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ELECTRON MICROSCOPIC AND CYTOCHEMICAL OBSERVATIONS OF MAST CELLS CONTAINING MELANOSOMES IN BLUE NEVUS

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Abstract. In an attempt to characterize the functional relationship of mast cells to melanocytes with special regard to melanogenic activity, electron-microscopic studies were undertaken. Combined ultrastrutural and cytochemical observations disclosed no evidence of autochthonous melanogenesis by mast cells distributed within the skin lesions of blue nevus. Melanosomes in the cells were found mostly accumulated in the form of melanosome complexes inside membrane-bounded vesicles. They were thought to be taken up by way of heterophagocytosis for degradation rather than derived from the biosynthetic activities of these cells. The present findings are based on a definitive criterion for distinguishing melanosomes from mast cell granules. They are consistent with our previous reports indicating that mast cells in the human skin are capable of ingesting extraneous materials. In addition, acid phosphatase activity was demonstrated in mast cell granules.

One of the current topics in the investigations into the functional properties of mast cells is that the cells are capable of phagocytizing extraneous materials. Padawer (15, 16) amply demonstrated that zymosan, a polysaccharide residue of yeast cells, and thorium dioxide administered *in situ* were avidly incorporated by mast cells of the rat. Padawer (17), in a more recent paper, has reported pox virus phagocytosis by the cells. In our previous papers, observations on dermal melanocytosis and blue nevus were described which indicated that a few melanosomes in the form of membrane-bounded aggregations were present in mast cells of these skin lesions (21, 22).

In the course of further studies on the same lesions, we were impressed by the presence of fully melanized melanosomes either intimately associated with mast cell granules or lying singly in the intergranular loci of mast cells. These new facts prompted us to study the functional relationship of mast cells to melanocytes with reference to a possible melanogenic activity of mast cells.

MATERIALS AND METHODS

Biopsy specimens were obtained from skin lesions of 7 patients with blue nevus, since mast cells have been easily found in close association with melanophages and larger numbers of dermal melanocytes (24). The materials were prefixed at 4°C for 2 hours with 4% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4, followed by postfixation with 1% osmium tetroxide adjusted to pH 7.4 with the buffer. These were dehydrated in increasing concentrations of ethanol, passed through propylene oxide, and embedded in Epon 812. Ultrathin sections cut with a Porter-Blum ultramicrotome MT-2 were stained with uranyl acetate and lead citrate, and were examined in a Hitachi HS-8E electron microscope at an accelerating voltage of 50 kV.

For the cytochemical detection of dopa oxidase and tyrosinase activities, 50 μm thick sliced tissue sections were prepared with a Sorvall TC-2 tissue sectioner. These were prefixed in glutaraldehyde-cacodylate buffer for 30 minutes, and were incubated in 0.1% l-dopa as well as in 0.05% l-tyrosine plus 0.01% l-dopa solutions at 37°C for 6 and 10 hours using a Dubnoff metabolic shaking incubator. For acid phosphatase activity, the prefixed materials were subjected to incubation at 37°C for 30 minutes in Gomori's lead salt mixture. As a control for enzymic reactions, the tissues were treated in complete media without substrates.

RESULTS

Mast cells in blue nevus were observed to have irregular shapes with numerous microvillous projections. Their cytoplasm was occupied by an abundance of oval-shaped, discrete granules, which ranged from 0.3 to 1.5 μm in diameter (single-type granule). The granules were composed of a moderately electron-dense material. This material, revealing a granular or honeycomb-like appear-

