Arch Dermatol Res (1986) 278:352–362

Archives of Dermatological Research

Differential Analysis of Experimental Hypermelanosis Induced by UVB, PUVA, and Allergic Contact Dermatitis Using a Brownish Guinea Pig Model

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Summary. In moderately colored guinea-pig skin, UVB, PUVA, and allergic contact dermatitis were shown to induce hyperpigmentation that resembled the pigmentary changes observed in mongoloid human skin. Using this model, we examined the effects of chemical agents, including tyrosinase inhibitors and sunscreen agents, on the color changes induced by UV irradiation. The daily exposure of brownish guinea-pig skin to UVB irradiation at a variety of energies for 3 successive days induced clearly visible black pigmentation on the irradiated rectangular areas of the flank within a few days of irradiation, the maximum being reached about 1 week after irradiation, i.e., similar to the changes that occur in pigmented human skin. Split epidermal sheets prepared from untreated pigmented guinea pigs exhibited 200–400 melanocytes/mm^2; 1 week after UV irradiation, the applied areas show an increased number of strongly dopa-positive melanocytes with stout dendrites (800–1,000 cells/mm^2). UVA irradiation following an intraperitoneal injection of 8-methoxypsoralen (8-MOP) also produced black pigmentation 1 week after irradiation, and this was paralleled by a marked increase in the number of dopa-positive melanocytes in dopa-reacted split epidermal sheets. Allergic contact dermatitis produced by the application of 1-phenylazo-2-naphtol induced hyperpigmentation after an interval of about 14 days in 10 of the 21 allergy-acquiring animals examined. This induced pigmentation was accompanied by an increase in the number of dopa-positive melanocytes as compared to the number seen in controls. In contrast, allergic contact dermatitis produced by the application of dinitrochlorobenzene failed to induce such a high ratio of postpigmentation, with only 3 of the 21 allergy-acquiring animals showing hyperpigmentation and 5 showing depigmentation; in the latter, there was a slight decrease in the number of dopa-positive melanocytes. To study the preventive effect of tyrosine inhibitors on UVB-induced pigmentation, daily topical applications of these compounds were performed after three daily UVB irradiations. Treatment with 10% hydroquinone for 10 days interrupted UVB-induced pigmentation and resulted in a marked reduction in the number of epidermal melanocytes as compared to the number found in UVB-irradiated, untreated control skin.

Key words: Hyperpigmentation – UVB – UVA – Allergic contact dermatitis – Guinea pig

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Introduction

A number of animal models such as black-haired, light-skinned C-57 BL black mice [3, 4, 22, 26], DBA/2 mice [14], hairless mice [11] and monkeys [6] have been used for testing the effects of UV on melanogensis as well as the effects of various chemical agents which cause hyperpigmentation. In contrast to routinely used mice, the skin of the lightly colored guinea pigs used in our laboratory has a moderate number of melanocytes and melanosomes not only in pilary structures but also in the epidermis, i.e., similar to the distribution found in human skin. These animals show a good response to UV and chemicals. We have found this system to be readily reproducible and used it to clarify the differential mechanisms of hypermelanosis experimentally induced by UVB, PUVA, and allergic contact dermatitis. We also tested the therapeutic control of such induced hyperpigmentation by melanogenic inhibitors.

Materials and Methods

Experimental Procedure

Groups of five guinea pigs with brownish pigmentation (tortoiseshell guinea pigs) were used in the experiments. Five separate areas of the flank of each animal were exposed to UVB

light from an SE lamp (290–320 nm; Toshiba, Japan) for 5–15 min/day for 3 successive days (total energy, 900–2,700 mJ/cm^2 per day). The skin reaction, including pigmentation, scaling, and erythema, was observed over the next 18 days. Pigmentation was assessed according to the following scale: 0, no pigmentation; 1+, minimal visible pigmentation; 2+, moderate pigmentation; 3+, intense deep pigmentation. Scaling was assessed according to the following scale: 0, no scaling; 1+, slight scaling; 2+, moderate scaling; 3+, marked scaling. Erythema was assessed as follows: 0, no reaction; +, slight erythema; 1+, apparent erythema; 2+, moderate erythema with edema; 3+, crust or necrosis. In order to evaluate the time course of the UVB-induced pigmentation using histochemistry, six different rectangular areas of the same animals were daily exposed to UVB light (total energy, 900 mJ/cm^2 per day) for 3 days starting from Day, 0, 3, 5, 8, 10, or 17; on Day 17, biopsies were taken (with the animal under ether anesthesia) for the preparation of split epidermal sheets. To assay the preventive effect of tyrosinase inhibitors, the five separate areas on the flanks of other animals were irradiated with UVB daily for 2 days (total energy, 900 mJ/cm^2 per day), and 0.2 ml 10% tyrosinase inhibitor in ethanol was daily painted onto each of the irradiated areas for the next 13 days. A control irradiated area was treated with ethanol alone over the same period. Biopsies of the flank skin were taken 15 days after the clinical evaluation of induced or interrupted pigmentation.

For PUVA treatment, guinea pigs received an intraperitoneal injection of 3 mg/kg methoxypsoralen (8-MOP) 30 min prior to UVA exposure (dose, 252–2,268 mJ at 365 nm) using an FL20S-BLB lamp (Toshiba, Japan). To test the effect of sunscreen agents on UVB-induced pigmentation, the experimental animals were treated with 5% para aminobenzoic acid (PABA) in ethanol on five separate areas and, 10 min later, were irradiated once with 0.2–1.04 J/cm^2 UVB light. This process was repeated daily for 4 days. Test sites were observed 14 days later to determine the minimal melanogenic dose (MMD), and biopsies were taken for the preparation of split epidermal sheets in order to evaluate the melanocyte population. The MMD was determined as being the smallest dose required to produce minimally visible pigmentation at 14 days. The sun-protection factors (SPFS) were calculated by dividing the dose required to produce minimal tanning on the sunscreen-treated skin by the dose required to produce minimal tanning on untreated skin.

