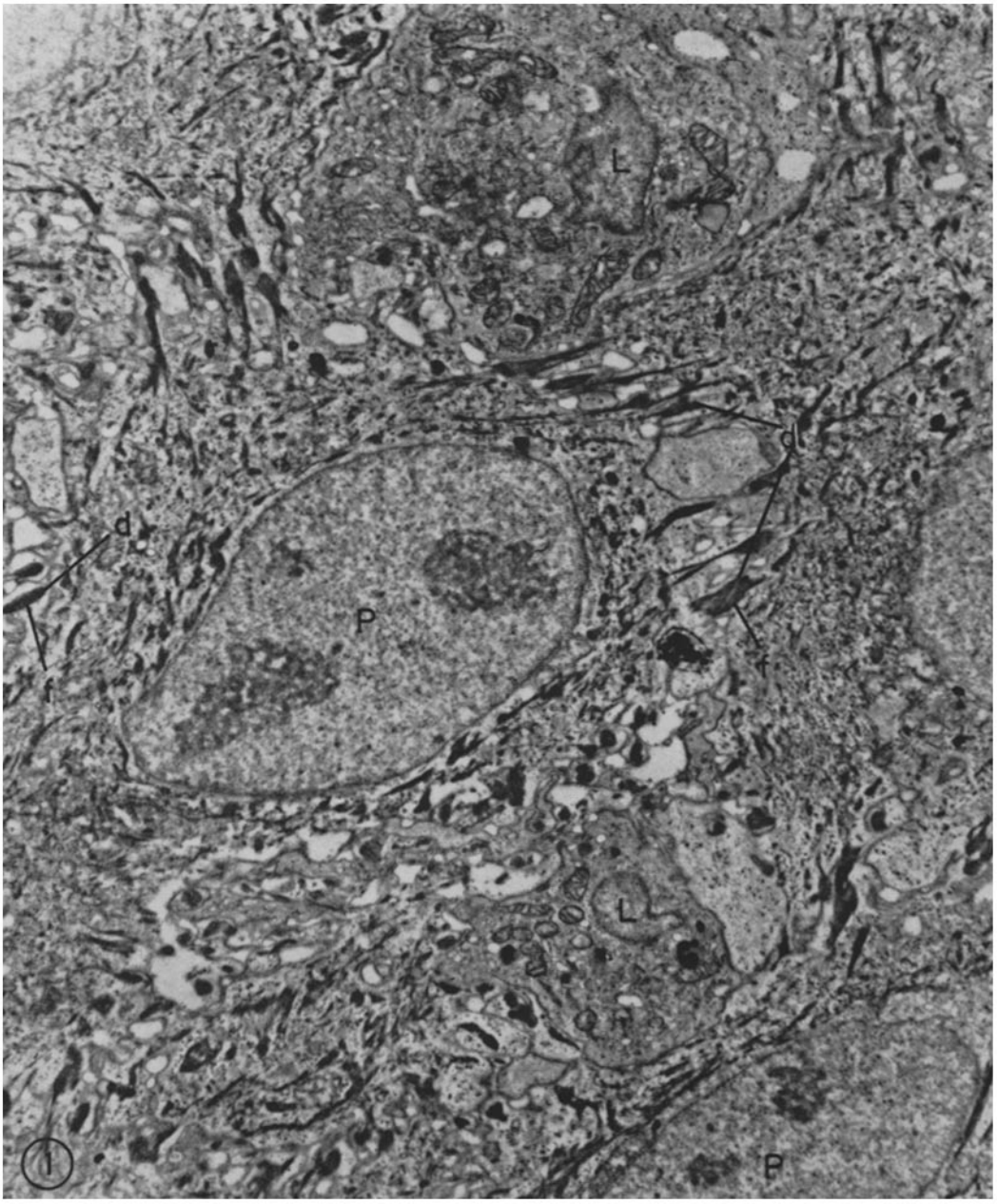

FIGURE 1 Two characteristic Langerhans cells (*L*) containing melanosomes in the prickle cell layer of human epidermis 24 hr after keratin layer stripping. In contrast to the surrounding prickle cells (*P*), the Langerhans cells do not possess desmosomes (*d*) or tonofilaments (*f*) and show an indented nucleus. Keratin layer up. × 8800.

