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ELECTRON MICROSCOPIC OBSERVATIONS OF MELANIN TRANSFER IN HUMAN SKIN

Evidence for Indirect Melanin Transfer

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Abstract. In an effort to provide information on the mechanisms responsible for melanin transfer in human skin, electron microscopic studies were undertaken. Comparative observations of the epidermis, dermo-epidermal junction, dermis and hair in normal and abnormal conditions are described. A few melanosomes in extracellular loci were noted adjacent to pigment cells, indicating that, in vivo, melanosomes are ejected by pigment cells. The released melanosomes may remain free for only a short while before they are taken up by receptor cells. We have termed this process "indirect melanin transfer". One of the characteristic features in the process is that the event seems to occur without any physical contact between pigment cells and receptor cells, such as occurs in the cellular organization of the epidermal melanin unit. The present findings would appear to suggest that this method of indirect transfer, in concert with cell-to-cell transfer or "direct melanin transfer", exists in human skin.

The routes of melanin transfer in human skin are twofold: transfer of melanosomes from epidermal melanocytes to a pool of neighboring keratinocytes (epidermal transfer), and a loss of the pigment from epidermal and dermal pigment cells with an accumulation in receptor cells in the dermis (pigmentary incontinence). Elucidation of these events in normal and abnormal conditions is a matter of considerable interest in the field of pigment cell biology. Previous electron microscopic observations of tyrosinase-positive albino gray hairs left us with the impression that the discharge of melanosomes by follicular melanocytes is associated with transfer of the pigment to melanophages (7). The present study was directed toward demonstrating the free melanosomes in extracellular loci and in obtaining further insight into the ultrastructural basis for melanin transfer.

MATERIALS AND METHODS

The skin during wound healing and the hair bulb can both serve as a useful model for the study of some of the processes in melanin transfer. Biopsy specimens of re-epithelialized skin were obtained from 4 patients with superficial burns. Black hair bulbs of the scalp were provided by 7 volunteers. Gray scalp hair bulbs were taken from 2 patients with tyrosinase-positive albinism. The source of dermal melanocytoma and pigmented nevi was described elsewhere (13, 14).

The materials were prefixed for 2 hours with 4% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4, followed by postfixation for 2 hours with 1% osmium tetroxide adjusted to pH 7.4 with the same buffer. The specimens 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 solutions, and examined in a Hitachi HS-8E electron microscope at an accelerating voltage of 50 kV.

RESULTS

Epidermis of re-epithelialized skin following burn. At the level of the suprabasal layer, single and highly electron-dense granules, identical in appearance and in size with melanosomes in melanocytic dendrites, were only rarely recognized in the extracellular space (Fig. 1). The melanosomes were also seen being enwrapped by pseudopod-like cytoplasmic projections of keratinocytes (Fig. 2). The intercellular space was apparently devoid of any spread of cytoplasmic elements, and the surrounding plasma membrane seemed to be intact.

Dermo-epidermal junction of re-epithelized skin. Basal lamina covering the junction was thicker than normal and fuzzy in outline. These changes resulted

