Scanning Microscopy

Volume 7 | Number 4 Article 17

10-25-1993

Reduction of Sunburn Damage to Skin by Topical Application of Vitamin E Acetate Following Exposure to Ultraviolet B Radiation: Effect of Delaying Application or of Reducing Concentration of Vitamin E Acetate Applied

John R. Trevithick
University of Western Ontario

David T. Shum
University of Western Ontario

Sahle Redae
University of Western Ontario

Kenneth P. Mitton
University of Western Ontario

Christopher Norley
University of Western Ontario

See next page for additional authors

Reduction of Sunburn Damage to Skin by Topical Application of Vitamin E Acetate Following Exposure to Ultraviolet B Radiation: Effect of Delaying Application or of Reducing Concentration of Vitamin E Acetate Applied

Authors

John R. Trevithick, David T. Shum, Sahle Redae, Kenneth P. Mitton, Christopher Norley, Stephen J. Karlik, Alan C. Groom, and E. E. Schmidt

Scanning Microscopy, Vol. 7, No. 4, 1993 (Pages 1269-1281)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/93$5.00+.00

REDUCTION OF SUNBURN DAMAGE TO SKIN BY TOPICAL APPLICATION OF VITAMIN E ACETATE FOLLOWING EXPOSURE TO ULTRAVIOLET B RADIATION: EFFECT OF DELAYING APPLICATION OR OF REDUCING CONCENTRATION OF VITAMIN E ACETATE APPLIED

John R. Trevithick*, David T. Shum[1], Sahle Redae, Kenneth P. Mitton, Christopher Norley[2], Stephen J. Karlik[2], Alan C. Groom[3], and E.E. Schmidt[3]

Departments of Biochemistry, [1]Pathology, [2]Diagnostic Radiology and Nuclear Medicine, [3]Medical Biophysics, Faculty of Medicine, University of Western Ontario, London, Ontario, Canada N6A 5C1

(Received for publication June 10, 1993, and in revised form October 25, 1993)

Abstract

The skin of the skh-1 mouse after ultraviolet B (280-320 nm, UVB) irradiation shows the pathological changes typical of sunburn damage: spongiosis (edematous spaces) around some cells, necrosis of keratinocytes, giving rise to sunburn cells, inflammatory infiltration of polymorphonuclear leucocytes, etc. In our previous study, these were accompanied by erythema, increased skin sensitivity, and edematous swelling. The topical application of tocopherol acetate (TA) immediately after the UVB exposure decreased these changes. In this paper, multiple measurements of the skin thickness were made at different locations along the magnetic resonance imaging (MRI) cross-sectional image of the skin. This permits effects to be quantified with (if desired) the contralateral half of the back serving as an internal control, either exposed (positive control) or unexposed (negative control). Topical application of TA resulted in an increase in the concentration of free tocopherol in the skin. No qualitative differences in ultrastructural appearance of the UVB-irradiated, TA-treated skin could be discerned by careful examination. *In vivo* high resolution video microscopy of blood flow in venules of the irradiated mouse ear revealed a large (tenfold) but not statistically significant decrease in stationary lymphocytes adhering to the venule walls. The delaying of the application of TA up to 8 hours after the termination of UVB irradiation still offered statistically significant protection as did immediate application of 5% TA in diluent Myritol 318 (Delios S, Henkel).

Key Words: Erythema, skin thickness, sunburn, ultraviolet B, antioxidants, vitamin E acetate, delay of application, concentration effect, ultrastructure of skin.

*Address for correspondence:
John R. Trevithick
Department of Biochemistry
Faculty of Medicine,
University of Western Ontario,
London, Ontario, Canada N6A 5C1
Telephone number: 519 661 3063
FAX number: 519 661 3175

Introduction

Sunburn is a painful experience for both humans and some domestic animals (Penny and Muirhead, 1986). Unfortunately, chronic ultraviolet (UV) exposure results in premature aging of the exposed skin and also increases the risk of skin cancer (Kligman and Kligman, 1986). Over-exposures can result in severe but local sunburn reactions with erythema, increased skin sensitivity to touch and edematous swelling of the skin, which are associated with necrosis of keratinocytes ("sunburn cell" formation) and inflammatory infiltration of the skin (Diffey *et al.*, 1984; Young, 1987; Lever, 1990).

This identifies the need for a satisfactory treatment in a topical vehicle which can be applied to the skin after UV exposure. Several groups have shown that topical application of antioxidants including tocopherol to the skin either before or after the UV exposure in model systems can reduce the severity of the sunburn reaction, which peaks during the first 24-48 hours (Black, 1974; Black and Chan, 1975; De Rios *et al.*, 1978; Roshchupkin *et al.*, 1979; Miyachi *et al.*, 1983; Potapenko *et al.*, 1983, 1984; Ohzawa *et al.*, 1984; LeCak, 1986; Moeller *et al.*, 1987, 1989; Khettab *et al.*, 1988; Bissett *et al.*, 1990; Gensler and Magdaleno, 1991; Record *et al.*, 1991; Trevithick *et al.*, 1992). Tocopherol in particular may be particularly suitable since it is hydrophobic, making it suitable for formulations which do not wash off. Unfortunately, free tocopherol is known to autooxidize easily (Burton and Ingold, 1989), which makes it unstable for long-term storage. For this reason, the acetate ester, in which the phenolic hydroxyl group of the chromanoxyl ring is protected from oxidation, is usually used when long-term stability is required. In addition, tocopherol occasionally causes erythema multiforme when applied topically two to three times a day for several days (Saperstein *et al.*, 1984). This severe skin inflammation would exacerbate the sunburn if it occurred.