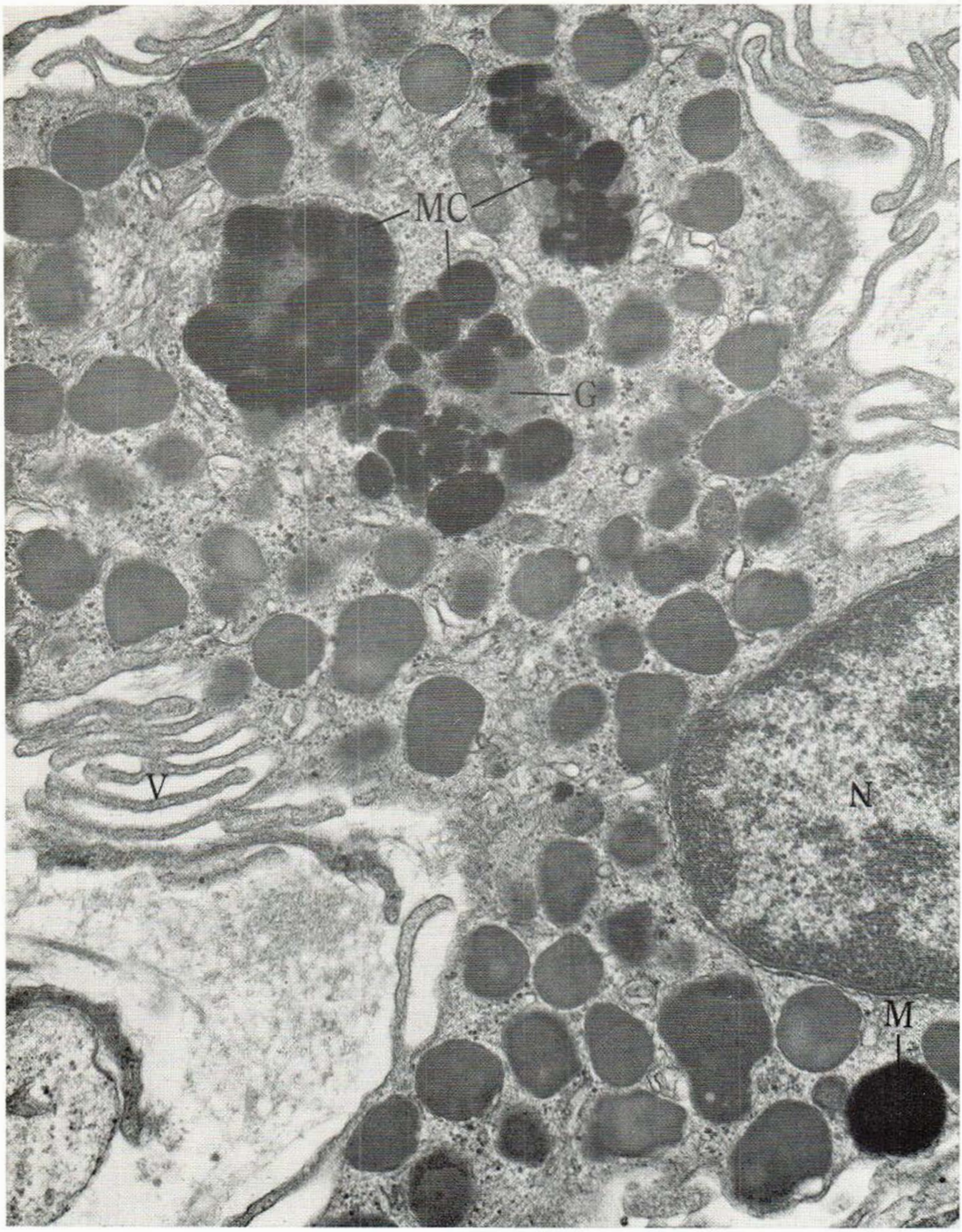

Fig. 1. Electronmicrograph showing portion of mast cell in blue nevus. Note conglomerates of melanosomes (*MC*) and mast cell granules (*G*) within membrane-limited vesicles located deep in cytoplasm. Melanosomes are seen broken up into small pieces with decreased electron density. A single and seemingly free melanosome (*M*) is present near cell membrane (*lower right*). No premelanosomes are recognizable, either in cytoplasm or in mast cell granules. Mast cell nucleus (*N*), microvillous projections (*V*). × 28 000.