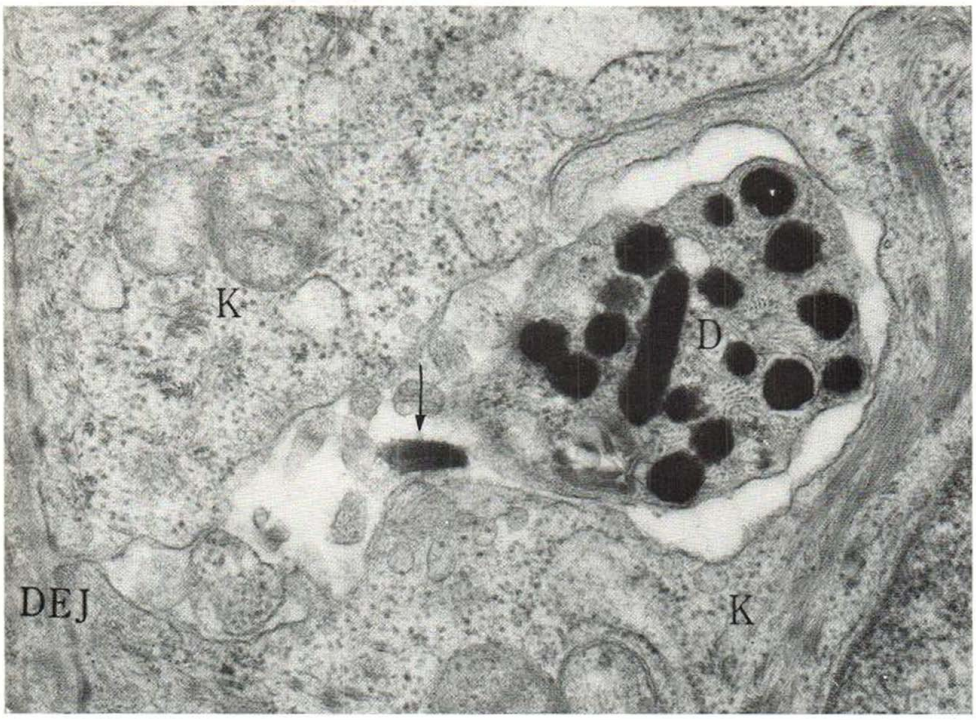
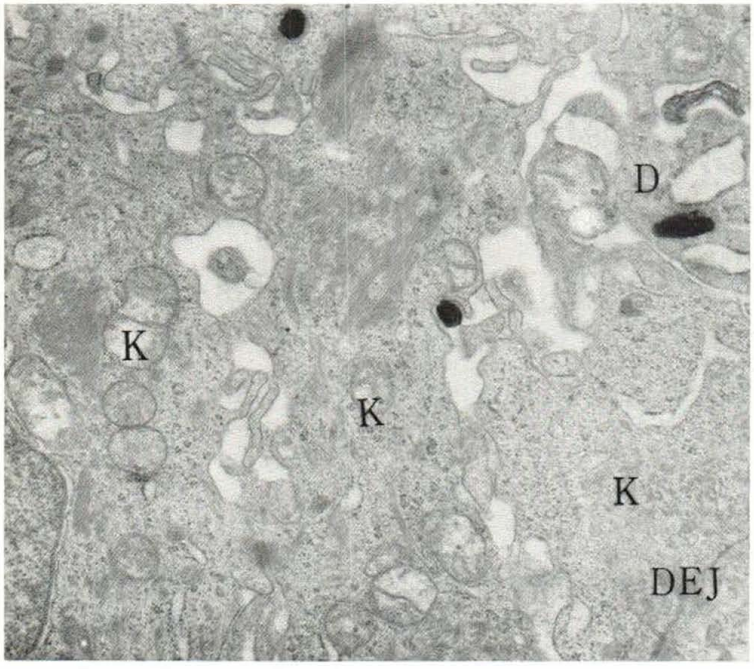


Fig. 1. Electron micrograph showing basal layer of re-epithelialized skin, 11 days after burn. Melanocytic dendrite loaded with melanosomes (*D*) and single free melanosome (vertical arrow) are seen. *DEJ*, dermo-epidermal junction; *K*, keratinocyte. ×37 000.



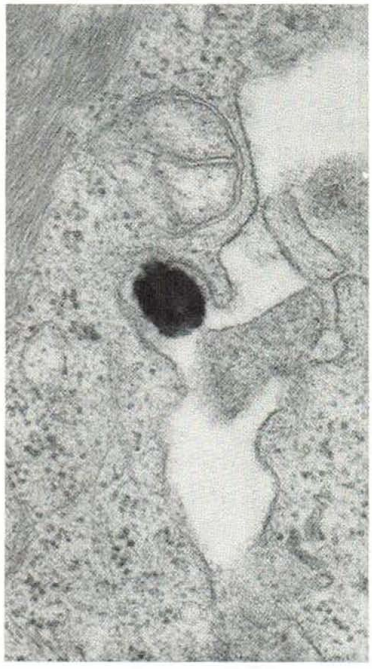


Fig. 2. Electron micrograph of suprabasal layer of re-epithelialized skin, 20 days after burn. *D*, melanocytic dendrite; *DEJ*, dermo-epidermal junction; *K*, keratinocyte. ×15 000. Accompanying micrograph is higher magnification of free melanosome being enwrapped by epidermal keratinocyte. ×45 000.

