Acta Dermatovener (Stockholm) 51: 353–357, 1971

# FORMALDEHYDE-INDUCED FLUORESCENCE OF EPIDERMAL MELANOCYTES AFTER A SINGLE DOSE OF ULTRAVIOLET IRRADIATION

G. Agrup, B. Falck and H. Rorsman

*From the Department of Dermatology and Institute of Anatomy and Histology, University of Lund, Lund, Sweden*

*Abstract.* The response of human melanocytes to UV-light was studied by the histochemical method of Falck & Hillarp in 6 subjects. Biopsies were taken 72 hours after a single exposure of forearm skin to 4 × MED. UV-light increased the distance between basal fluorescent melanocytes in 2 subjects and in 1 subject the basal fluorescent melanocytes disappeared. In 3 subjects the distance between basal melanocytes remained unchanged. The fluorescence intensity of observed melanocytes was increased in all cases after UV irradiation and dendrites became visible or more readily demonstrable.

The melanocytes of human epidermis synthetize increased amounts of melanin after stimulation with ultraviolet light of erythema-producing wavelength. There is evidence that ultraviolet light increases the number of functioning epidermal melanocytes (10, 11, 15, 16, 19, 20, 21). Other authors, however, have not been able to find any significant increase in the number of melanocytes (14), and it has even been reported that the number of basal melanocytes in the epidermis may decrease after exposure to ultraviolet light (9, 12).

The results obtained may seem contradictory, but the changes in melanocyte density may depend on several factors which were not identical in the experiments reported. Thus, the response of the melanocytes may vary with species, race and anatomical site of the exposed skin and possibly also with the UV-dose, the use of single or multiple UV-exposures, and the time between radiation and biopsy. Finally, the number of melanocytes observed depends on the techniques used. The widely used dopa reaction reveals tyrosinase activity in melanocytes, but a "dopa-premelanin reaction" may detect not only tyrosinase-active melanocytes, but also enzymically inactive or hypoactive melanocytes (11).

The histochemical method of Falck & Hillarp demonstrates a yellow or green fluorescence of epidermal melanocytes (4, 6) and the intensity of this fluorescence has been found to be increased in some conditions with increased pigmentation, e.g. after exposure to therapeutic X-rays (13) and in pregnancy (18). By contrast, the fluorescence intensity of epidermal melanocytes is low in genetically determined hyperpigmentation in negroes (1). The method of Falck & Hillarp detects catechol intermediates in pigment formation. Previous studies indicate that the fluorescence of Caucasian melanocytes may be a measure of cell function. The method has not previously been applied in studies on the effect of UV on melanocytes. The present study is concerned with the formaldehyde-induced fluorescence of melanocytes stimulated by ultraviolet light.

## MATERIAL AND METHODS

Six Caucasians, 2 women and 4 men, aged 24 to 52 years, were studied. All were healthy, but one man had an ulcer on his leg. All reacted normally to light. All subjects were given a 4 × MED dose of ultraviolet radiation from a set of Westinghouse sun lamps on a 2 × 2 cm area of the radial aspect of the forearm. 72 hours after the radiation, 2 mm punch biopsies were taken, two from the irradiated area and one from the non-irradiated skin, close to the exposed site. The biopsy specimens were dropped in propane cooled in liquid nitrogen, then dehydrated by freeze-drying, treated with paraformaldehyde at 80°C for 1 hour, embedded in paraffin and sectioned at 8 $\mu$. One specimen from the irradiated skin was not treated with paraformaldehyde, but otherwise treated in exactly the same way as the other 2 specimens. For