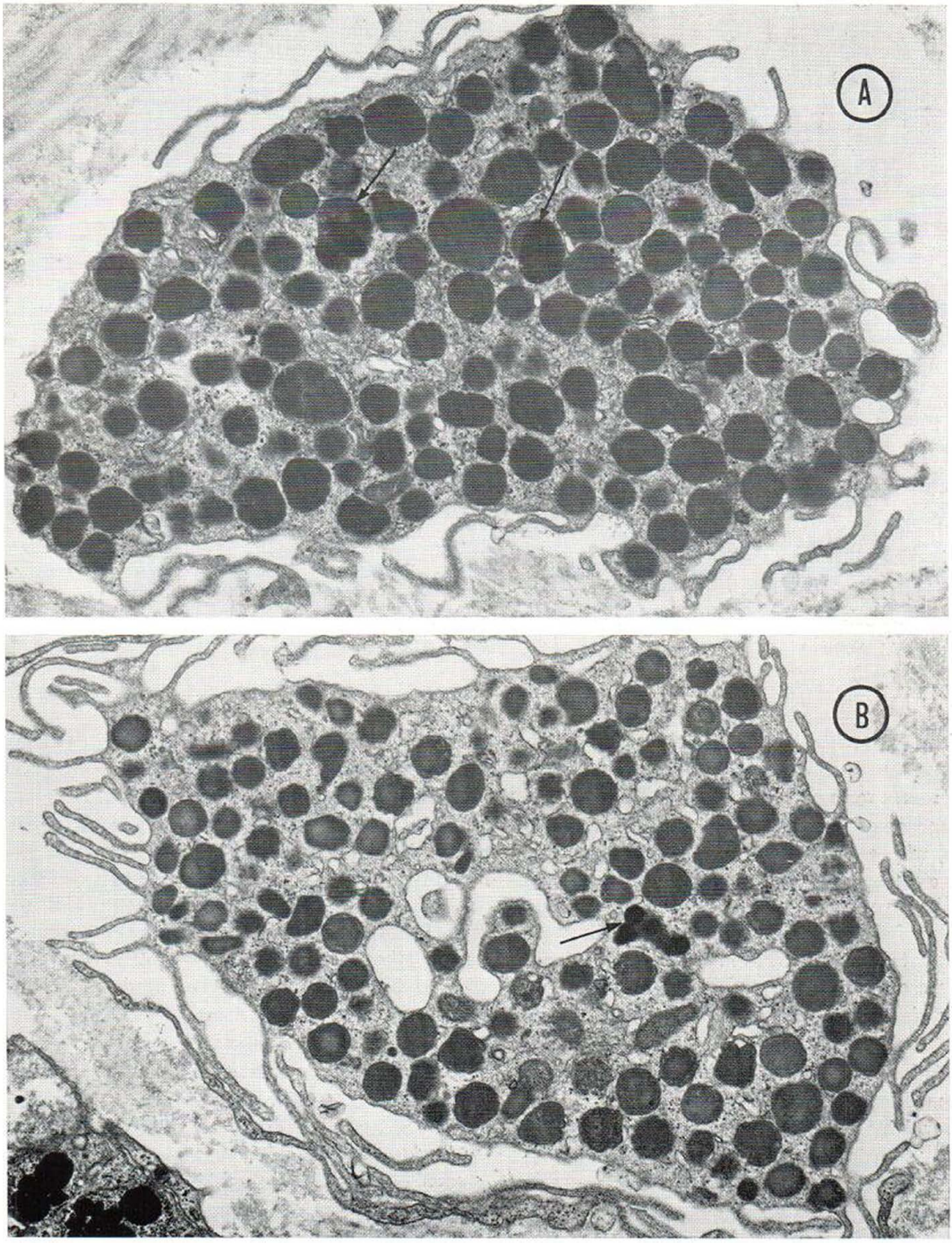

Fig. 2. Electronmicrographs of mast cells in blue nevus. A few melanosomes associated with mast cell granules are seen (*arrows*). Note no premelanosomal structures in intra- and extragranular site. × 17 000.