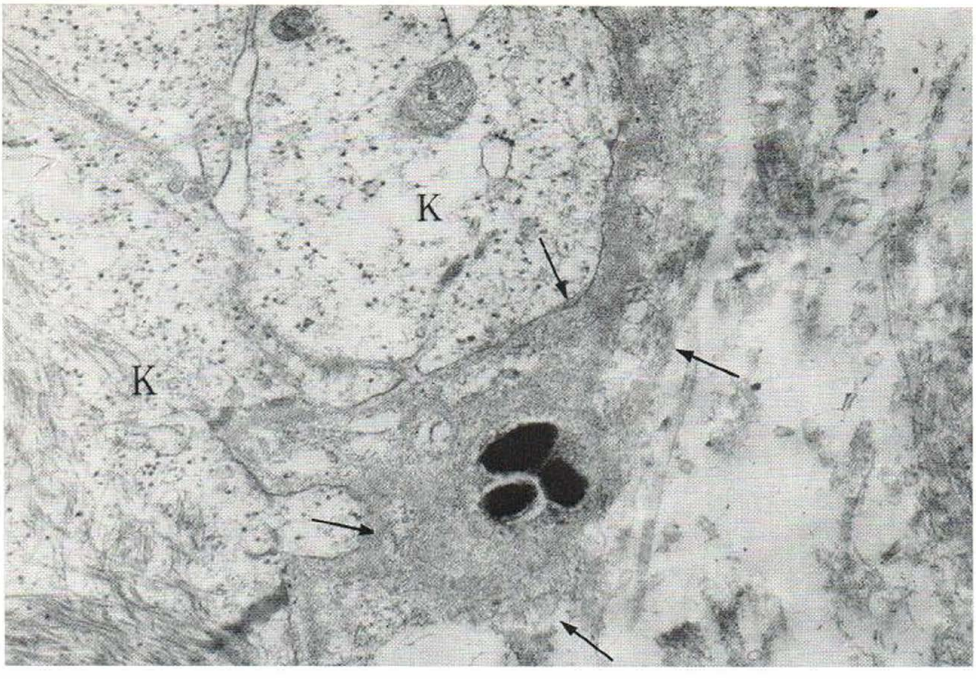


Fig. 3. Electron micrograph showing dermo-epidermal junction of re-epithelialized skin following burn. Note thick basal lamina-like structure (*arrows*) covering undersurface of epidermis, with a small cluster of melanosomes occurring in extracellular site. *K*, keratinocyte. ×30 000.

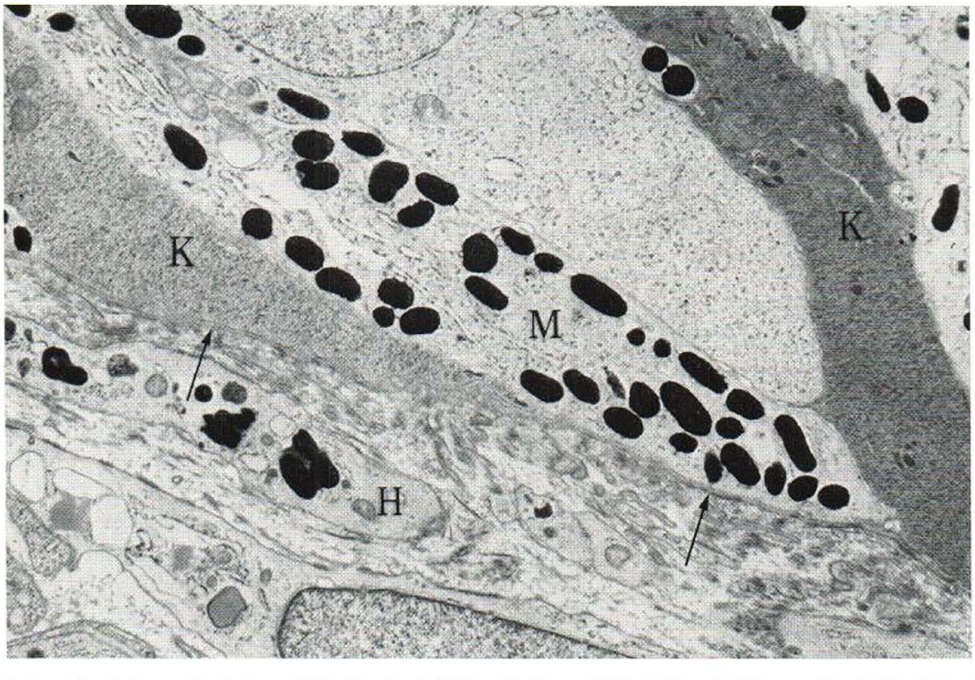


Fig. 4. Electron micrograph showing dermo-epidermal junction of normal black hair matrix during anagen. Thin, continuous sheet of basal lamina is seen (*arrows*). *H*, melanophage in hair dermal papilla; *K*, keratinocyte; *M*, follicular melanocyte. ×8 000.