Several studies have indicated that the concentration of free radicals in the skin is increased after UV radiation of the skin (Norins, 1962; Pathak and Stratton, 1968; Fuchs *et al.*, 1989) which provides a rationale for the effect of antioxidants, such as tocopherol, in

preventing sunburn damage to the skin. Such antioxidants would lower the concentration of free radicals in the skin, and this presumably would decrease the production of chemoattractants for leucocytes which result in both the erythema observed and the autocatalytic nature of the inflammatory process which is observed in sunburn (Luger, 1986; Roberts *et al.*, 1986). Concentrations of tocopherol reported in the skin are somewhat lower than those encountered in other organs (Fuchs *et al.*, 1989), and this may have a functional significance. Conversion of tocopherol acetate (TA) to the active free tocopherol requires that the acetate group protecting the phenolic hydroxyl be removed by esterases, a process which normally occurs in the gut during digestion (Burton and Ingold, 1989). Whether such a conversion could occur in the skin for topically applied TA was not known when this work began. In our previous studies (Trevithick *et al.*, 1992), after exposing the skin to UVB, we tested topical treatment with pure TA, a commonly used storage form of the antioxidant vitamin E. Tocopherol acetate at room temperature and skin temperature is a thick oil. Chemically, TA has the phenolic hydroxyl group protected.

The skh-1 mice used are frequently employed to evaluate sunscreens for sun protection factor (SPF, Diffey *et al.*, 1984; Kligman and Kligman, 1986).

The effectiveness of the treatment was evaluated previously by our group (Trevithick *et al.*, 1992) using three different parameters which were quantified: erythema (measured by an erythema meter, Diffey *et al.*, 1984), skin thickness and edematous swelling as measured by magnetic resonance imaging (MRI), and skin sensitivity as measured by a modified esthesiometric technique. Since these parameters appeared to vary similarly in response to UVB exposure and treatment with topical TA, in the experiments reported here we decided to monitor the effect of treatment by the decrease of skin thickening as a result of treatment, determined by MRI, since the desired result was obtained with half the back of one skh-1 mouse using the MRI technique. By contrast, measurements involving erythema index increases required 6-8 mice to obtain statistically valid results.

Initially, histology and visual observation of desquamation in the skh-1 mouse model system confirmed that UVB exposure resulted in 24 and 48 hour histopathological samples in typical keratinocyte necrosis ("sunburn cell" formation) and desquamation, which typically occurred during the period 6-10 days post-UVB. In these studies (Trevithick *et al.*, 1992) the topical application of vitamin E acetate was shown to result in significant reduction in each of the parameters measured: erythema, skin thickness and skin sensitivity, and earlier desquamation.

Several questions still remain to be answered, among these are the following:

(**1**) Does TA application to the skin result in a higher concentration of free tocopherol in the skin after the topical application of TA?

(**2**) Does the magnitude of the decrease in skin thickening which can be ascribed to TA treatment become less if the TA is applied at increasing time intervals after the UVB irradiation?

(**3**) Is the magnitude of the decrease in skin thickening related to the concentration of TA applied when a non-irritating diluent is used for the TA?

(**4**) Can half of the back serve as a contralateral control for estimation of the effectiveness of irradiation or treatment regimens by MRI?

In performing the experiments reported here we attempt to answer some of these questions. We also explored some of the ultrastructural changes in the skh-1 mouse skin after UVB exposure and/or TA treatment.

Methods

Animals

Hairless mice of the skh-1 strain obtained from Charles River were retired breeding males (approximately one year old at the time of purchase), and were kept for up to 9 months after purchase, giving an age of approximately 21 months for most of the mice described in the experiments below. Mice were housed individually in shoe-box cages, since the male mice were observed to fight and their skins became scratched when caged in groups of 3 or 4 animals. The mice were given water and Agway 3200 rodent chow *ad libitum*.

Irradiation

Irradiation to a total dose of 0.115 J/cm^2 and topical treatment with TA were performed as described previously (Trevithick *et al.*, 1992). Briefly, for UV exposure, mice were placed in one liter glass beakers (Corning, 10 cm diameter x 15 cm high) containing 100 ml dry measure of cedar chips (2-3 mm square). Mice were anesthetized using valium and pentobarbital prior to exposure to UVB for defined periods of time. The dose of UVB was calculated from the fluence measured at the same location using a UVB meter (UVX Digital Radiometer, UVP, San Gabriel, CA). For these measurements, the UVB (310 nm) sensor was placed in the same position and at the same distance (25.5 cm) from the source as the mouse. The UVB source was four Phillips TL40W/12 tubes placed in a standard four-tube fluorescent unit and operated at 110 volts (after initially starting at 140 V followed by a 10 minute warmup period to stabilize prior to irradiation of the mice). In most experiments, this resulted in a UVB level of approximately 450 μW/cm^2. Testing with the UVA sensor (360 nm peak) revealed that these tubes emit approximately 4 times as much UVB (310 nm peak irradiation) as UVA.

Half-back exposures and treatments

When desired, the anesthetized mice could serve as their own controls by shielding half of the back by two layers of loosely applied 18 mm wide masking tape (3M, London, Ontario). Testing with the UVB meter showed that this tape reduced the incident UVB light intensity by more than 98%. Alternatively, after the irradiation, half of the back could be treated with topical

vitamin E and the other half left untreated. As a control for any general effect on the skin thickness which might occur, the belly skin thickness was monitored and in no case did any increase in thickness occur in this unexposed area.

Application of treatments

Vitamin E acetate (VEA, D-α-tocopherol acetate), a clear, thick oil at room temperature, was applied at a concentration of approximately 5 mg/cm^2 by dipping a cotton-tipped wooden applicator stick into a jar containing the TA (either pure or diluted in Myritol 318, also known as Delios S, Henkel, Kankakee, IL), removing excess oily liquid on the edge of the jar, and gently rolling the liquid-soaked cotton applicator longitudinally along the back of the mouse in a pattern so that the entire back received a uniform shiny coat of liquid containing VEA. For half-back exposures the treatment was restricted to the half of the back to the right or left of the center line, as appropriate (Table 1, Figure 1). The shiny surface remaining after the coating permitted accurate application of the liquid within only the desired area.

The dose of UVB administered (0.115 J/cm^2) can be calculated to be 3.3-3.8 times the minimum erythema dose (MED) depending on whether one uses Kligman's value of 0.035 J/cm^2 (Kligman *et al.*, 1982) or Lowe's value of 0.03 J/cm^2 (Lowe, 1981).