ance, sometimes contained crystalloid structures with regular spacing of 120 to 170 Å. In some granules the contents seemed to form spiral lamellae. The single-type granules were occasionally to have undergone coalescence to form compound-type granules. Golgi apparatus, endoplasmic

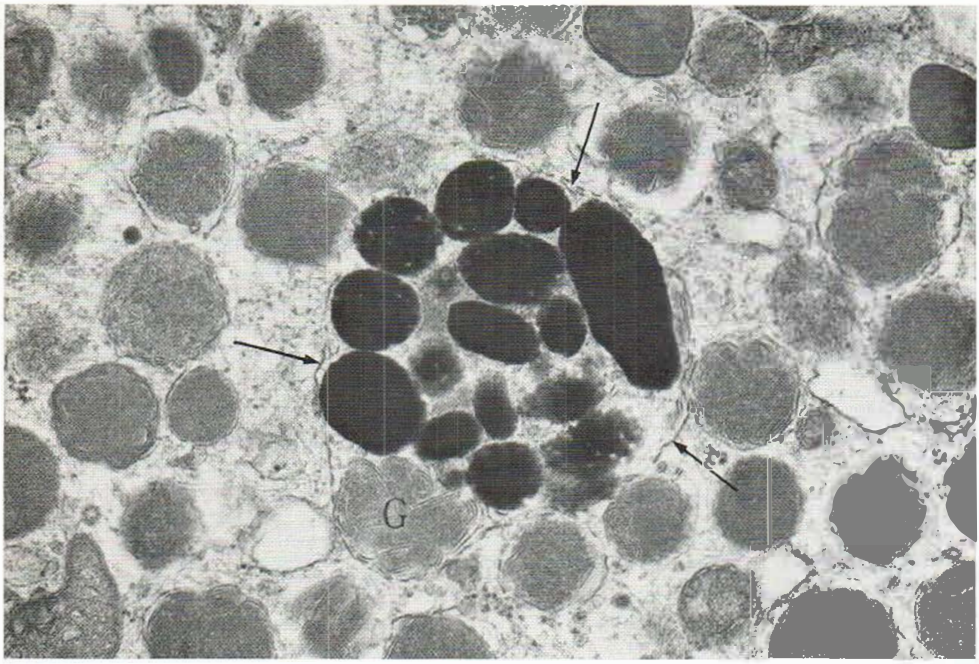

Fig. 3. Electronmicrograph showing large melanosome complex associated with mast cell granule (*G*) within membrane-limited structure (*arrow*). Note oval, electron-lucent bodies of melanosomes. × 33 000.

reticulum, mitochondria, and delicate filaments were poorly developed. Mast cells containing melanosomes were extremely sparse (Figs. 1–4, 6).

Melanosomes found in mast cells were oval in outline, with a diameter 0.5 to 1.5 μm. They had a high electron density concealing internal structures; however, most of them exhibited round granules of lower electron opacity which were approximately 200 to 400 Å in size. These melanosomes were morphologically comparable to those seen in dermal melanocytes. The melanosomes in mast cells were observed to be mostly accumulated in the form of aggregates confined within membrane-bounded vesicles which included masses which were regarded as mast cell granules (Figs. 1, 3, 4, 6). They were usually located deep in the cytoplasm of the cells. One or two melanosomes tightly associated with mast cell granules were less frequently encountered, and the melanosomes were seemingly unchanged in shape and in electron density (Figs. 2, 4, 6). Single melanosomes were occasionally noted to be unassociated with mast cell granules. These were usually larger than 1 μm in diameter (Fig. 1). The single melanosomes were situated in juxtaposition to the plasma membrane. No premelanosomes in the developmental stages were ever identified in mast cells examined.