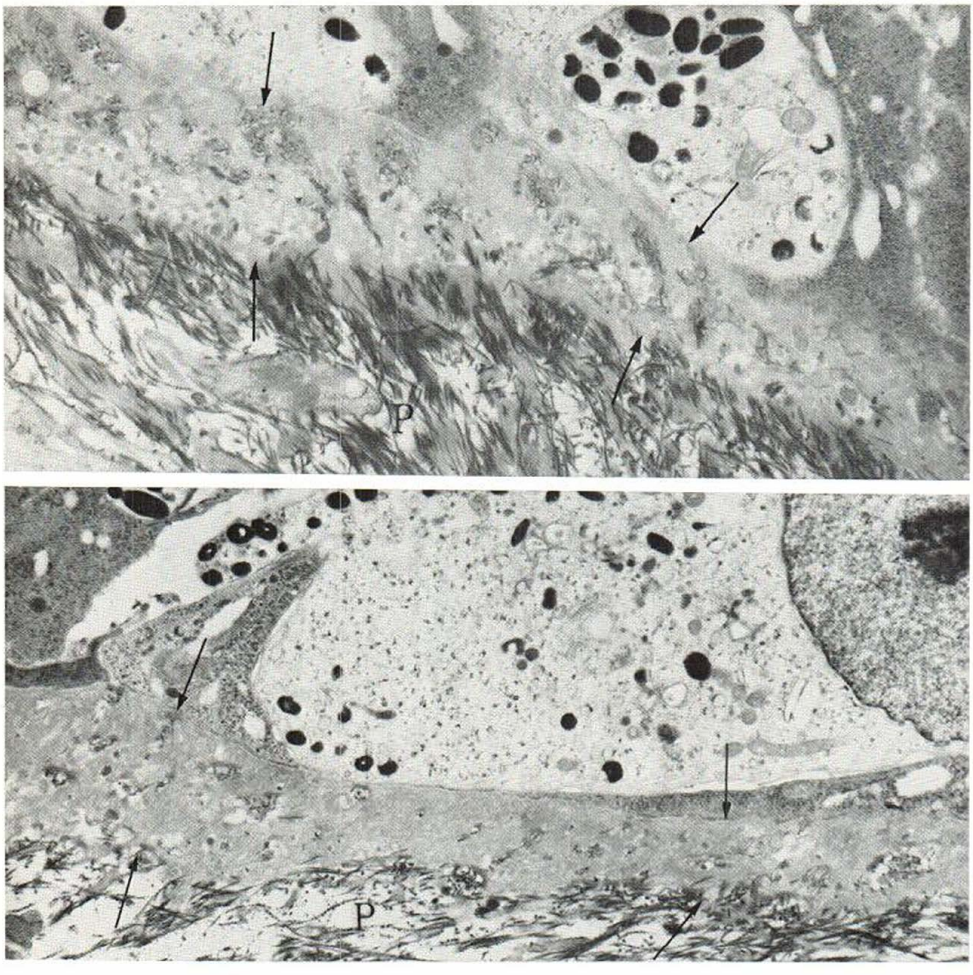


Fig. 5. Electron micrographs showing dermo-epidermal junction of normal black hair matrix during anagen. Note thick basal lamina-like structure containing numerous round or oval bodies of varying electron opacity (*arrows*). *P*, hair dermal papilla. × 8 000.

from the accumulation of fine fibrillar structures. Within the involved basal lamina, melanosomes, obviously free in extracellular loci, were present singly or in small clusters (Fig. 3). No focal interruption in continuity in the basal lamina that could provide passage for melanocytic dendrites and melanosomes has been found. No free melanosomes were observed in the subjacent dermis.

Dermo-epidermal junction of hair bulb in anagen. Hair bulbs during anagen were selected for this study. The basal lamina underlying the melanocytic zone of hair bulb usually consists of a thick, continuous sheet. On the dermal side, anchor fibrils connecting the basal lamina to the dermal papilla were not present (Fig. 4). In 4 specimens out of 9, the basal lamina appeared to show focal thickening up to more than 10 μm in depth. This change was accompanied by an accumulation of numerous round or oval bodies of varying electron opacity (Figs. 5, 6, 7, 8) as well as free melanosomes lying free in extracellular sites (Figs. 6, 7, 8). The melanosomes were observed to occur singly (Figs. 6, 8), in small clusters (Fig. 7), and in large aggregates (Fig. 8). They were scattered throughout the interlacing mesh of fibrillar structures, subjacent to and apart from follicular melanocytes. Fragmentation of plasma membrane was not present.