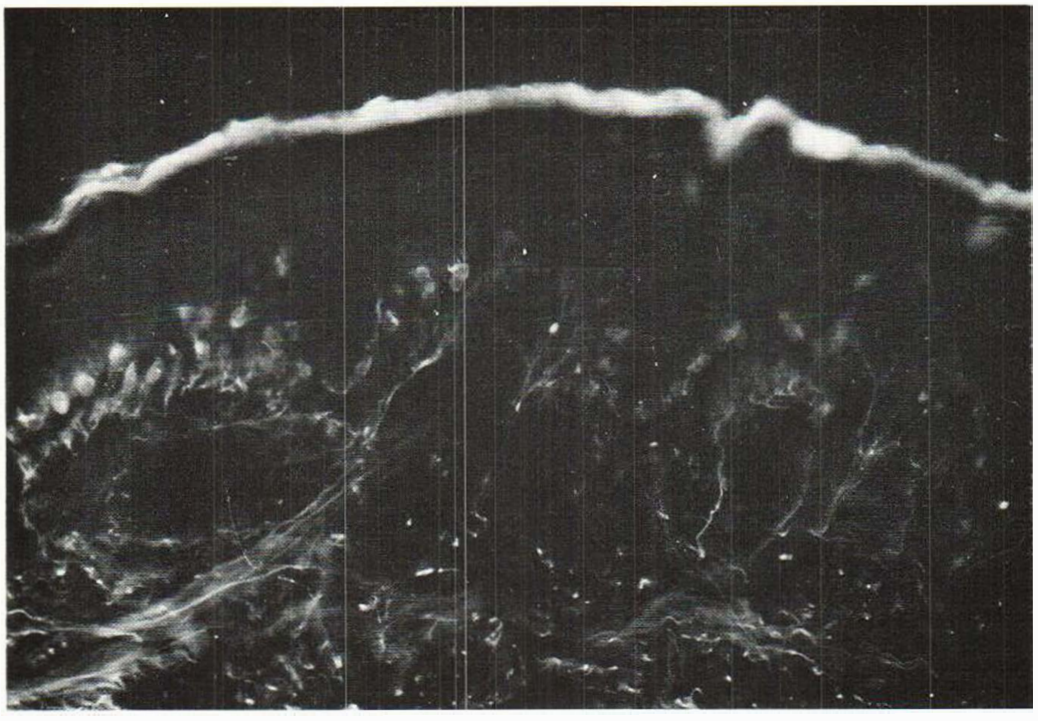

*Fig. 1.* Weakly fluorescent basal melanocytes in skin not exposed to UV. ×940.

fluorescence microscopy, a dark-field condenser with oil immersion was used. All technical details are given by Falck & Owman (5).

## RESULTS

All subjects showed erythema of the irradiated skin. In 2 subjects there was also a pronounced infiltration. The spontaneous fluorescence of keratin and dermal fibres of the specimen of UV-irradiated skin, which was not treated with paraformaldehyde, was similar to that of untreated skin (4).

In the formaldehyde-treated specimens from skin that was *not exposed to UV* the number and fluorescence intensity as well as the morphology of the melanocytes differed in the subjects studied. In 5 cases the distance between individual epidermal melanocytes never exceeded 3 melanocyte diameters and was often much smaller. In the sixth case the distance between melanocytes was 1 to 6 diameters. The fluorescence intensity was low in the melanocytes of 5 subjects, but strong in the sixth. This subject was the one with longer distances between the melanocytes. In 3 cases fluorescent thin dendrites were seen, but 3 others had no fluorescent dendrites. In all cases the melanocytes were located in the basal layer of the epidermis, but in 1 subject some melanocytes were observed slightly above the basal layer.

In *UV-irradiated skin* the distance between the melanocytes compared with the non-irradiated skin remained unchanged in 3 subjects examined. In 2 subjects the distance between basal melanocytes was definitely increased in irradiated skin and in 1 case all the basal melanocytes had disappeared. In 5 subjects, including the one who had lost all basal melanocytes, some fluorescent melanocytes were observed just above the basal layer. The distance from the basal layer was generally not more than the diameter of a melanocyte.

In all cases the fluorescence intensity of melanocytes of irradiated skin was stronger than that of the melanocytes of control skin of the same subject.

