

A CASE OF HALO NEVUS WITH EFFETE MELANOCYTES

Ken Hashimoto

From the Veterans Administration Hospital, Memphis, Tennessee, and the Department of Medicine, Section of Dermatology, University of Tennessee College of Medicine, Memphis, Tennessee, USA

Abstract. Epidermal melanocytes in the depigmenting lesion were examined in one typical case of halo nevus. Vacuolated melanocytes found in the upper layers of epidermis were not, as observed in serial sections, connected to the basal lamina. These melanocytes contained individual as well as aggregated melanosomes in what appeared to be autophagosomes. Part of the cytoplasm of these melanocytes appeared condensed and segregated and their nuclei were often pyknotic. These melanocytes were considered to be degenerated cells which had been detached from the basal layer and were being shed from the skin. Although epidermal keratinocytes, particularly basal cells, were also vacuolated, Langerhans cells and non-specific dendritic cells were more or less intact. The total number of these dendritic cells seemed to be increased.

Key words: Halo nevus; Nevus; Melanocyte; Melanoma; Langerhans cell

In 1961, Birbeck et al. (1) reported that melanocytes disappear and Langerhans' cells (L-cells) increase in the lesion of vitiligo. They postulated that the

aged melanocytes became L-cells. This concept was reinforced by Breathnach (2), although subsequently he denied this relationship between melanocyte and L-cell (3). Zelickson & Mottaz (15) described in vitiligo the presence of dendritic non-keratinocytes which contain neither melanosomes nor L-cell granules. They call these cells "indeterminate cells", suggesting that these cells are non-committed matrix cells for both melanocyte and L-cell. Mishima, Kawasaki & Pinkus (11) described the same cells in vitiligo and named them " α -dendritic cells". They made a statistical analysis and concluded that although L-cells were increased in the basal layer, if the whole epidermis was scanned, α -dendritic cells, and not L-cells, were increased in number. It was noted that even α -dendritic cells eventually disappear as the lesion ages, whereas the L-cell population does not significantly change throughout the entire process of depigmentation (11).



Fig. 1. This piece of tissue was taken from the advancing border of depigmentation, Araldite-embedded, cut at 1 μ m and stained with 0.01% Azure B on a hot plate. Several clear cells are present in the epidermis but no nevus cells forming a nest. The subsequent serial sections cut out of this block (Figs. 2-5) represented the nevus cell-free "periphery" since

after thin sectioning, 1 μ m thick sections were cut to confirm the absence of nevus cells. Transferred melanin pigments are visible in the basal cells and lower Malpighian cells but not in clear cells. Several shrunken clear cells (*) are present near the granular cell layer. Some of the basal clear cells are vacuolated (arrows). $\times 400$.



Fig. 2. In this low magnification view, 3 melanocytes (M_1 , M_2 , M_3), 2 Langerhans cells (L-cells) (L_1 , L_2) and two unidentifiable cells ($?_1$, $?_2$) are labeled. $?_1$ might be an extension of L_2 and $?_2$ a lymphocyte. M_1 and M_2 are unusually located, within or just below the keratohyalin (k)-containing granular cells (G), and show severe degenerative changes such as vacuolization (M_1 , M_2) and coagulation of the cytoplasm (M_2). L-cells (L_1 , L_2), on the other hand, do not show dam-

age. Intercellular spaces, particularly those of the basal (B) and lower strata, are widely open (edematous). Arrow-marked area M_1 is enlarged in Fig. 3A and the arrow-marked area of M_2 in Fig. 3B, whereas a serial section of L_2 is enlarged in Figs. 4 and 5 to show the specific L-cell granules. B : basal cell. f : fibroblasts. H : horny cells. n : nucleus of M_2 . $\times 4\ 000$.



Fig. 3A. The arrow-marked areas of M₁ of Fig. 2 are enlarged. A coagulation necrosis (cn) or dense homogenization of a part of the cytoplasm and partial segregation are

observed. The presence of a laminated melanosome (arrow) identifies this cell as a melanocyte. V: cytoplasmic vacuole. $\times 111\ 500$.

