Glucocorticoid Effects on Contact Hypersensitivity and on the Cutaneous Response to Ultraviolet Light in the Mouse

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A single exposure to 254 nm ultraviolet irradiation (UV) can systemically suppress experimental sensitization to the simple allergen 2,4-dinitro, 1-chlorobenzene (DNCB) in the mouse. We show here that topical application at the site of irradiation of the 21-oic acid methyl ester derivative of the synthetic glucocorticoid triamcinolone acetonide (TAme) prevents UV suppression of sensitization. That is, mice painted with TAme at the site of UV exposure developed normal contact hypersensitivity (CH); mice exposed to UV only, like mice treated with the parent compound triamcinolone acetonide (TA), failed to be sensitized by DNCB applied to a distal site. TAme is inactivated rapidly by plasma esterases, so its effect is thought to be confined to the skin. Apparently, TAme blocked the cutaneous signal(s) for systemic suppression of CH. Histologically, irradiated skin exhibited mild inflammation and hyperproliferation, but these effects were greatly exaggerated and prolonged in the UV + TAme-treated skin, independent of sensitization at the distal site. The infiltrate consisted mostly of neutrophils and lacked the round cells characteristic of cell-mediated immunity. Apparently, normal immune suppression by UV prevented this vigorous reaction to irradiated skin. Applied together with DNCB, TAme blocked sensitization. It also prevented response to challenge by DNCB in previously sensitized animals. However, unlike the parent compound triamcinolone acetonide (TA), Budesonide or Beclomethasone diproprionate, each of which can penetrate the epidermis in active form, TAme had no effect on sensitization when applied at a distal site. Likewise, TAme did not affect plasma B (17-desoxycortisol) levels, whereas the other three compounds reduced plasma B tenfold, as expected of compounds causing adrenal-pituitary suppression. The results as a whole show that glucocorticoids can specifically inhibit cutaneous steps in induction of cell-mediated immunity or its suppression, and can, at the site of challenge, prevent its expression in CH. *J Invest Dermatol 90:366–371, 1988*

Ultraviolet irradiation (UV) of mouse skin causes tolerance to the placing of relatively immunogenic skin tumor grafts [13]. The extent of suppression of contact hypersensitivity (CH) by UV has been used to quantify immunological tolerance caused by UV [21,22,26]. Both UV inhibition of tumor graft rejection and UV suppression of CH are thought to result primarily from the expansion and activation of clones of suppressor T lymphocytes that prevent rejection of skin cells modified by chemicals or by neoplastic transformation. Psoralen plus 340–360 nm light, as well as UV in the "sunburn" spectrum or 254-nm UV alone, can cause tumorigenesis or tumor graft tolerance and suppress CH in mice (reviewed in Refs. 13 and 19) and can suppress contact hypersensitivity in humans [25,20]. Irradiated mouse skin secretes a low molecular weight protein that stimulates suppressor T cells in the spleen [26,24]. Also, the *cis* isomer of urocanic acid is released from irradiated mouse skin, presumably a photoproduct of the *trans* isomer normally present in the skin [6]. Ultraviolet photoproducts of purified urocanic acid also suppressed delayed hypersensitivity to herpesvirus in mice [22], so there may be more than one inducer or more than one cutaneous step in this process. Neither the mechanism of action nor the source of the active substance has been identified with certainty. It is also not known what normal physiological function, if any, is served by the suppressor cells.

