

A POSSIBLE DYSFUNCTION OF MELANOSOME TRANSFER IN LEPROSY: AN ELECTRON-MICROSCOPIC STUDY

W. Westerhof

*From the Department of Dermatology, University of Amsterdam,
Binnengasthuis, Amsterdam, The Netherlands*

Abstract. An E.M. study was carried out to investigate whether *Mycobacterium leprae* occur intracellularly in epidermal melanocytes. As this could not be confirmed, the selective killing of melanocytes by cytotoxic lymphocytes could not explain the hypopigmentation in types of leprosy with a relative good immune response. There were indications that these hypopigmented lesions resulted from a disturbed transfer of melanosomes from melanocytes to keratinocytes. Further research is in progress.

Key words: Hypopigmentation; Transfer of melanosomes; Leprosy

In all types of leprosy, whether the resistance to *Mycobacterium leprae* is relatively high or low, one of the first symptoms is macular hypopigmentation (1), most distinct in the pigmented skin. In highly resistant tuberculoid leprosy the hypopigmentation is distinct, whereas in early lepromatous leprosy the macules are vague. The degree of pigment loss more or less parallels the degree of increase in resistance (clinically defined).

Furthermore, it is known that the Schwann cell and the melanocyte, both originating from the neuroectoderm, have a high DOPA metabolism. DOPA seems to be essential for the multiplication of *M. leprae* (5). In studies made by Weddell et al. (6), Lumsden (4), and Dastur (2) the Schwann cell was identified as being the target cell in leprosy and, consequently, of great importance in the neuropathogenesis of leprosy.

On the basis of this close relationship, epidermal melanocytes could act as host cells for *M. leprae*, after which the infected melanocytes would be killed selectively by cytotoxic lymphocytes, thus causing hypopigmentation.

The aim of this study was therefore to investigate

whether the epidermal melanocytes do contain *M. leprae* in bacterial-positive cases. If this could be confirmed, one could assume that in bacterial-negative cases, prior to triggering of the immune mechanism, the melanocyte also contains *M. leprae*.

MATERIAL AND METHODS

Skin biopsy specimens were taken from 11 patients with untreated leprosy, ranging from tuberculoid to lepromatous (classified according to the Riddley-Jopling scale). The clinical diagnosis was confirmed in all cases by a histological diagnosis.

Control specimens were obtained from sites on the contralateral side of the body, where the skin looked normal.

The biopsies were cut into 1 mm cubes, fixed in glutaraldehyde, post-fixed in a Durcupan ACM mixture. Ultrathin skin sections were cut on a Reichert OM U2 ultra microtome and stained with lead citrate. The specimens were examined with a Zeiss EM 9S electron microscope.

RESULTS

No mycobacteria were found in epidermal melanocytes. In lepromatous skin they were abundant in the dermis, mainly in histiocytic cells (Fig. 1) and especially around the small blood vessels (Fig. 2). In normal and in pathological skin, the melanocytes were rather large and bulged towards the dermis. They had large numbers of dendrites spreading up almost to the granular layer. A striking phenomenon in skin biopsies from patients with tuberculoid leprosy was a relatively sparse population of melanosomes in the keratinocytes (Figs. 3, 4) in comparison with dense crowding of melanosomes in the melanocytes. It was noteworthy that this