When the specimens were subjected to treatment with dopa or tyrosine solution, no selective deposition of melanin was recognized on the mast cell granules or on the other structures of the cells (Fig. 4). With these cytochemical procedures, on the other hand, the dermal melanocytes were seen to have positive reaction products on the peripheral part of cisternae of each Golgi apparatus and also on premelanosomes (Fig. 5).

Acid phosphatase activity was visible on mast cell granules either associated or unassociated with melanosomes; some mast cell granules failed to react. The reaction product was a dense precipitate and was usually seen as a deposit in the peripheral area of the granules. In every instance it was possible to recognize the internal structure of the granules. Phagosomal vacuoles with melanosome complexes also exhibited loosely distributed acid phosphatase reaction products. In the intergranular sites no reaction was present (Fig. 6). No appreciable reaction was observed in the tissues treated with substrate-free media.

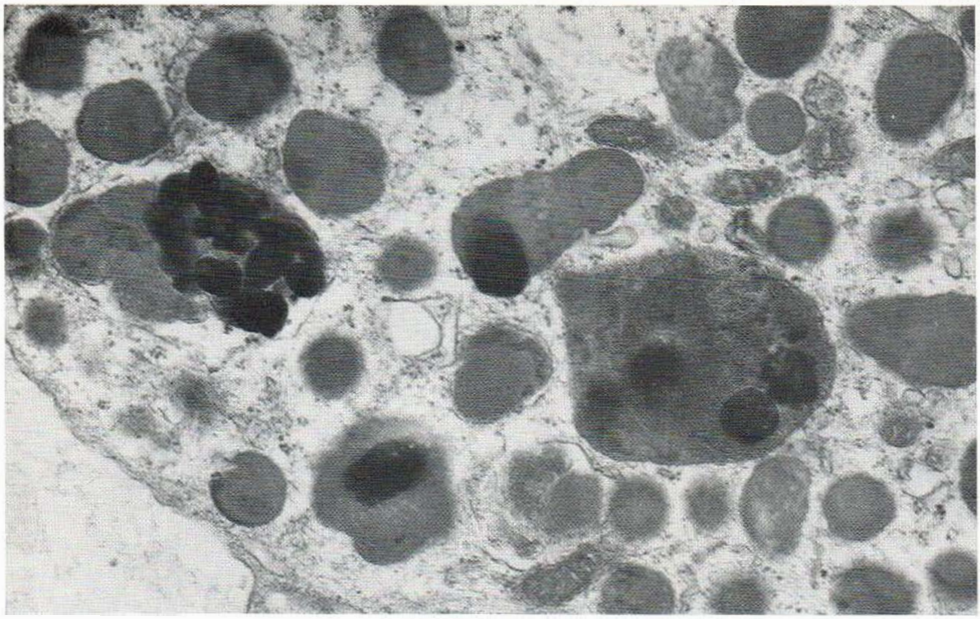

Fig. 4. Electronmicrograph showing mast cell stained for dopa oxidase. No selective reaction products can be seen in mast cell granules or in intergranular sites. × 28 000.

DISCUSSION

Morphologic observations on dermal melanocytes and mast cells in the dermis of the human skin reveal similar features in fine structures, especially the possessing of characteristic granulations. As to their histogenesis, Okun et al. suggested that mast cells and melanocytes are of similar lineage, and that the pluripotential connective