Magnetic resonance imaging (MRI)

For MRI, mice were anesthetized with sodium pentobarbital (30 mg/kg) with valium (5 mg/kg) as a preanesthetic. Imaging and skin thickness measurements were performed as described previously (Trevithick *et al.*, 1992) at 24 and/or 48 hours after the irradiation. Briefly, MRI (Karlik *et al.*, 1989) of anesthetized mice was used to measure skin thickness at the central back, by axial sectional images of 2 mm thickness of the thorax-upper abdomen using a Bruker MSL 80 imaging spectroscopy system (Figure 1). A 5.6 cm field of view and 256 matrix yielded an in-plane pixel dimension of 217 μm. The normal mouse skin was measured in an axial section positioned in the middle of the saddle hump of the mouse back (Figure 1A). The section showing minimum skin thickness (Fig. 1B, or in the sagittal view, Fig. 1A, the one in which the skin of the back was most parallel to the horizontal plane) was chosen for measurement: The downward curvature of the back on both sides of the saddle or hump, the highest point of the back of the prone resting mouse (as one proceeded in either posterior or anterior directions), increased the apparent thickness of the skin due to volume averaging, in images in sections adjacent to the one used to measure thickness of the skin. Thus, apparently thicker skin images were located on both sides of the section of minimum thickness (Figs. 1A and 1B). This selection could easily be made by comparing serial axial images and choosing the one with minimum skin thickness. For this section, the thickness was measured as illustrated in Figure 1C for the two sides, using a minimum of 8 different locations on the back skin, for each side of the back, as seen in the section. Typical measurements for either side of the back are shown, in a combined photograph (Fig. 1C), illustrating, for each side of the back, a typical measurement showing a point and a line, from which the perpendicular distance to the point is measured. The distances are illustrated for the upper half of the figure, by the line labelled "Y" and for the lower half of the figure, by the line labelled "Z". As is apparent from simple visual inspection, the distance "Y" (irradiated side) is much greater than the distance "Z" (side protected during irradiation with masking tape) and the skin on the upper irradiated half is thicker, but its outline less distinct than the unirradiated lower side of the image.

Since we developed this technique (Trevithick *et al.*, 1992), its utility in measuring skin thickness repeatedly on successive days by a non-invasive technique became apparent, since the skin thickness of the same animal could be followed initially on a daily basis, and later during the recovery period at weekly intervals for several weeks until the thickness returned to normal. Time-dependent responses were determined using multiple regression statistical modelling, with UV exposure, Vitamin E acetate (VEA) treatment and time as independent variables. The resolution of the technique, 0.2 mm per pixel combined with the distance measurement program (Bruker), permitted significant differences to be determined between skin thickness measurements made on different days using the axial sections of the mouse. To perform statistically significant comparisons, a minimum of 7 or more measurements was made per section, each at a different site of the centrally located back skin as described in the legend to Figure 1. The MRI skin thickness was measured for the skin in the region to the left or right of the midline of the back as described above (Trevithick *et al.*, 1992; and Figure 1). To perform statistically valid comparisons, a minimum of 7 or 8 measurements per section was obtained, per section or for the half-back tests, for the half of the back in question. Each measurement was made at a different spot in the section or half-back, as appropriate. The region used for measurements was as close to the midline as practical, in order to achieve consistency in measurement by comparing regions receiving similar UVB doses. Each measurement was documented by a computer printout of the image showing the position of the internal point and the tangential line used for this measurement (Fig. 1C). Since each area of the skin was shown to be independent in its UVB response when compared to adjacent areas, as shown by the shielding experiments and as known from the sharp delineation of sunburned areas observed at the edge of clothing, or as reported by Cole *et al.* (1983) for irradiated spots on mouse skin, contralateral halves of the back of each mouse could be used (Table 1) to determine the skin response to UVB or for different treatment regimens. Thus, one mouse could serve to determine skin thickening for two different exposure and treatment regimens (Fig. 1C).