In leukoderma acquisitum centrifugum (Sutton), i.e. halo nevus, several authors have noticed that the melanocytes were replaced by L-cells (5, 6, 8, 11, 12, 14). As in the case of vitiligo, Mishima et al. (11) reported that the L-cell population was increased in the basal layer of the lesion but the total number in the entire epidermis was unchanged.

These studies focused attention on the interrelationship between melanocyte, L-cell, and non-specific dendritic cell and a transformation of melanocyte into L-cell or other types of dendritic cells was explicitly or implicitly suggested. If such a transformation really occurs, one might find a cell or two which would contain both melanosomes and L-cell granules. However, the coexistence of (non-phagocytosed) melanosomes and L-cell granules in the same cell has never been convincingly demonstrated. Thus, the fate of the melanocytes could be

(i) that they become degenerated and die, or (ii) that melanocytes become dendritic cells which no longer produce melanosomes. Thus far, the first possibility has not been explored adequately.

In the course of electron microscopic studies of halo nevus (6) the first possibility was explored. A number of degenerated melanocytes was indeed found in the upper layers of the epidermis in the progressively depigmented (halo) areas surrounding a compound nevus. This observation supported the first hypothesis, that some melanocytes in halo nevus actually die, although it did not rule out entirely the concept of Masson and colleagues (10) that "effete melanocytes" become L-cells. In this report the "high level effete melanocytes" of halo nevus are described. The details of the degenerative mechanism of melanocytes have been published elsewhere (6) and therefore will not be emphasized.