One way to better scrutinize UV suppression of cellular immunity would be to experimentally dissociate the splenic from cutaneous effects of the ultraviolet irradiation, and to study each separately. The glucocorticoid fluocinolone acetonide has been shown to alter protein synthesis in cultured skin cells that have been exposed to UV light [23]. Irradiation of epidermal cell cultures with 254-nm UV increases production of a cytokine whose synthesis, or release, is prevented by hydrocortisone [15]. However, glucocorticoids can also cause atrophic changes in mouse skin [11] and depress the cellular immune system [2]. Topical glucocorticoids entering the circulation affect multiple internal sites, precluding a definitive analysis in vivo. The preparation of the methyl ester of the 21-oic acid of triamcinolone acetonide was recently described by one of us [9]. This compound, referred to here as triamcinolone acetonide 21-oic acid methyl ester (TAme), behaves much like TA in a granuloma inhibition bioassay [16], but it is converted by serum esterases to a form lacking all glucocorticoid activity. We report here studies of the effect of TAme on CH and its suppression by UV.

MATERIALS AND METHODS

Materials Mice, aged 4 mo, were secured from the Rockefeller University colony established in December 1983 from NIH Balb/

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Abbreviations:
B: 17-desoxycortisol,11-β, 21-dihydroxy-pregn-4-ene-3,20-dione
CH: contact hypersensitivity
DNCB: 1-chloro-2,4-dinitrobenzene
TA: 9 α-fluoro-11-β,16α,17α, 21-tetrahydroxy-pregna-1,4-diene-3,20-dione
TAme: TA 21-oic acid methyl ester
UV: ultraviolet light

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CAnN stock. DNCB (1-chloro-2, 4-dinitrobenzene) and TA (triamcinolone acetonide) were purchased from Sigma (St. Louis, Missouri); TAme (triamcinolone acetonide 21-oic acid methyl ester) was synthesized as described by Gorsline, Bradlow, and Sherman [9].

Irradiation To irradiate mice in groups, squares 2 cm on a side were cut in a cardboard mask, which was then set above a bank of two GEG15T8 low-pressure Hg lamps emitting primarily at 254 nm. We chose this lamp to minimize direct subepidermal effects of irradiation. 254-nm radiation penetrates the epidermis less deeply than sunlamp radiation, which is more commonly used to study UV suppression of CH. Incident fluence was 2.5–3.4 mW/cm² (as measured by an actinometrically calibrated Black Ray model J-225 shortwave UV monitor), depending on position above the lamp. Mice, anesthetized by injection with i.p. Nembutal (2 mg/kg in 0.5 ml saline), were fastened gently to the mask with tape for the 15–20 min exposure. Length of exposure was adjusted to ensure each mouse received the same cumulative exposure, 3 J/cm² (total, 12 J per mouse), to clipper-shaved skin on the lower abdomen. The timing was based on a series of preliminary studies which showed that this exposure gave the best results (data not shown).

Steroid Treatment Twenty or forty micrograms of TA or TAme, dissolved at a concentration of 0.2% in absolute USP ethanol, were spread over the unirradiated or UV-irradiated, shaved skin, with a microliter pipette (Rainin Pipetman P-20); the same dosage was used for both compounds because they differ only by a methyl group. Gorsline et al [9] showed that purified glucocorticoid receptor binds TA and TAme equally well, though it lacks affinity for the 21-oic acid. Receptor binding affinities and molecular weights were similar for Budesonide and Beclomethasone Diproprionate [18], so these drugs were also administered at the same dose as TAme.

DNCB Applications and Tests for Contact Hypersensitivity Animals were shaved on the lower back to expose about 4 cm² skin. For experimental sensitization, 20 μl of a 1% solution of DNCB in ethanol were applied to this shaved site. To ascertain the degree of contact hypersensitivity 4 days after the first sensitizing application,

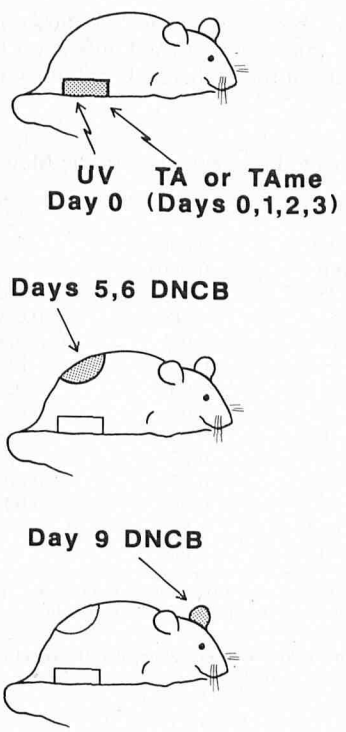

Figure 1. Schematic representation of the experimental procedures used in the experiments of Tables I and II.

