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Trichochromuria in Melanosis of Melanoma

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Two patients with metastasizing melanoma and diffuse melanosis have previously been reported to excrete large quantities of trichochromes in the urine. The present study describes 2 further melanoma patients with diffuse melanosis and trichochromuria. The hair of one of the patients which had been red in childhood and turned brown in adult age returned to red with the appearance of melanosis. Normal excretion of a methylated melanocytic metabolite, 6-hydroxy-5-methoxyindole-2-carboxylic acid, was observed in this patient, possibly indicating exhaustion of the methylating system. The other patient excreted large quantities of 6-hydroxy-5-methoxyindole-2-carboxylic acid. Both patients showed highly increased excretion of 5-S-cysteinyldopa. Both patients with melanosis exhibited fine electrone-dense granules in lysosomes of dermal histiocytes. The findings support the concept that trichochromes or similar pigments in dermal histiocytes are responsible for diffuse melanosis in melanoma patients. *Key words: Melanin; 5-S-Cysteinyldopa; 6-Hydroxy-5-methoxyindole-2-carboxylic acid; Methylation.* (Received April 16, 1986.)

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Diffuse melanosis may occur in metastasizing malignant melanoma, but it is a rare clinical sign in this disease. Among 161 patients with melanoma metastases examined for 5-S-cysteinyldopa excretion, 3 were found to have diffuse melanosis (1). Various explanations for the diffuse melanosis have been proposed. Fitzpatrick et al. (2) suggested that circulating melanin precursors are oxidized to melanin in the extracellular fluid or within histiocytes in the dermis. Silberberg et al. (3) considered the possibility that the melanin may be released from malignant melanoma cells and then secondarily deposited in the dermis via the circulation. Konrad & Wolff (4) and Schuler et al. (5) have produced evidence that diffuse melanosis may be the result of spread of single melanoma cells throughout the dermis. These cells may continue to produce melanosomes which eventually are deposited in dermal macrophages. Bork et al. and Adrian et al. (6, 7) made electron microscopic findings indicating that the pathogenesis of the diffuse slate blue colour was due primarily to pigment deposition within perivascular dermal macrophages. Agrup et al. (1) produced evidence that the diffuse melanosis in metastatic melanoma patients could be due to deposition of trichochromes, the simplest phaeomelanic pigments, in the tissue. In the present study the urinary excretion of 5-S-cysteinyldopa, trichochromes and of 6-hydroxy-5-methoxyindole-2-carboxylic acid was investigated in 2 melanoma patients with diffuse melanosis. 5-S-Cysteinyldopa is a precursor of phaemelanins and mixed type melanins and trichochromes represent the simplest phaeomelanins (8, 9). 6-Hydroxy-5-methoxyindole-2-carboxylic acid is a compound formed by methylation of one of the precursors of eumelanin (9). The hyperpigmented skin was examined by light and electron microscopy.

PATIENTS AND INVESTIGATIONS

The first patient was a 31-year-old man, who was redhaired in childhood. In adult age his hair colour changed to light brown. He was operated on for a cutaneous melanoma Clark III, localized to his right upper arm, 4 ½ years before the investigation. Four years later, 6 months before the investigaiton, melanoma metastases to the liver were diagnosed, and the patient received chemotherapy with DTIC®. Diffuse melanosis appeared about 2 months before our investigation, and at the same time the patient got redhaired again. The patient died 6 weeks after the investigations were done. Necropsy was not performed.

The second patient was a 67-year-old women who had 6 months earlier undergone surgery for a malignant melanoma of the back, Clark IV. She had evidence of metastases to the liver and lungs and showed metastasis in the skin and in lymph nodes and general grey-blue discolouration of the skin at the time of investigation. The patient had been treated with Interferon and cimetidine without effect on metastases. The patient died 6 weeks after the studies reported here.

24-hour urine specimens were collected in plastic bottles containing 50 ml of acetic acid and 1 g of sodium metabisulphite. Determinations of trichochromes, 5-S-cysteinyldopa, and of 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C) were carried out by methods previously described (1, 10, 11). Biopsies of pigmented skin were obtained and processed for light and electron microscopy.

Light microscopy

Sections (4 μm) from paraffin-embedded skin biopsies were stained with hematoxylin-eosin and by the Fontana-Massom method.

Electron microscopy

Biopsy specimens from hypermelanotic skin were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer with 0.1 M sucrose (pH 7.2) at 4°C. They were postfixed in 2% osmium tetroxide (adjusted to pH 7.2), stepwise dehydrated in ethanol and embedded in Agar resin 100. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Zeiss EM 10 at 60 kV.

RESULTS

The urinary excretion of the compounds studied is reported in Table I. Both patients showed strongly increased excretion of 5-S-cysteinyldopa. Trichochromes which are absent in the urine of healthy subjects and in most patients with melanoma metastasis were demonstrated in the urine of both patients. The urinary excretion of 6H5MI-2-C was 0.05 mg/24 h in the man, which is within the normal range (11). The woman showed pathologically increased excretion of 6H5MI-2-CA (1.4 mg/24 h).