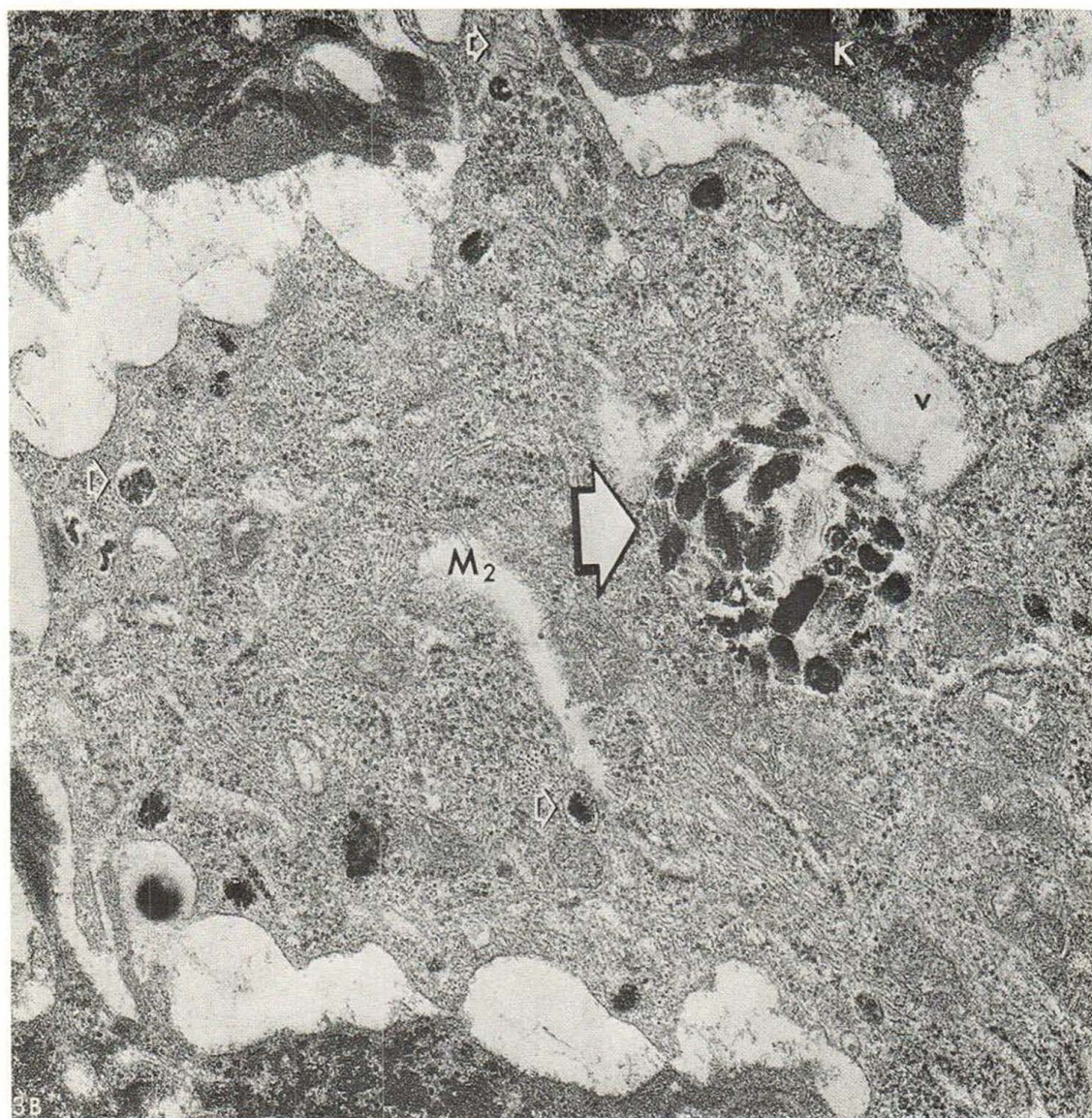


Fig. 3B. An aggregation of melanosomes, some of which appear to be fragmented, in one particular area (solid arrow) suggests that these melanosomes are being autophagocytosed. Immature melanosomes are present singly and sur-

rounded by membrane (hollow arrows). In both pictures there are many cytoplasmic vacuoles (*v*). *K*: keratinocyte. Both A and B: $\times 68\,000$.

MATERIALS AND METHODS

The specimens were excised under 1% procaine anesthesia from the left shoulder of an 11-year-old white male. He reported a depigmentation of skin surrounding a brown-black mole over a period of a few months. The tissue was immediately sliced into 1 mm³ blocks. Although each tissue piece was marked upon excision according to its topography as "center" or "periphery", a thick section was cut at 1 μ m from each Araldite block to confirm the area to be examined

in the subsequent thin sections. The "center" pieces contained the compound nevus and the "periphery" ones contained originally normal skin which was undergoing depigmentation. The "periphery" specimens contained the advancing border of depigmentation which was located approximately 5 mm from the central mole. The depigmenting border covered in the present study was approximately 2 $\times$ 2 mm; it was divided into 2 tissue blocks. The tissue blocks were fixed for 3 hours in 5% glutaraldehyde buffered to pH 7.4 with 1/10 M cacodylate. After an overnight rinse in the

