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Effects of ultraviolet irradiation on epidermal Langerhans cells in guinea-pigs

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Summary. There is good evidence to suggest that u.v. irradiation (UVB) of the skin causes a reduction in the numbers of normal epidermal Langerhans cells (LC) in man and mouse. Little information is available on the effects of u.v. radiation on LC in guinea-pig skin.

In this study, different dosages of UVB and UVC were applied to the ear skin of guinea-pigs. UVC irradiations were found more effective than UVB in causing depletions of ATPase-positive epidermal LC. Highly significant depletions, lasting 6 days after irradiation, were produced with little evidence of significant inflammatory reactions occurring in the epidermis.

INTRODUCTION

The effects of ultraviolet radiation (UVR) on epidermal Langerhans cells (LC) have been investigated quite extensively. Studies on the changes of the epidermal LC induced by the mid-wavelength UVR (UVB) have been reported primarily in man and mouse. Toews, Bergstresser & Streilein (1980) reported that the numbers of ATPase-positive LC in abdominal skin of the mouse were greatly reduced following UVB irradiations. Similar studies by Aberer *et al.* (1981), using a single dose of UVB to irradiate human and murine skin, demonstrated reduction of the LC numbers as assessed by immunofluorescent staining of Ia antigens, ATPase activity, or electron microscopy. In guinea-pigs, there are few reports dealing with the effects of UVR on epidermal LC. Fan, Schoenfeld & Hunter (1959) briefly mentioned the occurrence of a decrease in epidermal LC numbers with u.v. irradiation of guinea-pigs skin, but the u.v. source, dose and exposure method were unclear.

LC have recently been found to play an important role in immune responses of skin to many allergens and antigens (Stingl, 1980). They are the only epidermal cells that have functional characteristics similar to Ia-bearing macrophages, possessing Fc and C3 receptors and Ia antigens on their surface membranes. Furthermore, it has been demonstrated that LC, like macrophages, are capable of antigen presentation in a genetically restricted manner, eliciting antigen specific and allogeneic T lymphocyte proliferation (Stingl, 1980). It has been shown that the antigen-presenting function of LC in mice was impaired following ultraviolet irradiation of skin. In studies of contact allergic hypersensitivity reactions to dinitrofluorobenzene (DNFB) in mice, Toews *et al.* (1980) and Aberer *et al.* (1981) demonstrated the inability of the animals to be sensitized with DNFB following UVB irradiation. Similar experiments on the effects of UVB irradiation on dinitrochlorobenzene (DNCB) contact dermatitis in guinea pigs produced similar results (Morison *et al.*, 1981).

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Resistance to tick feeding has been shown to be an acquired, immunologically mediated phenomenon in guinea-pigs, associated with cutaneous hypersensitivity to tick antigens (Wikel, 1982). There is some evidence to support the hypothesis that epidermal LC play a role in tick resistance mechanisms (Allen, Khalil & Wikel, 1979; Nithiuthai & Allen, 1984).

The present study was designed to look for a suitable method of depleting ATPase-positive epidermal LC populations of guinea-pigs' ear skin for a period of at least 5 days, so that the effects of such LC depletion on the acquisition and expression of tick resistance could subsequently be assayed. Ultraviolet radiation from two different sources, each at different dosages was applied to the dorsal ear skin of guinea-pigs. Effects of such treatments on ATPase-positive epidermal LC were assessed and inflammatory changes in the epidermis induced by irradiations were monitored grossly and histologically.

MATERIALS AND METHODS

Animals

Thirty-two male albino Hartley outbred guinea-pigs, 300–350 g in weight, were supplied by Canadian Breeding Labs Ltd., Montreal. They were fed on a pellet diet and water supplemented with ascorbic acid (10–15 mg/day/animal). The animals were housed individually in metal cages in a room not exposed to sunlight.

