

ULTRASTRUCTURAL STUDY OF LEUKODERMIC SKIN IN WAARDENBURG-KLEIN SYNDROME

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Abstract. The skin of a patient with Waardenburg syndrome has been studied by electron microscope. In leukodermic skin, there were neither melanocytes nor indeterminate cells; Langerhans cells were normal in number and distribution. On the edge of the lesion, melanocytes were few and appeared degenerated, with abnormal melanosomes; indeterminate cells were observed in suprabasal areas. Healthy skin also showed melanosome abnormalities. These findings are discussed and compared with the characteristics of piebaldism.

Key words: Waardenburg; Achromia; Melanocytes; Ultrastructure

The Waardenburg-Klein syndrome (13, 7) is determined by a dominant gene with variable penetrance. It includes the following main characteristics; external displacement of the internal canthi, enlargement of the root of the nose, hyperplasia of the eyebrows which are confluent in the mid-line, total or partial heterochromia of the iris, white forelock, and congenital unilateral or bilateral deafness.

Other anomalies are sometimes observed, for instance leukoderma. Although this aspect of the disease has not been studied in detail, it was present in 12.5% of 180 patients found in a review of the literature (12). Achromia can be sited anywhere but is more usually found on the limbs. It presents as pigment-free spots that spread slowly to become confluent with their outlines broken up by islands of hyperpigmented skin.

On the basis of its clinical and genetic constitution, this leukoderma has been related to piebaldism (4). However, to our knowledge, no histochemical study has confirmed this hypothesis and ultrastructural studies of the skin are very few. Only the research of Anton-Lamprecht et al. (1) concentrated on this aspect and showed a very important reduction of achromic skin melanocytes and

melanocyte abnormalities in the hyperpigmented skin.

In this paper, we report an electron microscopic study of the skin of a patient with Waardenburg syndrome, undertaken to define its nosological ap-
purtenance and to investigate the mechanism underlying this particular form of leukoderma.

MATERIAL AND METHODS

A 29-year-old female whose clinical signs are reported elsewhere (10) presented with a typical Waardenburg-Klein syndrome: she had lateral displacement of the inner angles of the eye, with dystonia of the lower lachrymal orifices; enlargement of the root of the nose, hyperplasia of the eyebrows with median fusion, partial heterochromia of the iris, a white streak in her hair, bilateral congenital deafness being total on one side and partial on the other, diffuse hypertrichosis especially on the legs and the lumbar region (with normal endocrine status), plaques of leukoderma on the anterior surface on both legs, with their outlines broken up by islands of hyperpigmentation



Fig. 1. Achromic skin. Intra-epidermal mastocyte. $\times 6400$.