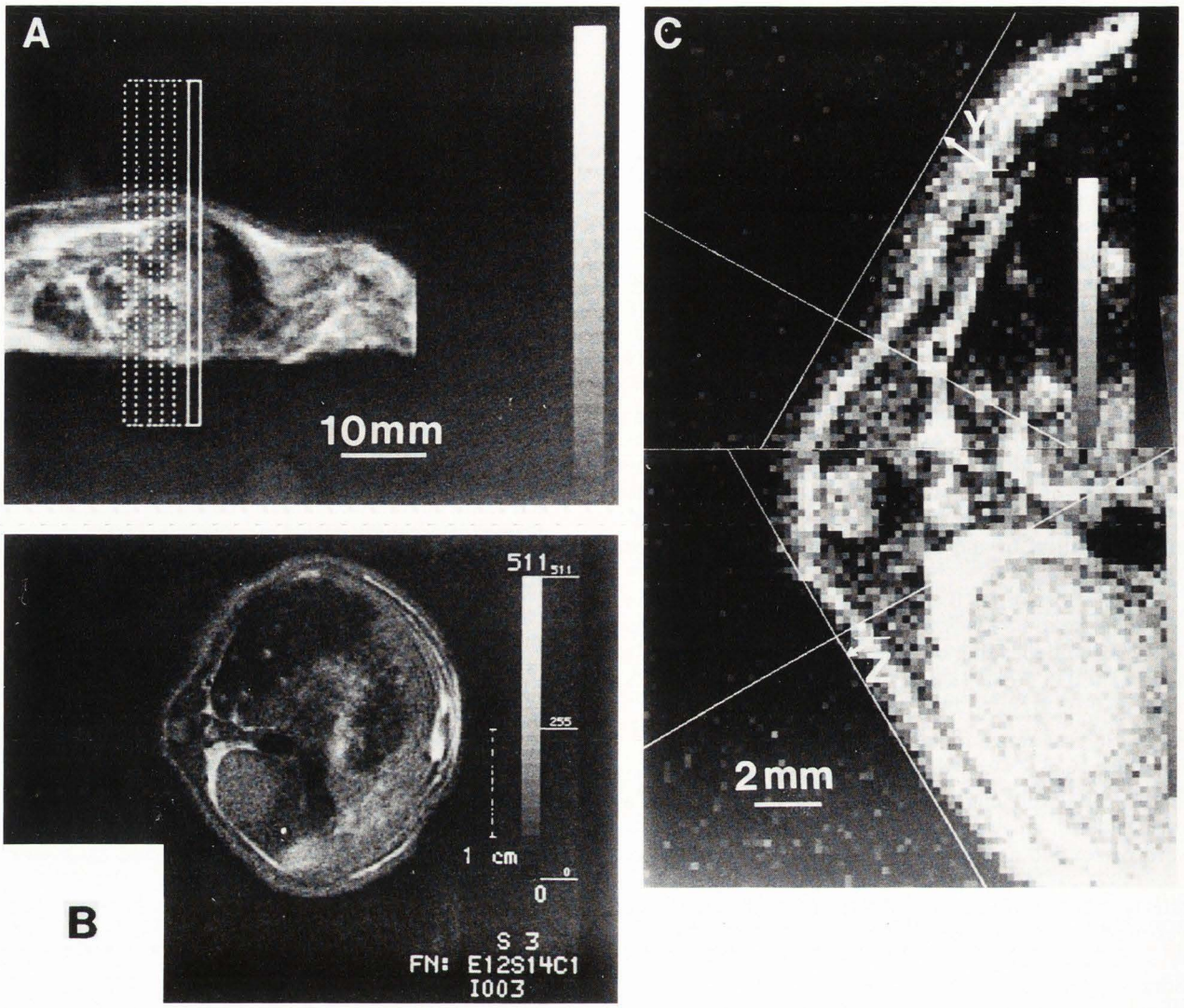

Figure 1. Magnetic resonance imaging of skh-1 mouse skin 24 hours after UVB exposure or sham exposure, illustrating how images and skin thickness measurements are obtained. **A**: Typical sagittal view of skh-1 mouse illustrating longitudinal sectional image with locations of 2 mm axial sections shown by superimposed rectangular frames in solid and dotted white lines. The slice thickness, shown by the distance between the white vertical lines, is 2 mm. The slices described in the text are numbered sequentially from the one outlined in a solid white line, which occurs at the anterior end (i.e., right to left on the photograph A). **B**: Sagittal image of third slice. **C**: Magnified view of axial image as used to obtain measurements of skin thickness. Using cursors in the distance measurement mode of the Bruker MSL80, the tangential lines for the upper and lower parts of the figure are positioned to just touch the outside edges of illuminated pixels delineating the outer layer of the mouse skin. Using a cursor, the points shown by the crosses are moved to a point delineating the inner boundary of the skin. At this boundary the light pixels of skin change to dark pixels, which do not form part of the skin. The boundary chosen is the point at which the black pixel penetrates furthest into the white or grey skin layer. Thus consistency is obtained in positioning both outside and inside boundaries similarly at the same (left) side of the layer whose thickness is being measured. The perpendicular distance Y (1.74 mm) from upper point to upper tangential line shown is calculated by the program. Similarly, for the lower line Z, 0.88 mm is the relevant distance for this measurement on the section shown, the distance from the point to the tangential line labelled which just touches the pixels delineating the skin outer surface in the lower half of the figure. To obtain average skin thickness values, this measurement is repeated by placing the cursor point at each appropriate dark point on the inside of the skin, and repositioning the line to give the best tangential line for the skin surface above that point, as explained in the text, until 7 to 8 measurements are obtained, for each side of the back. Measurements were usually taken in the area near to the midline or central area of the back, and out to approximately 0.5 cm on either side of the center, using the third axial section.

Table 1. Comparison of skin thickness of skh-1 mice determined after UVB exposure of half back or whole back.

Table 1A. Changes in thickness measured using MRI and comparison of statistical significance of any differences between whole and half back measurements.

Day After UVB	UV Dose J/cm^2	Half Back Skin mm x 100	Standard error (S.E.)	Mouse # L-left or R-right	Whole Back Skin mm x 100	S.E.	Mouse #
0	0	79*	±3.7	S14/L	86*	±3.4	K4
0	0	76*	±3.3	S14/R	76*	±3.0	K5
1	0	85*	±2.0	S14/L	82*	±2.6	K4
1	.115	131**	±3.6	s14/R	123**	±2.3	K1
1	.115	133*	±10	K15/R	129*	±6.5	K5
2	0	85*	±3.6	S14/L	87*	±2.4	K4
2	.115	148*	±8.5	S14/R	139*	±3.8	K1
2	.115	151**	±5.2	K15/R	134**	±5.9	K5

Probabilities of significant difference in skin thickness between whole and half back values (L-left or R-right) for the day of monitoring of MRI skin thickness : day 0, 1, or 2 after UVB exposure:

*No significant difference; **Significant difference, $p < 0.05$.

Significance of differences are evaluated between half back values and similarly treated mice in which whole back was treated, reading across in the same row from column 3 to column 6. Day 0 corresponds to initial skin thickness measured prior to UVB irradiation, while day 1 and day 2 are measurements obtained at 24 and 48 hour intervals after the UVB irradiation.

Table 1B. Percent change in skin thickness determined using whole back or half back measurements for L-left or R-right half back for same mice as compared in Table 1A.

DAY	UV J/cm^2	Mouse L-left or R-right Back	Percent change in skin thickness	UV J/cm^2	Mouse Whole Back	Percent change
1	0	S14 L	+7.5%	0	K4	-4.1%
1	0.115	S14 R	73.8%	0.115	K1	63.3%
1	0.115	K15 R	76.5%	0.115	K5	71.4%
2	0	S14 L	+7.9%	0	K4	+0.4%
2	0.115	S14 R	95.6%	0.115	K1	83.2%
2	0.115	K15 R	100.4%	0.115	K5	77.8%

The reproducibility of this measurement was tested (Table 1) by comparing different mice for which either whole back measurements or half-back measurements in which the area, for which MRI thickness was measured, was protected from UVB or exposed to UVB irradiation. In 6 of the 8 comparisons, the whole back measurements were not significantly different from those obtained using contralateral halves of the same mouse. Even in the one case (mouse K5) in which a statistically significant difference in thickness after UVB exposure was shown, similar trends of increased skin thickness after UVB exposure were evident. Although the actual thickness was different, this could be because of some other factor such as age, etc., since the other values were in agreement.