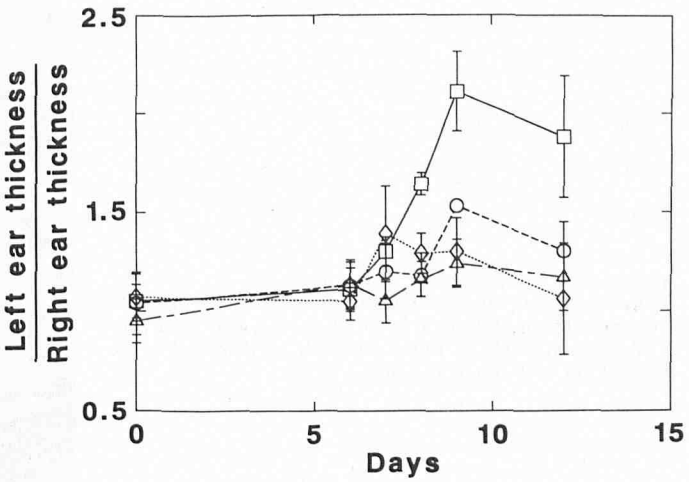

Figure 2. Effect of TAme on sensitization and response to challenge with DNCB. TAme was mixed with DNCB in ethanol, and the two compounds were applied together. The ratio of left to right ear thickness is shown (n = four mice per group; triplicate ratios for each mouse's ears at each time point). Error bars represent the standard deviation for each group of four mice. *Boxes:* DNCB only at both sensitization and challenge; *circles:* DNCB at challenge only, to test for DNCB irritation; *triangles:* DNCB at sensitization and challenge, TAme at sensitization; *diamonds:* DNCB at sensitization and challenge, TAme at challenge only.

5 μl of DNCB freshly dissolved at 2% in ethanol were applied to the inner and outer aspects of each animal's left ear (total, 50 μg). Ear thickness was measured just prior to this challenge and at 24-hour intervals thereafter with the aid of a dissecting microscope and a dial engineer's caliper (Sears, Craftsman).

All measurements of ear thickness were made in triplicate by an experienced measurer who did not know the identities of the groups of mice at the time of measurement. Statistical analysis was carried out using the BMDP P3D program mounted on a Vax 11/780 computer. There was no statistically significant difference between data calculated from measurements made by two independent observers.

Histology Abdominal skin, fixed in 10% formalin, was embedded in paraffin; microtome sections were stained with hematoxylin-eosin.

Experimental Design Two kinds of experiments are described in this paper: In the first paradigm (Figs 1 and 3; Tables I, II, and IV), ultraviolet-irradiated animals and unirradiated controls were painted with glucocorticoid only at the site of irradiation, mainly to evaluate glucocorticoid effects on the UV suppression of CH. The second paradigm pertains to glucocorticoid effects on the sensitization (Fig 2 and Table III) or elicitation (Fig 2) phases of CH itself when applied together with DNCB and on the ability of various glucocorticoids to influence CH when placed distal to the site of DNCB application (Table III).

RESULTS

Figure 1 schematically represents a typical experiment. Mice from a colony were caged in six groups. Three of the groups of mice were exposed to 3 J/cm² 254-nm light; others were shaved but not irradiated. Immediately following ultraviolet exposure (day 0), mice were painted with steroid or with vehicle (ethanol) at the site of irradiation. This treatment was repeated three times at approximately 24-h intervals. Animals to be sensitized were painted with 1% DNCB in ethanol on days 5 and 6. Pilot studies showed the test for contact hypersensitivity to be maximal four to five days after the first sensitizing treatment. We therefore chose day 9 to challenge for contact sensitivity by application to the left ear of DNCB in ethanol. Ear swelling was measurable on day 10, but peaked on day 11, 48 h after challenge. This paradigm was repeated several times with similar results.