U.v. light sources and u.v. irradiation of guinea-pigs

Guinea-pigs were divided into six groups and were subjected to different u.v. treatments. The right ears of animals were irradiated whereas the left ears were used as untreated controls. The u.v. irradiation was applied only to the dorsal surface of the animal's right ears which were shaved 24 hr before the irradiations. They were exposed to the radiations on a daily basis for 4 consecutive days. During the treatments, each animal was restrained and covered with a blue denim bag, leaving only the right ear exposed. Their right ears were maintained flat on adhesive cardboards to ensure uniform exposure of the dorsal surface to the radiations. The u.v. light sources and irradiation dosages were as follows.

(i) The mid-wavelength (UVB) light source (Westinghouse sunlamp, model FS20) consisted of three light tubes emitting wavelengths between 270 and 380 nm. The maximum emission was at 313 nm. The irradiance at the targets was $8{\cdot}59 \times 10^{-2}$ mW/cm^2 at a distance of 45 cm from the light source. Four groups of animals, groups I–IV, were exposed to the UVB radiation for 4, 8, 10 and 20 min/day with total exposure of 82, 165, 206 and 412 mJ/cm^2 respectively.

(ii) The short wavelength (UVC) light source (General Electric model G30T8) emitted wavelengths between 250 and 280 nm, with a peak emission at 254 nm. The irradiance at the target was $4{\cdot}42 \times 10^{-2}$ mW/cm^2 at a distance of 35 cm from the light source. The animals in Groups V and VI received UVC for 15 and 30 min/day giving a total exposure of 159 and 318 mJ/cm^2 respectively.

The u.v. energy output was measured using a light intensity measuring system, model 920 radiometer/photometer (Ealing Corporation, Massachusetts) connected to the extended u.v. microprobe detector placed in the sample position.

Skin biopsies

Five-millimeter punch biopsies were taken from the u.v.-exposed and non-exposed ears of the guinea pigs at day 0 (immediately), and days 2, 4 and 6 after the last exposure to radiations. Biopsies were kept on ice until processed the same day through histological and enzyme histochemical techniques.

Histology, enzyme histochemistry and assays of ATPase-positive LC in the epidermis

Epidermal sheets and skin sections were processed through the procedures previously described by Nithiuthai & Allen (1984).

Statistical analysis

The significance of differences in the mean densities of epidermal ATPase-positive LC populations of u.v.-exposed and non-exposed ears was determined using the paired Students' *t*-test.

RESULTS

Gross observations

None of the guinea-pigs exposed to UVB (4–10 min daily exposure) or UVC showed gross inflammatory reactions throughout the entire period of the repeated exposures. In guinea-pigs exposed to 20 min UVB daily, after the second exposure the exposed skin showed signs of erythema. Repeated exposure to the radiation on day 3 and day 4 induced stronger erythematous reactions followed by hyperkeratosis and scaling.