Sensitization Method

All procedures for the induction of an allergic reaction were performed using the cumulative contact-enhancement test (CCET) of Tsuchiya et al. [29]. In brief, an area on the back of tortoiseshell guinea pigs (weight, 250–300 g) was shaved with electric clippers and then with an electric razor. A patch of lint colth (2 × 4 cm) containing 0.2 ml test solution was applied to the shaved area for 24 h under closed conditions. The patch-test unit consisted of a lint cloth (2 × 4 cm) attached to 2-inch-wide Blenderm. After application, the unit was covered with two layers of Silky Tex (Tokyo Eizai). Both edges of the tape were then secured to the skin with Elatex (Tokyo Eizai) in order to prevent the unit from peeling off.

As sensitization procedures, the 24 h closed-patch test using 0.1% 1-phenylazo-2-naphthol (PAN) or 1% dinitrochlorobenzene (DNCB) in ethanol was performed in 21 animals every third day for 2 weeks (maximum, four applications). An injection of Freund's complete adjuvant was intradermally administered prior to the third closed-patch test. An untreated group of five animals was used as a control. Eleven days after the last patch test, challenge procedures were performed daily for 3–4 days by applying 0.01 ml ethanol solution containing 0.01%, 0.1%, or 0,5% PAN, or 0.3%, 0.5%, or 0.8% DNCB to a nontreated area on the back of guinea pig. The reaction was evaluated over a period of 43 days using the criteria already described, and biopsies were then taken in order to evaluate the melanocyte population.

Histochemical Procedure

Skin specimens (2.5 × 2.5 cm) were removed from the flanks of guinea pigs. The tissues were rinsed in 0.1 *M* phosphate buffer (pH 6.8) and incubated in 1 *M* sodium bromide for 5 h at 37° C. The epidermal sheets separated from the dermis were fixed in 10% cold neutral formalin for 30 min, washed twice with 0.1 *M* phosphate buffer (pH 6.8), and incubated in 0.1% dihydroxyphenylalanin(dopa) in 0.1 *M* phosphate buffer (pH 6.8) for 5 h. The number of melanocytes (per square millimeter) was counted using an Olympus-BHA microscope at a magnification of × 200. In each specimen, the number of melanocytes was calculated by averaging the numbers found in 50–100 fields.

Electron-Microscopic Procedure

Skin specimens were minced and fixed in 2.5% glutaraldehyde (GTA) in 0.1 *M* cacodylate buffer (pH 7.2) containing 1% sucrose for 2 h. They were then washed with cacodylate buffer containing 1% sucrose, and postfixed in 1% OsO_4 in the same cacodylate buffer. After dehydration in a graded series of alcohol (50%–100%), the sample were treated with propylene oxide and, after pretreatment in a mixture of propylene oxide and Epon, embedded in Epon. Thin sections were cut using a Porter-Blum MT-I ultratome, stained with lead citrate, and examined using a JOEL 100-CX electron microscope.

Results

Effect of UVB on Pigmented Guinea Pig

In tortoiseshell guinea pigs whose skin color was uniformly brown, diffuse brownish-yellow pigment was seen on the flanks of untreated animals (Fig. 3, colored plate). This color resembled the skin color of Mongolians. Daily exposure of the brownish-yellow skin to UVB irradiation at a variety of energies for 3 successive days induced black pigmentation on the irradiated rectangular areas of the flank (Fig. 3). Black pigmentation appeared on the 3rd day following UVB exposure and reached its maximum after about 10–15 days. This time course of pigmentation was similar to that observed when mongoloid skin is exposed to UVB irradiation. The appearance of UV-induced pigmentation depended on the extent of the original pigmentation of the guinea-pig skin as well as on the dose of UVB administered. Thus, in guinea pig skin with less pigment, the pigmentation tended to be weak or absent. At high UVB levels exceeding the minimum erythema dose (MED), marked scaling (probably caused by the rapid turnover rate) was occasionally observed a few days after the last UVB treatment, thus interrupting the appearance of the pigmentation (data not shown). We found that doses (0.9 J) of shortwave ultraviolet light which were noninflammatory when repeated at daily intervals induced more marked

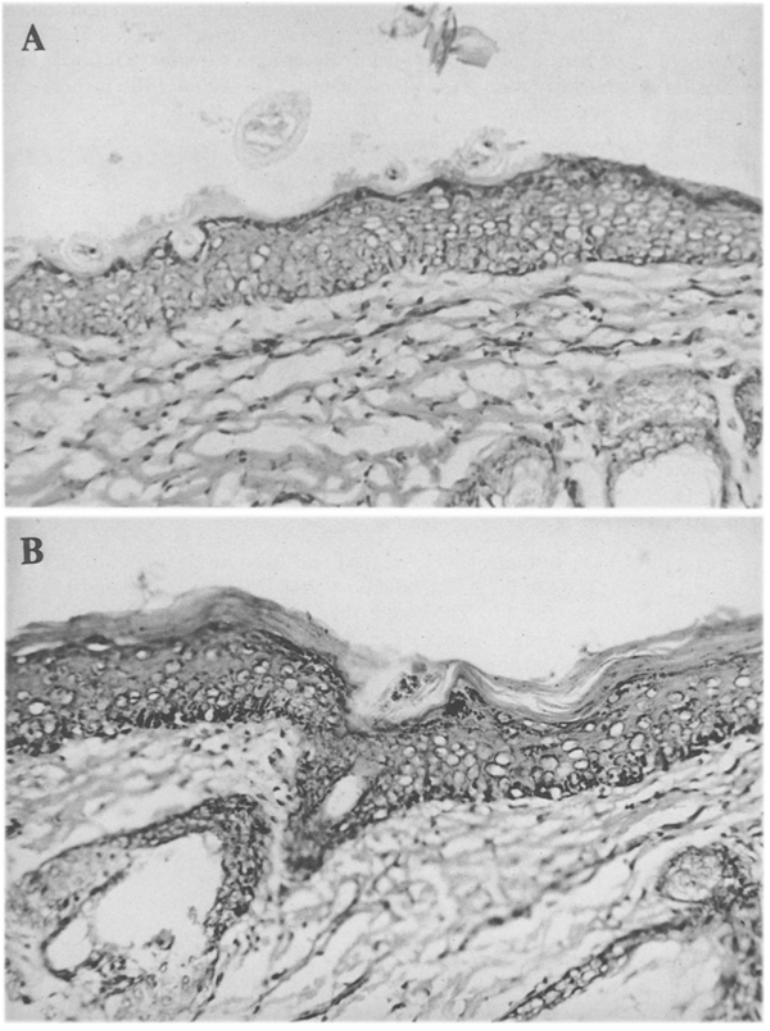

Fig. 1. Light microscopy of H&E-stained sections of non-UVB-irradiated control skin (**A**) and UVB-irradiated skin obtained 15 days later (**B**). × 540

pigmentation than a single exposure (2.7 J) to a large dose of inflammation-inducing light. Hematoxylin-and-eosin staining of cryostat sections of skin prepared on the 15th day after UVB treatment (Fig. 1) showed a large number of melanocytes with heavily pigmented granules, even in the absence of dopa reaction; in contrast, very few melanocytes were found in the skin of untreated pigmented guinea pigs.