Light microscopy

Hematoxylin-eosin-stained sections revealed barely visible changes. Elongated cells containing finely distributed yellow-brown pigment were seen in the corium between collagen fascicles and around capillary vessels. There was no significant inflammatory reaction. Epidermal melanocytes seemed normal in morphology and distribution. No tumour cells were observed.

In Fontana-Masson-stained sections rather numerous elongated cells were found in the corium, containing tightly packed fine pigment granules. These cells seemed diffusely distributed in the upper corium, especially concentrated in the upper stratum reticulare. In

Table I. *Urinary excretion of 5-S-cysteinyldopa (5-S-CD), 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-CA) and of trichochromes B and C (Trich)*

All values are mg/24 h

Patient	5-S-CD	6H5MI-2-CA	Trich
Man 31 years	96	0.05	2.8
Woman 67 years	48	1.4	0.3

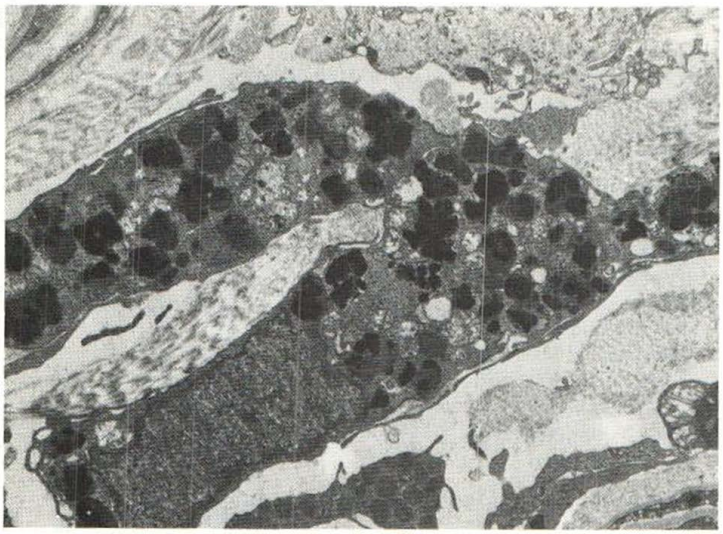

Fig. 1. Electron micrograph of 2 histiocytes in reticular dermis containing phagocytic vacuoles and accumulations of electrondense material. ×6560.

the deeper stratum reticulare they were mainly found around capillary vessels and often distributed in small groups. The epidermis showed a somewhat uneven basal pigment distribution.

Electron microscopy

The pigment-containing cells in the corium had ultrastructurally histiocytic character with phagocytic vacuoles and numerous lysosomes (Fig. 1). Some of the lysosomes contained fine electrondense granules mainly in their periphery. Several histiocytes contained round or oval but sometimes irregularly shaped (size 0.4–0.8 μm) accumulations of electrondense material, as a rule bounded by a thin single membrane. The electrondense material was finely stippled, coarsely granular or condensed to larger precipitates of varying shape (Figs. 2 and 3). Melanosomes could not be identified with certainty. Sometimes smaller

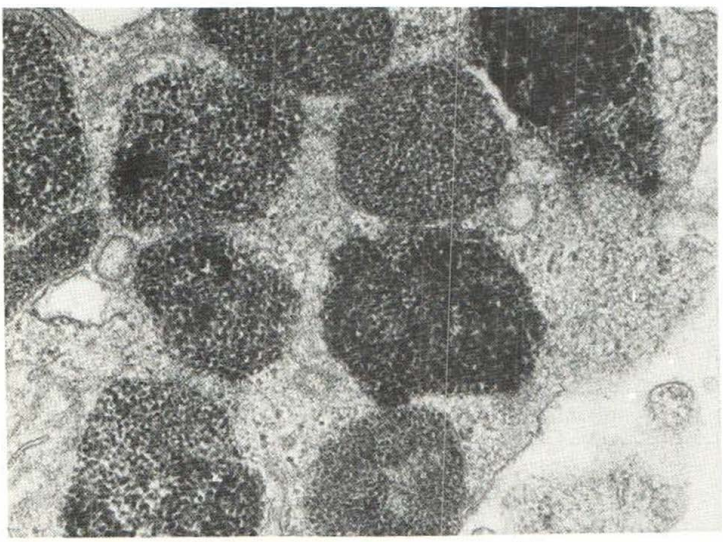

Fig. 2. Accumulations of finely stippled or coarsely granular electrondense material in a histiocyte. ×41600.