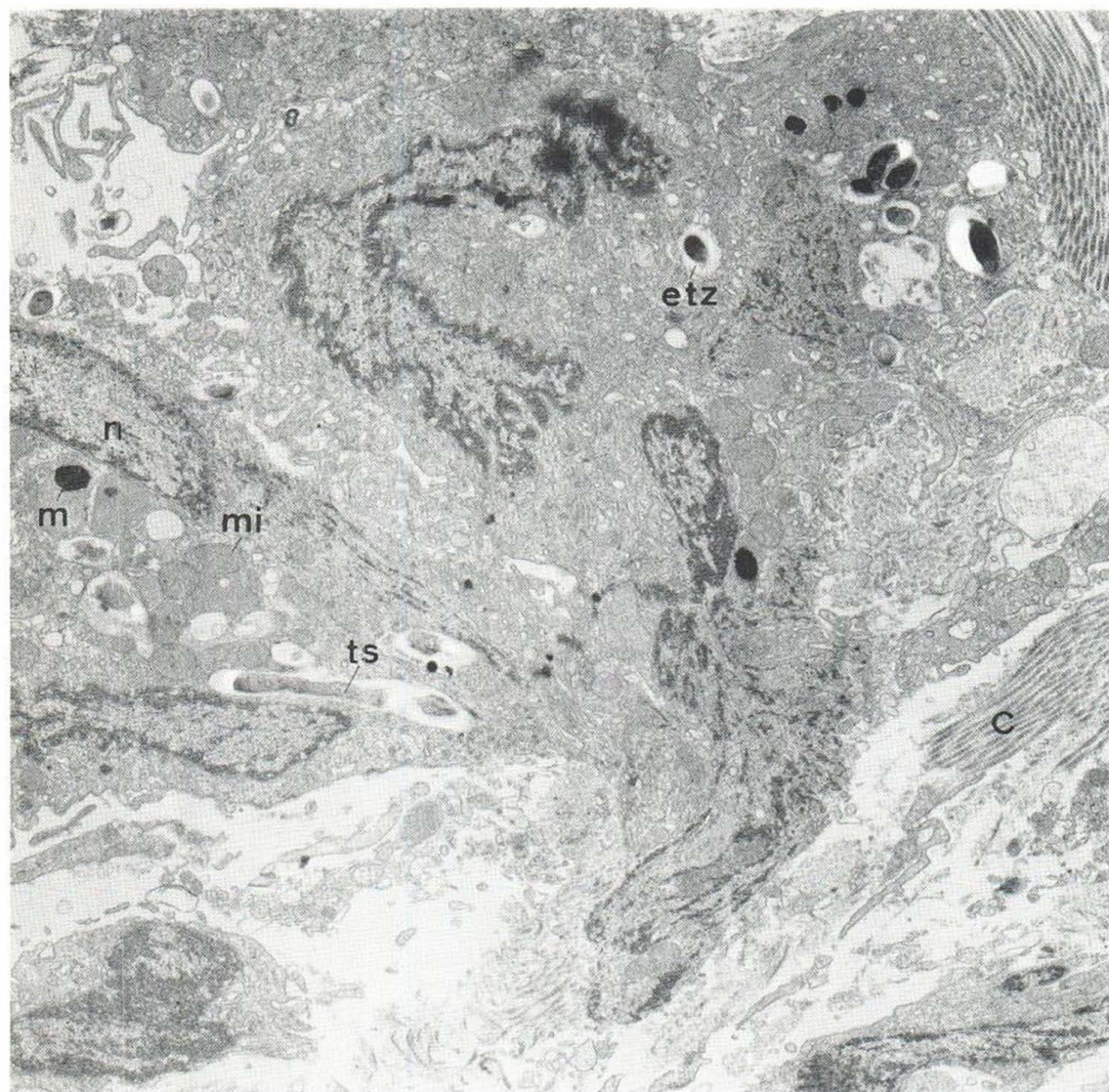


Fig. 1. The *Mycobacterium leprae* is surrounded by an electron-transparent zone (etz) which consists of an inert material (lipopolysaccharide) most likely of bacterial origin. Its consistency is less hard than the surrounding

structures. The artefacts (holes) in the etz which were caused by the ultra microtome, were unavoidable. $\times 12000$. n=nucleus of histiocytic cell; m=melanosome; ts=transverse section of *M. leprae*; C=collagen fibrils.