Figure 2 shows photomicrographs of dopa-reacted epidermal sheets on the 13th day after the last of three daily UVB irradiations. The irradiated skin contained strongly dopa-positive melanocytes with stout dendrites as well as clearly increased numbers of melanocytes as compared to nonirradiated skin. In the first 10 days following UVB exposure, the number of dopa-positive melanocytes in dopa-reacted split epidermal sheets increased from 450 to 800 cells/mm^2, reaching a maximum of 820 cells/mm^2 on the 17th day (Table 1), i.e., corresponding to the visible appearance of pigmentation.

Effect of Sunscreen Agents on UVB-Induced Pigmentation

The sunscreen agent, PABA was examined for its protective potential against UVB-induced pigmentation; daily topical applications were followed by UVB irradiation for 4 successive days. In the separate rectangular areas of skin examined, the UVB exposure ranged from 1.8×4 to 7.2×4 J, with the average MMD being about 2.47×4 J on the 7th day (determined in five animals). It was found that 5% PABA partially blocked the pigmentation reaction, although it almost completely inhibited the inflammatory reaction (see Table 1). The sun-protection factor of 5% PABA was calculated to be about 7.5 from the MMDs

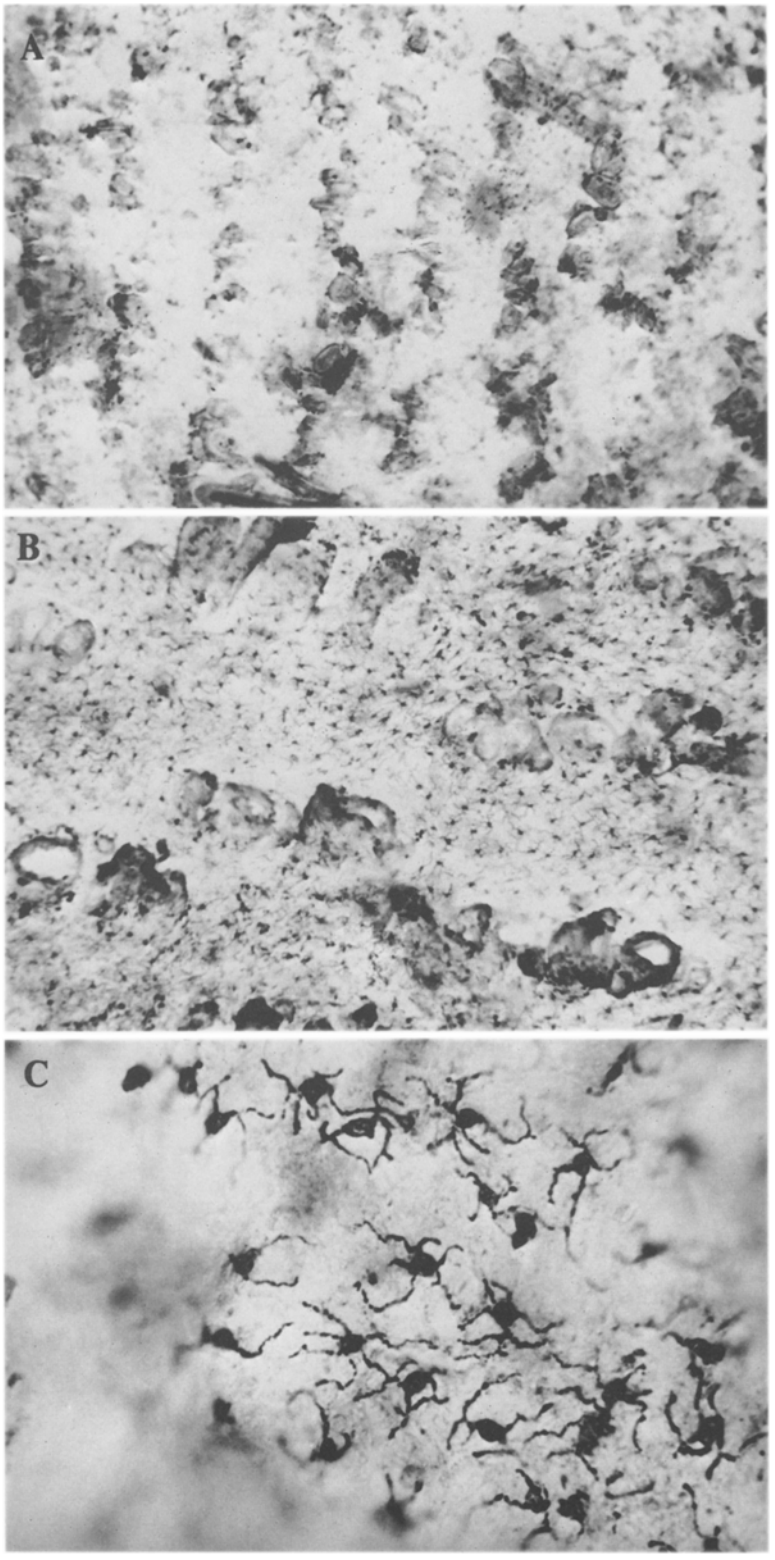

Fig. 2A – C. Distribution of epidermal melanocytes in the flank skin on the 13th day after the last UVB exposure. **A** Control unirradiated skin; **B, C** irradiated skin. Photomicrographs of dopa-incubated split epidermal sheets. **A, B** ×270; **C** ×1,080