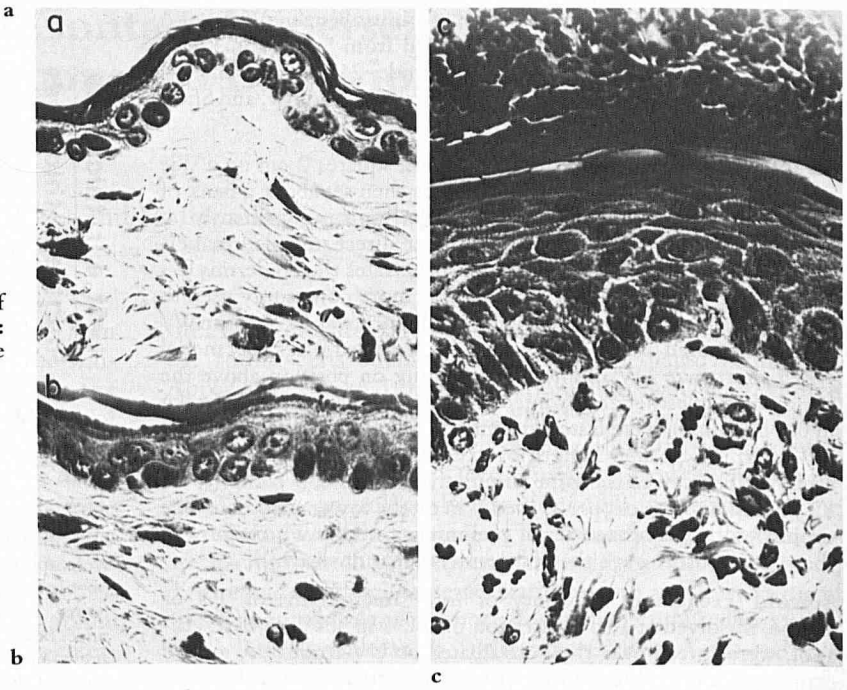

Figure 3. Photomicrographs of skin sections from mice of the experiment of Table I, killed on day 12; **a:** UV only; **b:** TAme only; **c:** UV + TAme. Methods are described in the text. 1 cm = 15 μm in all panels.

Sensitization caused the DNCB-treated back skin to thicken, redden, and indurate. This response was most pronounced in the controls, the TAme-only, and the UV + TAme groups. The average thickness of the left and right ears for most groups was 0.24 mm on day 9. After challenge, ears swelled according to group. The swelling was accompanied by a mild erythema, and visual estimate of the extent of the erythema and swelling generally correlated with the caliper measurements. Ears of sensitized controls swelled to about twice their normal thickness. Ears of unsensitized mice did not swell. The response measured here therefore was a consequence of contact hypersensitivity rather than primary irritation, which, at higher DNCB concentrations than those used here, also produces ear swelling. For instance, DNCB irritation was measurable in the controls of Fig 2. UV irradiation on day 0 suppressed the swelling for all but one animal whose response was delayed but about normal in magnitude. In sharp contrast, mice treated with TAme either following UV or without UV responded like the control mice, showing normal contact hypersensitivity. Mice that were treated with TA after UV, like those treated with TA alone on days 1–3, had ears 0.18 mm thick on day 9, about 0.06 mm thinner than those of other mice. When the right ear is taken as the baseline, both groups exhibited diminished CH, comparable to that in the UV-only group. Mice treated with TAme but not with UV, on the other hand, exhibited normal contact hypersensitivity; TAme is thought to act only at the site of application, while TA gains access to the circulation and may act at a distance. A second experiment confirmed the result that TAme completely reversed the effect of UV irradiation on the ability of mice to be sensitized by exposure to DNCB.