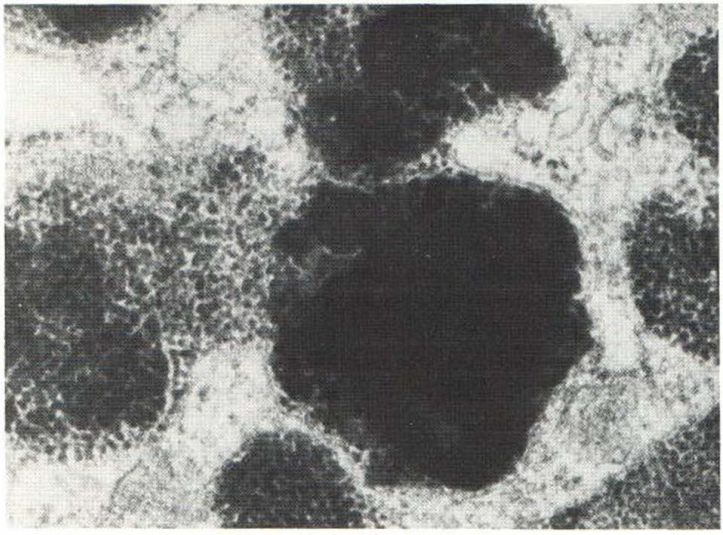

Fig. 3. Membrane-bound heavily condensed electron-dense material and free fine granules in cytoplasma. ×70 400.

collections of similar granular material were observed in the cytoplasm without any identifiable delineating membranes (Fig. 3).

The epidermal melanocytes contained melanosomes of normal appearance, and corresponding to the findings on light microscopy basal keratinocytes in some regions contained numerous melanosomes.

The microscopic findings were similar in both patients although more pronounced in the man.

DISCUSSION

Both patients excreted trichochromes B and C in the urine. Excretion of trichochromes is not seen in healthy subjects and has previously only been reported in 6 patients with advanced melanoma (1, 12).

In the first report on trichochromuria in melanoma the occurrence of diffuse melanosis in the patient was unknown to the authors (13). The patient was later reported as no. 1 in the paper by Agrup et al. (1). The patients described in the present paper increase the number of reported patients with trichochromuria to 8 and emphasizes the connection between general melanosis and trichochromuria which previously has been found in 2 patients (1, 12). The highest values of urinary trichochrome excretion in melanoma patients have been observed in the 4 patients with melanosis, 2 described in the previous (1, 12) and 2 in the present report. Trichochromes are soluble in acid and alkaline. The urinary excretion of trichochromes in patients with melanosis demonstrates that the trichochromes are formed and transported in the body under these pathologic conditions. It seems possible that trichochromes may be ingested by phagocytes and thus be the compounds responsible for the electron dense granules in the dermal phagocytes.

Trichochromes B and C have been demonstrated in red human hair (14) and in the tumour of a patient with melanoma metastasis and with urinary excretion of trichochromes (15). It is, however, not known if trichochromes are produced in melanocytes or if they appear as the result of metabolic events in other cells, like keratinocytes or macrophages, which may incorporate the precursors of trichochromes, i.e. cysteinyldopas.

The EM findings in our patients correspond closely to those reported by Adrian et al. (7). Their study did not contain any information on the excretion of trichochromes. The observations in the 2 patients with melanosis in the present study represent specific and unusual biochemical changes. Trichochromuria in melanosis has now been connected with the previously described electrone-dense granules in lysosomes of dermal histiocytes. The chemistry of trichochromes is well known and these defined pigments can be synthesized in vitro. Experimental studies on the possible formation of trichochromes in the skin from their precursors and studies on the reaction of the histiocytes to such pigments can provide further information on the pathogenesis of melanosis.

The occurrence of increased quantities of a O-methylated indolic compound 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-CA) in the urine of melanoma patients (16, 17) was confirmed in one of our patients. However, the observed normal quantities of 6H5MI-2-CA in the other patient with higher excretion of cysteinyldopa and of trichochromes may be of still greater biologic interest, since the observed normal amounts of this methylated compound may indicate that the O-methylation system may become overloaded in certain cases of advanced melanoma. It is well known that a large number of O-methylated products are formed from catecholic compounds and dihydroxyindoles in melanoma (9, 18), and it has also been demonstrated that S-adenosylmethionine, which substance is essential for O-methylation, decreases after high doses of L-dopa (19, 20). O-Methylation of cysteinyldopas and urinary excretion of such compounds in patients with melanoma have previously been reported (21, 22). But it seems possible that cysteinyldopas more than many other melanocytic metabolites remain non-methylated, since it has been demonstrated that methylation of 5-S-cysteinyldopa is catalysed extremely slowly by catechol-O-methyltransferase, despite the relatively high affinity of the enzyme for the substrate (23).

A remarkable change of hair colour was observed in the male patient. He had had red hair in childhood, but the hair had darkened and become brown with age. When melanosis developed in this patient the hair again became red.

The explanation for the observed change to the original red hair colour is not known. It seems however possible that the advanced melanoma disease may have induced protein malnutrition, which may induce colour changes; dark hair becomes lighter often reddish (24). However, the colour which developed in the patient was strikingly red and it seems possible that the colour change was in some way more closely related to specific metabolic events in diffuse melanosis.

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