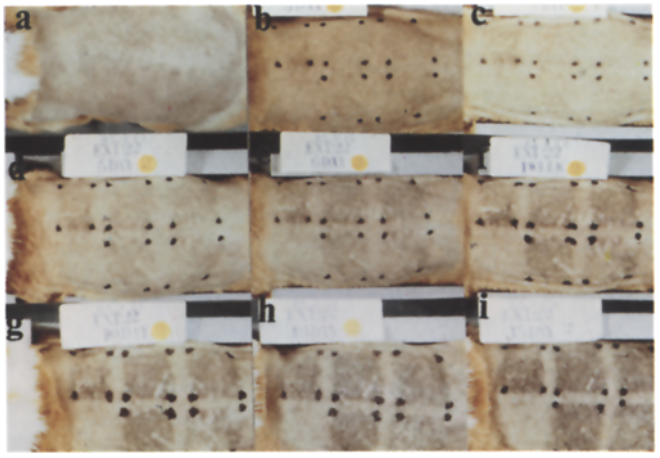

Fig. 3. Tortoise-shell guinea pigs with brownish-yellow pigmentation responded well to UVB irradiation: guinea-pig skin before UVB exposure (*a*); skin immediately after the 3rd UVB exposure (*b*) — three UVB exposure did not cause distinct erythema. Appearance of the skin is shown on Day 1 (*c*) and on Days 2 (*d*), 3 (*e*), 4 (*f*), 7 (*g*), 10 (*h*), and 12 (*i*). The hyperpigmentation on the rectangular areas that were exposed to UVB light is clearly visible

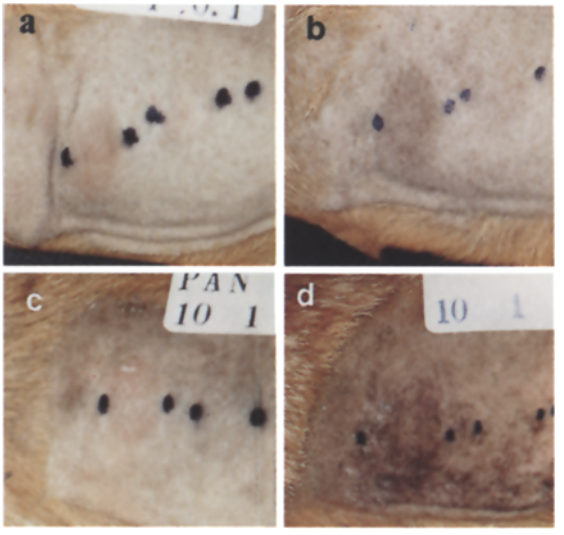

Fig. 5. Effect of PAN allergic reaction on epidermal pigmentation; *a*, allergic reaction observed after challenge with 0.5% PAN; *b*, pigmentation induced 43 days after the allergic reaction shown in *a*; *c*; allergic reaction observed after challenge with 0.1% PAN; *d*, pigmention induced after the allergic reaction shown in *c*

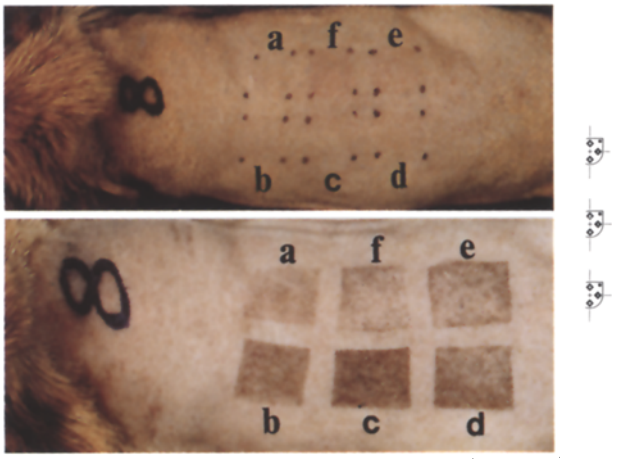

Fig. 4. Effect of PUVA treatment on epidermal pigmentation: *top* guinea-pig skin on the 2nd day after a single PUVA treatment. Slight erythema is visible on some rectangular areas which had been exposed to UVA (4.2 mW/cm^2) for 1 (*a*), 2 (*b*), 3 (*c*), 5 *d*), 7 (*e*), and 9 (*f*) min after an intraperitoneal injection of 8-MOP. *Bottom* Guinea-pig skin on the 22nd day after PUVA treatment. Marked pigmentation was induced in the treated rectangular areas in which the pigmentary reaction did not correspond to the intensity of the erythema reaction observed on the 2nd day. The optimum pigmentary skin response occurred after 3 min of exposure to UVA

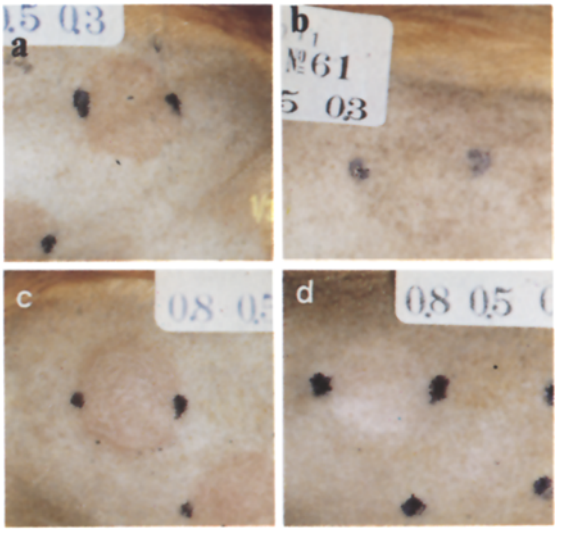

Fig. 6A–D. Effect of DNCB allergic reaction on epidermal pigmentation. **A** Allergic reaction observed after challenge with 0.3% DNCB; **B** weak pigmentation induced 43 days after the allergic reaction shown in **A**; **C** allergic reaction observed after challenge with 0.8% DNCB; **D** depigmentation exceeding the original color level induced 43 days after the allergic reaction shown in **C**

of control and treated skin. In split epidermal sheets obtained from a representative experimental animal, the number of dopa-positive melanocytes in areas exposed to less than the MMD dose was similar to that found in the non-UVB-irradiated control, but a marked increase in the number of melanocytes was found in areas exposed to more than the MMD dose (Table 1).