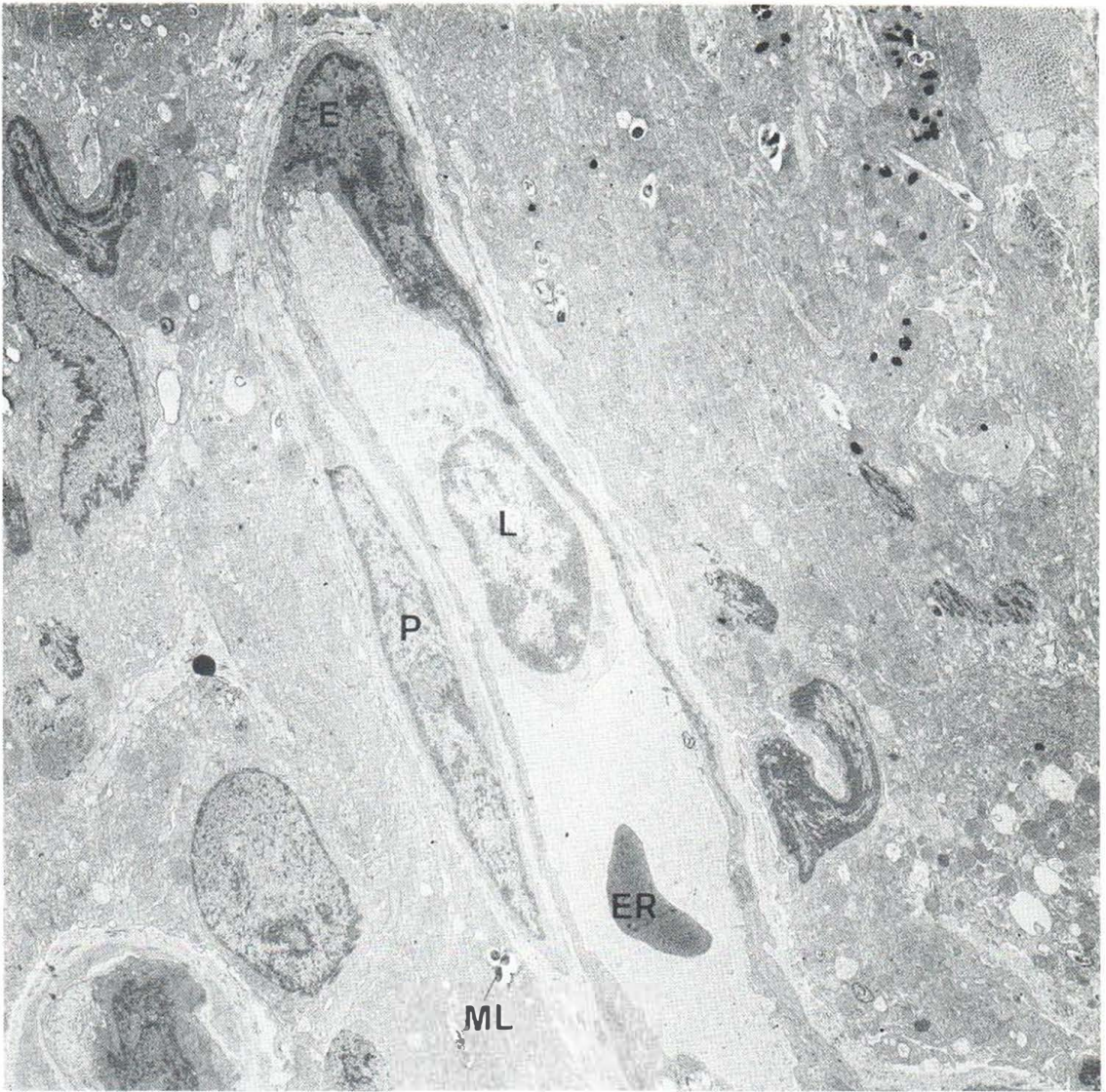


Fig. 2. Blood vessel in dermis of lepromatous skin. $\times 3850$. E=endothelial cell; P=pericyte; L=lymphocyte; ER=erythrocyte; ML=*M. leprae*.