Histology

Either after CO_2 euthanasia or immediately after MRI and sacrifice with an overdose of Somnotol, the back skin of the mouse was removed by dissection with scissors and either frozen in liquid nitrogen or fixed in 10% neutral buffered formalin for histopathology or 1% glutaraldehyde for electron microscopy embedding. The histological samples were dehydrated and embedded in paraffin for sectioning followed by haematoxylin-eosin staining, while the glutaraldehyde-fixed tissues were embedded in Epon for electron microscopy and sections were stained with lead citrate and uranyl acetate (Hayat, 1981), and examined in a Philips 410 electron microscope.

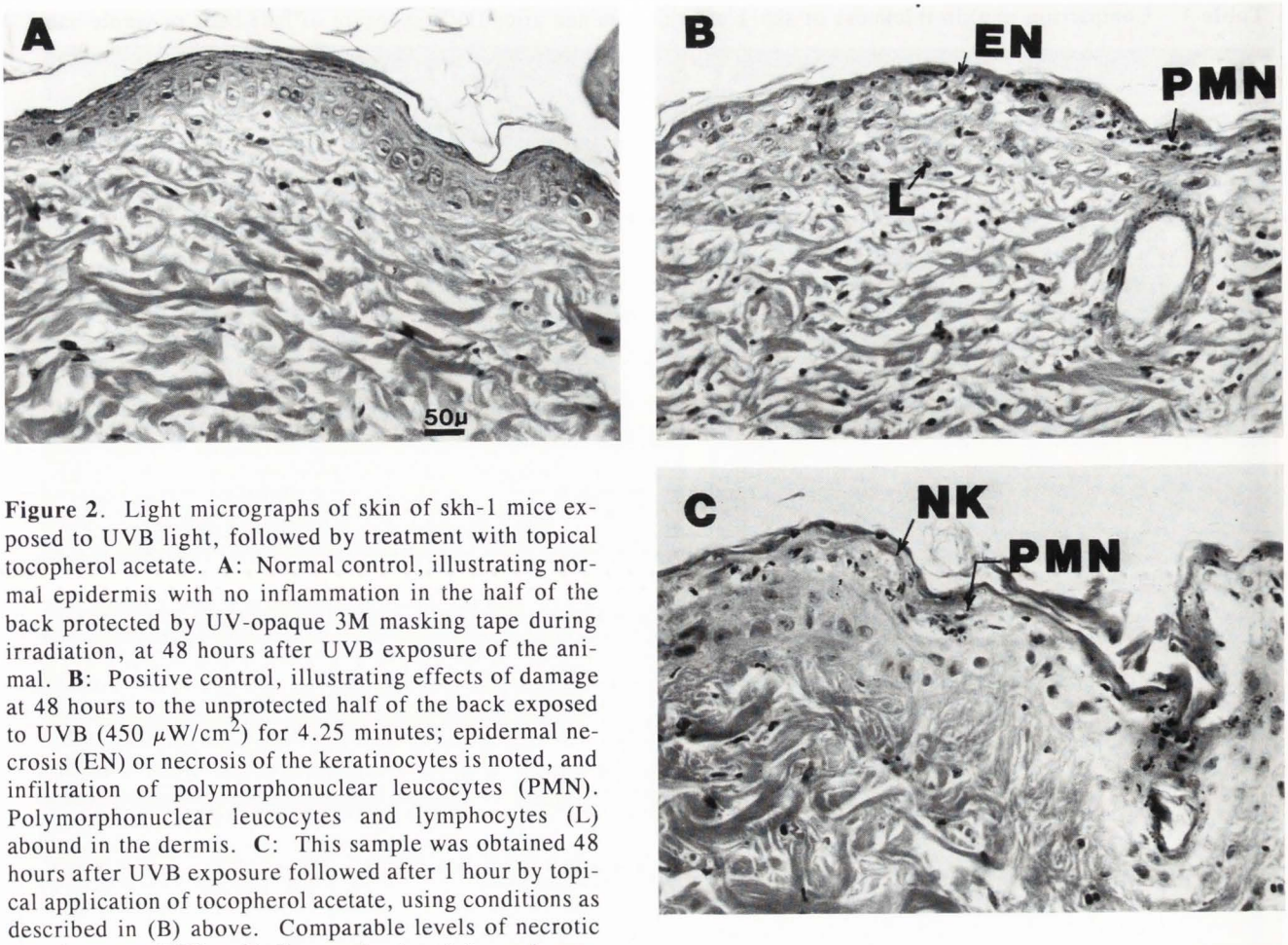

Figure 2. Light micrographs of skin of skh-1 mice exposed to UVB light, followed by treatment with topical tocopherol acetate. **A**: Normal control, illustrating normal epidermis with no inflammation in the half of the back protected by UV-opaque 3M masking tape during irradiation, at 48 hours after UVB exposure of the animal. **B**: Positive control, illustrating effects of damage at 48 hours to the unprotected half of the back exposed to UVB (450 $\mu W/cm^2$) for 4.25 minutes; epidermal necrosis (EN) or necrosis of the keratinocytes is noted, and infiltration of polymorphonuclear leucocytes (PMN). Polymorphonuclear leucocytes and lymphocytes (L) abound in the dermis. **C**: This sample was obtained 48 hours after UVB exposure followed after 1 hour by topical application of tocopherol acetate, using conditions as described in (B) above. Comparable levels of necrotic keratinocytes (NK) and inflammation involving polymorphonuclear leucocytes (PMN) were observed.

Tocopherol extraction

To extract tocopherol, skin samples (69-142 mg) previously frozen at -70°C were homogenized in 7% sodium dodecyl sulfate (SDS) in 50% aqueous ethanol containing butylated hydroxytoluene (BHT, 10 μM) and extracted three times with heptane (1.6 ml, each time) (Chou *et al.*, 1985). The extracts were pooled and evaporated under a stream of nitrogen. The residue was dissolved in 400 μl of methanol and aliquots of 45.6 μl injected using a Gilson Autoinjector on to a C18 HPLC column (5 μm ODS octadecyl 25 x 0.46 cm column with C18 guard column). The tocopherol was detected by a Bioanalytical Systems (BAS) glassy carbon electrode (LC-4B), set at 0.8 V. The mobile phase for the chromatographic separation was methanol (50%) acetic acid (3.4%) and water (1.6%), pH 4.5, and the flow rate was 1 ml/min. An internal standard (δ-tocopherol, 10 μg/ml, 0.020 ml) was used in all samples. Concentrations of α-tocopherol in each sample were calculated on a per unit weight basis from a standard curve constructed by running standard samples under identical conditions.