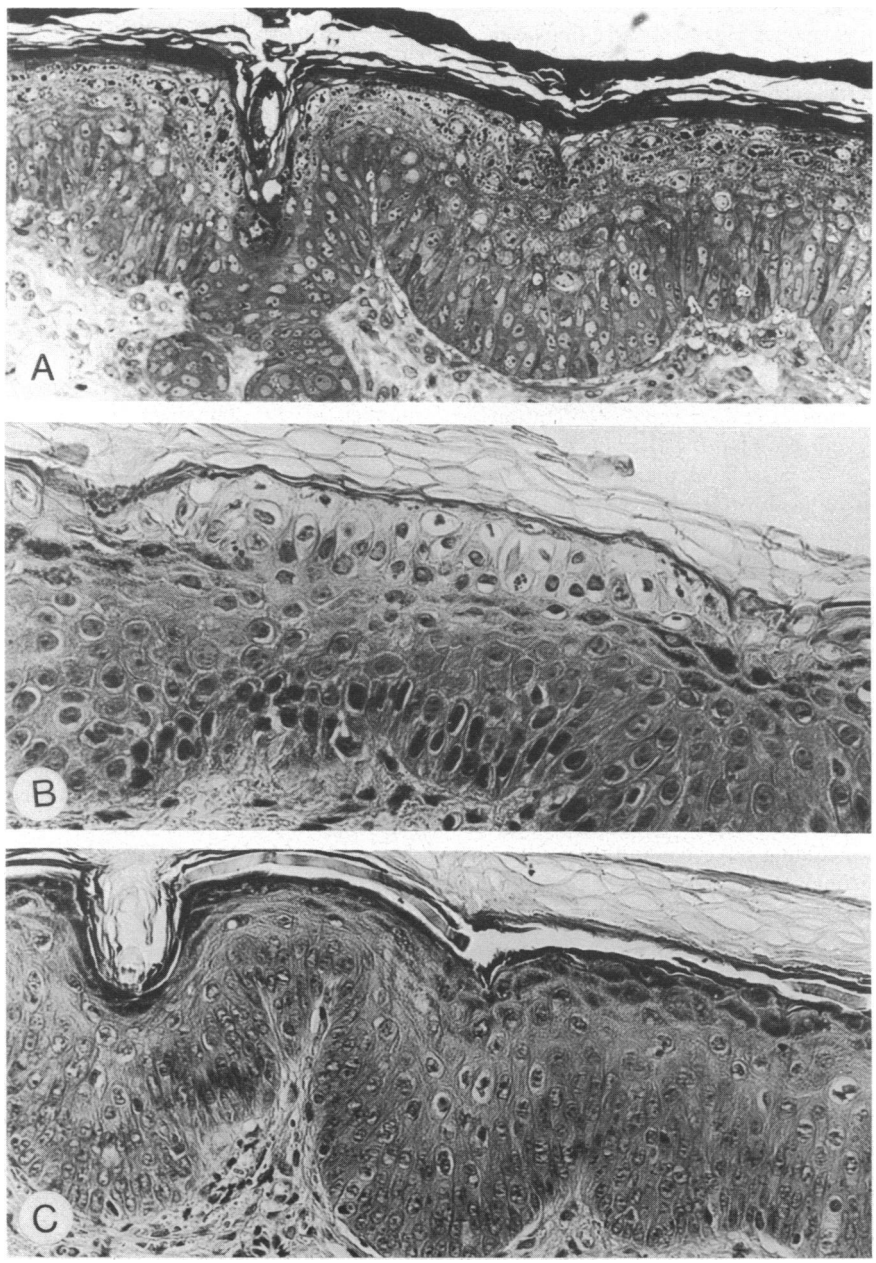

Figure 1. Transverse sections of guinea-pig epidermis (magnification × 520). (a) Normal (toluidine blue stain). (b) Day 0 following 4 consecutive daily exposures of UVC, 30 min/day (H & E stain). (c) Day 4 following 4 consecutive daily UVC exposures for 20 min/day (H & E stain).

Microscopic observations

Histology

In H & E, Giemsa and toluidine blue stained sections, the skin of control guinea pigs exhibited a thin layer of stratum corneum. The epidermis consisted of about 10 layers of epidermal cells. A thin prominent granular layer was visible (Fig. 1A).

In the skin sections of irradiated ears, histological evidence of epidermal changes was found. Using UVB, the epidermis was slightly thicker than normal with increased numbers of melanin granules. Thickening of the stratum corneum was evident. Following 20 min/day of UVB exposure, a few epidermal vesicles with pyknotic cells were obvious.

UVC at both dosages caused changes in epidermal histology. At day 0 following 30 min/day of UVC exposure for 4 consecutive days, rapid proliferation of keratinocytes occurred in basal and granular layers of the epidermis. In the basal layer, many large cells with dark nuclei surrounded by clear spaces were obvious (Fig. 1B). Also in the granular layer, large atypical epidermal cells containing small, dense and polymorphic nuclei and vacuolation were present. The stratum corneum was also thicker than normal. Four days after irradiation (Fig. 1C), most of these observed changes in the epidermis were absent. The cells in the basal lamina seemed to be normal in appearance. Some mitotic cells were seen in the suprabasal layer of the epidermis. The stratum corneum was still thicker than normal and contained cells with pyknotic nuclei. However, at day 6 after irradiation, the histology of the epidermis, including cell types and their arrangement, was indistinguishable from that of controls.

