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Effects of Aging and Chronic Sun Exposure on Melanocytes in Human Skin

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Both aging and sun exposure have well-documented effects on the human melanocyte system. Paired biopsies of habitually exposed and nonexposed skin from adjacnet anatomic sites were obtained from 8 donors aged 28 to 80 yr in order to study the combined effect of chronic actinic irradiation and chronologic aging. Density of dopa-positive melanocytes was roughly twofold higher in the exposed than in the nonexposed skin at all ages, suggesting an irreversible effect of sun exposure. Melanocyte density declined approximately 6 to 8% of the surviving population per decade in both sites. Dopapositivity of individual melanocytes was consistently greater in the chronically exposed skin than in the nonexposed skin of the same subject and did not vary with age. These data strengthen and expand earlier observations of age-related melanocyte changes, and explain the apparent paradox of a generalized increase in pigmentation and simultaneous decrease in melanocyte density which frequently accompany advancing age. In addition, the present study suggests that the principal effect of chronic sun exposure on the human pigmentary system is not premature "aging" as currently recognized histologically, but rather activation and/or proliferation of the exposed melanocytes.

The number of 3,4-dihydroxyphenylalanine (dopa)-reactive melanocytes in human skin is known to decrease with age by approximately 10 to 20% of the surviving population each decade [1–3], in nonsun-exposed areas (thigh and buttock) as well as in habitually sun-exposed areas (forehead). On the other hand, chronically sun-exposed skin of elderly individuals is characterized by mottled irregular pigmentation and frequently by generalized hyperpigmentation, as compared to younger individuals with a similar complexion [4,5]. The changes in melanocyte morphology and dopa-reactivity which correspond to the tan produced by short-term exposure to sunburn-spectrum ultraviolet light have been well studied [1,6], but those underlying the hyperpigmentation of aged, actinically altered skin have not.

The following study was undertaken to explore the apparent contradiction between decreasing melanocyte density and increasing pigmentation that occurs with age in sun-exposed skin and to document the melanocyte changes which result from chronic ultraviolet irradiation.

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MATERIALS AND METHODS

Patient Material

Eight adult male Caucasian volunteers aged 28 to 80 yr, known to be in good health and specifically not to have diabetes, were recruited for this study. All were life-long residents of New England and had similar histories of outdoor activities. No subject was taking medication other than multivitamins. On the basis of their past experience with tanning and sunburning, subjects were classified as skin type I–IV, according to Fitzpatrick's criteria [7]. Biopsies were performed during the winter and spring; all subjects denied recent sun exposure of the biopsied areas.

Four-millimeter diameter punch biopsies, using local 2% xylocaine anesthesia, were obtained from the normal-appearing skin of the medical and lateral aspects of the upper arm. Each biopsy was bisected and one-half was used for this study. Each specimen was coded by a third party prior to further processing so that the investigators did not know its anatomic site. Informed consent was obtained from all subjects.

Dopa-Procedure

The combined "skinsplitting" technique and dopa (L-3, 4-dihydroxyphenylalanine) incubation of Staricco and Pinkus was used [8]. Each skin specimen was incubated in 2 N NaBr at 37°C for 45 min and the epidermis was then removed from the dermis. The epidermis was incubated at 37°C in a 1:1000 solution of dopa (Hoffman-LaRoche, Inc., Nutley, New Jersey) buffered by Sorensen's phosphate buffer to pH 7.4, the solution was replaced hourly until dopa-positive melanocytes could easily be distinguished under the microscope from the normal background color of the epidermis, usually 2 to 4 hr. The epidermal pieces were fixed in 10% formol saline, counterstained with paracarmine, dehydrated, mounted on glass slides with the dermo-epidermal junction facing up, and examined and photographed with a Zeiss Ultraphot II. Epidermal melanocytes were counted in each specimen using the light microscope in 10 independent fields. The number of melanocytes was expressed per square millimeter of skin surface, following original methods of Billingham and Medawar [9] and Szabo [10]. Each ocular field was 0.14 mm^2 and an objective × 25 magnification was used.

RESULTS

In all subjects, the melanocyte density was greater on the habitually sun-exposed (lateral) aspect of the upper arm than on the nonexposed (medial) aspect (Table). In addition, the number of dopa-positive melanocytes per square millimeter decreased strikingly with age in both sets of biopsies (Fig 1). The rate of decline was 8% of the surviving melanocyte population per decade for the nonexposed skin. The rate of decline for melanocyte density in the chronically sun-exposed skin has been computed omitting the data points for subjects 7 and 8, biopsied in June when the ambient temperature permitted direct exposure of the arms to sunlight.