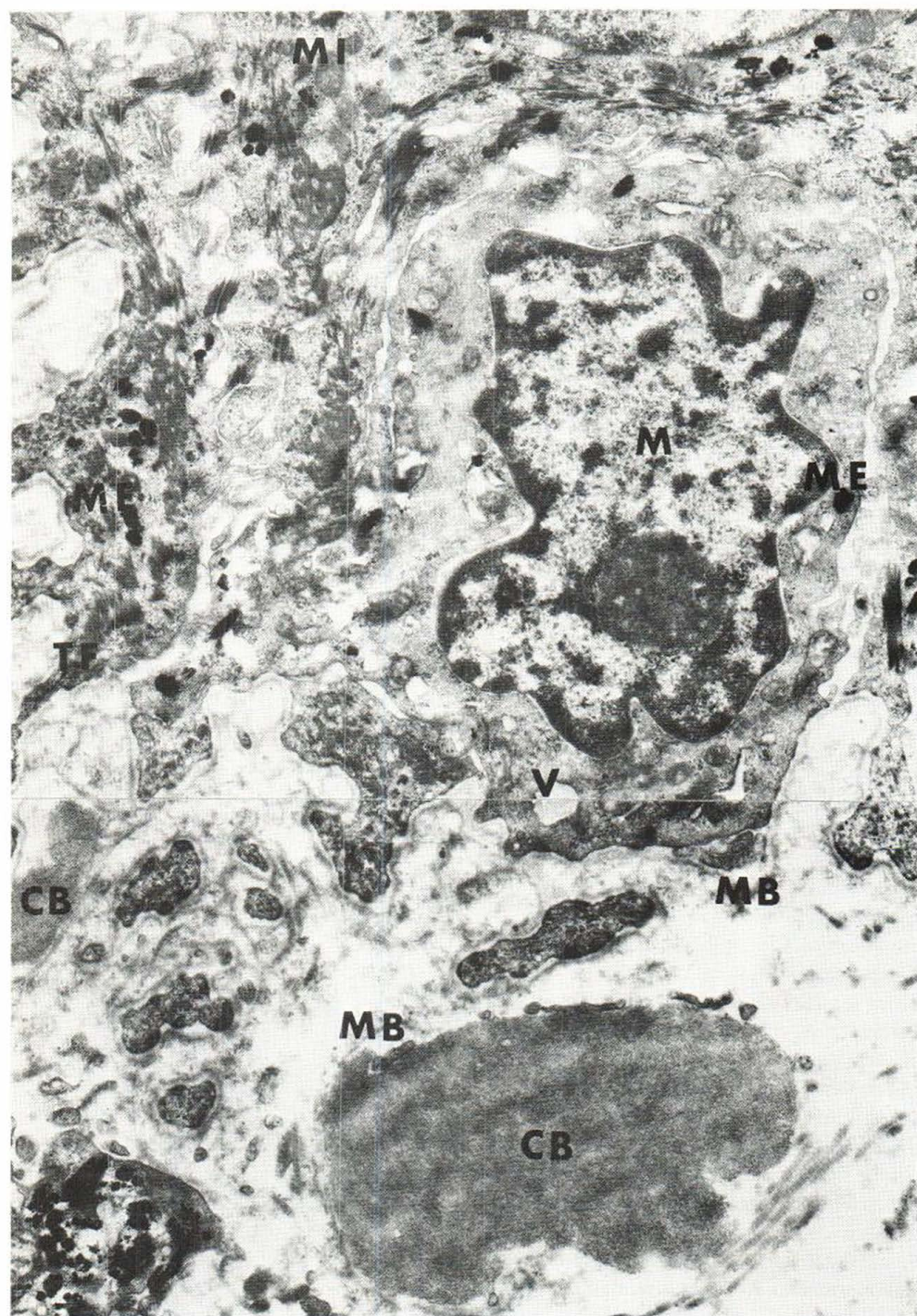


Fig. 2. Skin from the edge of the lesion. *M*, Melanocyte; *ME*, melanosome; *MI*, mitochondria; *TF*, tonofibrils. *CB*, colloid bodies; *BM*, basement membrane; *V*, vacuole; $\times 13\,700$.

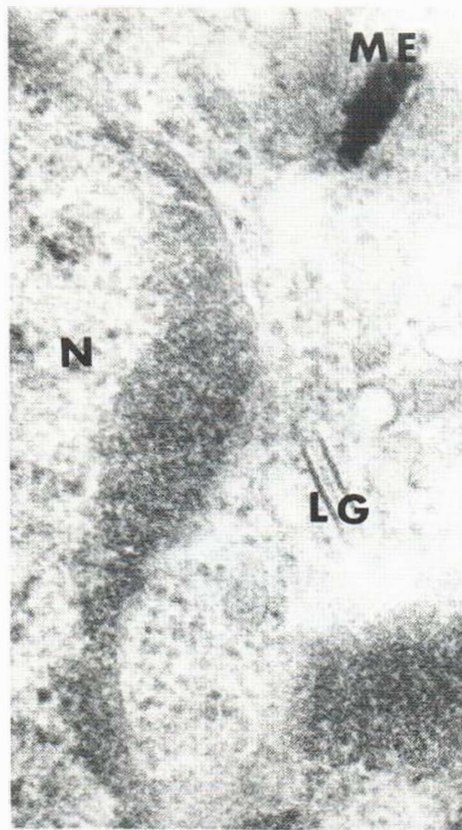


Fig. 3. Skin from the edge of the lesion. A Langerhans cell containing a melanosome. *N*, Nucleus; *ME*, melanosome; *LG*, Langerhans granule. $\times 80\,000$.

and a diffuse goitre since the age of 18. Her karyotype was normal and her H. L. A. type was: HL-A5 HL-A11 (Te 59); blood group: A1 Rh⁺. Genetic investigation covering four generations showed that 6 of the 13 members of the family had this disease. In 1972, she developed pruritus and this led to diagnosis of Hodgkin's disease.