Melanocyte dendrites were observed in all speci-

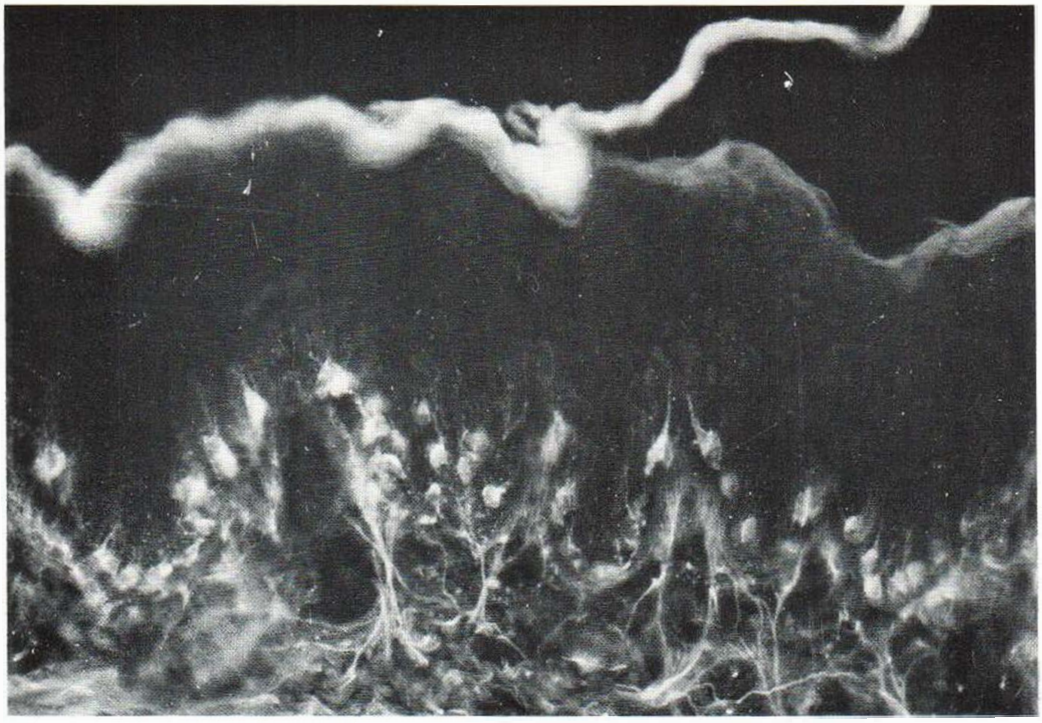

*Fig. 2.* Strongly fluorescent melanocytes in skin exposed to 4 × MED of UV-light. × 940.