Enzyme histochemistry (ATPase activity)

Control ears. The epidermal sections and sheets from control ears showed ATPase-positive LC located in the suprabasal layer of the epidermis with an even distribution and spacing (Nithiuthai & Allen, 1984). They exhibited many long dendritic processes radiating from their central bodies.

UVB-treated ears. In UVB-irradiated skin, no obvious changes in distribution and density of LC were seen in the animals exposed to radiation for 4 and 8 min daily exposures (Table 1, Fig. 2A, B, C). The LC counts revealed that numbers of the cells were constant throughout the 6-day period after the last u.v. exposure. Although in 8 and 10 min u.v.-exposed animals, the pattern of distribution, density and number of LC remained unchanged, these cells appeared to show morphological changes, with shorter and fewer dendritic processes at day 0 (Fig. 2b, c). Also, the central body of a few cells was apparently oblong or round. However, these changes were not evident at day 2 after the last exposure. Obvious morphological changes of the ATPase-positive LC occurred in the animals' skin exposed to UVB for 20 min/day (Fig. 2D). At the end of the 4-day exposure, the ATPase-positive LC showed somewhat uneven staining properties. The cells became rounded and the size was slightly larger than normal, dendritic processes were shortened and fewer in number. A significant reduction in numbers of LC was demonstrable at day 0 and day 2 following the last u.v. exposure, as shown in Table 1 ($P<0.001$). However, the number and morphology appeared to be normal by day 4.

Table 1. Effects of mid wavelength u.v. irradiation (UVB), on the epidermal ATPase-positive LC in guinea pigs after 4 consecutive daily exposures

	No. of LC/mm^2 (±SEM) following u.v. exposure on 4 consecutive days							
	4 min/day		8 min/day		10 min/day		20 min/day	
After last exposure	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated
0	1054±1·05	1108±1·27†	1413±0·87	1362±1·16†	1127±1·02	1008±0·96†	1298±0·72	913±0·77***
2	1331±0·76	1210±0·74†	1246±1·54	1225±1·19†	1301±0·20	1276±1·92†	1411±0·91	924±2·41***
4	1130±1·24	1028±1·51†	1334±1·04	1274±2·33†	1227±1·44	1204±2·82†	1276±1·70	1148±1·92†
6	1171±0·79	1149±1·95†	1296±0·66	1201±1·32†	1249±1·01	1179±2·31†	1205±1·24	1092±0·07†

*** $P<0.001$.

† No significant difference.