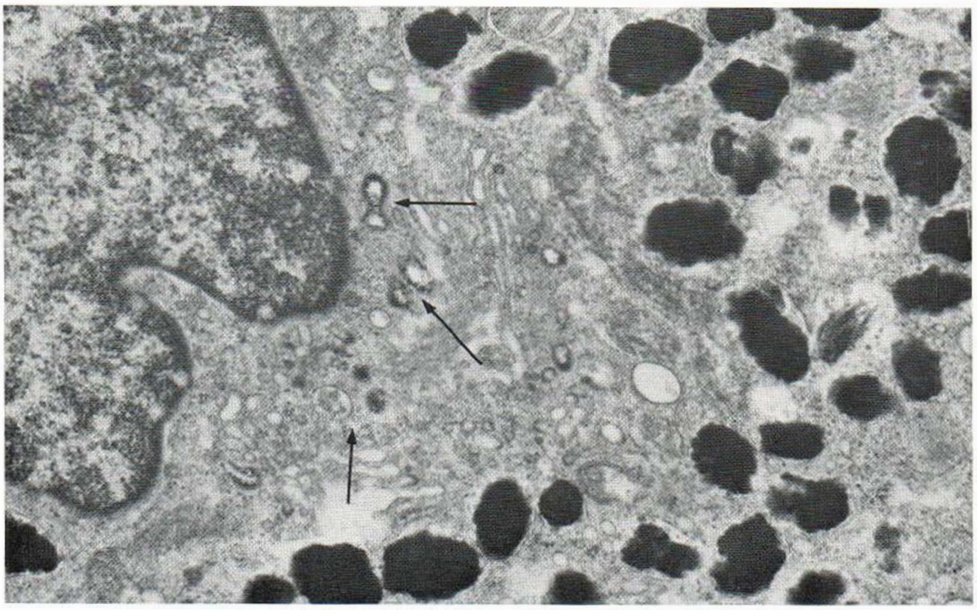

Fig. 5. Electronmicrograph showing dermal melanocyte stained for dopa oxidase. Selective deposition of melanin is seen on peripheral part of cisternae of Golgi apparatus and on premelanosomes (*arrows*). × 22 000.

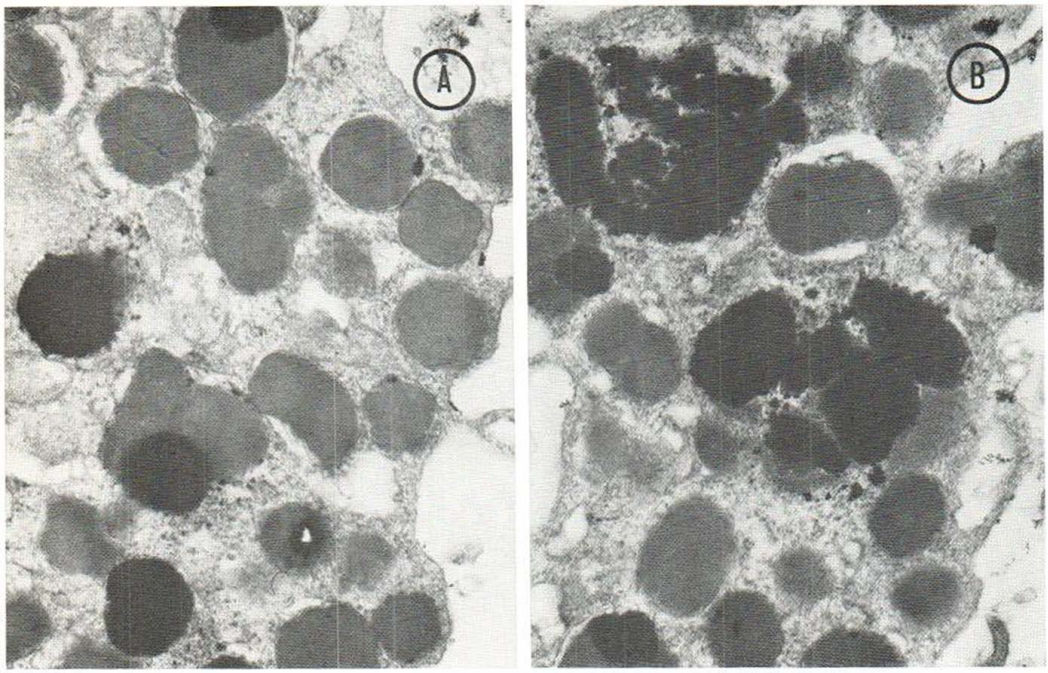

Fig. 6. Electronmicrographs of mast cells stained for acid phosphatase. Reaction products are seen on the peripheral area of mast cell granules both associated and unassociated with melanosomes. Phagosomes with melanosome complexes also exhibit positive reaction. × 28 000.

