

LENTIGINOSIS PROFUSA SYNDROME

IV. Giant Pigment Granules (Light Microscopy)

Victor J. Selmanowitz

From New York Medical College and Metropolitan Hospital Center, New York, N.Y., USA

Abstract. Ionically separated epidermal sheets from pigmentations of two patients with lentiginosis profusa were incubated in dopa (3,4 dihydroxyphenylalanine) and slide-mounted. Light microscopy revealed intracellular giant pigment granules which appeared like those seen with von Recklinghausen's disease.

Key words: Lentigo; Pigmentation disorders; Melanocytes; Melanosomes; Neurofibromatosis

Comparative aspects of the lentiginosis profusa syndrome (progressive cardiomyopathic lentiginosis, (11), multiple lentigines syndrome (4)) and von Recklinghausen's disease were previously discussed (13). For example, the cutaneous finding of hypermelanotic spots in the axillary vaults, thought to be pathognomonic of von Recklinghausen's disease (3), can also occur with lentiginosis profusa (13). This is not seen in all cases of either disorder (13) (personal observation of unpublished cases of lentiginosis profusa). The axillary and general body surface pigmentations tend to be of deeper coloration in lentiginosis profusa. However, in patients with lentiginosis profusa who have lesser cutaneous expression or in whom the lentigines have faded in later adulthood (14), differentiation of the brownish spots from those of von Recklinghausen's disease on the basis of intensity of hue can be difficult. (In a third condition of axillary spottiness, "acquired axillary and inguinal pigmentations", tardy development of dark melanotic macules in the axillae and groins has been reported in adults in whom no other evidence of either disorder was found (5, 16)).

Another finding which was considered rather specific for von Recklinghausen's disease is the observation by high power light microscopy of giant pigment granules in dopa (3, 4 dihydroxyphenylalanine)-incubated epidermis from the café-au-lait spots and the smaller melanotic macules (miniature

café-au-lait spots) which may be widespread in this disease (18, 7, 8). The same procedure was applied to the pigmentations of lentiginosis profusa to determine if they could be differentiated on this basis from the spots seen with neurofibromatosis.

PATIENTS, MATERIALS AND METHODS

Two patients, a female proband 20 years old and her also-affected mother 53 years old at the time of this study, were previously described in some detail (13, 14, 19). Most of the daughter's pigmentations ranged in color and size from tiny tan dots to dark brown spots up to 5 mm in diameter. Occasional dark pigmentations were much larger, up to 5 cm in long axis, and these tended to have scalloped borders suggesting overlap with or accretion of the smaller spots (Fig. 1). The mother's lentigines were equally variable in size but of faded color.

Representative smaller pigmentations and samples of larger ones were removed using 2 to 4 mm cylindrical (Orentreich) punches. The plugs of skin were immersed in 2 N NaBr solution for 4 to 5 hours, after which the epidermis was peeled off. The circular epidermal sheets were incubated at 37°C in buffered dopa solution and subsequently slide-fixed in accordance with a previously described procedure (17, 12). For anticipated negative and positive controls, respective punch biopsies of café-au-lait spots from two normal adults and two adults having neurofibromatosis were processed in the same manner (7, 8).

RESULTS

The epidermal sheet preparations of the pigmentations of lentiginosis profusa reflected the microscopic changes seen in conventional cross section (13). Change in the pattern of epidermal ridges in the epidermal mounts corresponded with elongation and clubbing of the rete ridges seen in cross section (Fig. 2). Melanocytes occurred with increased frequency along the ridges. On the whole, the morphology of the melanocytes appeared normal and

characteristic for the appropriate cutaneous region. The usual melanocyte had long dendrites and an angular perikaryon. In heavily pigmented portions the melanocytes were not easily distinguishable from the pigmented keratinocytes.

Giant pigment granules were found in the epidermis of pigmentations of various sizes in both patients with lentiginosis profusa. The giant pigment granules appeared as darkly stained circular sections of spherical globules but were occasionally ellipsoidal. The larger ones occupied a major portion of melanocytic or keratinocytic cytoplasm. Sometimes they were multiple in a single cell (Fig. 3). An occasional giant pigment granule stained to a lesser degree so that internal stippled structuring of blackish dots or of clear microglobules with darkly stained perimeters was discernible (Fig. 4).