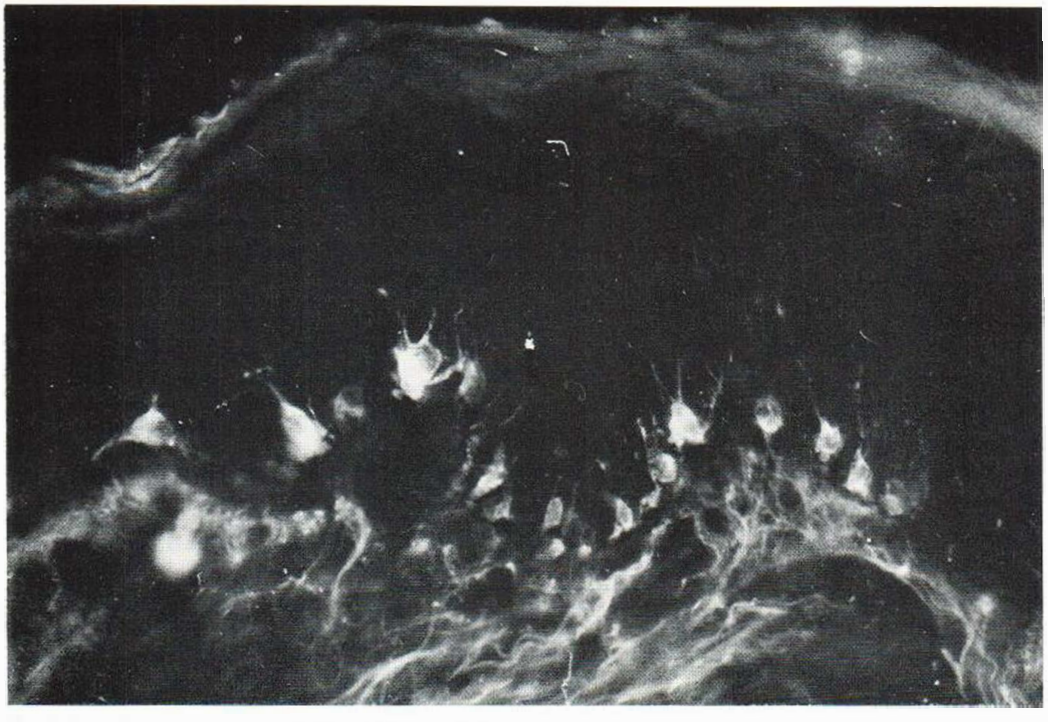

*Fig. 3.* Suprabasally located melanocytes with coarse dendrites in skin exposed to 4 × MED of UV-light. × 1 300.

mens of irradiated skin. The dendrites seen in irradiated skin were invariably more distinct and longer than those in control skin.

## DISCUSSION

It is generally thought that UV-stimulation increases the tyrosinase activity of melanocytes. Since the method of Falck & Hillarp detects dopa and/or other intermediates in the melanin synthesis (17), it was assumed that UV-light would increase the population of fluorescent melanocytes. The longer distance between melanocytes of UV-irradiated skin of 2 subjects and the total disappearance of basal fluorescent melanocytes in 1 subject were therefore unexpected findings. It is possible that the UV-exposure given induced inactivity of some melanocytes, but a rapid copolymerization of formed intermediates in some activated cells could also explain the diminished number of fluorescent melanocytes.

Furthermore, a high melanin content might quench the fluorescence of fluorophores present. This has been considered a possible explanation for the absence of fluorescence in negro melanocytes with a high melanin content (1), but the pigmentation of melanocytes was not strikingly increased in our UV-irradiated subjects.

The increased fluorescence intensity of the melanocytes in UV-irradiated skin shows that dopa, dopa peptides or sulphur-containing dopa compounds are formed in increased amounts after exposure to UV.

Ultrastructural studies of dopa and tyrosine reactions 72 hours after UV-irradiation have revealed reaction products, especially in the smooth endoplasmic reticulum, closely associated with the Golgi apparatus and in the Golgi saccules as well as in intracytoplasmic vesicles of all sizes, ranging from 50 nm to 500 nm in diameter (8).

Our method does not permit examination of the ultrastructural localization of the fluorophores. In melanoma cells the fluorescence is mainly localized to distinct granules (3), but it is quite possible that compounds giving fluorescence may occur also outside any cell organelles.

The finding of fluorescent melanocytes in the upper part of the basal layer or just above the basal layer in irradiated skin is in agreement with the previous observation by Fan et al. (7) of dopa-positive melanocytes at this level.

The epidermis of irradiated skin was not markedly wrinkled, but the higher incidence of suprabasally located fluorescent melanocytes may, of course, be due to tangential sectioning of basally located melanocytes in more vertically oriented dermal-epidermal junctions (2).

## ACKNOWLEDGEMENTS

This investigation has been supported by grants from The Swedish Medical Research Council (B71-14X-712-06A and B71-14X-56-07A) and from The Swedish Cancer Society (67-111).