Pigment cell lesions in the dermis. In blue nevi and pigmented nevi, the pigment cells formed closely packed masses. Each pigment cell was

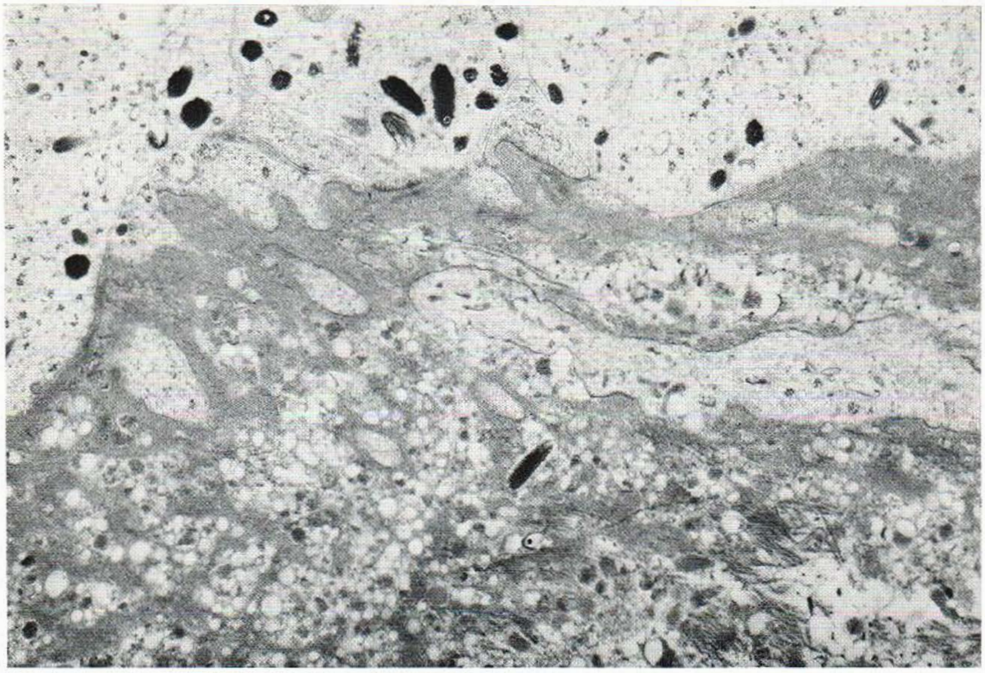


Fig. 6. Electron micrographs showing dermo-epidermal junction of tyrosinase-positive albino gray hair matrix. Within thick basal lamina-like structure, a single free melanosome is seen. × 11 500.