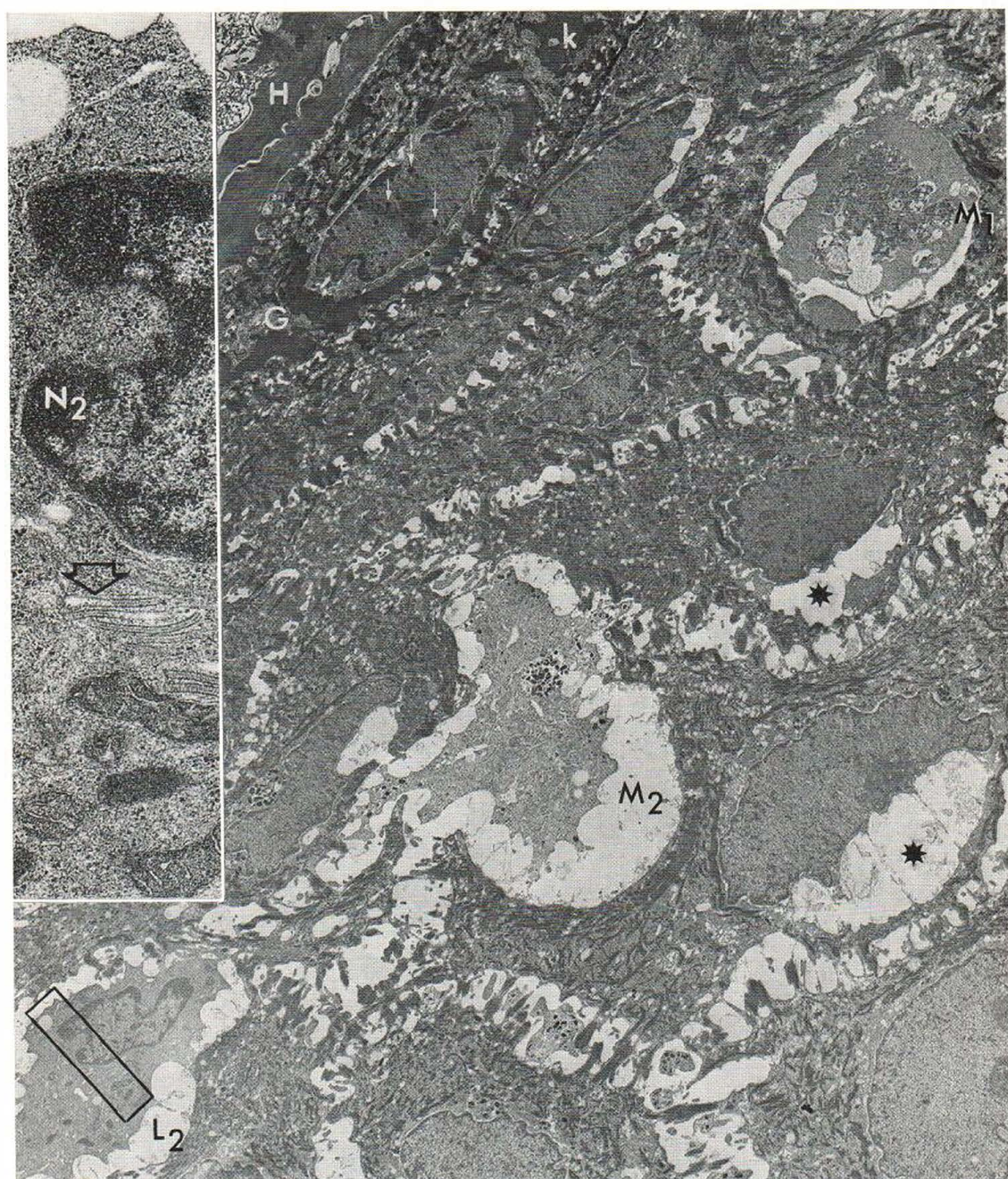


Fig. 4. The section serial to that shown in Fig. 2. M_1 shows more severe vacuolar and coagulation degeneration. M_2 also contains many vacuoles and remains in the same position between M_1 and L_2 . It does not extend toward the basal layer. Rather, we lost the nucleus which was present in Fig. 3; it is unlikely that this cell attaches to the basal lamina in further serial sections. L_2 contains a large number of L-cell

granules (arrow in inset). Keratohyalin granule (k)-containing granular cells and horny cells (H) are just above these cells. Not only the intercellular edema but also severe degenerative changes (*) are seen in Malpighian cells. The rectangle at L_2 indicates the area enlarged in the inset. N_2 : nucleus of L_2 . $\times 8\,000$. Inset: $\times 61\,000$.