The giant pigment granules were more clearly distinguishable in the mother's lesions and were numerous in particular foci of given specimens rather than being evenly distributed. They were not seen in every one of the daughter's pigmented spots and finding them in the positive spots sometimes required diligent search.

Darkly stained giant pigment granules which could not be differentiated by light microscopy from those in the lentiginosis profusa pigmentations were observed in the café-au-lait spots of both

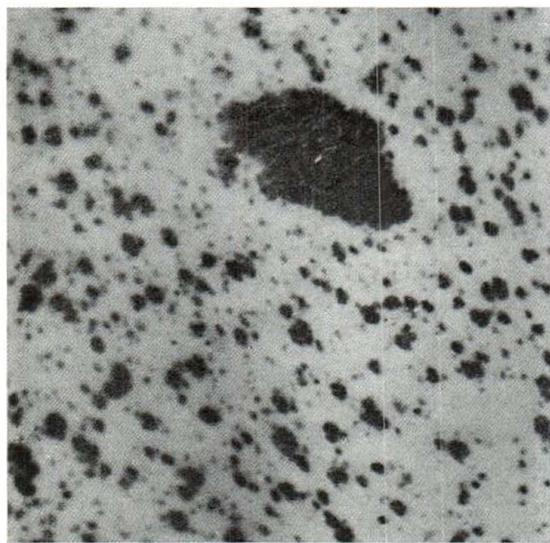


Fig. 1. Size-range of lentigines over scapular area. The large pigmentation (biopsy site in its center) has scalloped borders and satellite lentigines.

Acta Dermatovenere (Stockholm) 55



Fig. 2. Top: Cross section has rete ridges of normal appearing skin on left half and the elongated, thickened and hyperpigmented ridges of a lentigo on the right half. Bottom: Epidermal whole mount shows thickened and hyperpigmented epidermal ridges of a small, tan lentigo in the right half of the specimen. Dopa, $\times 35$.

patients with neurofibromatosis. They were not found in the café-au-lait spots of the normal controls.

DISCUSSION

The giant pigment granules may be related to some proliferative aspect of melanocytes. They occur in the pigmentations of von Recklinhausen's disease and lentiginosis profusa where the number of melanocytes per unit surface area tends to be increased (7, 8, 13). The frequency of dopa-positive melanocytes is not increased in the café-au-lait spots of normal persons (7, 8) and generally this is the case with the pigmentations of Albright's syndrome (2). Giant pigment granules are not expected in such café-au-lait spots (7, 8) and were found in only one of ten patients with Albright's syndrome (2).

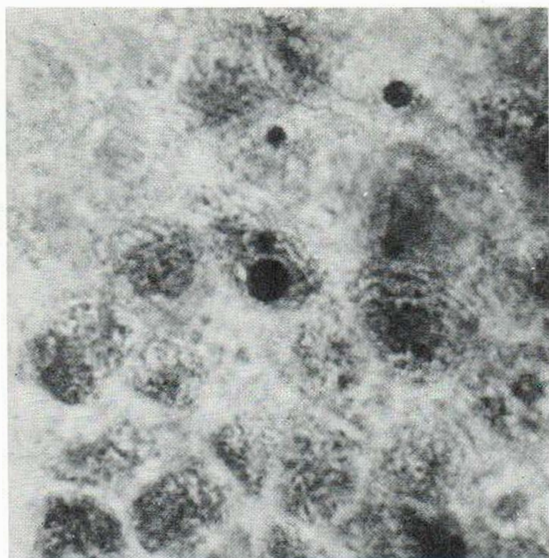


Fig. 3. Two giant pigment granules in central cell and one each in two cells above center. Dopa, $\times 1150$ ($\times 2.5$ photoenlargement).

The assumption that macromelanosomes (the giant pigment granules) are synthesized in the melanocytes and are then transferred to the keratinocytes, as postulated for von Recklinghausen's disease (6), can also be made for lentiginosis profusa. It remains to be determined whether pigmented cells in the dermis of lentiginosis profusa spots, be they melanocytic *thèques* or scattered melanophages (which, the author has observed, may congregate about nerve bundles) (13) also contain the giant pigment granules. It seems probable that these cells would contain them.