Examination of the paired specimens for each subject revealed consistently stronger dopapositivity or darker staining for melanocytes in the chronically sun-exposed skin (Fig 2). Intensity of the dopa stain did not correlate with donor age in either exposed or nonexposed specimens. For 7 of the 8 subjects, there was little or no difference in size and dendricity between the melanocytes in chronically sun-exposed and nonexposed skin. Only in subject 2, aged 71 yr, were the melanocytes larger

Density of dopa-positive melanocytes

Subject	Age	Skin type	Melanocytes/mm^2 ± SE mean, SD	
			Nonexposed[a]	Exposed[b]
1	74	III	635 ± 30, 100	1265 ± 60, 120
2	71	III	335 ± 15, 50	935 ± 25, 80
3	61	II	785 ± 20, 65	1560 ± 35, 115
4	56	III	935 ± 20, 70	1415 ± 40, 120
5	80	II	565 ± 20, 60	1220 ± 25, 75
6	56	III	795 ± 25, 80	1470 ± 20, 55
7	41	II	1255 ± 20, 60	2335 ± 35, 110
8	28	II	915 ± 25, 85	3075 ± 45, 140

[a] Nonexposed: medial aspect of upper arm.
[b] Exposed: lateral aspect of upper arm.

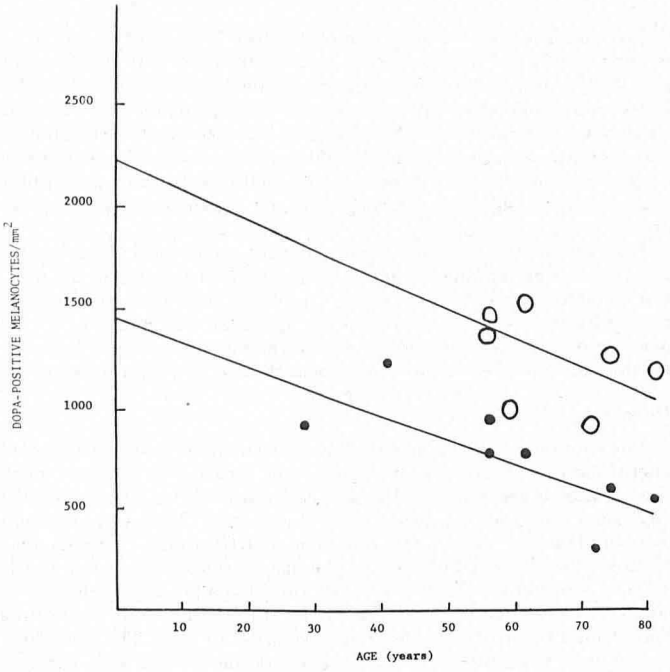


FIG 1. Relationship between age and melanocyte density in nonexposed skin (*closed dots*) and chronically sun-exposed skin (*open dots*), corrected for possible recent sun exposure. y = −12 + 1475, correlation coefficient = −.75, degree of certainity >95%, for nonexposed skin; y = −14.5 + 2275, correlation coefficient = −.76, degree of certainity >90%, for exposed skin.