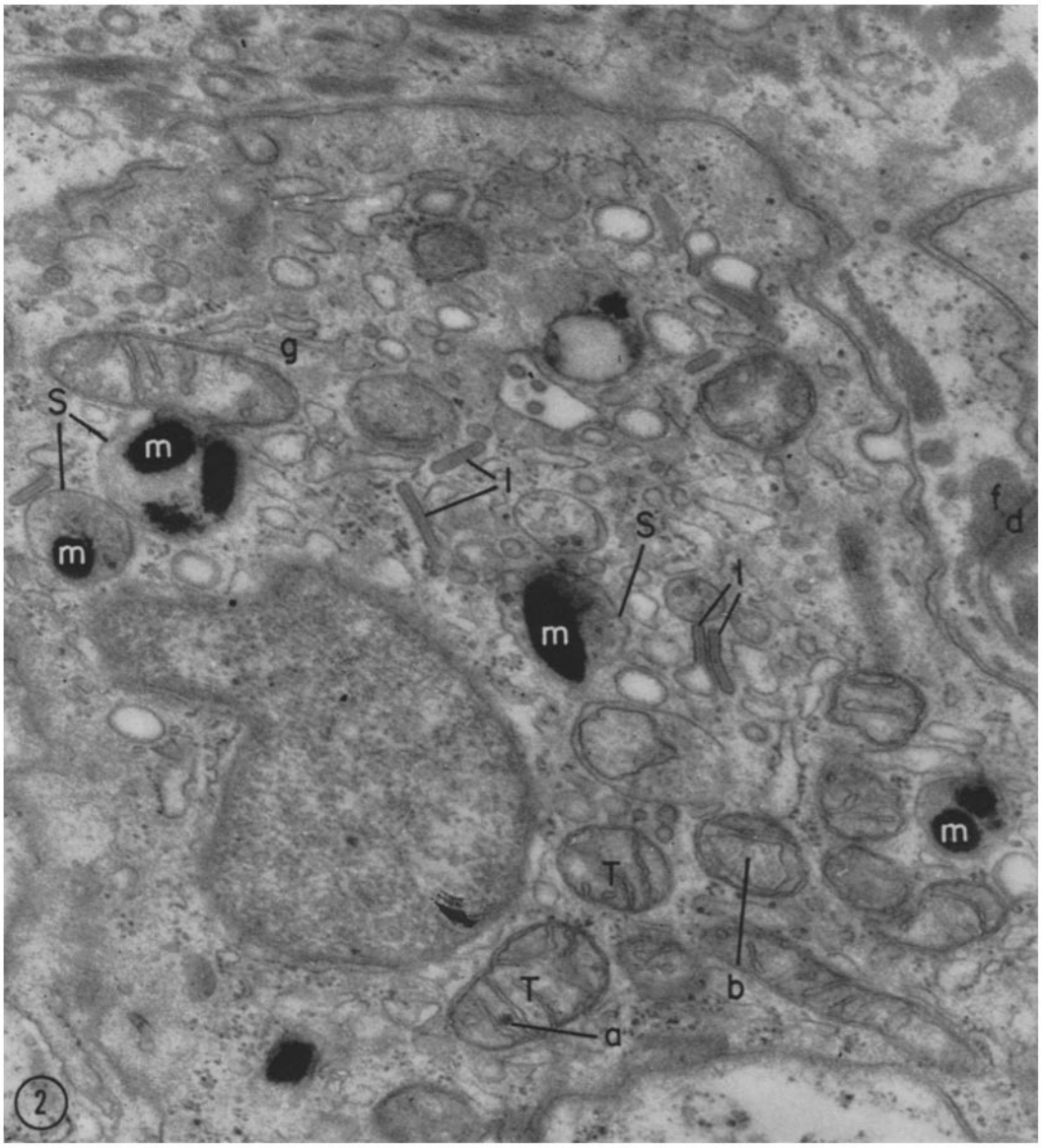

FIGURE 2 Higher magnification of a Langerhans cell shown in Fig. 1, demonstrating that melanosomes (*m*) are uniformly and maximally electron opaque and engulfed within phagocytic vacuoles (*S*) whereas the characteristic Langerhans cell granules (*l*) are seen in various developmental stages in the vicinity of the Golgi region (*g*). Desmosomes (*d*) and tonofilaments (*f*) of surrounding prickle cells are seen. A moderately electron-opaque particle (*a*) having a diameter of approximately 500 A and many smaller particles (*b*) having diameters of approximately 120 A are seen in the mitochondria (*T*). × 43,500.

often appear rod-shaped in thin sections, the occasional appearance of rounded bodies with internal structure (Fig. 3) suggests that Langerhans cell granules are three-dimensionally disc-shaped. Furthermore, the cytoplasm of Langerhans cells (Figs. 2 to 4) exhibits various developmental stages in the Golgi region, from small vesicles to vesicles with outgrowths of characteristically disc-

