THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 60, No. 5

Printed in U.S.A.

CHANGES IN MELANOSOME DISTRIBUTION IN CAUCASOID SKIN FOLLOWING TOPICAL APPLICATION OF NITROGEN MUSTARD*

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ABSTRACT

Hyperpigmentation resulting from topical application of nitrogen mustard to skin of Caucasoid and Negroid patients with mycosis fungoides and psoriasis was studied using electron microscopic techniques. In Negroids the melanosomes retained normal nonaggregated patterns but increased in number. In Caucasoids treatments resulted in nonaggregation of melanosomes within keratinocytes without a concomitant increase in melanosome size.

Nitrogen mustard (HN2, mechlorethamine) dissolved in water and applied topically to human skin, causes complete regression of cutaneous lesions of the lymphoma mycosis fungoides, a disease characterized by malignant hyperplasia of lymphoreticular cells [1]. The drug also causes clearing of lesions of psoriasis [2, 3], a disorder characterized by benign epidermal hyperplasia. In both Caucasoid and Negroid patients with these diseases, skin exposed to HN2 may become deeply hyperpigmented [1]. Clinically normal skin as well as previously diseased skin shows this pigmentary response. The mechanism whereby HN2 exerts this hyperpigmenting effect is unknown, for it occurs in the absence of any clinical sign of irritation or inflammation. The extreme reactivity of mechlorethamine makes it unlikely that the drug penetrates the epidermis chemically unchanged. The ultrastructural study reported in this paper reveals that in Negroids topical application of HN2 is associated with a simple increase in the number of melanosomes in keratinocytes; in Caucasoids, however, an increase in number of melanosomes is also accompanied by a striking change in their arrangement and distribution.

MATERIALS AND METHODS

Small biopsies of skin (2 mm diameter) were placed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 hr, washed in buffer, postfixed in a 1:2 mixture of OsO_4 in collidine [4], dehydrated through alcohol and propylene oxide, and embedded in araldite. One-μ plastic sections stained with Azure II were examined with the light microscope and appropriate areas selected. Ultrathin sections were cut with a diamond knife, stained in uranyl acetate and lead citrate, and examined in an AEI Corinth electron microscope.

Manuscript received November 27, 1972; in revised form January 18, 1973; accepted for publication January 23, 1973.

This study was supported by Grants No. 1 PO1 CA 11536 from The National Cancer Institute, and No. 1 PO1 AM 15515 from The National Institute of Arthritis and Metabolic Diseases.

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RESULTS

Biopsies were taken from normal skin that had never been involved with disease and from sites of previous lesions of either mycosis fungoides or psoriasis that had regressed with treatment. In all cases, the areas had been treated with HN2 for periods of 6 weeks to 3 years at a rate of 1 to 5 times weekly. Skin areas treated with HN2 received approximately 10 μgm of drug per 15 cm^2 at each application. All patients were receiving treatment at the time of biopsy. All biopsies were taken from distinctly hyperpigmented areas (Fig. 1). Six patients (4 Caucasoid, 2 Negroid) had mycosis fungoides and two patients (1 Caucasoid, 1 Negroid) had psoriasis.

Ultrastructural examination of the skin revealed surprisingly little evidence of any toxic effect of this alkylating agent. Except for changes in melanosome characteristics, alterations in other skin components were found infrequently and varied from patient to patient. Occasionally, keratinocytes showed enlarged nucleoli, infolding of the nuclear membrane, some small cytoplasmic vacuoles, and arrangement of tonofilaments at the extreme cell periphery. Rarely, a dead, dyskeratotic cell was found in the basal layer. In the hyperpigmented sites, melanocytes seemed to be increased in number in Caucasoids, were often very prominent, and extended down into the papillary dermis. Occasional cells contained large cytoplasmic vacuoles (Fig. 2). Most striking was the prominent alteration in melanosome distribution within the keratinocytes of Caucasoid skin. For reference purposes, aggregated and nonaggregated melanosomes of normal Caucasoid and Negroid skin are shown in Figures 3 and 4, respectively. In Caucasoids, individual melanosomes ranged from .3–.5 μm in length and were arranged in membrane-bound groups of two or more. In Negroids, the size range was .5–.8 μm and aggregation was minimal or absent. In hyperpigmented areas from all five Caucasoid patients, both from normal and previously diseased skin, the melanosomes within keratinocytes were mainly nonaggregated, as in Negroids, and were no longer arranged in membrane-bound groups