Data from the above experiments are shown in Tables I and II. The average ear thickness ratios and differences for each group are given as a percent of the positive control in columns 6 and 7. We

Table I. Interaction of Corticoids and Ultraviolet Radiation on Contact Hypersensitivity in the Mouse

DNCB	UV	Steroid	n	Day	l/r	l-r	p_a[a]	p_b[a]	p_c[a]
+	—	—	5	10	100[b]	100[b]	0.0001	—	0.001
			5	11	100	100	0.0001	—	0.001
+	+	—	4	10	72	25	NS	0.0001	NS
			4	11	70	31	NS	0.0001	NS
+	—	+	2	10	110	113	0.07	NS	0.001
		Tame	2	11	98	91	0.005	NS	0.006
+	—	+	2	10	94	69	0.05	0.03	0.05
		TA	2	11	63	16	0.05	0.02	NS
+	+	+	5	10	100	110	0.05	NS	0.05
		Tame	5	11	90	90	0.05	NS	0.05
+	+	+	5	10	82	45	0.05	0.009	0.02
		TA	5	11	79	45	0.05	0.001	0.02
—	—	—	4	10	64	64	NS	—	—
			4	11	71	54	NS	—	—

DNCB 200 μg per application at sensitization; 50 μg at challenge. TA or TAme 40 μg per application. The day 9 values were all statistically insignificant. In all cases both the drug and the hormone were applied to the left ear. The data shown are for the thickness of the left ear divided by the thickness of the right ear (l/r) or for the difference between these values (l-r). Each measurement was made in triplicate.

[a] The t-test results showed similar levels of significance ($P < 0.05$) using l/r or l-r values as the test parameter. In no case was there a discrepancy between the results obtained using either parameter

p_a = side to side p value. Untreated ear as negatve control.
p_b = Test group vs Positive Control (DNCB only)
p_c = Test group vs Negative Control (No pretreatment)

[b] Percentage of positive control (DNCB only); same for l-r.

Table II. Interaction of Corticoids and UV on Contact Hypersensitivty in the Mouse

Group	DNCB	UV	TAme	n	Day	l/r	l-r	p_a	p_b
	Front								
1	+	—	—	9	11	100	100	0.0001 1–2	0.0001
2	+	+	—	8	11	71	14	0.0001 2–3	0.04
3	+	+	+	12	11	98	87	NS 1–3	0.0001
	Apex								
1	+	—	—	9	11	100	100	0.0001 1–2	0.0001
2	+	+	—	8	11	66	6.2	0.0001 2–3	NS
3	+	+	+	12	11	95	89	NS 1–3	0.0001

Ear thickness was measured either on the front or at the apex. DNCB and steroids were given as described in Table I. The l/r and l-r were calculated as percent of the positive control; l/r and l-r are as defined in Table I; n = number of animals in each group.
[a] p_a = intergroup probability; p_b = side to side probability

also performed a one-tailed *t* test on the correlated ear measurements. We applied three tests for significance: comparison of the average values for left and right ears within each group (p_a); each group against the positive control (p_b); and each group against the negative control group (p_c). The results of the tests for significance are summarized in Table I, columns 8–10. When the values are smallest (i.e., when the data are most significantly different), the differences are largest. The ultraviolet suppression of sensitization is readily apparent in the UV-only group. The TAme-only and UV + TAme animals responded like the positive control animals. This shows that treatment with TAme immediately after ultraviolet irradiation prevented UV suppression of CH. This effect was more pronounced in the experiment of Table II, where larger numbers of Balb/C mice were used. The TA-treated animals exhibited a more complex response pattern: those treated with TA but not with UV responded much like the negative controls (see Table III); UV + TA animals were also closer to the negative controls, but were not so close to the negative controls as the UV only group. There was no additivity between the UV effect and the TA effect.