tissue cells which migrated into epithelium differentiate into melanocytes, Langerhans cells or into mast cells (14), and that under certain conditions mast cells represent a transitional phase in the differentiation to melanocytes (10, 11). According to contemporary views, it is definitely established that melanocytes stem from the neural crest (19), while the chief source of mast cells is generally thought to be fixed, undifferentiated mesenchymal cells abundant in the connective tissue (2, 3, 20). Under the conditions of the present studies, there were no convincing sequential steps suggesting mast cell transformation into melanocytes. At the moment the hypothesis proposed by Okun et al. (10, 11, 14) that melanocytes and mast cells have a common connective tissue stem cells does not seem objectively supported.

Okun et al. (10, 11, 14) postulated that the cytoplasmic granules of mast cells sometimes had an ultrastructural resemblance to melanin granules, and they proposed an intergraded sequence between melanosomes of normal epidermal melanocytes and normal mast cell granules. However, the detailed investigations into the ontogeny and morphology of melanin-containing organelles and mast cell granules (4, 5, 6) would appear to indicate an essential difference. Furthermore, Okun et al. (10, 11, 12, 13) have reported that mast cells possess the enzymic potential to oxidize dopa or tyrosine to melanin. If this were a phenomenon occurring regularly within intact mast cells of the human skin, the enzymic activities responsible for melanogenesis would be demonstrated with the usual techniques applied to mast cells. The negative reaction of mast cells incubated in dopa and in tyrosine (8), and the absence of premelanosomal structures in the cells (4, 6, 21) lends no support to the view that mast cell does biosynthesize melanin (14). Altogether, it seems most probable that mast cells and melanocytes in the human skin represent two independent and distinct cell types. The reasons why and the degree to what extent mast cells affect increase of skin pigmentation remain uncertain (1, 9, 18, 27).

In this context, it could be argued that melanosomes synthesized in melanocytes might be heterophagocytized by mast cells. The observations would appear to indicate that a possible sequence of events may occur as follows: (*a*) melanosomes originate in the adjacent dermal melanocytes and

are first engulfed by mast cells, (*b*) subsequently the ingested melanosomes accumulate, forming aggregates within phagosomes in an intergranular locus, or (*c*) they associate with mast cell granules, and these undergo a fusion. Melanosomes are thus ultimately enclosed within secondary lysosomes where gradual degradation occurs. In the present study, configurations suggesting active phagocytic uptake of cytoplasm of surrounding dermal melanocytes or melanophages on the part of mast cells have not been observed.

Toda et al. (25) and Wolff et al. (26) have indicated that the size of the melanosomes is a determining factor in the mode of uptake and storage: melanosomes larger than 0.8 μm in diameter are observed to be incorporated singly, whereas smaller ones are aggregated in groups within phagosomes of the receiving cells. This evidence corresponds well with the rare occurrence of single larger melanosomes in mast cells.

Another finding worth considering is the demonstration of acid phosphatase activity in mast cells. The nature and subcellular localization of the reaction are in agreement with the report of Hoffmeister & Rupec (7) who examined mast cells from the human vaginal mucous membrane. The biological significance of the enzyme in mast cell granules remains obscure. Fujita et al. (4) and Hashimoto et al. (6) thought that mast cell granules were morphologically and histochemically related to lysosome. Setoguti (23), on the other hand, has pointed out dense bodies having the features of lysosome in the Golgi area of newt mast cells. It is of considerable interest to note that zymosan was found to be rapidly digested by macrophages, though it was still morphologically unaltered several days after association with mast cell granules of the rat (15). Thus it is still uncertain whether the single- and compound-type mast cell granules are closely related to primary lysosomes.

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