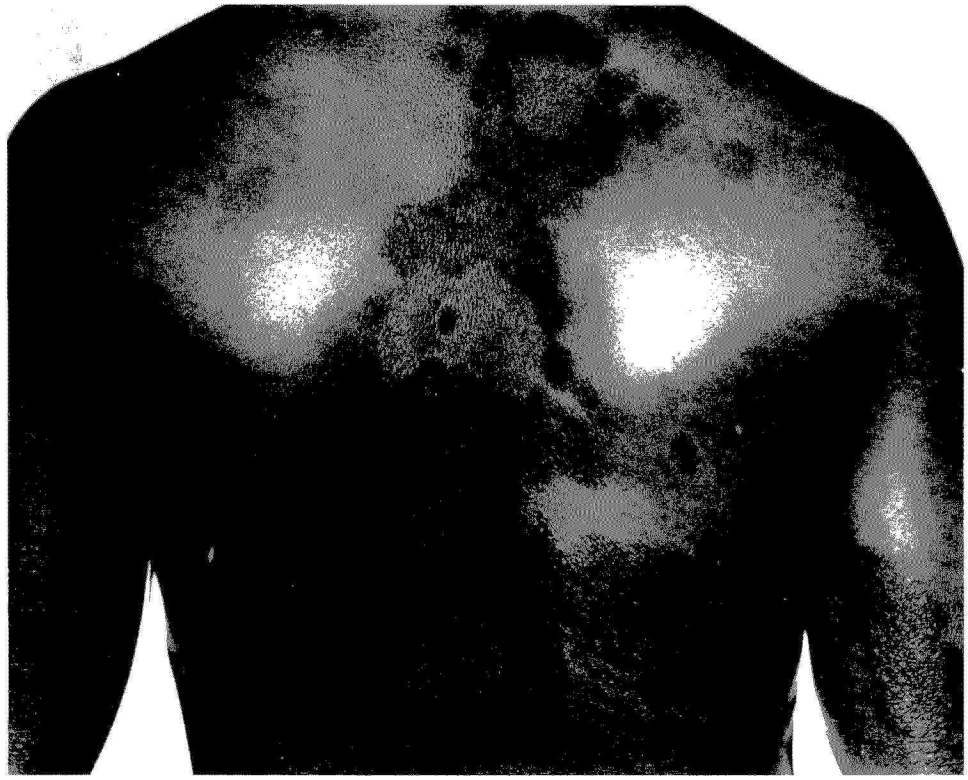

FIG. 1. Hyperpigmented areas (sites of previous lesions) on back of a patient treated with topical nitrogen mustard.