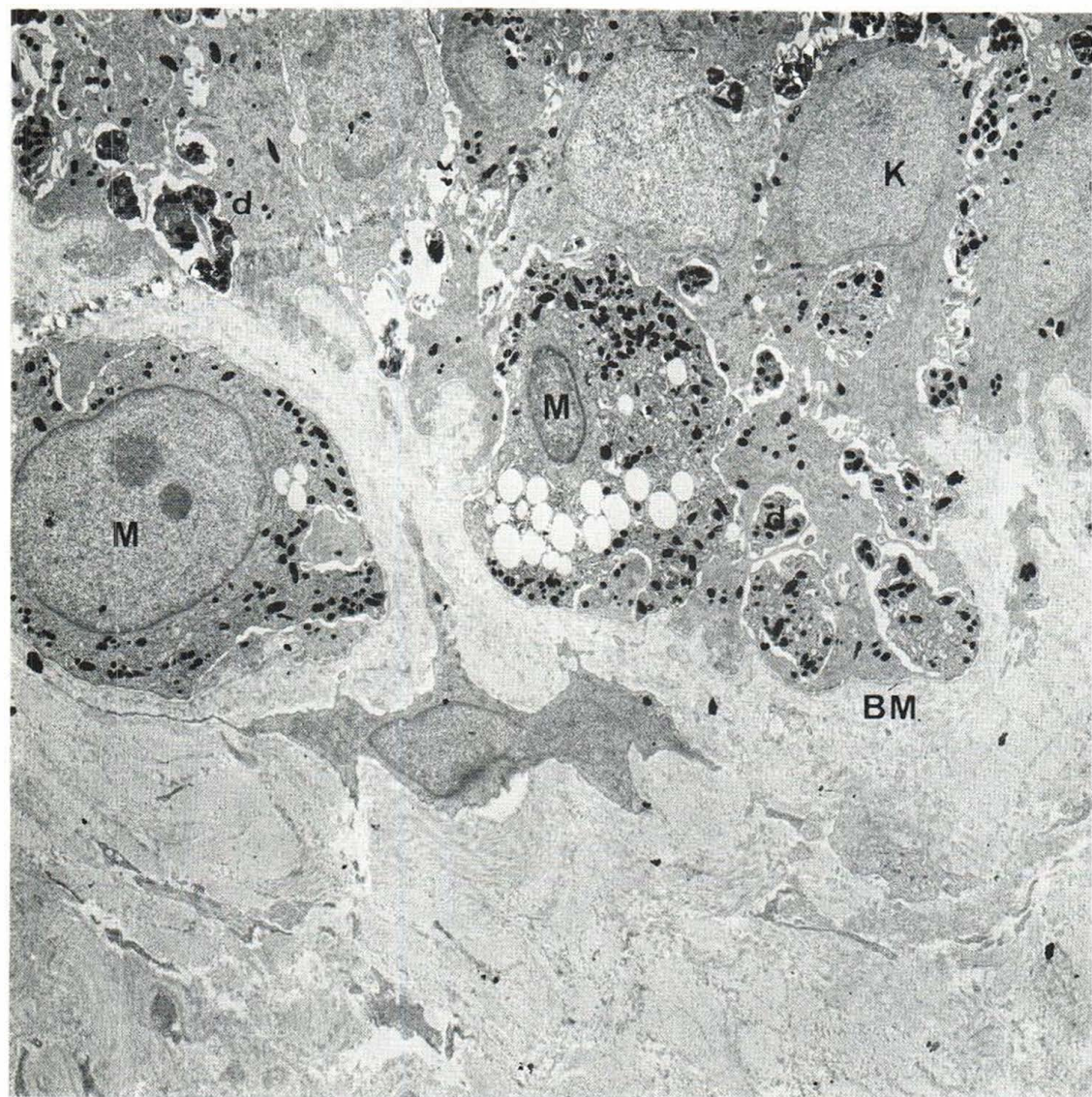


Fig. 3. Dermo-epidermal junction (tuberculous skin). The ratio between the melanosome content of melanocytes and keratinocytes is approximately 9:1. $\times 3850$.

M=melanocyte; *d*=dendrite of melanocyte; *K*=keratinocyte; *BM*=basement membrane.