In the above experiment, TA could have acted systemically or at the skin to prevent CH. We therefore tested for the effect of TAme on CH at the site of sensitization. On day 0, the shaved backs of C57Bl/K6S mice were painted with DNCB; with TAme; or with both compounds. On day 5, the animals were tested for contact hypersensitivity by application of DNCB or of TAme + DNCB. Although lacking inhibitory effect when applied distal to the site of sensitization (Tables I and II), the data in Fig 2 show that TAme prevented CH when applied to the back at the time of sensitization and also prevented ear swelling when applied to the ear at the time of challenge. This indicates that the inhibitory effect of TA when applied distal to the site of sensitization (Tables I and III) was probably due to systemic absorption of this drug and consequent action at a distance.

To further test the idea that glucocorticoids might affect sensitization both at the site of application and by systemic action, we performed an experiment with the topical glucocorticoids Beclomethasone and Budesonide as well as with TAme and TA. Like TA, but unlike TAme, Beclomethasone and Budesonide are metabolized slowly by serum and liver enzymes [1]. Mice were sensitized on their backs, and glucocorticoid was applied either with the DNCB at the site of sensitization (B group animals) or to a distal site (the abdomen; group A animals). Glucocorticoid treatment was continued on days 1–3. On day 5, two mice from each group were bled just prior to challenge to test for circulating B (17-desoxycortisol); B is the predominant circulating glucocorticoid of mice. Ear thickness was measured, and there were no significant differences between the groups. The mice were then challenged, and ear thickness was measured again on day 7. Data for B (in μg/ml) and for the averaged ratio and difference of left and right ear thicknesses as a percent of the positive control group are shown in Table III. The data show that Budesonide, Beclomethasone, and TA all suppressed plasma B at least tenfold, whereas TAme had no effect. The drugs that suppressed plasma B also abolished CH, there being no statistically significant difference between these data and the negative controls. The suppression of CH was similar in magnitude whether the drug was applied to the abdomen or to the back. On the other

Table III. Effect of Glucocorticoids on Sensitization to DNCB

	Negative Control	Beclomethasone		Budesonide		TA		TAme		Positive Control
N[c]	5	4	4	4	4	4	4	4	4	4
Group	1	2	3	4	5	6	7	8	9	10
DNCB[a]	—	+	+	+	+	+	+	+	+	+
Steroid[b]	—	A	B	A	B	A	B	A	B	—
B level[c]	12.7	0.61	1.25	0.2	0.0	0.14	0.0	11.3	14.9	6.2
Mean l/r day 7	70	73	71	70	73	71	72	120[d]	86[d]	100[d]
Mean l-r day 7	12	19	22	12	21	20	37	171[d]	47[d]	100[d]

The 1/r and 1-r were calculated as described in Table I. Day 5 values were not significant in all cases.
[a] DNCB: 200 μg per application at sensitization; 50 μg at challenge; glucocorticoids 20 μg per application.
[b] B = steroid applied on the back; A = steroid applied to the abdomen.
[c] B values μg/mL; N = Number of mice per group.
[d] $p_{1-8} < 0.001$; $p_{1-10} < 0.06$; p_{1-9} NS; p_{9-10} NS; p_{8-10} = NS; $p_{1-8} < 0.0005$; $p_{1-10} < 0.03$; p_{1-9} NS; $p_{8-10} < 0.06$; p_{9-10} NS; all other cases were not significantly different from the negative control value.