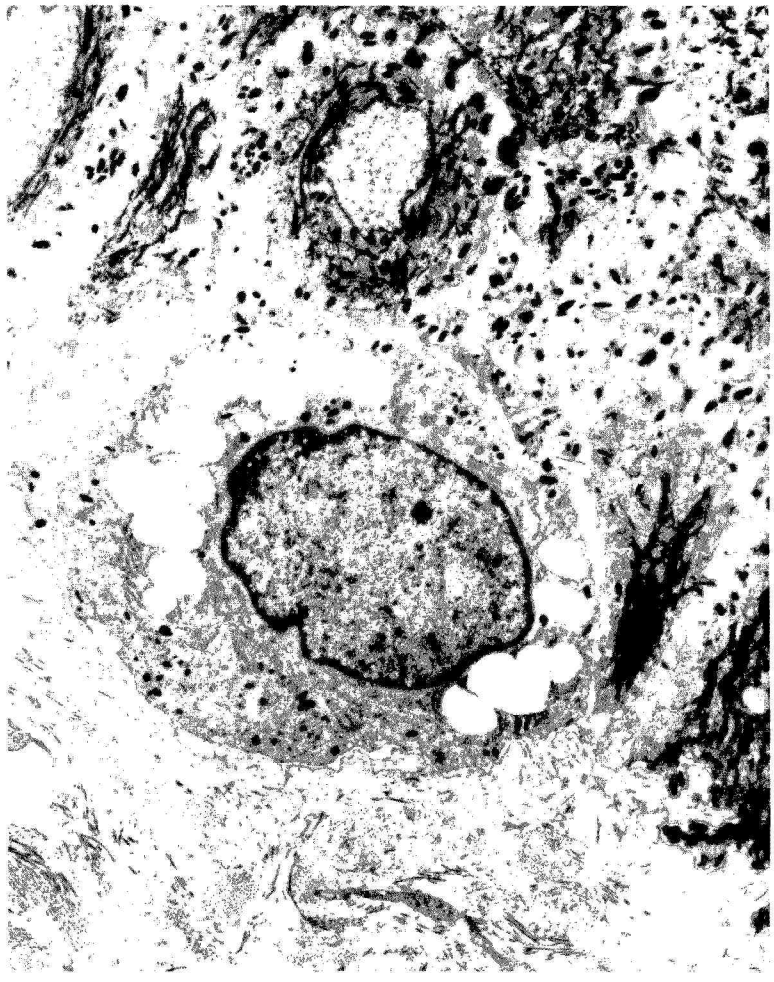

FIG. 2. Electron micrograph showing cytoplasmic vacuoles in melanocyte of patient treated with topical nitrogen mustard. × 4500.

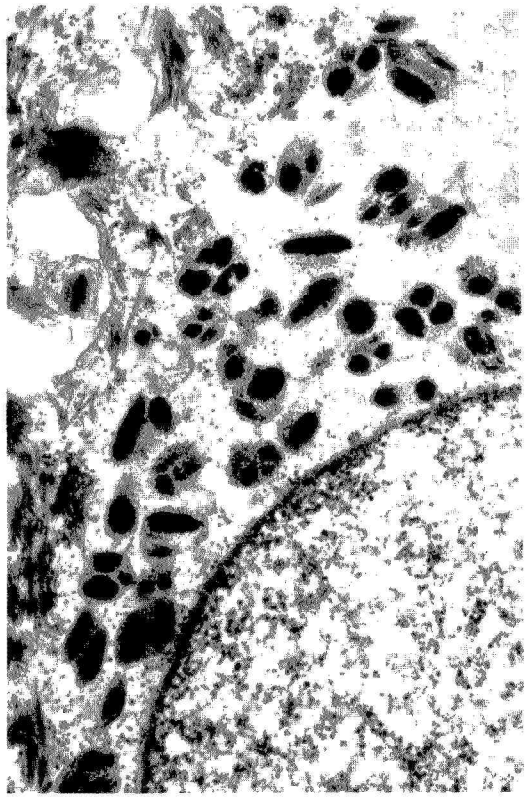

FIG. 3. Electron micrograph showing normal melanosome complexes in keratinocyte of Caucasoid skin. × 33,000.

(Figs. 5, 6). Most melanosomes were completely melanized and their greatest length was only .5 μm. An increase in the number of melanosomes was apparent in most cases but attempts were not made to quantitate this observation. Hyperpigmentation in Negroids was due to an increased number of normally nonaggregated melanosomes whose size was not increased over that of controls.