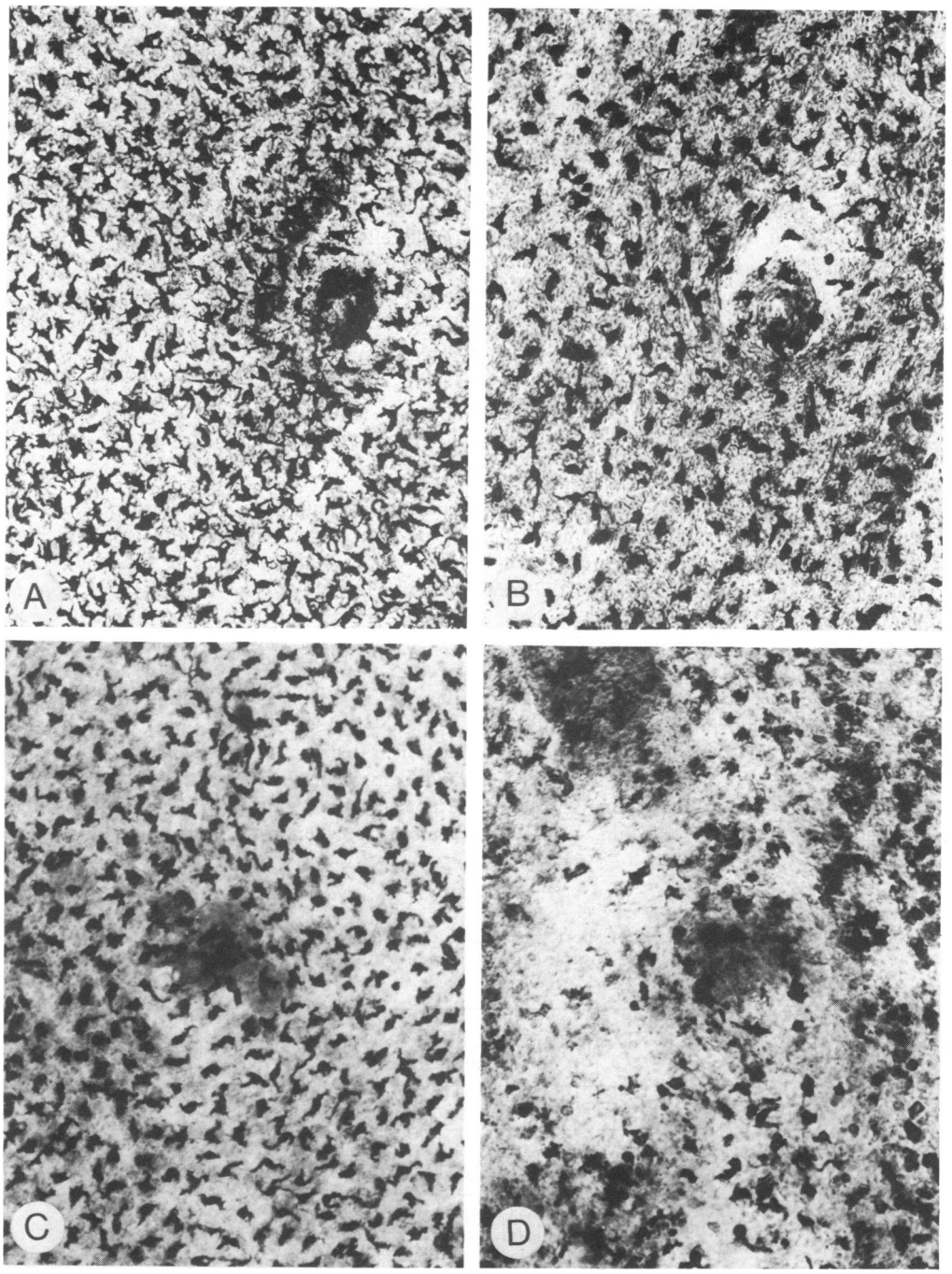

Figure 2. ATPase-positive Langerhans cell distribution in epidermal sheets of guinea-pigs at day 0 following UVB exposure on 4 consecutive days (magnification × 148). (a) 4 min/day. (b) 8 min/day. (c) 10 min/day. (d) 20 min/day.

UVC-treated ears. UVC irradiations induced a profound effect on the epidermal ATPase-positive LC with alterations in their numbers, morphology and distribution. Both groups of animals (Group V 15 min/day, and Group VI 30 min/day of u.v. exposure) showed significant decreases in the numbers of LC as shown in Table 2. The reduction in the LC population lasted for 6 days after the last u.v. exposure and the remaining ATPase-positive LC exhibited abnormal morphology. At day 0, the numbers of LC fell dramatically from the normal of 1312 and 1352 cells/mm^2 to 623 and 754 cells/mm^2 in treated ears Groups V and VI respectively. Many of these ATPase-positive cells were not recognizable as normal LC; most of their dendritic processes were absent or attenuated (Fig. 3A, B). The cells became smaller, round or elongate and unevenly distributed. Some were found in suprabasal layers of the epidermis but many were located in the upper granular layer which was abnormally close to the stratum corneum (Fig. 3B). The epidermis seemed to be thicker than normal. Granulated keratinocytes were evident, providing a darker granular background of apparent ATPase activity in the epidermal sheets of treated animals. This appearance was seen in both groups of treated animals but it was more prominent in the animals exposed for 30 min/day. Two days following the last u.v. exposure, changes in morphology and density of ATPase-positive epidermal LC were similar to those at day 0.