Table IV. Summary of Histological Data

	Day 3				Day 12				
	S	Tm	UV + Tm	UV	Tm	TA	UV + TA	UV + Tm	UV
Inflammatory changes									
Spongiosis			+++	+				++	
Infiltrate									
Neutrophilic									
Dermal			++	++				++	
Epidermal			++					++	
Mononuclear	+			+					
Hyperplastic changes									
Dermal								+	
Epidermal									
Hyperkeratosis	+	+		+			+	+++	+
Hypergranulosis				+				+++	+
Acanthosis								+++	
Atrophic changes									
Dermal						++	++		
Epidermal						++			
Necrosis			++	++			+		

S = shaved control; Tm = triamcinolone acetonide 21-oic acid methyl ester; TA = triamcinolone acetonide; UV = ultraviolet.

hand, TAme had no effect on CH when applied to the abdomen; in fact there was an apparent stimulation as compared to the positive control. However, TAme applied to the back prevented development of CH. The effect was less than that of the other glucocorticoids, but the data were not statistically different from the negative controls. Thin-layer chromatography of the various mixtures held for 2 days at 37°C revealed no evidence for chemical reactivity between the glucocorticoids and DNCB or ethanol (not shown). The data show that glucocorticoids can prevent sensitization at the site of application of DNCB or at a distal site, but in the latter case, only if they are able to penetrate the epidermis in an active form and act systemically.

Some animals of the experiment shown in Table I were killed on day 3 or day 12 for histological examination of the UV-exposed portion of their abdominal skin. The evaluation is summarized in Table IV; selected photomicrographs are shown in Fig 3. The skin of UV-only mice on day 3 contained a diffuse dermal infiltrate, consisting of about 90% neutrophils and 10% monocytes and macrophages. The inflammatory changes were not accompanied by erythema and were in other ways characteristic of UV-exposed mouse skin [4]. The dermis of TAme-treated, UV-irradiated mice was infiltrated by polymorphonuclear leukocytes. Macroscopically, there was induration and purulent crusting. The infiltrate persisted for at least 12 days in the TAme plus UV-treated mice, when it had subsided in the UV-only group (Figs 3*A*, 3*C*). There was no apparent influx of mononuclear cells in the UV + TAme skin, suggesting that the cellular immune system was unresponsive to UV damage. The other striking histological finding in the UV + TAme-treated animals was the intense epidermal hyperplasia, accompanied by acanthosis and hyperkeratosis. In many places, the epidermal thickness exceeded 20 nucleated cells. Normal mouse belly skin is 1–3 nucleated cells thick. This prolonged and exaggerated response did not occur in the UV-only and TAme-only groups (Fig 3*B*), so it is a result of interaction of the two treatments. The TAme-only group, in contrast, had a normal epidermis, accompanied by mild follicular hyperplasia.

DISCUSSION

We chose TAme for these studies because unlike its parent compound TA, it is hydrolyzed by nonspecific esterases to an acid lacking glucocorticoid activity. TAme and TA were equally effective at inhibiting local granuloma formation in the rat [9]. This makes unlikely the possibility that the strikingly different behavior of the two drugs in our experiments arose from dose effects. Rather, the action of TAme appears to be confined to the epidermis, as predicted from the sensitivity of TAme to nonspecific esterases. Glucocorticoids that can penetrate the skin in an active form can influence CH or the response to UV systemically: for instance, TA applied to the belly prevented sensitization on the backs of unirradiated animals (Tables I and III). However, UV + TA-animals became sensitized, in low degree, on subsequent exposure to DNCB, as though the systemic effect of TA opposed its effect on the irradiated skin (Table I). The histological evidence of steroid-induced epidermal atrophy, dermal fibrosis, and adnexal atrophy (Table IV) and the decrease in baseline ear thickness from 0.24 to 0.18 mm further evince the multiple cutaneous and systemic effects of the parent compound, TA. Consequently, from these data alone one cannot estimate which part of the response to TA was systemic and which part was local. Unlike TA, TAme did not modulate CH or prevent its suppression by UV when applied distal to the sensitizing or challenge treatment (Tables I and III). Skin treated with TAme alone appeared normal (Table IV; Fig 4). However, when applied simultaneously at the same site as the DNCB, TAme prevented both sensitization and response to challenge (Fig 2; Table III). These results demonstrate that glucocorticoid-sensitive site(s) in the skin exist both in the early events of sensitization and, much later, in the elicitation of CH upon re-exposure to the allergen.