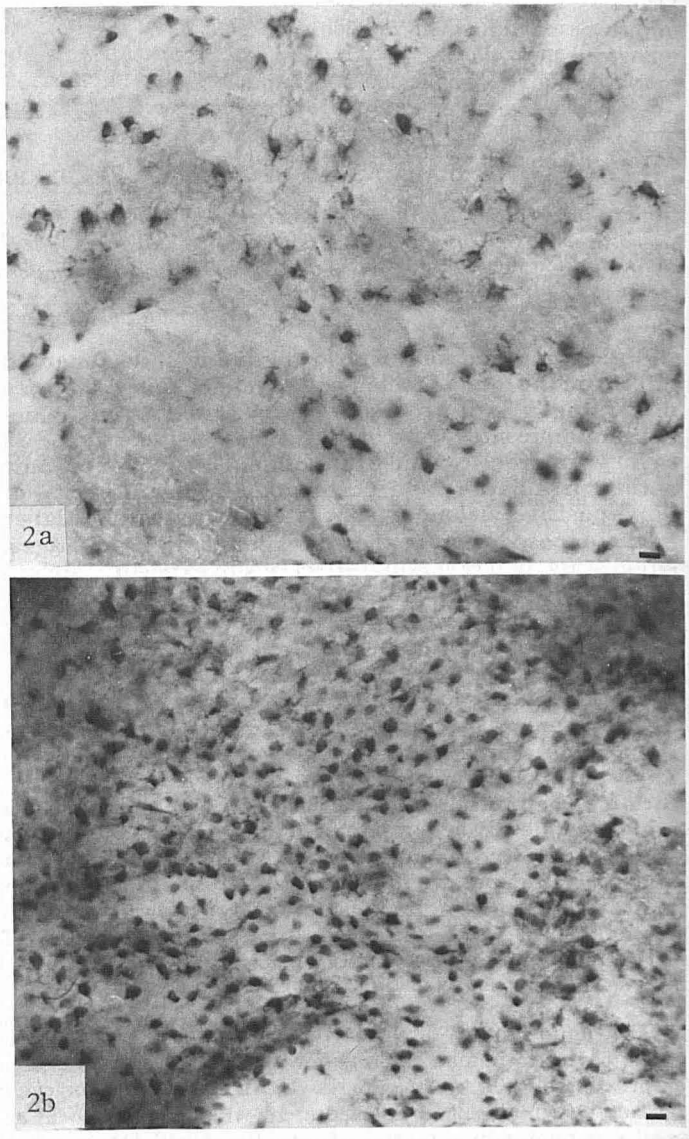


FIG 2. Dopa-incubated "split skin" preparations of the upper arm of subject 3, age 61. *a*, Nonexposed skin. *b*, Chronically sun-exposed skin. Note stronger dopa positivity of melanocytes in (*b*). No difference in size or dendricity is seen. *Scale* bars 10 μm.

and more dendritic in the chronically sun-exposed specimen (Fig 3). The variability in size, dendricity, and staining among neighboring melanocytes was similar in each specimen; there was no discernible correlation with either donor age or biopsy site.

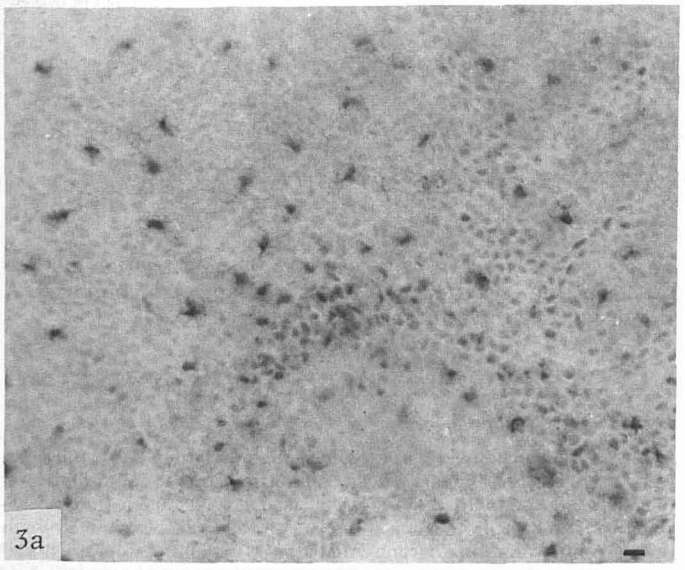
Four of the subjects were skin type II and four were skin type III. There was no correlation between age and skin type (Table).

DISCUSSION

Studies of age-associated changes are heir to several methodological problems [11]. All gerontologic studies of the human melanocyte system, including the present one, are cross-sectional analyses, which compare individuals of different ages to each other, rather than longitudinal analyses, which compare the same individuals to themselves at different ages. The present study differs from previous work in this field, however, in that paired coded biopsies were obtained from single donors, all of similar complexion and life-long residents of the same geographic area with comparable histories of past sun exposure. In contrast, skin specimens for the former studies [1,2] were collected incidentally during major surgical procedures, from only one site per donor in most cases. It was not possible to control for donor complexion or amount of prior sun-exposure, nor could the specimens be processed "blindly." The important controls employed in the present study greatly strengthen the conclusions which can be drawn from this small group of subjects examined at a single point in time.

Our data confirm earlier findings [1–3] that the populations of dopa-positive melanocytes in human skin gradually decreases with age throughout adulthood. The rate of decline noted for our subjects, 8% per decade in nonexposed areas, is not significantly less than the 10 to 20% rates measured previously. Moreover, the present study establishes that melanocyte density is approximately twofold higher in chronically sun-exposed skin and shows a decline in density with advancing age similar to that in nonexposed skin. This suggestion was also inherent in a single earlier investigation employing 2 groups of 12 subjects in whom forehead or thigh specimens were studied [2].