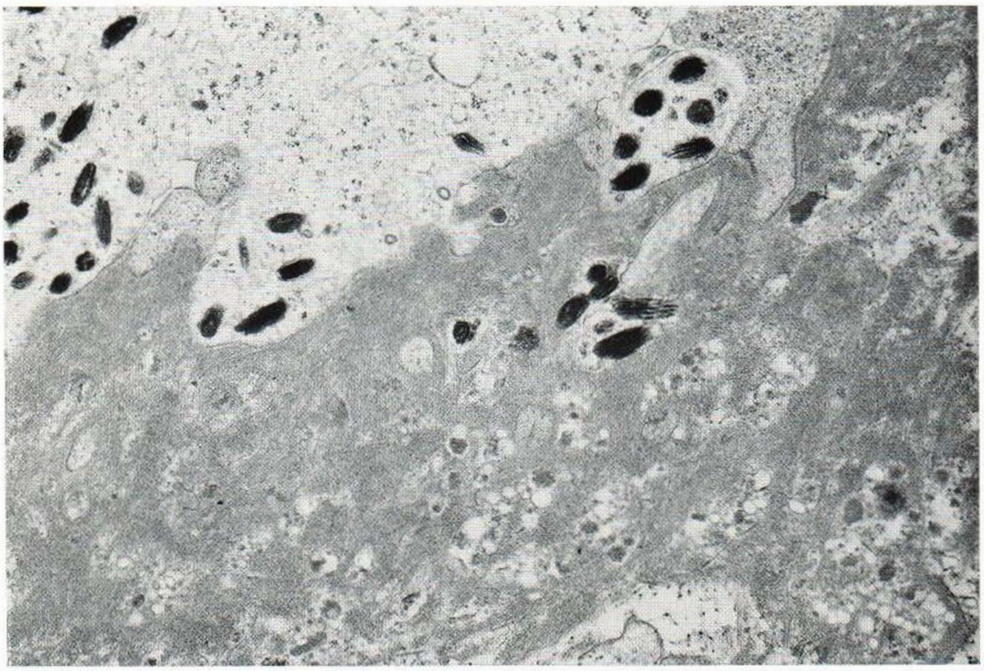


Fig. 7. Electron micrograph showing dermo-epidermal junction of tyrosinase-positive albino gray hair. Note a small aggregate of free melanosomes. × 11 500.