The ultrastructural study was made on the achromic skin, on the border of an achromic lesion, and on healthy skin of normal colour taken 4 cm from the lesions. The biopsy specimens were taken from the leg under local anaesthesia and were fixed in 3% glutaraldehyde for 1 h, then post-fixed in osmic acid for 1 h, dehydrated in alcohol, embedded in Epon and cut on a Reichert ultramicrotome. The sections were stained with uranyl acetate and lead citrate and then examined with a Philips EM 300 electron microscope.

RESULTS

1. In the achromic skin, there is no melanocyte; no melanosome is visible within keratinocytes. Langerhans cells are normal in number and distributed

throughout the thickness of the epidermis; there is no indeterminate cell. An intra-epidermal mastocyte is observed above the basal layer, with peripheral protoplasmic digitations and dense granules having an average diameter of 150 nm (Fig. 1). The dermo epidermal junction shows no abnormality and only a very small number of colloid bodies are visible in the superficial dermis at a distance from the basement membrane.

2. At the edge of the lesion, there are numerous abnormalities (Figs. 2, 3, 4, 5). The melanocytes, reduced in number, are impaired; the nucleus is often contorted, with a large nucleolus and predominant peripheral dense chromatin; the cytoplasm is filamentous, with a few vacuoles containing granular, not very dense material. The melanosomes and premelanosomes are few in number and are sometimes found inside the vacuoles surrounded by a clear halo (Fig. 4); though some of

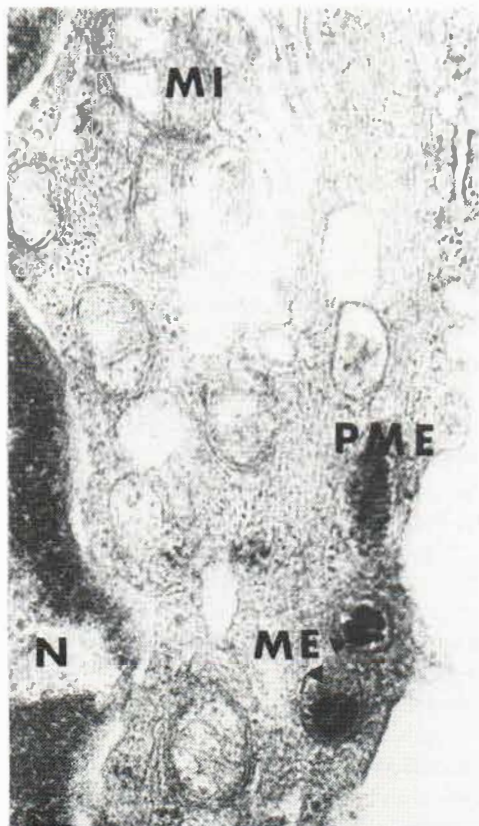


Fig. 4. Skin from the edge of the lesion. Melanosomes of varying appearance in a melanocyte. *N*, Nucleus; *MI*, mitochondria; *ME*, melanosome; *PME*, premelanosome. $\times 63\,000$.