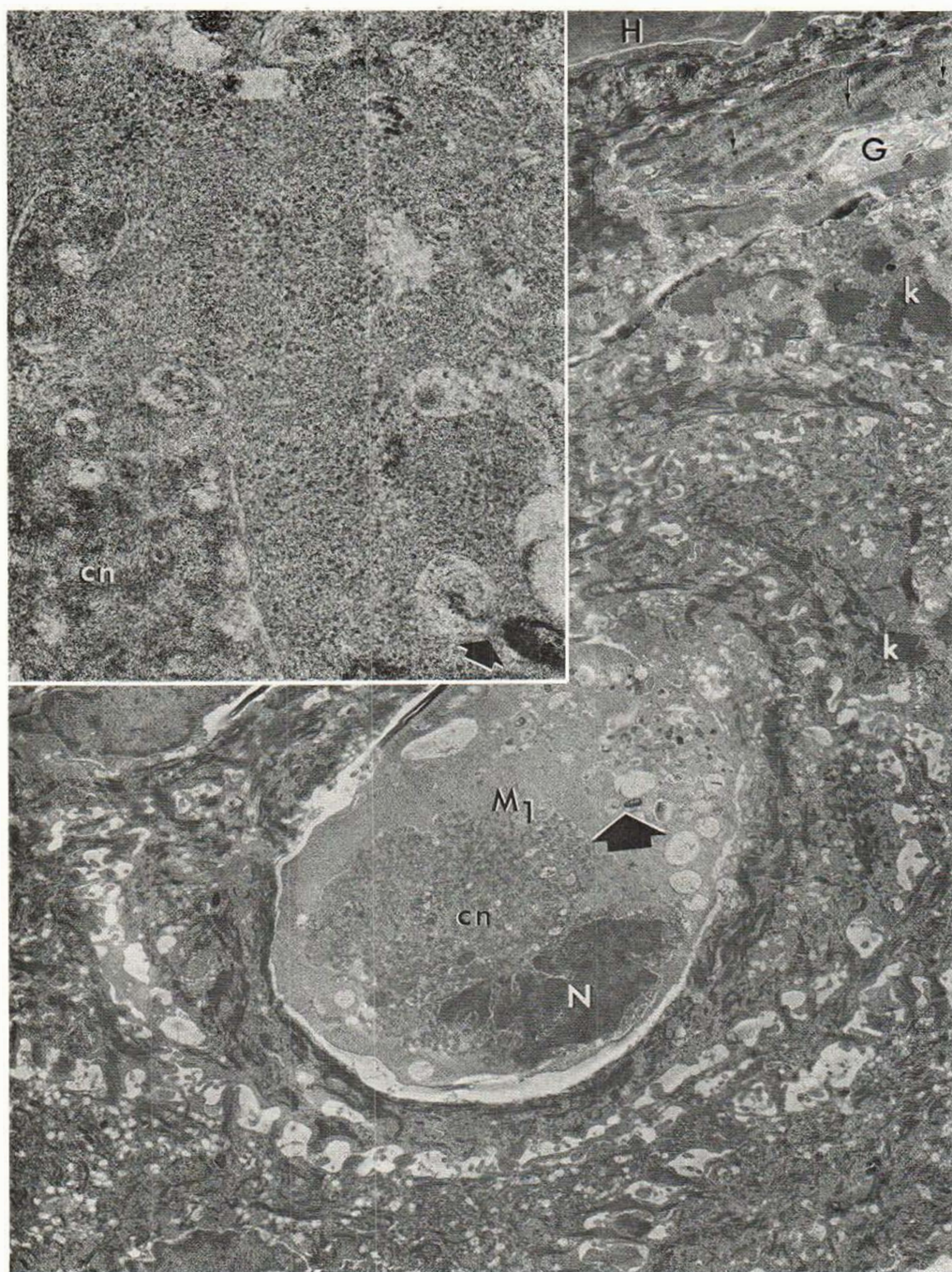


Fig. 5. Section serial to that shown in Fig. 4. Pyknotic nucleus (N_1) is now visible in the same cell (M_1) shown in Figs. 2, 3, and 4. A part of the cytoplasm is enlarged in the inset and broad arrows in both pictures point to the same area. Melanosomes in various stages of maturation are seen (broad arrows). Many immature ones appear to be degenerating in

vacuoles. Coagulation necrosis (cn) or dense homogenization of the cytoplasm is enlarged in the inset. This melanocyte is located among granular cells which contain cytoplasmic (k) and intranuclear (thin arrows) keratohyalin granules. $\times 12\,000$. Inset: $\times 60\,000$.