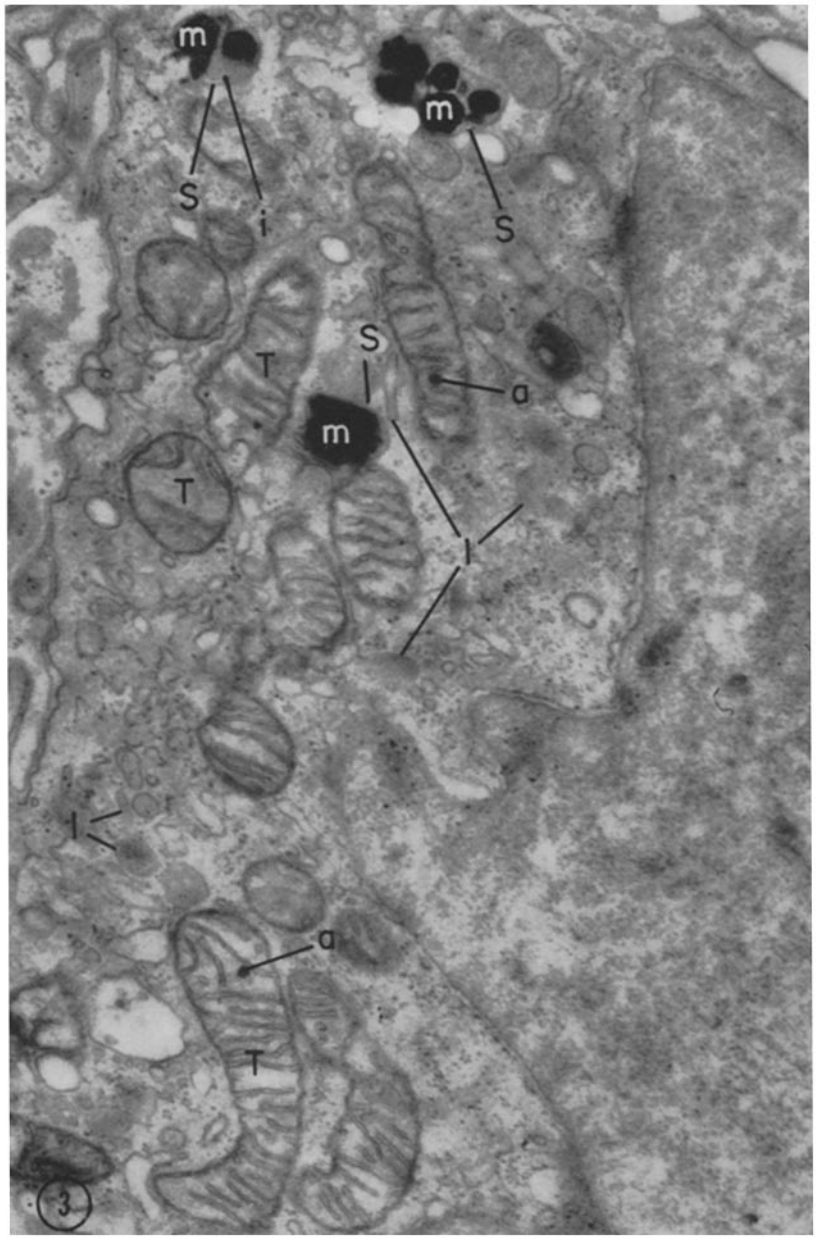

FIGURE 3 The cytoplasm of a Langerhans cell from epidermis 24 hr after keratin layer stripping, showing the presence of various developmental stages of Langerhans cell granules (*l*) free in the cytoplasm whereas melanosomes (*m*) are fully mature and are engulfed within phagocytic vacuoles (*S*). A closely aligned particle interface (*i*) is seen continuous with the matrix of phagocytic vacuoles and concentric with the limiting membrane. Moderately electron-opaque particles (*a*) approximately 500 A in diameter are seen within several mitochondria (*T*). × 29,500.