At day 4 after irradiation, many uneven dark patches of fine pigment, possibly melanin, were evident (Fig. 3C). Some cells exhibited more dendritic processes than at day 0. However, numbers of ATPase-positive cells/mm^2 remained significantly decreased and unchanged from day 0 (Table 2). The stratum corneum was obviously much thicker than normal and contained irregular deposits of ATPase positive material (Fig. 3D).

At day 6, dendritic ATPase-positive cells were obvious in some areas of the epidermal sheets but most of the cells remained abnormal in appearance. Hence, these morphological changes existed over the period of 6 days of the observations. The numbers of cells recorded in epidermal sheets from UVC treated ears (Table 2) included all ATPase-positive cells, whether or not they showed normal LC morphology.

DISCUSSION

Our study attempted to demonstrate the effects of UVB and UVC radiations on the epidermal ATPase-positive LC of guinea-pigs' skin. Using a gold chloride staining technique, Fan *et al.* (1959) were able to demonstrate a reduction in the number of epidermal LC in guinea-pigs' ear skin after u.v. exposure; in this experiment, however, the light source, dose and duration of u.v. irradiation application was unclear.

In UVB-treated guinea-pigs, our observations demonstrated that changes in both LC morphology and density occurred only after the animals were exposed to UVB at a total dose of 412 mJ/cm^2. These changes were demonstrable at day 0 and day 2, but not

Table 2. Effects of short wavelength u.v. irradiation (UVC) on the epidermal ATPase-positive LC in guinea-pigs after 4 consecutive daily exposures

	No. of LC/mm^2 (±SEM) following u.v. exposure on 4 consecutive days			
	15 min/day		30 min/day	
Days after last exposure	Control	Irradiated	Control	Irradiated
0	1312±1·07	623±1·12***	1352±1·63	754±1·49***
2	1297±1·05	546±1·79***	1460±0·86	492±0·89***
4	1263±0·93	614±1·07***	1380±0·84	723±1·45***
6	1200±0·76	898±1·90***	1360±0·67	682±1·01***