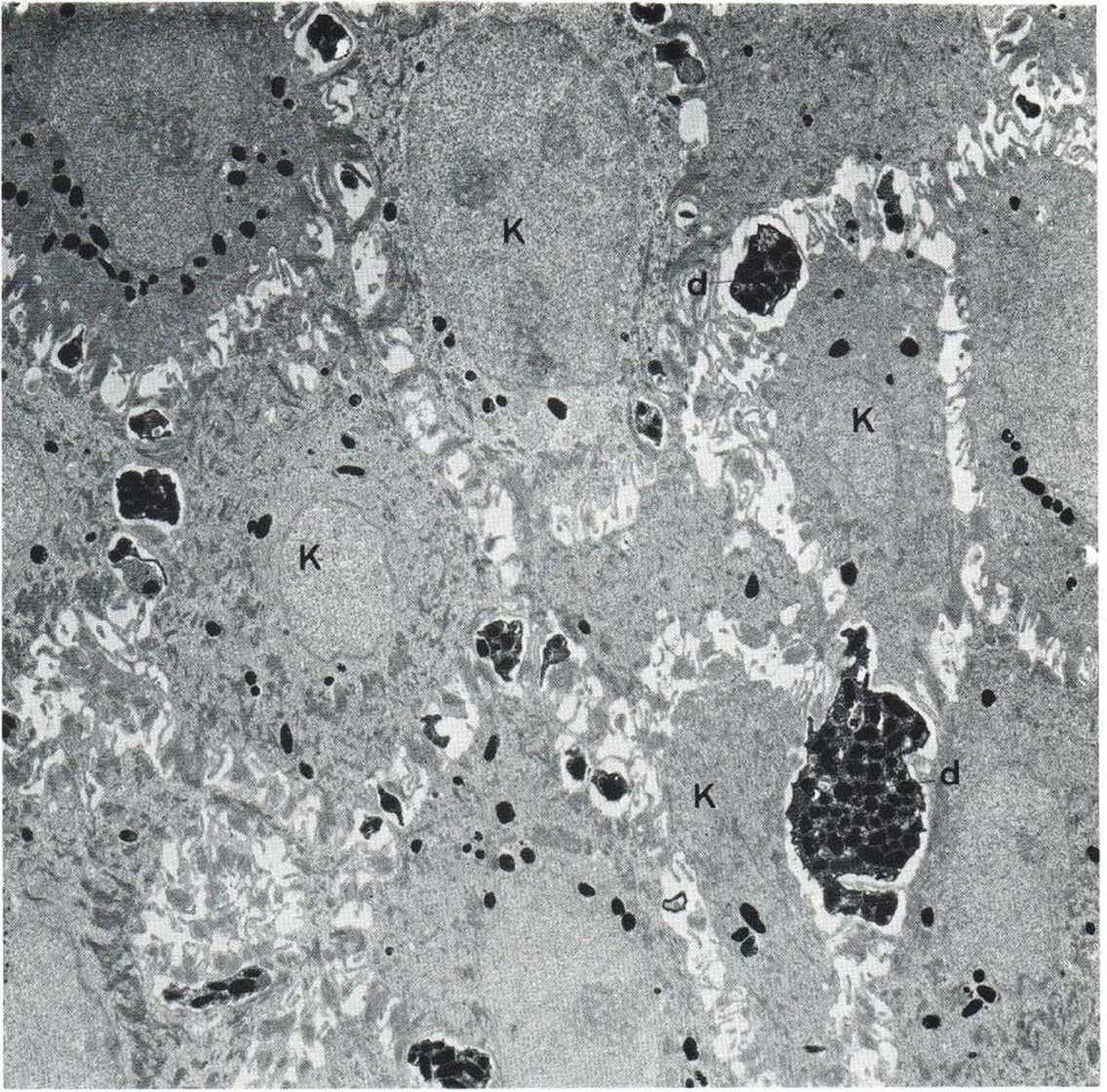


Fig. 4. Higher magnification, to illustrate the crowding of melanosomes in keratinocytes. $\times 7075$. *d*=dendrite of melanocytes; *K*=keratinocytes.