Giant pigment granules have not been found in every tested case of von Recklinghausen's disease (7, 15). A single cutaneous biopsy may not be sufficient to rule out their occurrence in a given patient. It has also been suggested that the epidermal giant pigment granules may be less common in young patients (15). These problems are reminiscent of the findings here in lentiginosis profusa: the giant pigment granules were not as evident in the daughter (often obscured by the overall heavy pigmentation); they were not found in every one of her pigmented lesions; and they were unevenly distributed and so could be missed if only certain parts were examined.

Electron microscopic study of pigmentations in a 22-year-old white male with multiple lentiginosis syndrome revealed that keratinocytes were glutted with melanosomes and that melanosomes in the

melanocytes occurred either in packets or individually (10). Electron microscopic study of pigmentations in the patients reported here (now in progress, in association with Steven Victor & George Szabo) has revealed irregularity in contour of the abundant melanosomes. However, the most impressive feature was the presence of macromelanosomes in melanocytes and also in keratinocytes. These macromelanosomes were similar in ultrastructure to certain ones seen in von Recklinghausen's disease (6) and consisted of electron-lucent and electron-dense particles. At the periphery of some of the giant melanosomes the particles seemed to have diffused out from the main body. Illustrations and further details will be published.

There are recent reports of other pigmentary irregularities where giant pigment granules were found. Brief mention was made of light microscopic observation of giant pigment granules in ordinary lentigines (6). Giant melanosomes were observed in nevus spilus with ultrastructure similar to macromelanosomes of von Recklinghausen's disease (6). In a publication as early as 1930 (1), there was indication of enlarged pigment granules in epidermal basal cells and melanoblasts in the course of melanomatous transformation. (Certain granular melanosomes observed by electron microscopy in the café-au-lait spots of von Recklinghausen's disease have also been found in human and mouse melanomas (6).)

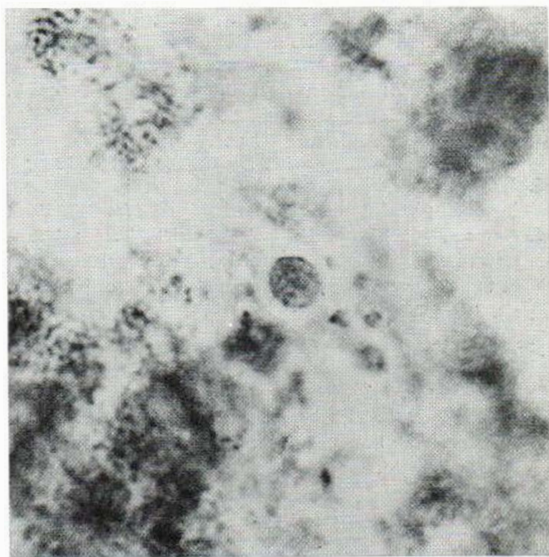


Fig. 4. Stippled giant pigment granule near center of field. Dopa, $\times 1150$ ($\times 2.5$ photoenlargement).

In addition to conditions where hyperpigmented spots are prominent, other disorders marked by pigmentary dilution come into play (9). Very large melanin granules (giant melanosomes) have been found in skin and eye in patients with Chediak-Higashi syndrome (and in the animal model for this disorder, the Aleutian mink) (21). Though early melanosomes of human Chediak-Higashi syndrome are sometimes very large, an orderly lamellated membrane substructure may be observed (20). Additionally, the membrane pattern in maturing Chediak-Higashi syndrome melanosomes, as evidenced by increasing melanization, suggests some fusion of smaller forms (20). There also appear to be ultrastructural differences between the giant pigment granules of the Aleutian mink and those seen in von Recklinghausen's disease (6) and lentiginosis profusa. The full significance of giant pigment granules in the various situations in which they have been and will be found remains to be clarified.

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V. J. Selmanowitz, M.D.
Orentreich Medical Group
909 Fifth Avenue
New York, N.Y. 10021
USA