DISCUSSION

Racial differences in color among humans are due not to quantitative differences in the number of melanocytes in skin but apparently to differences in the melanosomes, i.e., number, size, distribution within the cell, and degrees of melanization [5]. Normally, melanocytes transfer melanosomes to the keratinocytes, but the fate of melanosomes differs thereafter according to race. In Caucasoids, Mongoloids, and American Indians, melanosomes are contained in groups of two or more within membrane-bound packages or phagosomes in the cytoplasm [6, 7]. These phagosomes contain acid phosphatase and apparently are lysosomes in which melanosomes are degraded [7, 8]. In Negroids, melanosomes remain unaggregated in the cytoplasm. Toda et al [9] found that hyperpigmentation in Caucasoids following treatment of skin with a combination of psoralen and ultraviolet light (UVL) was associated with an increase in number and size of melanosomes as well as a change from the aggregated to a nonaggregated state. Whereas most melanosomes in Caucasoid skin measured less than 0.8 μm in the long axis before treatment (when they were aggregated), following treatment the average length was 1.2 μm. It was concluded that aggregation of melanosomes was a size-dependent phenomenon [9, 10] and that the combination of UVL and psoralen could alter the normal genetic racial expression. Since the effect persisted up to 6 months, it was proposed that the treatment caused long-term gene derepression.

The present study shows that part of the basis of hyperpigmentation for HN2 is similar to that of UVL and psoralen in that melanosomes in Caucasoids remain unaggregated in the keratinocyte. However, in contrast to findings of Toda et al [9], the unaggregated melanosomes following HN2 were about the same size as aggregated ones in controls and all were less than 0.8 μm in the long axis. Since there was no change in size following HN2, it appears that size of melanosomes need not be the sole determining factor in aggregation. It should be noted that in the present study, although melanosomes in Negroids were larger than those in Caucasoids, the very large melanosomes in Negroids reported by Toda et al [9] were not seen.

FIG. 4. Electron micrograph of nonaggregated melanosomes within keratinocyte of normal Negroid skin. × 33,000.