The only previous systematic attempt to investigate the effect of ultraviolet exposure on melanocyte number and morphology concerned short-term effects [1]. Immediately after 10 to 15 sun-lamp exposures delivered over a two-week period, biopsies of irradiated and control, nonirradiated buttock skin were compared in each of 12 subjects, aged 29 to 65 yr. The plot of melanocyte density versus donor age for these exposed



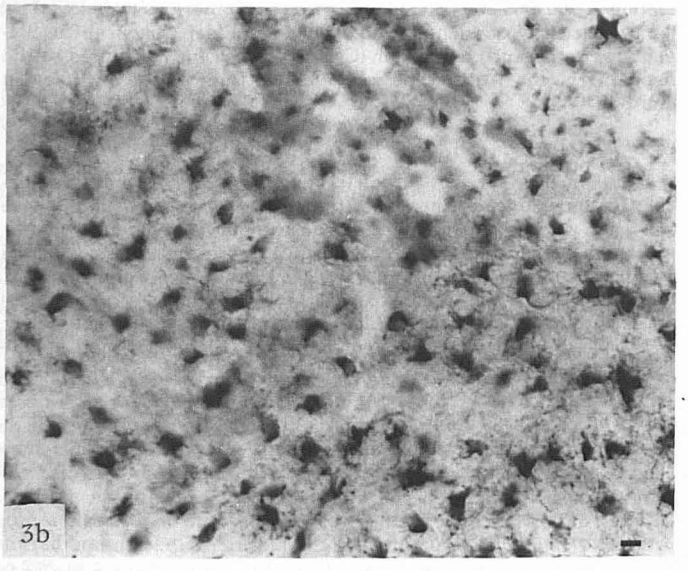


FIG 3. Dopa-incubated "split skin" preparations of the upper arm of subject 2, age 71. *a*, Nonexposed skin. *b*, Chronically sun-exposed skin. Note larger size of melanocytes and prominent dendricity in (*b*). *Scale* bars 10 μm.

and nonexposed sites strikingly resembles Fig 1 of the present study, except that the melanocyte density is considerably less at all ages in chronically exposed skin than in acutely exposed skin. Comparison of the data in these 2 studies underlines the possibility that, despite their denials, subjects 7 and 8 had exposed the outer arm to sun during the month preceding their biopsies, as their values fall along the regression line for age versus melanocyte density in recently irradiated skin.

This study strongly suggests that repeated sun exposure can irreversibly alter the number of dopa positive melanocytes in human skin. At least 6 of the 8 subjects had no ultraviolet irradiation of the upper arm for approximately 6 mo prior to examination of the skin, and the 3 oldest subjects denied any substantial sun exposure of the area for many years. Furthermore, our data may help to explain the "regional distribution of melanocytes" first described 20 yr ago [12] and referenced in standard texts with the implication that these differences are inherent to the human body [13–15]. Review of the conventional diagram reveals that, with the exception of the male genitalia, adult melanocyte density is roughly proportional to the expected cumulative sun exposure, being highest on the face, intermediate on the distal extremities, and lowest on the trunk. The fact that the medial and lateral arm, anatomically adjacent sites with markedly different cumulative sun exposure, yielded such different values in the present study further implies that the previously measured "regional variations" were spurious.

At the clinical level, the paradox of generalized hyperpigmentation in aged habitually sun-exposed skin (when compared to skin of a younger person with similar complexion) which occurs despite a decreased melanocyte density, may be explained by greater dopa-positivity of the individual chronically irradiated melanocytes.

Unfortunately, the dopa-staining technique employed in this and earlier investigations cannot distinguish between a change in melanocyte number and a change in tyrosinase activity of a constant number. More sophisticated histologic or biochemical techniques will be necessary to resolve this problem.

Finally, it must be noted that the major age-associated change in human pigmentary system, a decline in the melanocyte density apparent after dopa-staining, is not accentuated in habitually exposed skin. Melanocyte density is indeed twofold higher, not lower, that in adjacent nonexposed skin. Therefore, at least for melanocyte populations, chronic sun exposure does not accelerate aging as currently defined histologically, but rather appears to have a net stimulatory effect on exposed cells.

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