In vivo microscopy

In vivo microscopy of the mouse ear after UVB and TA treatment was performed to investigate how the leucocytes behaved in the microcirculation. Leucocytes were observed as described previously (Schmidt *et al.*, 1990). The skin of the ear near the ear margin was optically coupled to the microscope objective by immersion oil. Several different sites were observed to obtain average readings for numbers of rolling and stationary leucocytes per 1000 μm^2 of vessel wall area.

Results

As previously noted, sunburn damage to the skin after the exposure to UVB light resulted in an appearance we previously termed "elephant skin" (Trevithick *et al.*, 1992), which Cole *et al.* (1983) called visible edema. Protection of one side of the back of the mouse by a double thickness of loosely attached masking tape which absorbed 98% of the incident UVB, resulted in a sharp delineation between the erythema and the visible edema of the irradiated area and the normal appearance of the unirradiated protected side. Measurement of the MRI skin thickness of the UVB-exposed areas showed: (1) no significant increases in thickness of the tape-

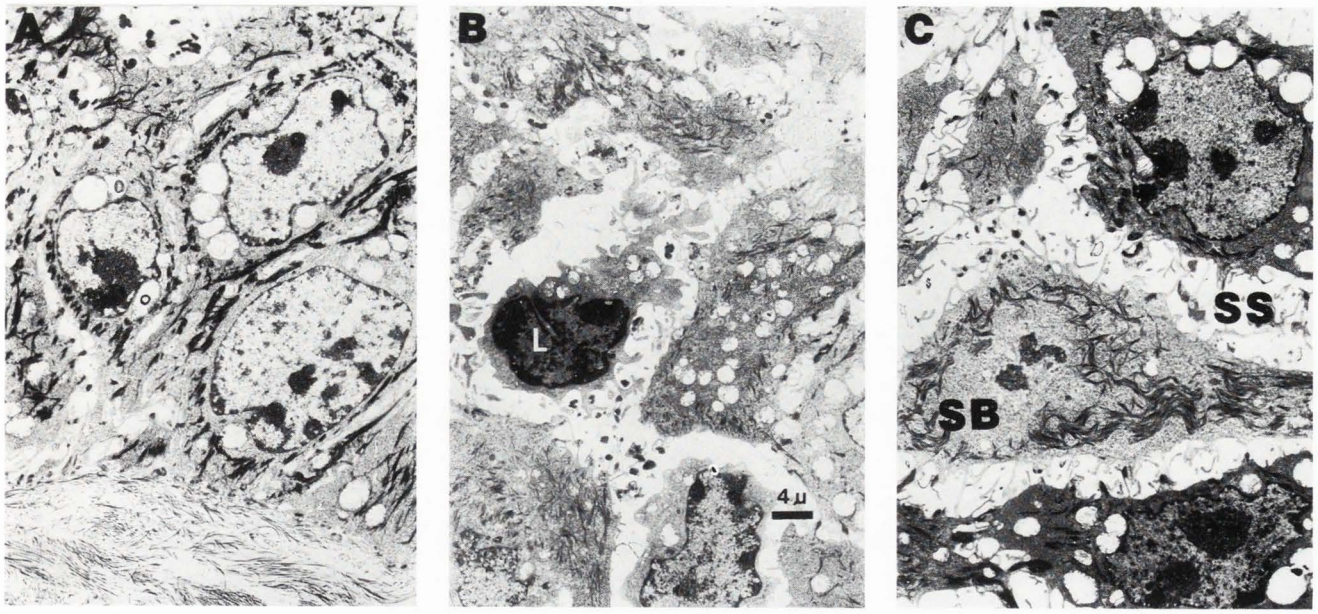

Figure 3. Electron micrographs of skin of skh-1 mice exposed to 450 $\mu W/cm^2$ for 4.25 minutes, illustrating typical changes observed 48 hours after UVB exposure. **A**: Normal control: Normal skin, from the half of the back protected by UV-opaque 3M masking tape while anesthetized during UVB exposure as described in Figure 1B. This photograph illustrates the typical appearance of normal skin, showing squamous cells and dermal collagen. **B**: UVB-exposed skin of a different area of (B) above, illustrating infiltration of inflammatory cells: a lymphocyte is seen in the center of the micrograph. These changes are part of the sunburn tissue response after UVB exposure of the skin. **C**: UVB-exposed skin from contralateral unprotected half of back of skh-1 mouse shown in (A) above, illustrating single cell necrosis of the keratinocytes with dissolution of the nuclear membrane of the center cell. This is typical of a photo-toxic reaction.

protected unirradiated skin (Table 1, Figure 1), when compared to the unshielded unirradiated areas, and (**2**) significant increases in thickness when the irradiated areas were compared to the shielded contralateral half or to the unshielded unirradiated mice.

Dissection of the skin for histopathological evaluation showed by light microscopy (Figure 2) and electron microscopy (Figure 3) the presence of necrotic keratocytes,"sunburn cells", along with inflammatory infiltration of polymorphonuclear leucocytes and lymphocytes; the space between the cells was filled with edematous fluid. The histopathological and ultrastructural features of the histopathological controls and irradiated and/or treated skin samples were typical of those described in the literature for skin: for normal skin, squamous cells and collagen fibers, etc. No significant changes were associated with TA application. Following UVB irradiation, there were polymorphonuclear leucocytes and to a lesser extent, lymphocytes infiltrating both the epidermis and dermis. Necrotic sunburn cells, along with spongiosis (frequent edematous spaces, surrounding the sunburn cells and some other cells), were observed in histology slides and by electron microscope examination. There was vascular dilatation. These changes were consistent with a larger change in the erythema index observed for the same mouse.

Table 2. Average α-Tocopherol concentration of mouse skin samples (data shown are average concentration $\pm$ standard deviation).