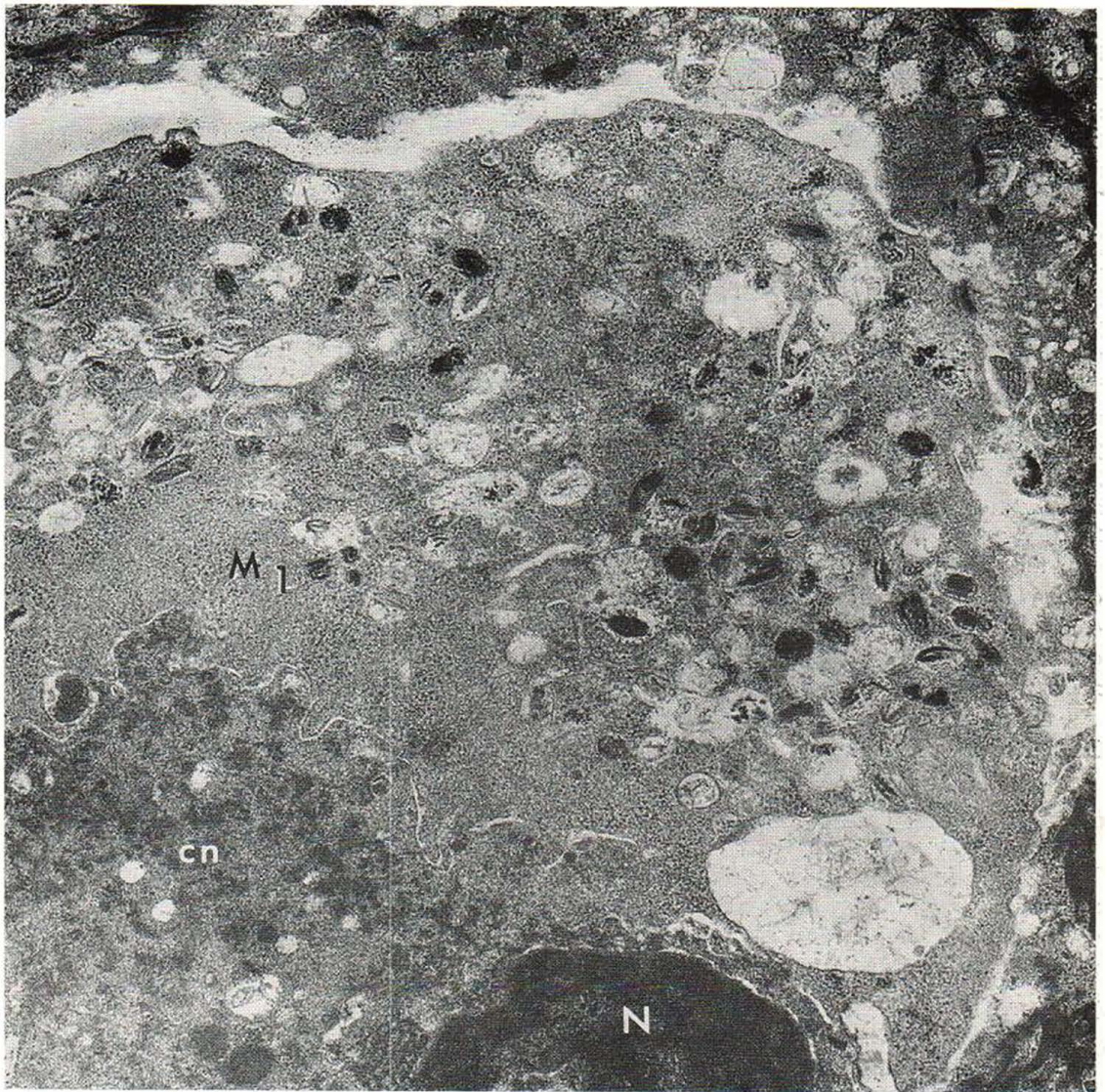


Fig. 6. The section cut serially to that shown in Fig. 5 contains numerous individual melanosomes in M_1 . N : pyknotic nucleus. cn : coagulation of cytoplasm. $\times 42\,000$.

same buffer, the tissue blocks were osmicated with 1% osmic acid in the same buffer for 30 minutes. Graded concentrations of ethanol (50% through absolute) and propylene oxide were used for dehydration and Araldite for embedding. Thin sections were cut at 400–600 Å in a Porter-Blum ultramicrotome and stained with 15% uranyl acetate in 50% methanol and then lead citrate (13). Stained sections were observed in an Hitachi HU-12 electron microscope at 125/kV.