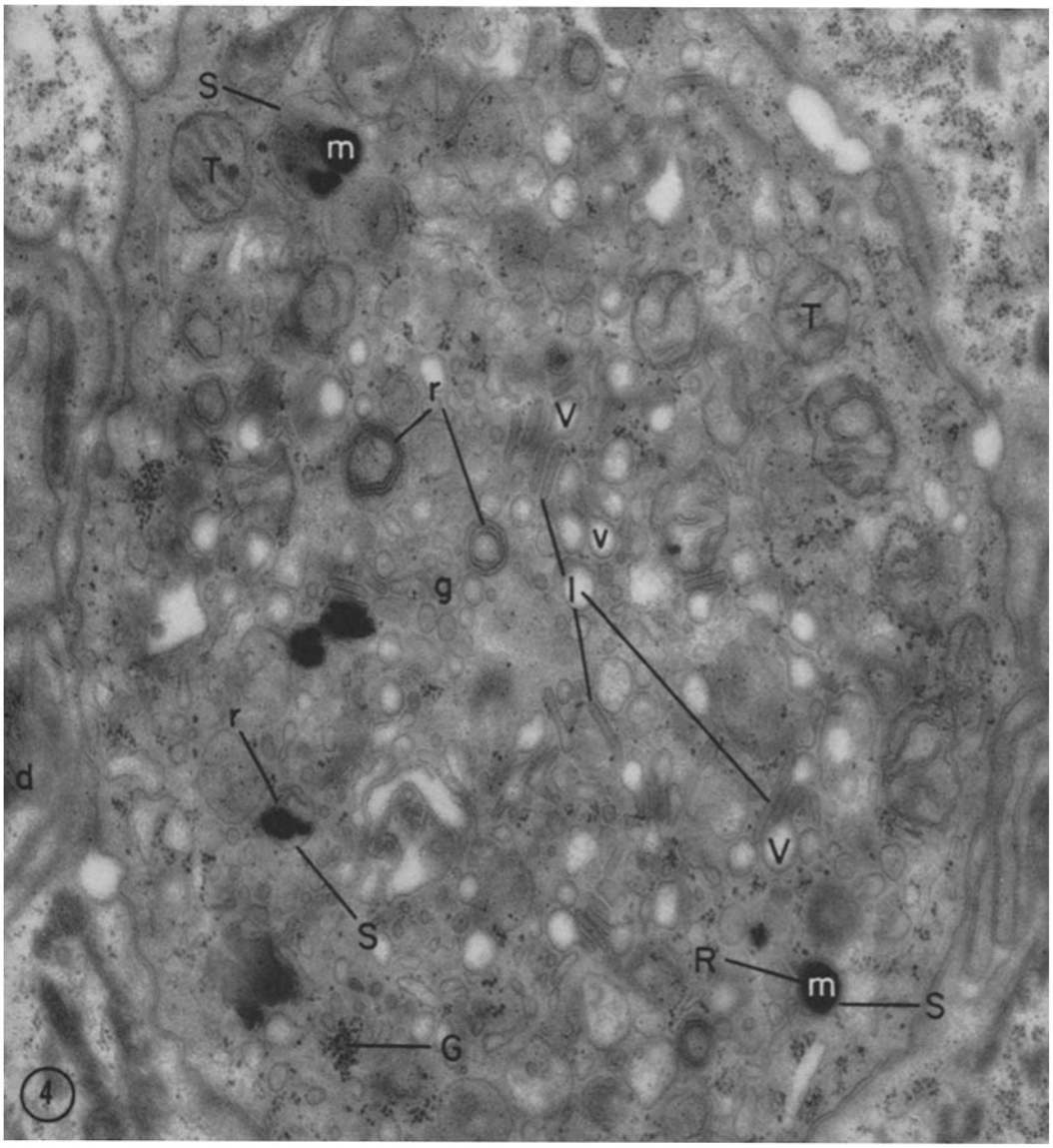

FIGURE 4 Langerhans cell in prickle cell layer 18 hr after keratin layer stripping, showing various developmental stages of Langerhans cell granules in the Golgi region (*g*), from small vesicles (*v*) to disc-shaped organelles (*l*), with or without continuous vesicles (*V*). Melanosomes (*m*) are maximally electron opaque and engulfed within phagocytic vacuoles (*S*). The phagocytic vacuoles often exhibit multilayered concentric inner membranes (*r*) which at times can be shown to be membranes (*R*) surrounding melanosomes engulfed within the phagocytic vacuoles. *T*, mitochondria; *G*, glycogen particles; *d*, desmosomes. × 32,500.

shaped bodies and further to mature disc-shaped organelles, thus indicating active synthesis of Langerhans cell granules.

The Langerhans cells in the specimens taken 18 and 24 hr after tape stripping do contain melanosomes. However, no developmental stages of melanosomes have been seen. All melanosomes are of uniform and maximal electron opacity and are

surrounded by membranes, a fact which suggests that they lie within phagocytic vacuoles. Often, several melanosomes are within one vacuole in which multilayered inner membranes (Fig. 4) are seen. Some of these inner membranes appear to surround melanosomes engulfed within the phagocytic vacuoles. A closely aligned particle interface (12) also can be seen which is continuous with the matrix and concentric with the limiting membrane of the phagocytic vacuole (Fig. 3). This interface can be contrasted with the above-described inner membranes.