*** $P<0·001$.

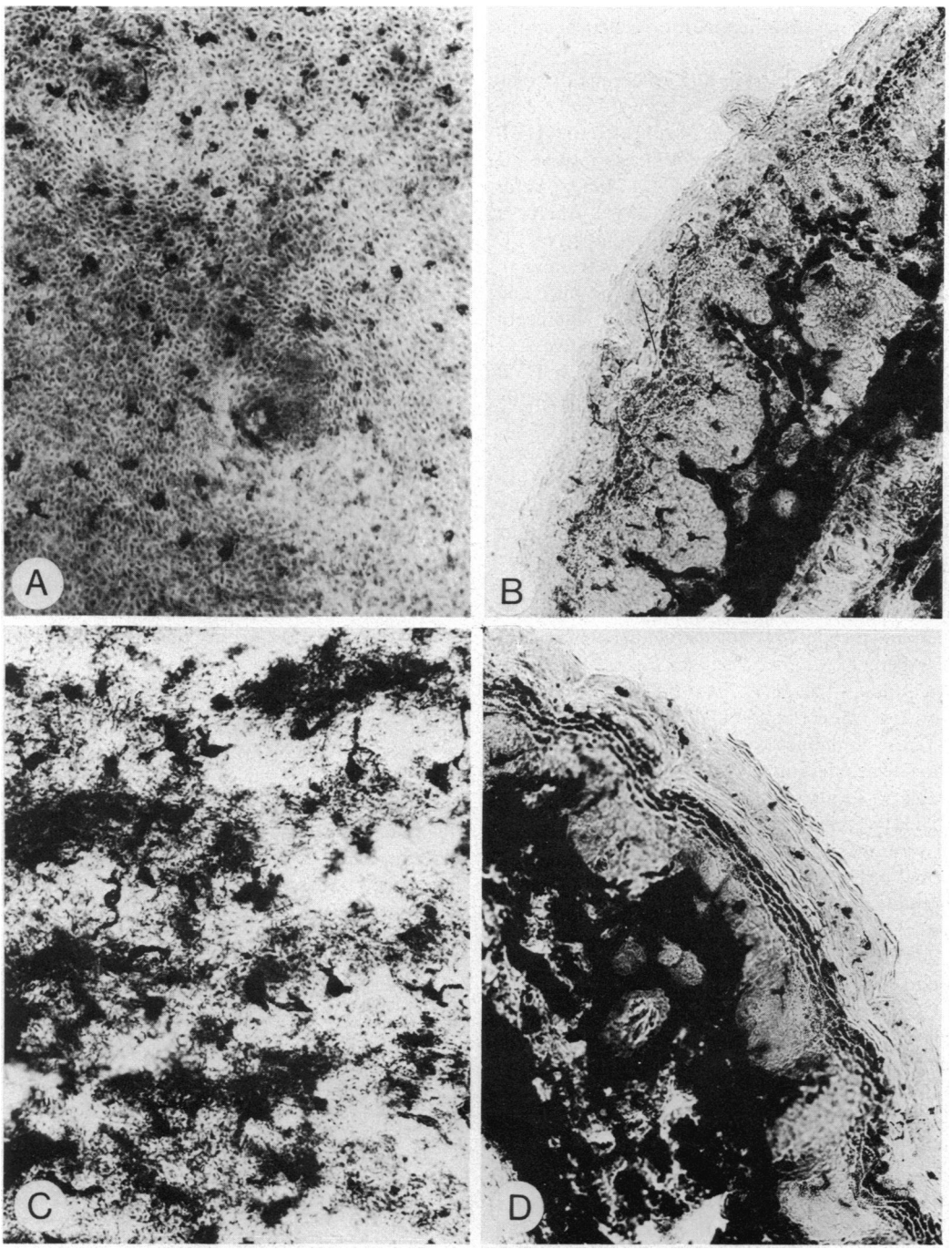

Figure 3. Distribution of epidermal ATPase-positive Langerhans cells of guinea-pigs after UVC exposure for 4 consecutive days, 30 min/day (magnification × 148). (a) Epidermal sheet (day 0). (b) Cross section (day 0). (c) Epidermal sheet (day 4). (d) Cross section (day 4).

at day 4 and day 6 after the last u.v. exposure, and this dose caused marked inflammatory changes in the exposed ears.

The responses of the skin and epidermis of some species to UVB irradiations have been studied extensively. Many of these studies have indicated that UVB is capable of depleting epidermal ATPase-positive LC in mice, hamsters and man (Toews *et al.*, 1980; Aberer *et al.*, 1981; Streilein & Bergstresser, 1981). Aberer *et al.* (1981) studied the effects of UVB irradiation on LC populations in the skin, using single doses from 15 to 100 mJ/cm^2 and from 15 to 200 mJ/cm^2 in mice and man, respectively. They demonstrated significant reductions of Ia-positive and ATPase-positive LC. Toews *et al.* (1980) reported that 4 daily UVB exposures, providing a total dose of 40 mJ/cm^2, greatly reduced the number of epidermal ATPase-positive LC in the abdominal skin of mice. Streilein & Bergstresser (1981) showed a reduction of ATPase-positive LC in the body wall skin of hamsters after 4 consecutive daily UVB exposures. These results are similar to our findings with guinea-pigs.

Furthermore, it has been indicated that UVB irradiation in mice caused changes not only in numbers and morphology but also changes in function of LC (Aberer *et al.*, 1982; Perry & Green, 1982). In an *in-vitro* study, Aberer *et al.* (1982) showed that UVB-treated mouse epidermal cells, as stimulator cells, failed to induce syngeneic and allogeneic T lymphocyte proliferation. Also Perry & Greene (1982) were able to demonstrate delayed-type hypersensitivity (DTH) responses to trinitrophenyl (TNP) in mice which were recipients of syngeneic TNP-conjugated epidermal cells, but not in mice which had received TNP-conjugated epidermal cells from UVB treated donors.

In UVC-treated skin, the results here showed profound effects of UVC on epidermal ATPase-positive LC. A highly significant reduction in ATPase-positive LC numbers and abnormal morphology of the remaining cells were evident over a period of 6 days after the last exposure. Nordlund, Ackles & Lerner (1981), however, using anti-Ia serum and indirect immunofluorescence to identify LC, indicated that UVC or UVB irradiation of mice at relatively very low dosages (1900 μJ/cm^2 and 2920 μJ/cm^2 respectively) resulted in increased densities of LC in the epidermis.