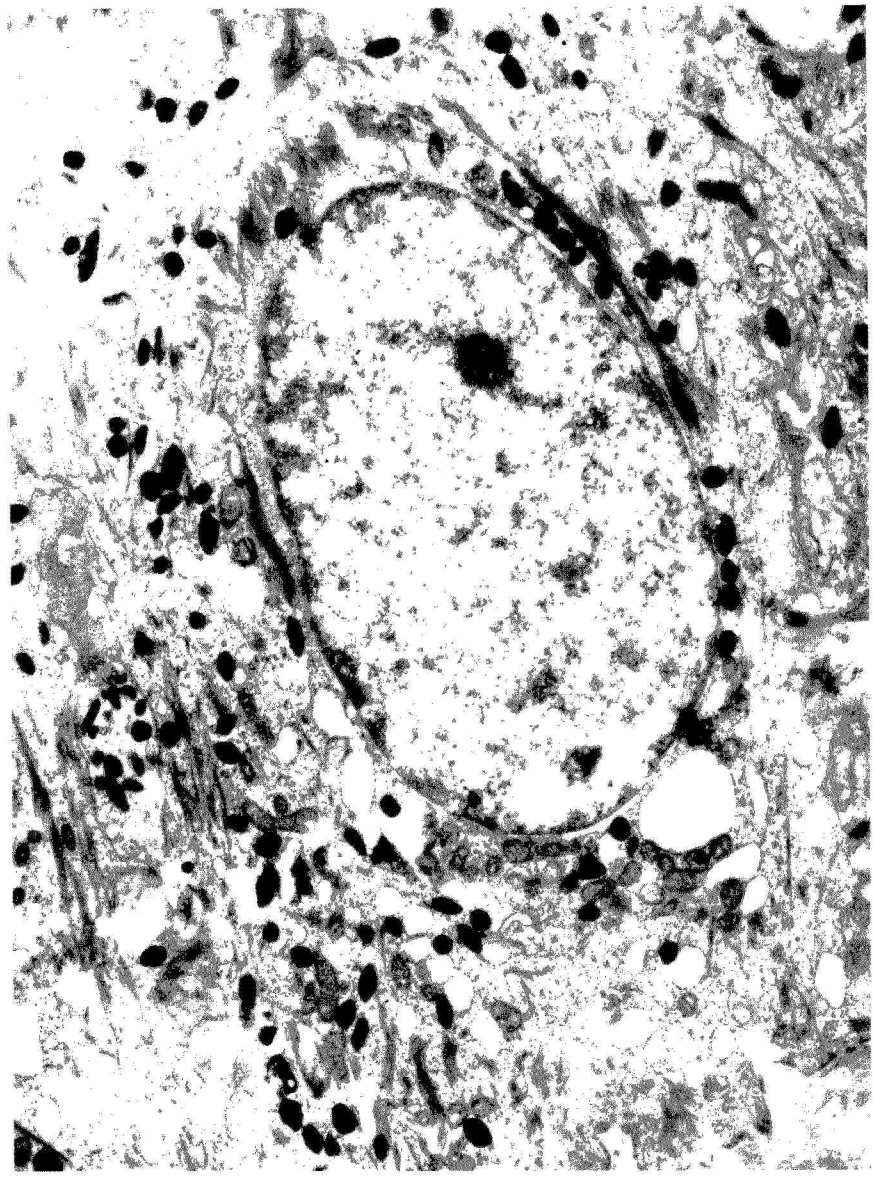

FIG. 5. Low-power electron micrograph showing nonaggregated melanosomes within keratinocytes of Caucasoid skin following topical nitrogen mustard. Small cytoplasmic vacuoles may represent sign of toxic effect of drug. × 12,000.

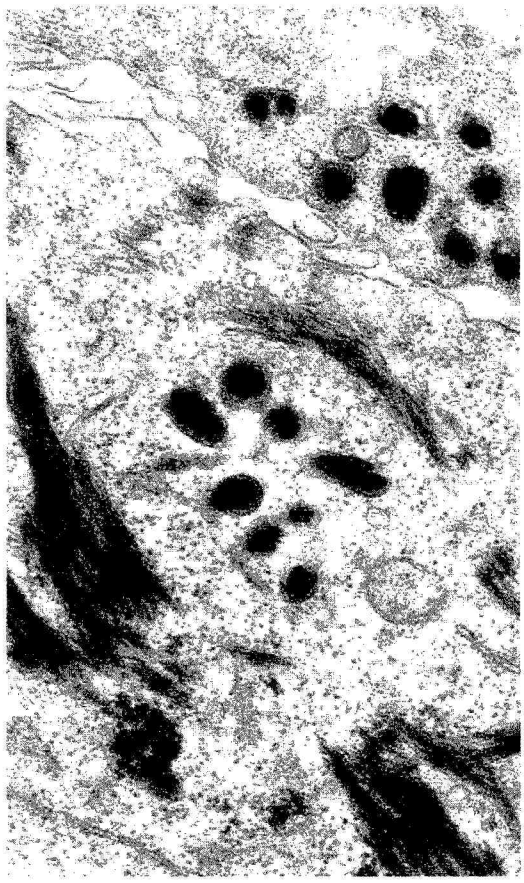

FIG. 6. Higher-power electron micrograph showing mainly nonaggregated melanosomes in keratinocytes of Caucasoid skin treated with nitrogen mustard. Compare with Negroid skin in Fig. 4. × 33,000.

The reason for alteration of melanosome aggregation in the present study is not clear. Whereas studies using psoralen and UVL were of brief duration but had long-lasting effects, those with topical nitrogen mustard were of longer duration and were still taking place at the time of biopsy. The simplest explanation would be that HN2 exerts a continuous toxic effect and that failure to aggregate melanosomes is related to functionally impaired keratinocytes. Evidence against this explanation, however, includes absence of clinical signs of pathologic change such as erythema, vesiculation, etc. coupled with a paucity of significant ultrastructural signs of cell damage. An alternative reason for failure of aggregation of small melanosomes ($<0.8\ \mu m$) might be that HN2 has a selective effect on the aggregation mechanism whereas psoralen and UVL affect both size and aggregation concomitantly. Increased rate of melanosome production coupled to lack of ability of keratinocytes to aggregate them represents another possible but unlikely explanation. Regardless of the mechanism, the present study provides additional evidence that the racial characteristics of melanosome packaging can be altered by experimental methods.

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