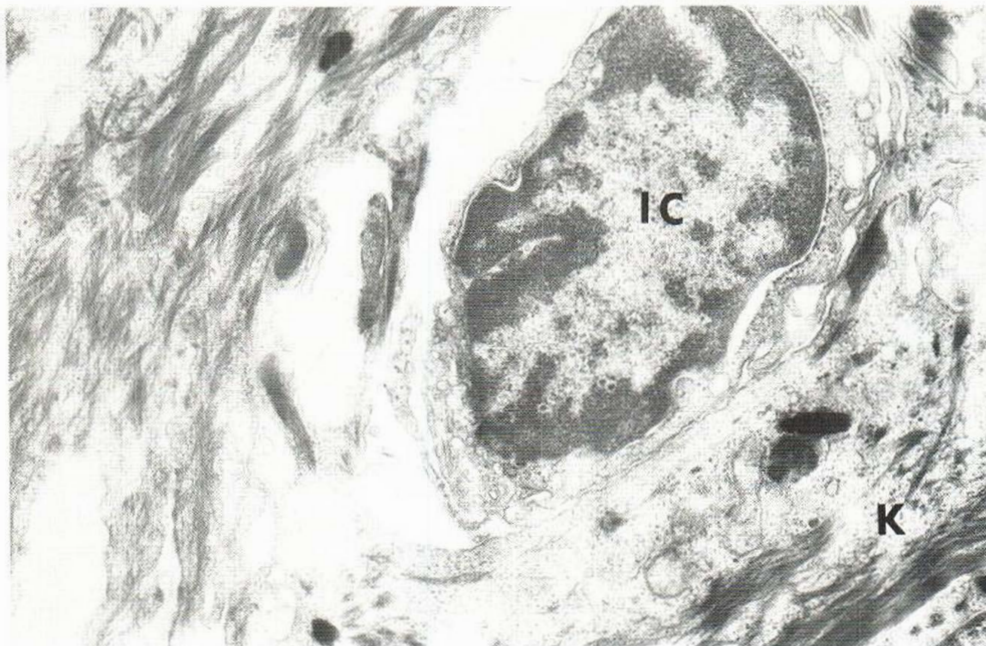


Fig. 5. Skin from the edge of the lesion. Indeterminate cell looking like a lymphocyte in the deeper part of epidermis. K, Keratinocyte; IC, indeterminate cell. $\times 18\,000$.

them have a normal structure, others are granular and heterogeneous. Within keratinocytes, melanosomes are numerous, variable in size, located at the periphery of the cell. Langerhans cells are normal in number and visible throughout the length of the malpighian layer; some of them contain melanosomes (Fig. 3). In the deeper part of epidermis, some cells lacking characteristic organelles cannot be identified; some look like lymphocytes (Fig. 5).

Abnormalities are observed at the dermo-epidermal junction close to melanocytes. The basement membrane frequently forms loops which hem in fragments of keratinocytes or fibrillary typical colloid bodies. Far from the epidermis, fragments of basement membrane are seen around some colloid bodies (Fig. 2). However, the onion bulb leaf-like folding of the basement membrane as seen around the lesion in vitiligo (11) is not present in this patient's skin.

3. In normal skin, away from the lesions, numerous melanocytes appear to be normal, though some have some abnormalities; their general appearance is fairly close to normal, although their dendrites are less developed and not so numerous; large melanosomes with irregular outlines are broken up

and heterogeneous (Fig. 6). Premelanosomes sometimes display their characteristic striped appearance, but fairly numerous rounded and granular formations, small in size and of fairly low contrast, can be seen. We have interpreted them as being abnormal premelanosomes.

DISCUSSION

Our observations have enabled us to classify the leukoderma as being of the Waardenburg-Klein syndrome, along with vitiligo and piebaldism, in the achromias with few or no melanocytes. We were unable to find any melanocytes or melanosomes in the achromic skin; our observations are similar to those of Anton-Lamprecht et al. (1) who found in hypopigmented skin a very considerable reduction of melanocytes containing poorly melanised melanosomes. In healthy skin of normal colour, we too found melanosome abnormalities. Though the premelanosomes had a granular appearance, there were also very large melanosomes of heterogeneous structure and irregular outline. In the hyperpigmented zone, Anton-Lamprecht et al. (1) ob-