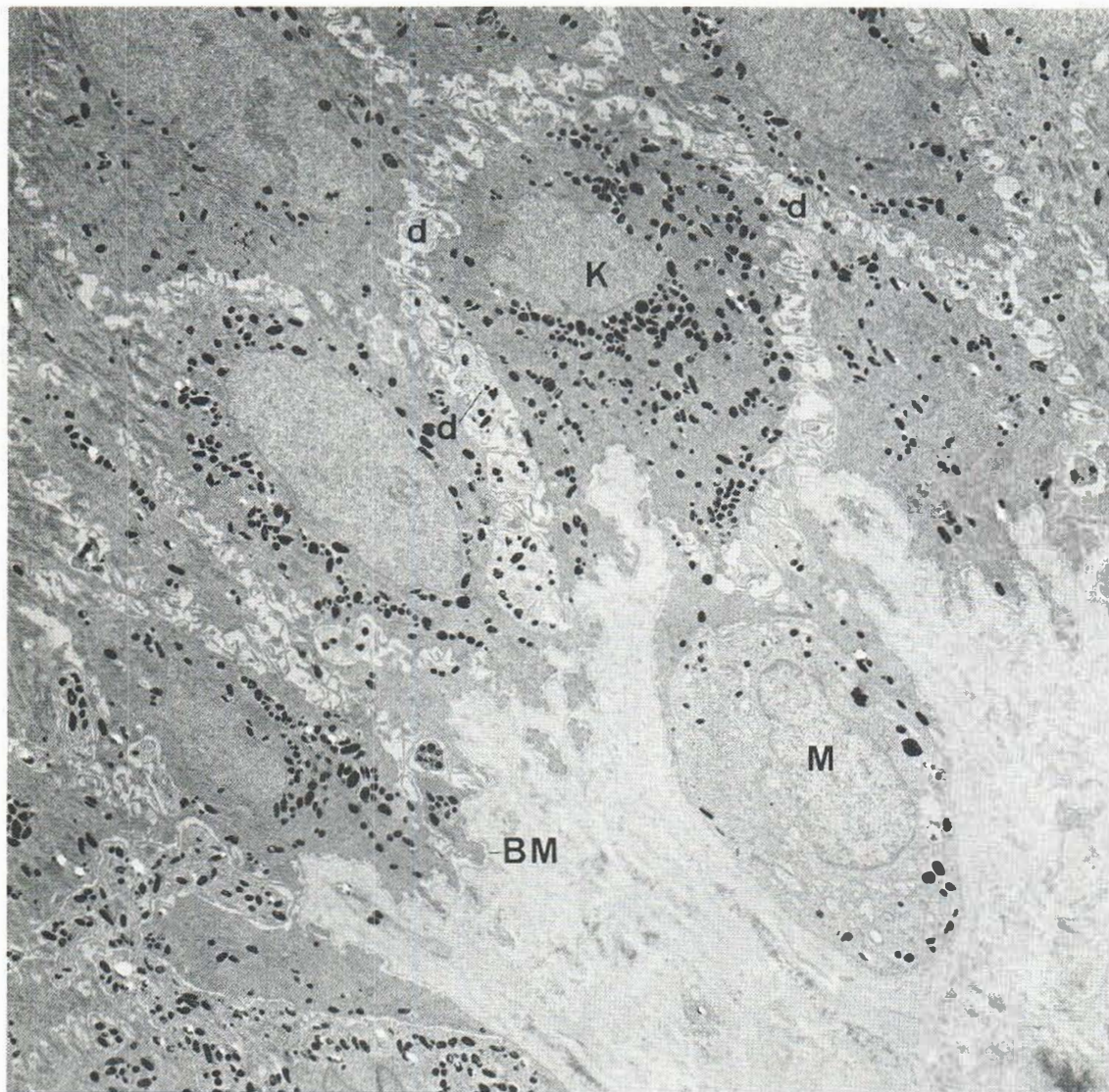


Fig. 5. Normal skin of patient (as in Fig. 4) with tuberculous leprosy at the dermo-epidermal junction. The ratio between the melanosome content of melanocytes and

keratinocytes is here approximately 2:9. $\times 9450$. *M* = melanocyte; *d* = dendrite of melanocyte; *K* = keratinocyte; *BM* = basement membrane.