The reasons for the differences in the published results from u.v.-irradiated animals have been discussed by several authors and probably depend on a number of factors: the dose of u.v. exposure, the site of skin treated, the criteria used for establishing minimal erythema doses, stray radiation, wavelength of radiation, and species of animals used. It has been suggested that the selective penetration of different wavelengths of u.v. radiation may be due to the thickness, composition and morphology of the stratum corneum of the animals, since radiation must pass through the stratum corneum before reaching the viable cells in the epidermis (Anderson & Parrish, 1981). A large decrease in depth of u.v. penetration can be ascribed to even a small increase in thickness of the stratum corneum. Therefore, variation in skin thickness in different species of animals or in the same species may be reflected in different results.

In conclusion, in our experiments designed to find a suitable wavelength of u.v. radiation to deplete ATPase-positive LC in the epidermis of guinea-pigs' ears for a period of at least 5 days, UVC radiation treatments were shown to cause dramatic and prolonged depletion of numbers of morphologically normal ATPase-positive epidermal LC in guinea-pigs, without causing gross inflammatory responses of the skin. Considering the results of Aberer *et al.* (1982) and Perry & Greene (1982), it is probable that such UVC treatment of guinea-pigs may also reduce the immunological functions of the LC.

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REFERENCES

ABERER W., SCHULER G., STINGL G. HONIGSMAN H. & WOLFF K. (1981) Ultraviolet light depletes surface markers of Langerhans cells. *J. invest. Dermatol.* **76,** 202.

ABERER W., STINGL G., STINGL-GAZZE L.A. & WOLFF K. (1982) Langerhans cells as stimulator cells in the murine primary epidermal cell–lymphocyte reaction: alteration by UV-B irradiation. *J. invest. Dermatol.* **79,** 129.

ALLEN J.R., KHALIL H.M. & WIKEL S.K. (1979) Langerhans cells trap tick salivary gland antigen in tick-resistant guinea pigs. *J. Immunol.* **122,** 563.

ANDERSON R.R. & PARRISH J.A. (1981) The optics of human skin. *J. invest. Dermatol.* **77,** 13.

FAN J., SCHOENFELD R.J. & HUNTER R. (1959). A study of the epidermal clear cells with special reference to their

relationship to the cells of Langerhans. *J. invest. Dermatol.* **32,** 445.

MORISON W., PARRISH J.A., WOEHLER M.E. & BLOCK K.J. (1981) The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. I. UVB radiation. *Br. J. Dermatol.* **104,** 161.

NORDLUND J.J., ACKLES A.E. & LERNER A.B. (1981) The effects of ultraviolet light and certain drugs on Ia-bearing Langerhans cells in murine epidermis. *Cell. Immunol.* **60,** 50.

NITHIUTHAI S. & ALLEN J.R. (1984) Significant changes in epidermal Langerhans cells of guinea-pigs infested with ticks (*Dermacentor andersoni*). *Immunology*, **51,** 133.

PERRY L.L. & GREENE M.I. (1982) Antigen presentation by epidermal Langerhans cells: Loss of function following ultraviolet (UV) irradiation *in vivo*. *Clin. Immunol. Immunopathol.* **24,** 204.

STINGL G. (1980) New aspects of Langerhans' cell function: Review. *Int. J. Dermatol.* **19,** 189.

STREILEIN J. & BERGSTRESSER P.R. (1981) Langerhans cell function dictates induction of contact hypersensitivity or unresponsiveness to DNFB in syrian hamsters. *J. invest. Dermatol.* **77,** 272.

TOEWS G.B., BERGSTRESSER P.R. & STREILEIN J.W. (1980) Epidermal Langerhans cell density determined whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* **124,** 455.

WIKEL S.K. (1982) Immune responses to arthropods and their products. *Ann. Rev. Entomol.* **27,** 21.