Treatment Group	Tocopherol[1]
VEA Treated & UV-B Exposed	$3.3 \pm 1^{**++}$
UV-B Exposed	$1.2 \pm 0.3^{**+}$
VEA Treated & Sham Exposed	$2.1 \pm 0.3^{*++}$
Sham Exposed	$1.04 \pm 0.08^{*+}$

[1]in ng/mg skin;
**significant difference ($p << 0.01$);
*significant difference ($p < 0.01$);
$^{++}$no significant difference ($p > 0.05$);
$^{+}$significant difference ($p < 0.05$).
#each treatment group consisted of 3 skh-1 mice. Mice were sacrificed by CO_2 euthanasia and the skin immediately dissected from the central back and frozen immediately in liquid nitrogen. Frozen skin samples were weighed and extracted as described in the text.

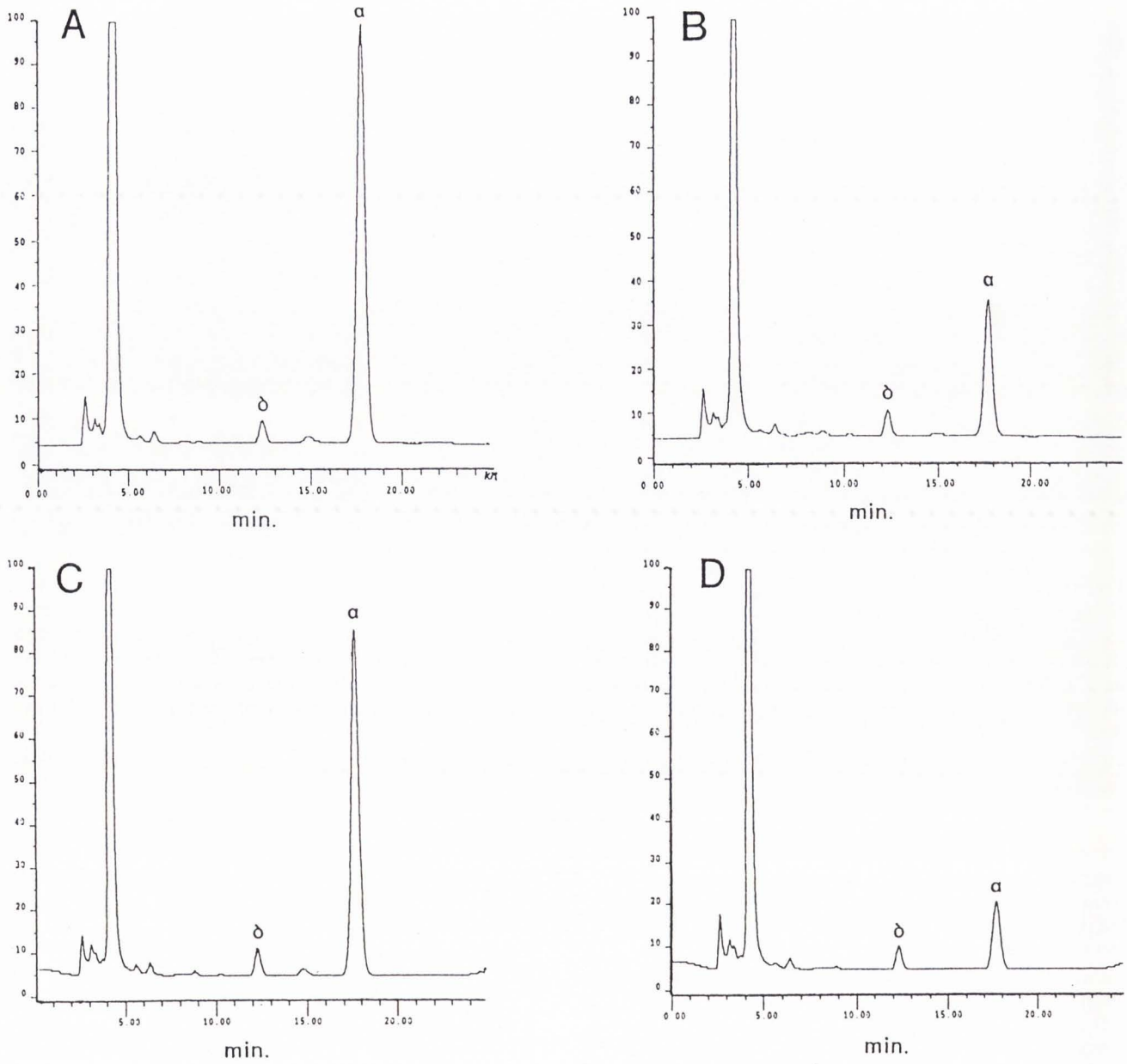

Figure 4. High performance liquid chromatography (HPLC) of extracted skin samples using electrochemical detection of tocopherols, after topical application of d-α-tocopherol acetate. Using this technique, only free, unesterified tocopherols are detected. All samples analyzed had an internal standard of δ-tocopherol added. Conditions of the analysis were: glassy carbon electrode, +0.800 V, column: Octadecyl D-5 column (B&J) 25x 0.46 cm; solvent phase: methanol/ acetic acid/water (95/3.4/1.6, v/v/v), with adjustment of the pH to 4.95 with sodium hydroxide prior to methanol addition; flow rate: 1.0 ml/min; injection volume 45.6 μl; reading of 100 (full scale) of ordinate indicates a signal of 76 mV. Chromatograms shown were obtained from skin extracts prepared as described in the text 24 hours after the following treatments: **A**: irradiated, topically treated with vitamin E acetate; **B**: irradiated control, treated with corn oil; **C**: non-irradiated, topically treated with vitamin E acetate; and **D**: non-irradiated control, topically treated with corn oil.

In vivo microscopy has been used previously (Schmidt *et al.*, 1990) to investigate the numbers of rolling and adhering lymphocytes in venules of the splenic microcirculation. In normal mouse ear, UVB irradiation resulted in an increase in numbers of rolling white cells (2.5 fold) and stationary cells (3.6 fold) in venules, while the combination of UVB followed by TA application resulted in no increase in rolling cells and a ten-fold decrease in stationary cells. Although the number of fields counted for the last result (three) was not large

Table 3. Time of treatment after UVB irradiation for 4.25 minutes.

A: Thickness in mm x 100.