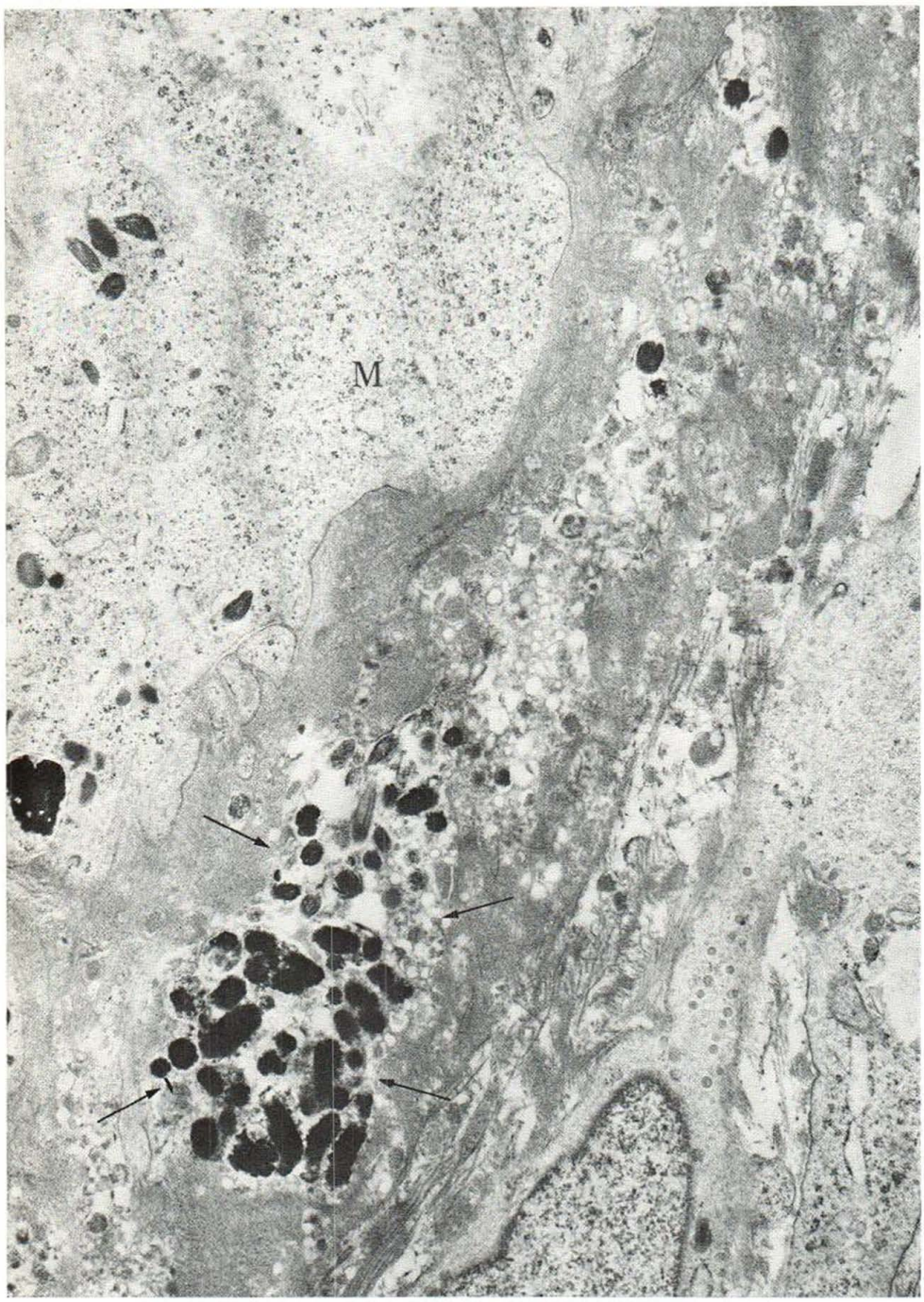


Fig. 8. Electron micrograph showing aggregation of a large number of free melanosomes (*arrows*) within thick basal lamina-like structure. Single, free melanosomes are also noted in upper right corner. Mesenchymal type of cell is present subjacent to basal lamina. In upper left corner is part of follicular melanocyte (*M*), containing a small number of melanosomes. × 14 000.

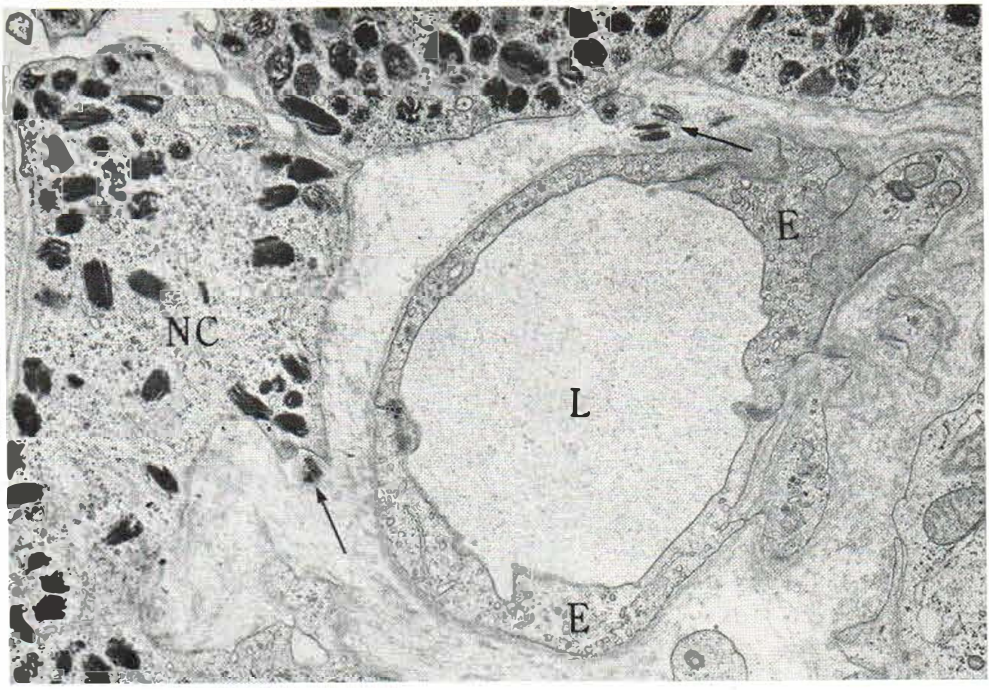


Fig. 9. Electron micrograph showing nevus cell lesion in the dermis. A small number of free melanosomes are present (*arrows*). *E*, endothelial cell; *L*, vascular lumen; *NC*, nevus cell. × 12 000.