Typical melanocytes containing various developmental stages of melanosomes were present in the basal layer of epidermis removed after stripping. No cell in the process of melanosome synthesis has been found to contain Langerhans cell granules, either free in its cytoplasm or within phagocytic vacuoles.

No structures suggesting that Langerhans cells may be intraepidermal nerve endings were seen in this electron microscope study.

DISCUSSION

It has long been proposed that the Langerhans cell occupies a place in the life history of the melanocyte, but direct proof is lacking. A recent hypothesis (7) claiming that the melanocyte is a melanogenic phase in the life history of the Langerhans cell is based on conditions in vitiligo. In contrast to congenital albinism, in which melanocytes synthesize structurally normal melanosomes, but do not achieve melanization, the acquired form of cutaneous depigmentation, vitiligo, exhibits replacement of melanocytes by cells synthesizing Langerhans cell–type organelles and possessing other characteristics similar if not identical to Langerhans cells (13).

The presence of melanosomes or melanin granules within Langerhans cells can be explained in three ways: (*a*) the melanosomes are being synthesized concomitantly with Langerhans cell granules; (*b*) the melanosomes were transferred to the Langerhans cell from external sources; and (*c*) the melanosomes were previously synthesized by the cell which is now synthesizing Langerhans cell granules and is segregating its own melanosomes within phagocytic vacuoles.

Simultaneous synthesis of melanosomes and Langerhans cell granules has been reported (3). However, the electron micrographs published with that report suggest that the melanosomes are aggregated within phagocytic vacuoles rather than occurring individually in various developmental stages as seen in active melanin synthesis. Furthermore, it seems likely that the red hair–type premelanosomes previously reported in Langerhans cells (14) are actually phagocytic vacuoles containing multilayered inner membranes (Fig. 4). There seems to be agreement that Langerhans cells can synthesize phagocytic vacuoles (3, 4), but the presence of engulfed melanosomes within these organelles has not been previously noted in the normal skin. The present finding that all melanosomes in Langerhans cells are within vacuoles structurally corresponding to lysosomes and do not show developmental stages seems to rule out active synthesis of melanosomes in these cells at the time of observation after stripping. It does not differentiate between the extracellular or intracellular origins of the segregated granules.

I cannot rule out the possibility that the granules were acquired by the penetration or invagination of Langerhans cells by melanocyte dendrites, as suggested in vitiligo (15), similar to the mechanism by which melanin is transferred into keratinocytes (16). The multilayered membranes of the phagocytic vacuoles (Fig. 4) may represent the intrinsic structure of phagocytic vacuoles or may be derived from the plasma membrane of pinched-off melanocytic dendrites.

However, in normal control epidermis, melanosomes have not been found in Langerhans cells, nor phagocytic vacuoles in melanocytes. Also, no melanocytes were found to engulf Langerhans cell granules within phagocytic vacuoles 18 to 24 hr after stripping.

Present evidence suggests that under normal physiological conditions the melanocyte discharges all of its melanosomes into the surrounding keratinocytes. After ceasing melanin biosynthesis the melanocyte ascends into the higher levels of the epidermis and acquires the new ability of synthesizing Langerhans cell granules and forming phagocytic vacuoles. This conversion of melanocytes into Langerhans cells can be accelerated by keratin stripping. Under these experimental conditions junctional melanocytes seem to ascend into the higher layers before they can discharge all their melanosomes into keratinocytes which also lose their normal intercellular contacts (8). The granules remaining in the ascended Langerhans cell then become the objects of autophagic engulfment.

SUMMARY

The presence of melanosomes within vacuoles resembling lysosomes, is induced in the cytoplasm of active Langerhans cells of human epidermis by keratin layer stripping. It is suggested that the synthesis of Langerhans cell granules begins after the cessation of melanosome biosynthesis in the cytoplasm of melanocytes, and that this turnover process from melanocyte to Langerhans cell is accelerated in the epidermis during the period of forced regeneration induced by stripping.

There is no evidence that Langerhans cells are intraepidermal nerve endings.

Addendum

The term "melanosome" as used here follows the recommendations (17) of the committee on Pigment Cell Terminology, Sixth International Pigment Cell Conference. This term therefore includes the former term "melanin granule."

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