Table 1. Changes in the number of melanocytes caused by UVB, PUVA, and allergic contact dermatitis, and the corresponding clinical appearance of pigmentation

Treatment	UV irradiation (min)	Time after treatment (days)	Degree of pigmentation	Number of melanocytes/mm^2 (mean ± SD)
UVB (3 mW/cm^2 × 3)	5	0	−	444 ± 60
	5	3	±	578 ± 88
	5	5	+	592 ± 100
	5	8	+	706 ± 72
	5	10	+	802 ± 90
	5	17	++	821 ± 154
UVB (3 mW/cm^2) × 4	5	7	++	1,067 ± 80
(UVB + 5% PABA) × 4	10	7	±	250 ± 34
(UVB + 5% PABA) × 4	15	7	±	305 ± 42
(UVB + 5% PABA) × 4	20	7	++	361 ± 31
(UVB + 5% PABA) × 4	30	7	++	425 ± 46
(UVB + 5% PABA) × 4	40	7	++	863 ± 100
No UVB	0	−	−	241 ± 43
UVB (3 mW/cm^2) × 3	5	14	++	855 ± 118
UVB × 3 + EtOH	5	14	++	739 ± 129
UVB × 3 + HQ	5	14	−	190 ± 33
UVB × 3 + TBC	5	14	Depigmentation	0
8-MOP without UVA	−	22	−	213 ± 14
	1	22	−	475 ± 76
8-MOP + UVA (4.2mW/cm^2)	3	22	++	715 ± 45
	9	22	+	213 ± 16
Not PAN sensitized				
Untreated control		43	−	226 ± 21
0.01% PAN		43	−	251 ± 28
0.5% PAN		43	−	281 ± 12
PAN sensitized				
Untreated control		43	−	270 ± 26
0.01% PAN		43	−	259 ± 28
0.1% PAN		43	−	283 ± 47
0.5% PAN		43	++	342 ± 47
No DNCB sensitization				
Untreated control		43	−	228 ± 51
0.3% DNCB		43	−	233 ± 14
0.8% DNCB		43	−	251 ± 25
DNCB sensitization				
Untreated control		43	−	280 ± 14
0.3% DNCB		43	−	255 ± 25
0.5% DNCB		43	−	233 ± 12
0.8% DNCB		43	Depigmentation	220 ± 31

HQ, Hydroquinone; TBC, t-butylcatechol

Effect of UVA/8-MOP Treatment on Pigmentation

A single UVA irradiation (252 − 2,268 mJ) performed 30 min after an intraperitoneal injection of 8-MOP (3 − 18 mg/kg body weight) produced cutaneous phototoxic reactions of varying intensity (erythema, edema, and necrosis) depending on the doses of UVA and 8-MOP administered. At a dose (3 mg/kg 8-MOP; 756 mJ) which produced only minimal inflammation of the skin, pigmentation increased about 1 week after a single PUVA treatment, reaching its maximum after 22 days (Fig. 4). In contrast, PUVA in doses (12 mg/kg 8-MOP; 342 mJ) sufficiently large to cause a severe inflammatory reaction in the skin seemed to be injurious to pigment cells, for such doses resulted in hypopigmentation exceeding the original skin color

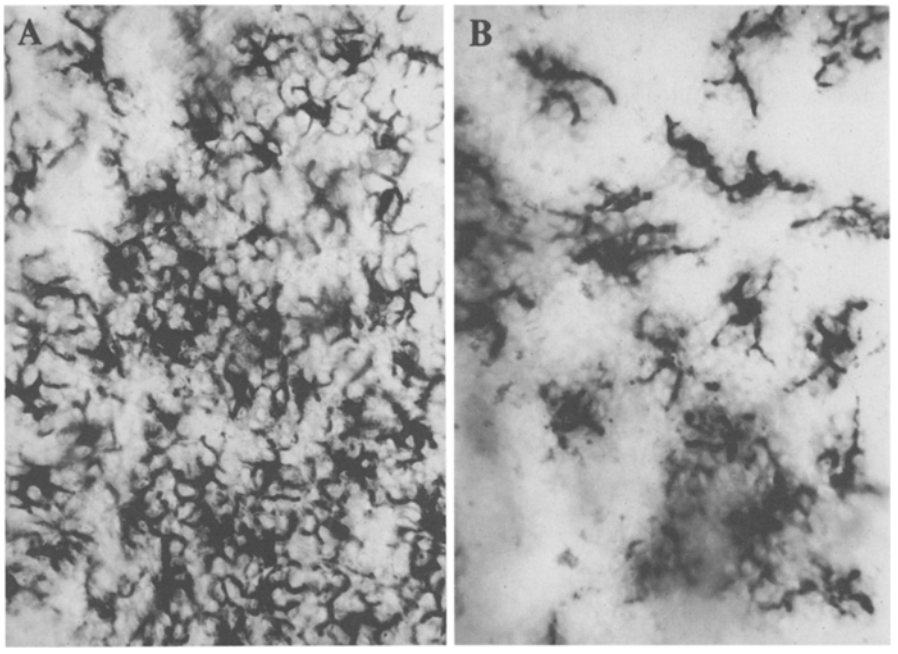

Fig. 7A, B. Distribution of epidermal melanocytes in flank skin. **A** Epidermis on the 22nd day after PUVA treatment; **B** untreated control epidermis. ×870

(data not shown). Dopa staining of split epidermal sheets derived from PUVA-treated skin (Fig. 7) exhibited an inreased number of dopa-positive melanocytes as compared to the untreated control (Table 1).

Effect of PAN Allergy on Pigmentation

In order to evaluate the effect of allergic reactions on pigmentation, we compared PAN and DNCB with regard to the degree of hyperpigmentation which occurred after their allergic reactions. The PAN-allergy reaction induced using the CCET method caused hyperpigmentation that began to appear within 14 days, reaching its maximum intensity about 40 days after the induction of the allergic reaction, as shown by the appearance of erythema and edema following the challenge application of PAN (Fig. 5). Of the 21 animals that exhibited a PAN-allergic reaction, 10 also had hyperpigmentation. In nonsensitized groups, no inflammatory or subsequent pigmentary reactions occurred following the application of PAN. In contrast, the DNCB-allergic reaction failed to induce such a high ratio of postpigmentation, with only 3 of the 21 allergy-acquiring animals showing hyperpigmentation and 5 showing depigmentation (Fig. 6). Even when pigmentation was induced by DNCB allergy, its intensity was relatively weak as compared to that produced by PAN. This hyperpigmentation seemed to be selective for allergic reactions, since an acquired allergic reaction (as manifested by erythema) was a prerequisite for its appearance in all cases. In no case did a suberythematous challenge dose of PAN to animals which had acquired allergy induce hyperpigmentation. In hyperpigmented areas caused by PAN allergy, there was a slight but significant increase in the number of dopa-positive melanocytes in split epidermal sheets (Fig. 8A; Table 1), while the size of hypopigmented areas induced by DNCB allergy showed a slight decrease in these cells (Table 1). Hematoxylin-and-eosin sections of hyperpigmented areas caused by PAN allergy (Fig. 8B) revealed melanin granules dropping into the upper dermis, and electron microscopy showed these to be phagocytosed melanosomes within the macrophage (Fig. 8C). The appearance of such dermal melanophages seemed to occur more frequently in PAN-induced hyperpigmentation than in DNCB-induced pigmentation.