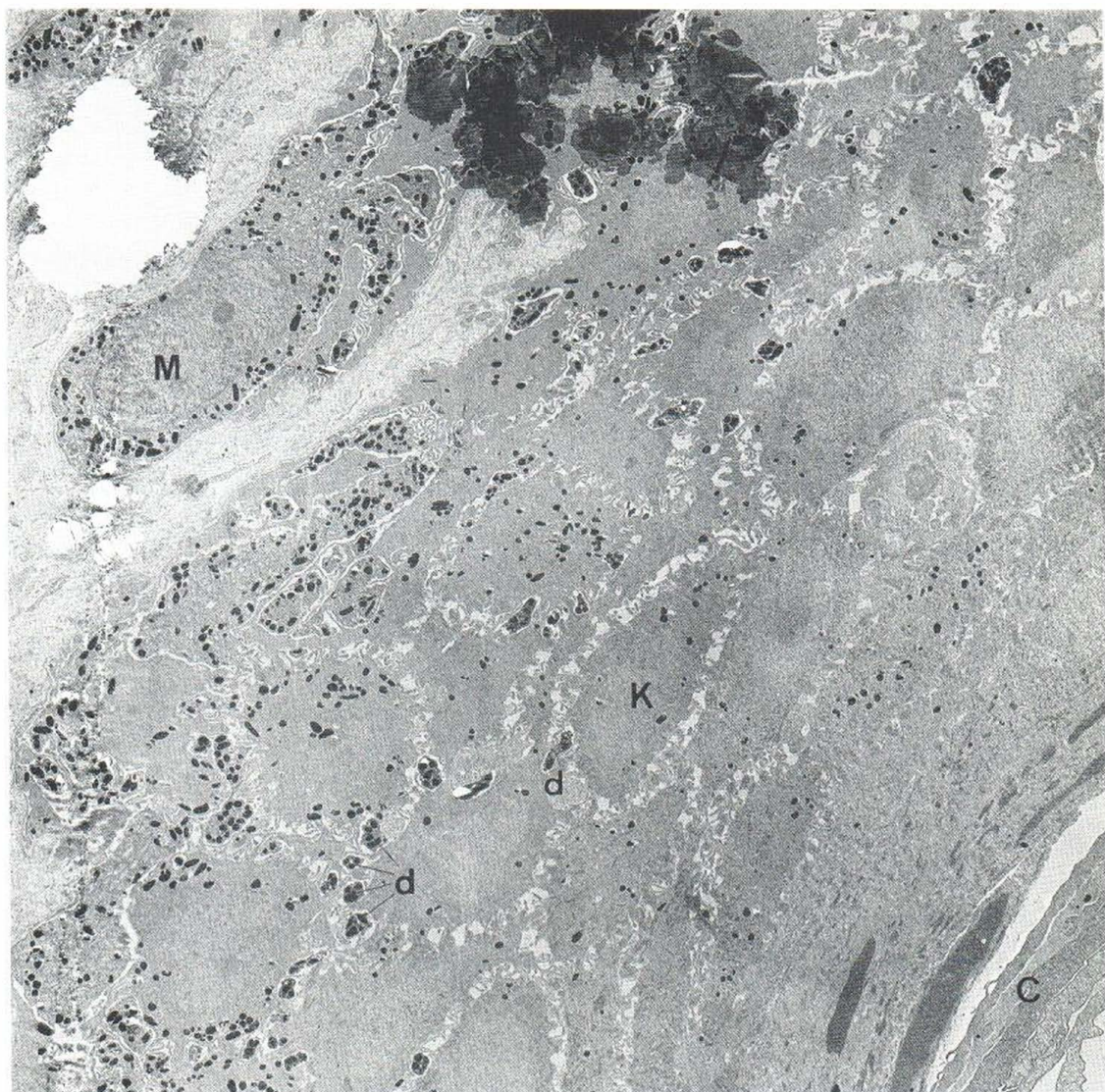


Fig. 6. Low power cross section of the epidermis of a tuberculous patient. Dendrites can be seen in the upper spinular layer. $\times 1300$. *M*=melanocyte; *K*=keratinocyte