## REFERENCES

1. Agrup, G., Falck, B., Olivecrona, H. & Rorsman, H.: Formaldehyde-induced fluorescence of epidermal melanocytes of Caucasian and negro skin. Acta Dermatovener (Stockholm) *51:* 350, 1971.
2. Becker, S. W., Jr, Fitzpatrick, Th. B. & Montgomery, H.: Human melanogenesis: Cytology and histology of pigment cells (melanodendrocytes). Arch Derm (Chicago) *65:* 511, 1952.
3. Ehinger, B., Falck, B., Jacobsson, S. & Rorsman, H.: Formaldehyde-induced fluorescence of intranuclear bodies in melanoma cells. Brit J Derm *81:* 115, 1969.
4. Falck, B., Jacobson, S., Olivecrona, H. & Rorsman, H.: Fluorescent dopa reaction of nevi and melanomas. Arch Derm (Chicago) *94:* 363, 1966.
5. Falck, B. & Owman, C.: A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. Acta Univ Lund, Sectio II, no. 7, 1965.
6. Falck, B. & Rorsman, H.: Observation on the adrenergic innervation of the skin. Experientia *19:* 205, 1963.
7. Fan, J., Schoenfeld, R. J. & Hunter, R.: Study of epidermal clear cells with special reference to their relationship to cells of Langerhans. J Invest Derm *32:* 445, 1959.
8. Hunter, J. A. A., Mottaz, J. H. & Zelickson, A. S.: Melanogenesis: Ultrastructural histochemical observations on ultraviolet irradiated human melanocytes. J Invest Derm *54:* 213, 1970.
9. Miller-Milinska, A. & Staricco, R. G.: Quantitative response of the melanocytes to ultraviolet light. J Invest Derm *41:* 7, 1963.
10. Mishima, Y. & Tanay, A.: The effect of $\alpha$-methyldopa and ultraviolet irradiation on melanogenesis. Dermatologica (Basel) *136:* 105, 1968.
11. Mishima, Y. & Widlan, S.: Enzymically active and inactive melanocyte populations and ultraviolet irradiation: Combined dopa-premelanin reaction and electron microscopy. J Invest Derm *49:* 273, 1967.
12. Mitchell, R. E.: The effect of prolonged solar radiation on melanocytes of the human epidermis. J Invest Derm *41:* 199, 1963.
13. Olivecrona, H. & Rorsman, H.: The effect of roentgen irradiation on the specific fluorescence of epidermal

melanocytes. Acta Dermatovener (Stockholm) *46:* 403, 1966.

14. Pathak, M. A., Sinesi, S. J. & Szabó, G.: The effect of a single dose of ultraviolet radiation on epidermal melanocytes. J Invest Derm *45:* 520, 1965.
15. Quevedo, W. C., Szabó, G., Virks, J. & Sinesi, S. J.: Melanocyte populations in UV-irradiated human skin. J Invest Derm *45:* 295, 1965.
16. Quevedo, W. C., Szabó, G. & Virks, J.: Influence of age and UV on the populations of dopa-positive melanocytes in human skin. J Invest Derm *52:* 287, 1969.
17. Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Fluorometry of a dopa peptide and its thioether. Acta Dermatovener (Stockholm) *51:* 179, 1971.
18 Rorsman, H. & Sjöberg, N.-O.: Increased formaldehydeinduced fluorescence of epidermal melanocytes in pregnancy. Acia Dermatovener (Stockholm) *51:* 358, 1971.
19. Snell, R. S.: The effect of ultraviolet irradiation on melanogenesis. J Invest Derm *40:* 127, 1963.
20. Wolff, K. & Winkelmann, R. K.: Nonpigmentary enzymes of the melanocyte—Langerhans cell system. *In* Advances in Biology of Skin, vol. VIII, The Pigmentary System (ed. W. Montagna & F. Hu). Pergamon Press, Glasgow, 1967.
21. Zelickson, A. S. & Mottaz, J.: The effect of sunlight on human epidermis. Arch Derm (Chicago) *101:* 312, 1970.

*Received April 5, 1971*

H. Rorsman, M.D.
Department of Dermatology
University Hospital
S-221 85 Lund
Sweden