RESULTS

The tissue from the “periphery” of the lesion showed a number of dendritic clear cells (Fig. 1). They were

located not only in the basal layer but also scattered through the epidermis, including the granular layer. None of these cells contained visible melanin granules. Although basal cells in the vicinity of some of the clear cells contained a large amount of dark pigment (Fig. 1), identification of the majority of these cells was difficult. No detectable amount of cellular infiltration was seen in the dermis (Fig. 1). In many of the clear cells, particularly in the upper layers of the epidermis, the cytoplasm appeared shrunken and clear spaces surrounded them (Fig. 1).

Electron microscopic examination revealed that the clear cells were of three types (Fig. 2): (i) melanocytes, (ii) L-cells, and (iii) unidentified cells. Melanosome-containing melanocytes were diminished in the basal layer; they were frequently encountered above the basal layer, including the granular layers. These "high-level melanocytes" showed various kinds of degeneration such as vacuolization (Figs. 3A, 3B, 4, 5, 6), coagulation, and segregation of part of the cytoplasm (Figs. 3A, 5, 6), and autophagocytosis of melanosomes (Fig. 3B). Degenerative changes were also apparent in keratinocytes (Fig. 4). On the other hand, L-cells found in the neighborhood of the degenerating melanocytes appeared normal except for a few vacuoles (Figs. 2, 4). Serial sections failed to connect these "high-level melanocytes" to the basal lamina (see M_1 in Figs. 2, 4, 5).

In the tissue from the center of the lesion was a cluster of degenerating nevus cells in both the epidermis and the dermis. In the upper layers of the epidermis there were vacuolated and shriveled melanocytes. It was, however, difficult to determine whether these "high-level, effete melanocytes" originated from normal melanocytes or nevus cells.

DISCUSSION

In an active junctional nevus or early malignant melanoma, nevus cells are often seen in the upper epidermis (9). The effete melanocytes considered in the present study do not belong to that group. They are from the periphery of the lesion, i.e. from the basal layer of the previously normal epidermis which surrounded the compound nevus. The survey picture (Fig. 1) does not show a clustered nevus cell nest in the area examined.

The neural crest origin of the epidermal melanocytes seems to have been established (3) and generally accepted. How the epidermal melanocyte system maintains its population is less well established; in fact, little is known about the turnover of the normal epidermal melanocytes. In vitiligo and halo nevus, despite such a dramatic disappearance, the fate of the epidermal melanocytes has never been clarified. In this study, the demise or fate of these cells, i.e. a degeneration and probable shedding from the epidermis, was demonstrated.

The other mechanisms of melanocyte disappearance, however, cannot be dismissed entirely. The hypothesis to which Swanson et al. (14) subscribed

is that melanocyte (4) or melanoblast (16), as a stem cell, gives rise to two daughter cells, one melanocyte and one L-cell; only the L-cell then matures and the melanocyte is destroyed. If the L-cells are increased in number, as they found, one might expect to see mitotic melanocytes produce L-cells. Mitosis of neither melanocyte nor L-cell was observed in the present study. Mishima and his co-workers (11) reported that in halo nevus, as in vitiligo, α -dendritic cells increased in inverse ratio to the decreased melanocytes, whereas the total number of both types of cells was maintained the same. This might suggest the possibility that melanocytes became α -dendritic cells. However, since the source of α -dendritic cells, i.e. melanocytes, is shown to diminish by death, α -dendritic cells should be increased out of the proportion of one to one melanocyte conversion to α -dendritic cell, either by an accelerated rate of division, or by supply from the dermis. As mentioned above, mitosis of neither melanocyte nor L-cell was observed in the present study. In this respect, it is interesting that Kurosumi & Suzuki (8) showed the presence of dermal L-cells in the dermal infiltration of halo nevus. One may wonder if so-called α -dendritic cells or indeterminate cells are in reality L-cells in which L-cell granules either were not produced or failed to be detected. A mesenchymal or histiocytic origin of L-cells was previously proposed by Hashimoto & Tarnowski (7) together with evidence that the L-cell can cross the epidermal-dermal border.

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K. Hashimoto, M.D.
Department of Medicine
Section of Dermatology
Veterans Administration Hospital
1030 Jefferson Avenue
Memphis, Tennessee 38104
USA