of spinular layer; *d*=dendrite of melanocyte; *C*=horny layer.

phenomenon was reversed in the non-pathological skin areas of the same patient (Fig. 5). Inflammatory cells were seldom seen invading the epidermis. These cells, most likely lymphocytes, were never seen in close contact with melanocytes.

DISCUSSION

The original hypothesis that hypopigmentation is a result of melanocyte killing by cytotoxic lymphocytes has had to be rejected. Although not an all-or-none phenomenon, the crowding of melanosomes in melanocytes and the relative absence in keratinocytes were relatively constant findings in tuberculoid skin. A statistical investigation is needed, however, to confirm these observations.

At the same time, DOPA-stained epidermal sheets and silver-stained sections of the same specimens need to be examined at the light microscopic level in order to study their melanocyte density and morphology as compared with the situation in normal skin.

Klaus (3) found in DOPA-stained epidermal sheets of tuberculous skin a normal density of melanocytes with shortened dendrites (unpublished data). However, in the present study there was no indication that the dendrites are shortened (Fig. 6). Disturbed transfer of melanosomes from melanocytes to keratinocytes could be due to 1) retraction of dendrites, 2) edema and inflammatory cells in the intercellular spaces, 3) shorter contact between melanocytes and keratinocytes, 4) other reactions, yet unknown.

The very rare occurrence of these inflammatory cells in the epidermis in which no difference could be noted between tuberculous and lepromatous skin, can certainly not account for the disturbance in the exchange of melanosomes. It is obvious that other, unknown reactions must be involved in the

disturbed transfer of melanosomes from melanocytes to keratinocytes.

Hypopigmented skin in tuberculous leprosy might prove a good model for the study of this phenomenon.

ACKNOWLEDGEMENTS

This study was supported by the Netherlands Leprosy Relief Association and the Gastmann Wichers Foundation.

REFERENCES

1. Cochrane, R. G.: *Leprosy in Theory and Practice* (ed. R. G. Cochrane & T. Davey), pp. 251-279, John Wright & Sons Ltd., Bristol, 1964.
2. Dastur, D. K., Ramamohan, Y. & Shah, J. S.: Ultrastructure of lepromatous nerves. *Neural pathogenesis in leprosy*. *Int J Leprosy* 41: 47, 1972.
3. Klaus, S.: Dermatological Department, Yale University, personal communication on the joint meeting of ESDR and SID in June 1975, Amsterdam.
4. Lumsden, C. E.: Leprosy and the Schwann cell in vivo and in vitro. *In Leprosy in Theory and Practice* (ed. R. G. Cochrane & T. Davey), 2nd ed., pp. 221-250. John Wright & Sons Ltd, Bristol, 1964.
5. Prabhakaran, K., Harris, E. B. & Kirchheimer, W. F.: The nature of the phenolase enzyme in *Mycobacterium leprae*: structure activity relationships of substrates and comparison with other copper proteins and enzymes. *Microbios* 5: 273, 1972.
6. Weddell, A. G. M., Palmer, E., Rees, R. W. J. & Jamison, D. G.: Experimental observations related to the histopathology of leprosy. *In The Pathogenesis of Leprosy* (ed. G. E. W. Wolstenholme & M. O'Connor), pp. 3-15. Ciba Foundation Study Group no. 15. J & E Churchill, London, 1963.

Received June 16, 1976

W. Westerhof, M.D.
Department of Dermatology
Binnengasthuis
Amsterdam-C
The Netherlands