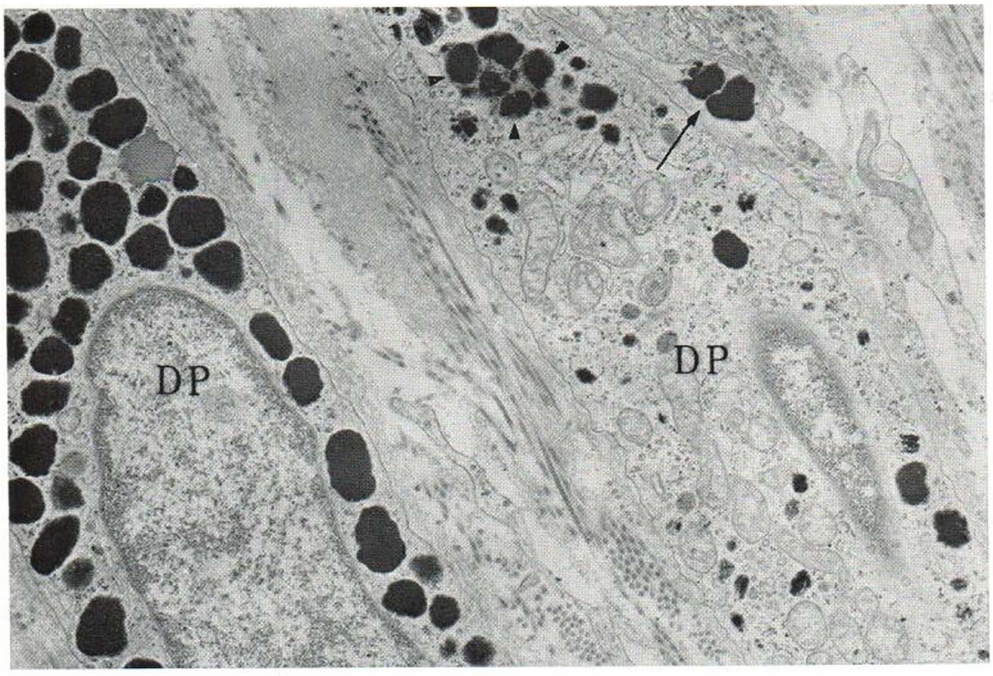


Fig. 10. Electron micrograph showing dermal melanocytes (*DP*) in blue nevi. They contain melanosomes in various stages of development. Two free melanosomes occurring in extracellular location are seen in upper right corner of micrograph (*arrow*). Arrowheads indicate melanosome complex in dermal melanocyte. × 12 000.

apparently covered with a thin basal lamina-like structure, and it could be distinguished from melanophages. Rare melanosomes, identical in ultrastructure with those seen in pigment cells, were noted in adjacent extracellular sites (Figs. 9, 10).

DISCUSSION

The dynamics of melanin transfer are currently being studied by many investigators and the majority of data have been concerned with epidermal transfer. According to contemporary views, the events in the in vitro model involve melanocytic dendrites first touching then entering keratinocytes. After the tips of the dendrites, which contain melanosomes, are pinched off within the keratinocytes, the dendrites withdraw (4, 5). Comparable findings in vivo that terminal segments of melanocytic dendrites penetrate the keratinocytes and are there nipped off have been confirmed (2, 9). However, these investigators failed to shed light on whether the process is accomplished by inoculation in which an active role is taken by melanocytes (4, 8), or by cytophagocytosis on the part of keratinocytes (9, 11, 17), or by a combination of both (5, 12).

It is somewhat surprising that so little data is at present available to support satisfactory explanations of pigmentary incontinence. Hypotheses postulated for the events are rather conflicting: (*a*) melanogenesis by a mesenchymal type of cell, such as mast cells (10), (*b*) transformation of keratinocytes into melanophages (6), (*c*) transformation of pigment cells into melanophages (3, 10), (*d*) inoculation into melanophages by pigment cells, and (*e*) cytophagocytosis of pigment cells by melanophages (1, 16). An outstanding feature of all the hypotheses mentioned is that epidermal transfer and pigmentary incontinence seem to be explained on the basis of two general systems. The first is the system of cell-to-cell transfer or "direct melanin transfer" and the second is of cell transformation.

Although the present study is based upon pathological materials, the ultrastructural findings all indicated a basically similar pattern of melanin transfer, both in the epidermis and in the dermis. The verification of melanosomes occurring free in the extracellular loci may suggest that melanin ejection of pigment cells is operative during melanin transfer in human skin, be it of epidermal transfer or of pigmentary incontinence. There can be little doubt that the fundamental mechanism is possibly "indirect melanin transfer", on the part of pigment cells (7, 15, 18), but not "direct melanin transfer". It seems, therefore, that physical contact between donor cells and receptor cells or cell transformation is no longer believed to be a primary prerequisite in the process of melanin transfer.

At present it seems less pertinent to place emphasis on the method of cell transformation in melanin transfer, since it is not adequately understood whether once fully differentiated pigment cells and keratinocytes, which have established specific functions, can readily transform into melanophages. The actual method of melanin transfer may not be dependent on one general method alone, but in view of the cellular partnership between donor and receptor cells, it may be reasonably conceived that direct transfer seems to predominate in the processes of epidermal transfer, while indirect transfer may be seen mostly in pigmentary incontinence. It may be concluded that the systems of direct and indirect transfer would be two basic mechanisms responsible for melanin transfer.

No confirmatory evidence of such indirect transfer has yet been obtained in normal physiological conditions. Further comparative studies on normal skin are now under active investigation.

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