

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Cancer Society (project 626-B78-07XA and 626-B78-07P), the Walter, Ellen, and Lennart Hesselman Foundation for Scientific Research, and the Edvard Weiland Foundation for Scientific Research.

REFERENCES

1. Agrup, G., Hansson, C., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Stable free radicals in phaeomelanins. Communications from the Department of Anatomy, University of Lund, Sweden, No. 4, 1976.
2. Blois, M. S., Zahlan, A. B. & Maling, J. E.: Electron spin resonance studies on melanin. *Biophys J* 4: 471, 1964.
3. Bruenger, F. W., Stover, B. J. & Atherton, D. R.: The incorporation of various metal ions into *in vivo*- and *in vitro*-produced melanin. *Radiat Res* 32: 1, 1967.
4. Chioccare, F., Novellino, E. & Prota, G.: Trimeric aldolization product of 1,4-benzothiazine. *J C S Chem Comm*, 1977, 50.
5. Commoner, B., Townsend, J. & Pake, G. E.: Free radicals in biological materials. *Nature* 174: 689, 1954.
6. Cotzias, G. C., Papavasiliou, P. S. & Miller, S. T.: Manganese in melanin. *Nature* 201: 1228, 1964.
7. Felix, C. C., Hyde, J. S., Sama, T. & Sealy, R. C.: Interactions of melanin with metal ions. ESR evidence for chelate complexes of metal ions with free radicals. *J Am Chem Soc* 100: 3922, 1978.
8. Mason, H. S., Ingram, D. J. E. & Allen, B.: The free radical property of melanins. *Arch Biochem Biophys* 86: 225, 1960.
9. McGarvey, B. R.: Line widths in the paramagnetic resonance of transition ions in solution. *J Phys Chem* 61: 1232, 1957.
10. Prota, G.: Structure and biogenesis of phaeomelanins. In *Pigmentation: Its Genesis and Biologic Control* (ed. Vernon Riley), p. 615. Appleton-Century-Crofts (Meredith Corporation), New York, 1972.
11. Prota, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Phaeomelanin pigments from a human melanoma. *Experientia* 32: 970, 1976.
12. Prota, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Occurrence of trichochromes in the urine of a melanoma patient. *Experientia* 32: 1122, 1976.
13. Sacchi, S., Lanzi, G. & Zanotti, L.: Electron spin resonance research on human hairs under varying physiological and experimental conditions. In *Advances in Biology of Skin* (ed. W. Montagna & R. L. Dobson), vol. 9. Hair Growth, p. 169. Pergamon Press, Oxford, 1969.

Diffuse Melanosis and Trichochromuria in Malignant Melanoma

G. Agrup, P. Agrup, C. Hansson, H. Rorsman, and E. Rosengren

Departments of Dermatology, Radiotherapy (Malmö) and Pharmacology, University of Lund, Sweden

Received February 28, 1979

Abstract. A previous finding of pronounced excretion of trichochromes in a patient with metastasizing melanoma and diffuse melanosis prompted this study on trichochromes in another patient with these two features. The patient now reported also excreted large amounts of trichochromes. It is suggested that diffuse melanosis in metastatic melanoma patients is due to deposition of trichochromes in the tissue.

Diffuse melanosis is uncommon in metastasizing malignant melanoma. Among 161 patients with melanoma metastasis examined by us for 5-S-cysteinyldopa excretion, 3 were found to have diffuse melanosis. In a study on trichochromes in the urine of 16 melanoma patients with metastasis we observed a very high value in one patient, who also exhibited diffuse melanosis (1). Since, like diffuse melanosis, pronounced trichochrome excretion is rare, we assumed that the occurrence of both phenomena in the same patient suggested a biological relationship. The urine of another of the 3 patients who developed diffuse melanosis was also examined, and the findings support our assumption that trichochromuria and diffuse melanosis are closely related.

MATERIAL AND METHODS

The patient investigated was a 43-year-old brown-haired man suffering from widespread melanoma metastasis. A history of a pigmented, bleeding skin tumour which had healed spontaneously antedated the appearance of lymph node metastases by 8 years. The first metastasis appeared 2 years before our investigation, and during the intervening period the patient received surgical treatment, chemotherapy with DTIC® and Bleomycin, and radiotherapy. Diffuse melanosis became widespread during the 2 months before our investigation. At the time of our study the patient showed multiple lymph node metastases and a liver scintigram consistent with liver metastases. Histology and cytology of involved lymph nodes showed histiocyte-like melanoma cells, with most of the melanin in macrophages. He died 1 month after the investigation of trichochromes with symptoms of abdominal metastasis. Necropsy was not done.