Preventive Effect of Tyrosinase Inhibitors on UVB-Induced Pigmentation

In our system, the tyrosinase inhibitors, azelaic acid, adipic acid, 4-tertiary butyl catechol (TBC), and hydroquinone (HQ), were topically applied for 13 successive days after the last UV irradiation. Daily applications of 10% HQ in ethanol for 13 days inhibited the appearance of UVB-induced pigmentation as compared to UVB-irradiated, untreated skin, but the control pigmented color was maintained (Fig. 9A, B). TBC treatment also prevented the induction of increased pigmentation by UVB exposure, but resulted in hypopigmentation that exceeded the control pigmented color; no remaining dopa-positive

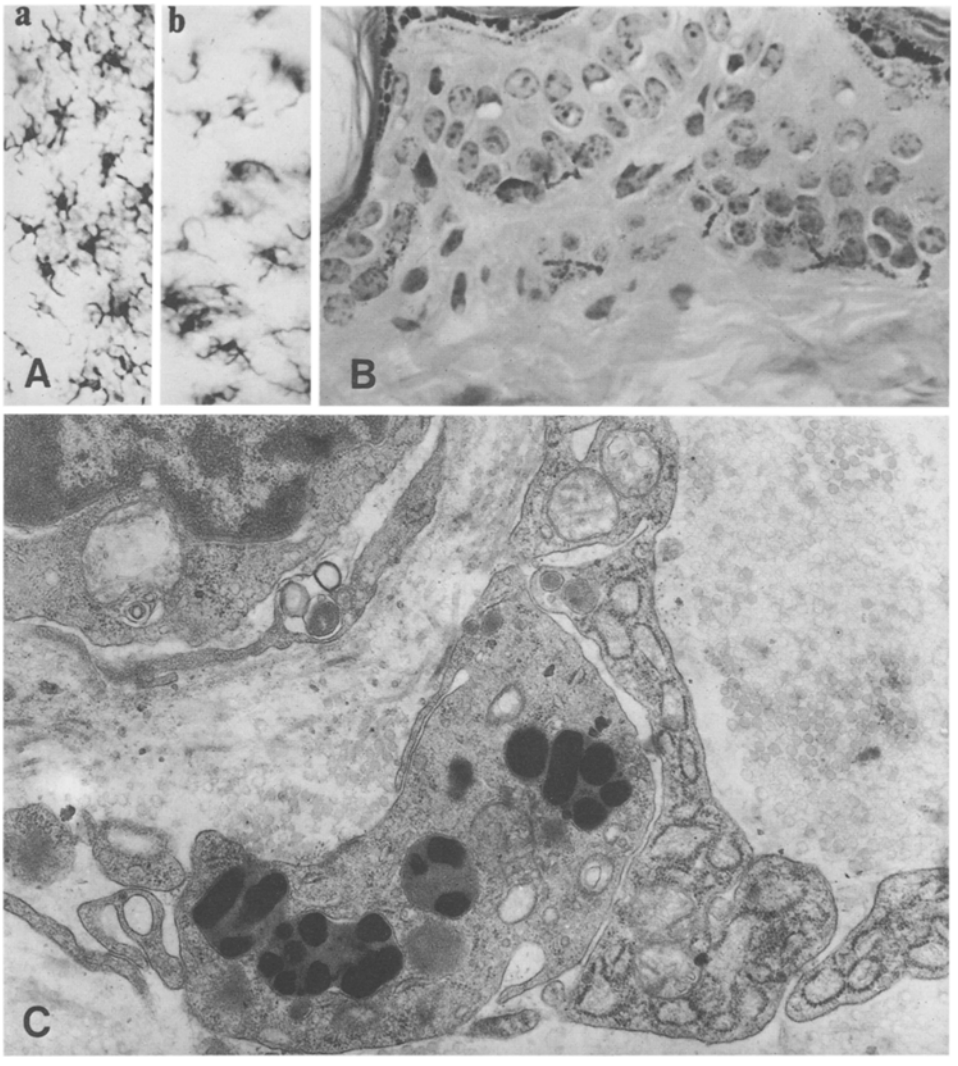

Fig. 8. A Distribution of epidermal malanocytes in flank skin: *a*, epidermis 43 days after PAN allergic reaction (×530); *b*, untreated control epidermis (×530). **B** H&E-stained section of PAN-induced hyperpigmented skin; melanin granules appear to be dropping into the upper dermis (×920). **C** Electron micrograph of melanophages in PAN-induced hyperpigmented skins fixed in GTA-OsO_4. ×18,000

melanocytes were found in epidermal-sheet preparations obtained after the appearance of severe cutaneous irritation (Fig. 10; Table 1). In contrast, 10% azelaic acid and adipic acid had no effect or only a very weak preventive effect on UVB-induced pigmentation (Fig. 9A, B). The application of ethanol alone had no preventive effect on UVB-induced pigmentation. Split epidermal sheets prepared from UVB-irradiated and UVB/ethanol-treated guinea-pig skin obtained on the 12th day after the last UVB irradiation contained strongly dopa-positive melanocytes with well-developed dendrites; the number of melanocytes found in this skin was almost twice as great as that found in nonirradiated control skin (Table 1). In contrast, in the HQ-treated rectangular area which was irradiated daily for 2 days prior to the application of HQ, the number of melanocytes was markedly reduced as compared to the number found in the irradiated, untreated rectangular area (Fig. 9C; Table 1). Consistent with the fact that the color of UV-irradiated, HQ-treated skin and untreated skin was the same, the number of melanocytes in UVB/HQ treated skin was comparable to that found in untreated epidermis; however, the structure of the melanocytes in HQ-treated epidermis appeared to be damaged when compared to the melanocytes observed in control and untreated, UV-irradiated skin (Fig. 9C).