When applied to irradiated skin, TAme prevented the UV suppression of CH (Table I). From the above, it seems likely that TAme blocked UV suppression of CH at the site of irradiation. For example, TAme might block all cell responses by poisoning cell machinery or by vasoconstriction. In this case, one would expect less change in irradiated, TAme-treated skin than in irradiated skin not treated with steroid, and atrophy or other abnormalities in the epidermis of unirradiated, TAme-treated animals. This exact pattern was indeed seen with TA. Rather, TAme had no discernible effect on unirradiated skin, there being no evidence for adnexal atrophy. However, when applied to irradiated skin TAme enhanced markedly neutrophilic infiltration and epidermal hyperplasia. The simplest interpretation is that TAme directly prevented an epidermal signal from suppressing these histological changes in irradiated skin and from inducing UV suppression of CH. The nature of this signal remains obscure. Our data do not permit a distinction between the specific explanations reviewed by Morison [19]. Our data favor the view that the glucocorticoid-sensitive synthesis or release of one or more substances from irradiated epidermis or superficial dermis causes the systemic suppression of CH. Possible glucocorticoid-sen-

sitive sites include the keratinocyte [15], the Langerhans' cell [3], and cutaneous fibroblasts [23].

Cell surface antigen changes following UV [7] or PUVA [5] could lead to immune recognition of the epidermis as foreign. The UV suppression of CH could prevent rejection of sunburned skin, at the risk of the long-term consequence of elevated skin tumor susceptibility. The intense infiltration, hyperproliferation, and purulent crusting in the UV + TAme group may either reveal the mouse's response to UV-damaged skin in the absence of normal immune suppression or to some ancillary effect of the steroid. This response was attenuated in the UV-only group, presumably by immune suppression, and was absent in the UV + TA group, presumably from the dermal, pituitary, or splenic effects of the steroid. As the infiltrate and hyperplasia were absent in the TAme-only group (Fig 3), however, these were not caused by simple drug effects such as irritation. However, the response did not resemble either graft rejection or contact hypersensitivity microscopically, in that mononuclear cells were not abundant [17]. The polymorphonuclear infiltrate could be a response to cell breakdown products or to bacterial antigens. As this inflammation was not seen in the UV + TA animals (Table IV), however, it is likely that subepidermal conditions needed for the response were eliminated by TA. Conceivably, the neutrophils or irradiated epidermal cells secrete a growth factor. γ-Interferon secretion from macrophages or T-lymphocytes has been implicated in acanthotic changes during CH reactions [12] and in active psoriasis [10]. Our data provide preliminary evidence that acanthosis does not require T-lymphocyte or macrophage infiltrate. In view of the above as well as the observation that TAme at the site of application can prevent sensitization in contact hypersensitivity (Fig 2 and Table III), it seems likely that several cutaneous mechanisms were affected by TAme, including the stimulation of helper T cells potentially responsive to UV-modified skin. It should be emphasized that we detected no glucocorticoid effects beyond the site of TAme application, yet TAme abolished UV suppression of CH. The simplest explanation again is that TAme prevented synthesis of a suppressor substance or its release from the irradiated skin to which TAme was applied, or caused the synthesis or release of a suppressor antagonist.

The data as a whole indicate that TAme-treated epidermis was ineffective at provoking or suppressing cell mediated immunity. This local paralysis of cellular immunity caused no apparent toxicity, nor did it affect sensitization or challenge at a distal site. Local effects in experiments with the other glucocorticoids were masked by concomitant systemic effects of these drugs.

Ultraviolet-induced immune suppression prevents rejection of tumor tissue, thus allowing ultraviolet carcinogenesis [8]. Our data suggest that a steroid with the properties of TAme could some day be of clinical value as a potent, exclusively local immunomodulator.

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