24-hour urine specimens were collected in plastic bottles containing 50 ml of acetic acid and 1 g of sodium metabisulphite. The 5-S-cysteinyl-dopa concentration was determined by methods previously described (4).

Isolation of trichochromes from the melanoma urine was carried out as follows.

A 100-ml sample of urine was adjusted to pH 13 with 5 M NaOH, and left at room temperature for 72 h under an oxygen current to convert cysteinyl-dopa and intermediate products of pigment formation into stable, colourless benzothiazine derivatives. The oxidized urine was then acidified to pH 1 with 6 M HCl, and passed on a column (2×10 cm) of Dowex 50 W, H⁺ form. After washing with M HCl (150 ml) and water the column was eluted with 0.5 M NaOH to give a brownish-yellow trichochrome-containing fraction. This was acidified, boiled for 15 min, and passed on another column (2×5 cm) of Dowex 50 W, H⁺ form. The column was washed with 100 ml M HCl and H₂O to pH 5, and with 0.2 M sodium acetate to pH 7. The material on the column was then eluted with 0.1 M NaOH, acidified, and evaporated. The residue was purified by chromatography on a column (1.5×20 cm) of Sephadex LH-20 using methanol-2 M HCl (95:5) as eluent. Under these conditions a sharply defined red band appeared which was collected and evaporated to dryness. The residue was dissolved in a minimum volume of 0.1 M HCl. The resulting solution was acidified to 2 M HCl, analysed spectrophotometrically at 530 nm to determine the total yield of trichochromes, and then fractionated by paper chromatography on Whatman 3MM with isopropanol-formic acid-concentrated HCl (30:70:1).

RESULTS AND DISCUSSION

The patient showed a 5-S-cysteinyl-dopa excretion of 40 mg/24 h. The excretion of trichochromes B and C was 5.7 mg/24 h. The excretion rate of 5-S-cysteinyl-dopa and of trichochromes was thus extremely high.

Among 17 patients with melanoma metastasis so far studied by us for trichochromes, 6 have shown trichochrome excretion in the urine, but only 2 of them have excreted very large amounts (9.0 mg and 5.7 mg). A striking clinical finding in these two patients was the presence of diffuse melanosis of the skin. The patient with diffuse melanosis and pronounced trichochromuria reported earlier was red-haired, but the one now presented had brown hair. Excretion of large amounts of trichochromes in melanoma does not therefore seem to be related to the patient's hair colour. Higher or similar 5-S-cysteinyl-dopa excretion has been noted in other patients with small or very small amounts of trichochromes in the urine, and diffuse melanosis is thus not related to the excretion of 5-S-cysteinyl-dopa (1).

Various explanations for the rare diffuse melanosis in advanced melanoma disease have been proposed. Fitzpatrick et al. (2) suggested that circulating melanin precursors are oxidized to melanin within the extracellular fluid or within histiocytes in the dermis. Silberberg et al. (5) also 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 (3) described a patient with dissemination of tumour cells within the dermis. They considered the melanosis to be due to melanosomes derived from the melanoma cells. Most of the pigment in the dermis was found in macrophages.

Our findings of pronounced renal excretion of trichochromes in diffuse melanosis suggests that this simple phaeomelanin may be responsible for the pigmentation in diffuse melanosis. Owing to the insolubility of decarboxy trichochromes B and C at physiological pH levels, it is reasonable to assume that trichochromes may be deposited in the tissues, thus giving rise to the diffuse pigmentation.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Cancer Society (projects 626-B78-07XA and 626-B78-07P), the Walter, Ellen, and Lennart Hesselman Foundation for Scientific Research, and the Edvard Weller Foundation for Scientific Research.

REFERENCES

1. Agrup, G., Lindbladh, C., Protá, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Trichochromes in the urine of melanoma patients. *J Invest Dermatol* 70: 90, 1978.
2. Fitzpatrick, Th. B., Montgomery, H. & Lerner, A. B.: Pathogenesis of generalized dermal pigmentation secondary to malignant melanoma and melanuria. *J Invest Dermatol* 22: 163, 1954.
3. Konrad, K. & Wolff, K.: Pathogenesis of diffuse melanosis secondary to malignant melanoma. *Br J Dermatol* 91: 635, 1974.
4. Rorsman, H., Rosengren, A.-M. & Rosengren, E.: A sensitive method for determination of 5-S-cysteinyl-dopa. *Acta Dermatovener (Stockholm)* 53: 248, 1973.
5. Silberberg, I., Kopf, A. W. & Gumpert, S. L.: Diffuse melanosis in malignant melanoma. *Arch Dermatol* 97: 671, 1968.