Discussion

Experimental clinical models for human cutaneous hyperpigmentary disorders have been needed for many years. We considered that parallel and synchronized analysis of clinical pigmentation and the microscopic evaluation of the number of melanosomes present in keratinocytes as well as in populations of

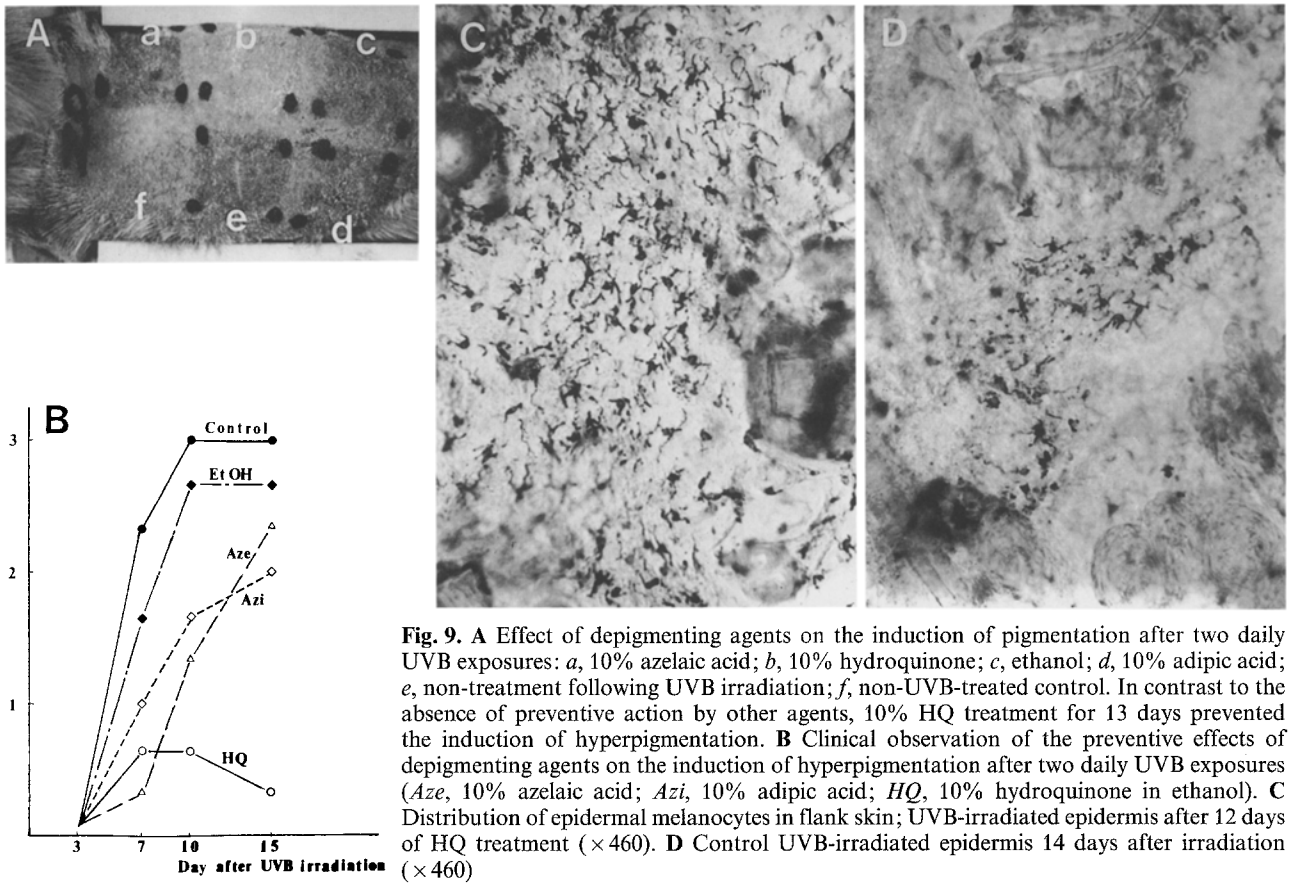

Fig. 9. **A** Effect of depigmenting agents on the induction of pigmentation after two daily UVB exposures: *a*, 10% azelaic acid; *b*, 10% hydroquinone; *c*, ethanol; *d*, 10% adipic acid; *e*, non-treatment following UVB irradiation; *f*, non-UVB-treated control. In contrast to the absence of preventive action by other agents, 10% HQ treatment for 13 days prevented the induction of hyperpigmentation. **B** Clinical observation of the preventive effects of depigmenting agents on the induction of hyperpigmentation after two daily UVB exposures (*Aze*, 10% azelaic acid; *Azi*, 10% adipic acid; *HQ*, 10% hydroquinone in ethanol). **C** Distribution of epidermal melanocytes in flank skin; UVB-irradiated epidermis after 12 days of HQ treatment (×460). **D** Control UVB-irradiated epidermis 14 days after irradiation (×460)

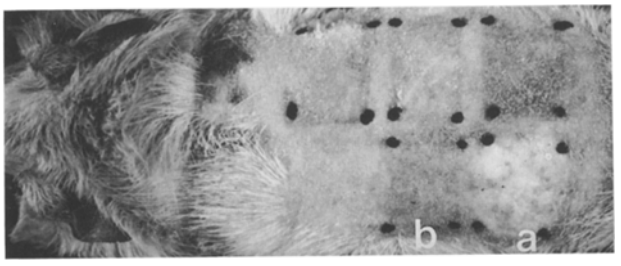

Fig. 10. Effect of 4-tertiary butyl catechol on the induction of hyperpigmentation after two daily UVB exposures. There is a marked depigmentation exceeding the original color level after severe inflammation. *a*, area after 14 days of treatment with 10% 4-tertiary butyl catechol; *b*, EtOH-treated area on the 14th day of treatment

dopa-positive melanocytes might be a useful combination for dermatopharmacological investigations of pigmentary disorders. Our present study revealed that, in the skin of brownish-yellow guinea pigs, UVB exposure, PUVA treatment, and PAN- and DNCB-induced allergic contact dermatitis, all of which are considered to be stimulants of cutaneous hyperpigmentation, produce clearly visible pigmentation which resembles that seen in human skin i.e., with an increased population of dopa-positive melanocytes in the epidermis. Furthermore, this melanogenic stimulation induced by UVB exposure can be suppressed by the subsequent application of tyrosinase inhibitors, e.g., HQ, as well as by pretreatment with sunscreen agents such as PABA; both of these suppressive effects are not accompanied by a substantial increase in the number of melanocytes.