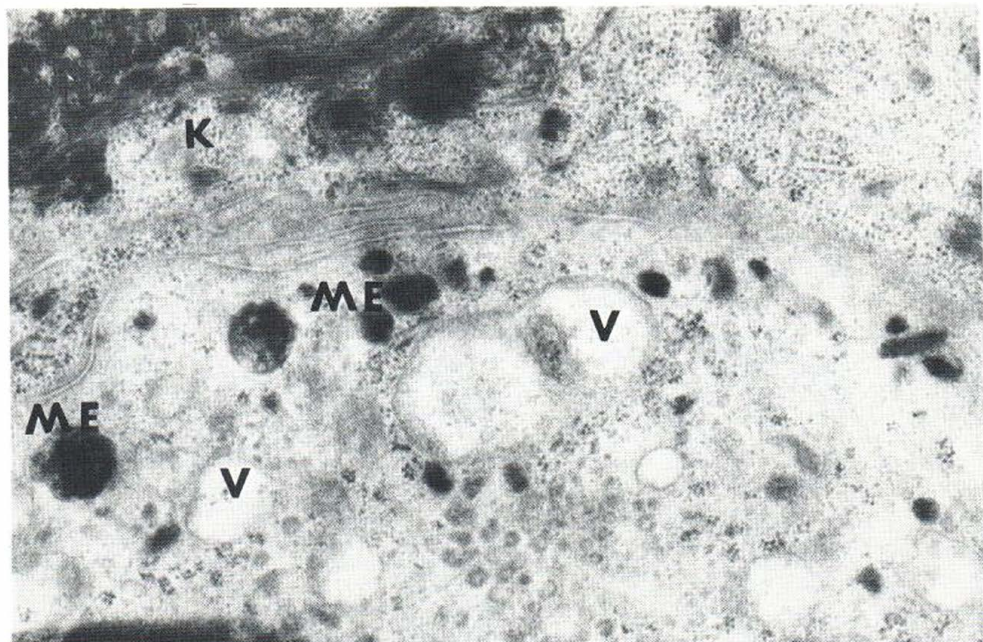


Fig. 6. Normal skin. Melanocyte with abnormal melanosomes (ME). V, Vacuole; K, keratinocyte. $\times 30\,000$.

served very large melanocytes having increased melanin biosynthesis.

When our observations are compared with those found in piebaldism, numerous similarities are noted:

(i) In the achromic skin of piebaldism, Comings & Odland (4) and Breatnach et al. (2) failed to find any melanocytes whereas Grupper et al. (5) did find some, but only very few. When studying serial sections Jimbow et al. (6) found only a single melanocyte, containing very few spherical melanosomes which were of granular appearance and not melanised. Recently, Nagao et al. (9) have observed an intra-epidermal mastocyte.

(ii) In the hypopigmented skin in piebaldism, Breatnach et al. (2) observed a very small number of melanocytes which contained dense spherical voluminous melanosomes surrounded by a clear halo. Grupper et al. (5), studying the edge of the lesion which is "more and less pigmented", observed melanosomes variable in size and degenerated granular premelanosomes surrounded by a clear zone.

(iii) In the hyperpigmented zone, the melanosomes were numerous (4, 6).

(iv) Whereas Comings et al. (4) found normal melanocytes in the normal skin of piebaldism,

Breatnach et al. (2) observed some degenerated melanocytes with abnormal melanosomes.

These observations indicate that in piebaldism, as also in the Waardenburg-Klein syndrome, though the melanocytes are absent in the achromic skin, in the hyperpigmented areas, and in the border of the lesion some melanocytes appeared containing abnormal melanosomes. These abnormalities do not always seem to be pathognomonic, inasmuch as we have observed large masses of pigment surrounded by a clear halo in the skin surrounding the vitiligo lesion (11).

The observation of abnormalities in healthy skin melanocytes in the Waardenburg-Klein syndrome and in piebaldism (2) must be stressed.

The pathogenesis of this achromia seems to be less clear than is suggested by most authors, who consider that melanocytes are replaced by Langerhans cells. In achromic skin, we have not observed in the basal layer any increase in the Langerhans cell population, whose number and distribution were normal. On the edge of the lesion, numerous cells without rods could not be identified as Langerhans cells; we prefer to use the term 'indeterminate' for these cells. It seems quite certain that Langerhans cells can phagocytize melanosomes.

In considering the mechanism of the depigmentation, it is necessary to take into account our observations on normal skin and studies in animal systems. In normal skin, melanisation is complete or abnormal in certain melanocytes. Mayer & Green (8), observing mice with achromic plaques, considered that the achromia in these animals was due to the inability of the melanoblast to migrate in the skin or to differentiate into normal melanocytes in some regions of the skin where the environmental conditions were hostile to the differentiation of the melanoblast. It remains to be determined which are the areas of skin where the melanocytes are abnormal and this will require systematic ultrastructural study of the normal skin. It is not possible to consider this syndrome as a variant of the first branchial arch syndrome (3) in view of the very numerous visceral malformations that have been observed, particularly affecting the cardiovascular and nervous system. Nevertheless, it can be ascribed to a genetic abnormality affecting the development of the neural crest during embryogenesis.

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