UV Pigmentation

PUVA treatment is known to be a very effective way of inducing pigmentation in normal and vitiligious human skin [24, 25] as well as in the ears [22] and tail skin [3] of mice; this treatment also produces a variety of pigment freckles [17] or increases in other types of pigmentation when used in psoriasis therapy [15].

In our animal model, a single PUVA treatment, i.e., UVA irradiation and systemic intraperitoneal administration of 8-MOP, induced a clear increase in the visible pigmentation and in the epidermal melanocyte population, thus resembling the responses seen after

human PUVA therapy. In our system, a single UVA irradiation alone induced neither melanocyte stimulation nor inflammatory changes. It has been pointed out that PUVA treatment not only inhibits keratinocyte mitoses (psoriasis therapy [15]) but also stimulates the proliferation of dopa-positive melanocytes (vitiligo therapy [24]). These melanocytes have a predetermined function to protect keratinocytes and other cutaneous elements from solar damage, thus suggesting that melanocytes and keratinocytes have a genetically programmed differential sensitivity to PUVA treatment. An example of such a situation in reverse has recently been found in similar dendritic Langerhans cells [10, 21), which lose their Ia-antigen and ATPase activity after UV irradiation. With regard to PUVA therapy for vitiligo, it is interesting to note that lower doses of suberythematous PUVA induce much darker hyperpigmentation than higher doses of erythematous UVA irradiation. We are currently investigating this induction of hyperpigmentation in human skin, but it is possible that too high a dose of UVA may inhibit the melanogenic function of melanocytes and may also induce higher excretion of melanosomes as a result of accentuated keratinocyte turnover or desquamation.

Allergic-Dermatitis-Induced Pigmentation

In humans, especially mongoloids, hyperpigmentation due to an allergic reaction is known as Riehl melanosis [16]; this has been found to be caused by chemical allergens such as the cosmetic red pigment, PAN. Our study of PAN-induced allergic contact dermatitis revealed that this allergic reaction can stimulate the proliferation of melanocytes, so that posthyperpigmentation first appears after a relatively long interval of 14 days. Furthermore, it seems likely that PAN allergy is essentially different from DNCB allergy, because with regard to the induction of posthyperpigmentation, PAN is three times more potent than DNCB, and dermal melanophages are found only after PAN-allergic reactions. In contrast to UVB- or PUVA-induced hyperpigmentation, which occurs even in the absence of visible inflammation, allergy-induced hyperpigmentation is induced through an inflammatory process after a long interval.

Pigmentation Mechanisms

The skin-pigmentation process involves three steps, i.e., the proliferation of pigment cells [27], the synthesis and activation [12, 19] of tyrosinase, and the transfer of melanosomes to keratinocytes [23]; the last may be closely related to the epidermal turnover rate. The recent findings [28] that, in hairless mice, only 1% of dopa-positive melanocytes exhibit DNA synthesis after UVB irradiation is in accordance with our previous findings [18] that UVB-induced hyperpigmentation is caused by the activation of inactive melanocytes and by an increase in the melanocyte population as a result of mitoses. Data obtained from the present experiments indicate that it takes as little as 3 – 5 days following a UVB dose of less than the MED for the number of pigment cells to exhibit an approximately twofold increase.

Moreover, histological sections of hyperpigmented skin exhibited no substantial changes with regard to epidermal thickness, indicating no induction of a hyperproliferative effect. Thus, it is possible that UVB irradiation also converts dopa-negative cells to dopa-positive cells in our experimental model. In contrast, pigmentation induced by PAN allergic dermatitis reaches its maximum about 40 days after the onset of inflammation, suggesting a different mechanism than that of UVB-induced hyperpigmentation. It may be suggested that the stimulatory effect of UV on melanocytes is due selectively to the protective function of melanocytes against solar damage, while allergic hyperpigmentation is a rather nonspecific secondary effect of the preceding inflammatory reaction, in which the repair of cell damage results in the selective development of pigmentation. Available evidence indicates that the proliferation of epidermal melanocytes in culture systems requires growth-factor-like substances, e.g., phorbol esters [5]; also, some agents such as 5-fluorouracil [30] that inhibit keratinocyte mitosis can stimulate melanocytes and result in hyperpigmentation when topically applied. Thus, melanocytes and keratinocytes exhibit differential growth mechanisms which interfere with each other to control the epidermal-pigmentation system.

Depigmenting Chemicals

A wide range of compounds are known to act as depigmenting agents when topically applied to genetically programmed cutaneous pigment, e.g., in black guinea pigs. These compounds have a selective cytotoxic action on functioning melanocytes, which leads to a reduction in the number of melanocytes. Outbreaks of occupational leukoderma have been shown to be the result of contact with melanocytotoxic compounds such as monobenzyl ether of HQ [7], *p*-tertiary butylphenol [1], *p*-tertiary amylphenol [2], and 4-tertiary butyl catechol [8]. Jimbow et al. [13] have reported that, when 2% – 5% HQ was applied for 3 weeks to the skin of black guinea pigs, their pigment was completely lost due to the cytoxic effect of HQ on melanocytes. In our system, the depigmentation caused by HQ treatment did not exceed the original

color of the brownish-yellow guinea pigs. This difference may be due to be different melanogenic status of black and brownish-yellow guinea-pig skin. In addition, the topical application of dicarboxylic acids, e.g., azelaic acid, which act as competitive inhibitors of tyrosinase in vitro [20], has little preventive effect on UVB-induced hyperpigmentation. With regard to the marked preventive effect of HQ as compared to azelaic acid and adipic acid, it seems reasonable to assume that a higher tyrosinase level in melanocytes induces increased levels of HQ-metabolized hydroxyhydroquinone [9], which results in the prevention of an increase in the population of dopa-positive melanocytes.

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Received March 25, 1985