Time of Treatment Time Applied (hour)	After UVB Day 0	Day 1	Day 2
0	---	103.56 ± 3.6	
1	79.14 ± 2.6	107.1 ± 1.3	118.2 ± 2.5
2	85.2 ± 3.6	95.8 ± 5.2	111.7 ± 3.4
4	85.5 ± 2.8	115.5 ± 5.2	---
8	84.1 ± 2.4	93.6 ± 7.3	---
Not Applied (NA)	75.3 ± 3.8	130.9 ± 3.2	145.0 ± 9.9

B: Percentage difference from skin thickness with probabilities of significant differences over the time intervals shown, from negative and positive controls.

	Skin thickness differences as % from negative control					
Time of Treatment After UVB	Day 1	P < from negative control	P < from positive control	Day 2	P < from negative control	P < from positive control
0	131%	.253 (0-1 hr)	0.001 (0 hr-NA)			
1	135%			149%	0.0001 (0-2 day)	0.011 (1 hr-NA)
2	112%	0.089 (0-1 day)	0.0001 (2 hr-NA)	131%	0.002 (1-2 day)	0.016 (2 hr-NA)
4	135%	0.001 0-1 day)	0.029 (4 hr-NA)	---		
8	171%	0.274 (0-1 day)	0.001 (0 hr-NA)	---		
Not Applied	171%	0.001 (0-1 day)	0.001 (0 hr-NA)	196%	0.0001 (0-2 day)	

C: Regression analysis of skin thickness increase as dependent variable with time of treatment and time after UV exposure as independent variables

Increase/day of skin thickness (mm x 100)	Increase/day/hour delay of Treatment (mm x 100)
17.83 ± 1.81 ($p < 0.00001$)	0.12 ± 0.03 ($p < 0.0002$)

enough to give statistical significance to this decrease, the trend is consistent with the result found for the difference in erythema index changes between the UVB-irradiated skin, when TA-treated and untreated skin were compared.

Tocopherol concentration in the skin (Table 2 and Figure 4) at 24 hours after topical application of TA, determined as described in the **Methods** section above, increased three-fold in the UVB-exposed skin after topical TA treatment, from a level about 1 μg/mg skin to 3.3 μg/mg skin, and in the sham-irradiated skin, a doubling of tocopherol concentration to 2.1 μg/mg skin was found.

The results on the effect of delaying application of the TA up to 8 hours are presented in Table 3. The skin thickness of the untreated irradiated area was compared to the thickness of the untreated sham-irradiated skin at 1 and 2 days after UVB irradiation. After 1 day, the skin thickness had increased less in every TA-treated skin sample than in the untreated UV-irradiated skin. When analyzed by linear regression using the SPSSPC statistical analysis program (Norusis, 1988), the increase

Table 4. Percent of tocopherol acetate in topical medium applied to skin after UVB irradiation.

A: Thickness in (mm x 100) at time indicated after UVB exposure.

Percent Tocopherol Acetate	Day 0	Day 1
0	75.3 ± 3.28	131.2 ± 2.8
5	---	98.7 ± 3.4
100	---	103.6 ± 3.6

B: Percentage increase in thickness from zero time control prior to irradiation and treatment, at day 1 after exposure and treatment.

Percent Tocopherol Acetate Applied	Percent differences from skin thickness at zero time prior to irradiation and treatment. Probability of difference from zero time or after treatment		
0	174%	0.0001 (0-1 day)	
5	131%	0.0001 (0-5%)	0.004 (day 0, 0% - day 1, 5%)
100	137.6%	0.37 (5% - 100%)	0.001 (0 - 100 %)

C: Results of regression analysis of tocopherol acetate application.

Change in skin thickness in mm per day / percent tocopherol acetate in medium

-0.1833×10^{-2} ($p < 0.0179$)

D: Reduction in skin thickness compared to untreated (Positive Control).

Percent Tocopherol Acetate	Increased by Percent	Increased to Percent
0	0%	100%
5	58%	42%
100	49%	51%

in skin thickness was 0.178 ± 0.02 mm ($p < 0.00001$) per day. The portion of the increase due to delay of treatment application was 0.0012 ± 0.0003 mm ($p < 0.0002$) per hour of delayed application per day, so that, for an 8-hour delay the additional amount attributable to the delay would be 0.0096 mm (5% of the total increase). To predict the increase for a longer delay would not be valid, although it can be extrapolated that a 24-hour delay would result in an additional 0.0288 mm increase in skin thickness (15% of total thickness).

The results of investigating the effect of decreasing the percent of TA in the oil applied to the skin immediately after the UVB exposure by applying TA diluted with Delios S (Myritol 318) are presented in Table 4. Regression analysis of the effect of concentration of tocopherol in the medium on the thickness increase indicates a decrease in thickness of 0.18334 ± 0.076 mm x 10^{-2} times the percent of tocopherol in the oil applied ($p < 0.0179$). Either treatment was successful in causing a decrease in skin thickness by comparison to the untreated irradiated skin: for 5% TA, a 58% decrease, and for pure TA, a 49% decrease.

Discussion

Uptake of radioactive tocopherol by the skin had been shown to occur by Kamimura and Matsuzawa (1968), but they did not show that conversion of the TA to free tocopherol, the active form, took place in the skin. Neither did they determine the level of free tocopherol in the skin, or whether it changed in response to topical application of TA. Thus, these experiments are the first to demonstrate that an elevation of tocopherol concentration actually occurs in the skin following a single application of pure topical TA. As pointed out in the **Introduction**, this conversion must be performed by esterases which convert TA to free tocopherol (Burton and Ingold, 1989). TA is an oily liquid which is a water-insoluble, non-swelling amphiphile, as are triglycerides and cholesterol, both of which form important components of cell membranes (Traber *et al.*, 1993). Since TA is soluble in lipids of the cell membrane, and can be taken into cells by diffusion across the cell membrane, it can enter the cell where cytoplasmic esterases can hydrolyse it. It was previously speculated

by Pascoe and Reed (1987) that this route operates in cells. Recently, Packer's group (Fuchs *et al.*, 1989) has published data showing a level of vitamin E in the skin of 2.2 ng/mg skin. This concentration is of similar magnitude to the 1.04 to 1.4 ng/mg skin we observed.

Treatment with TA has been previously shown to reduce the increase in erythema index in a statistically significant manner (Trevithick *et al.*, 1992). These experiments were performed with the primary objective of determining the tocopherol concentration in the skin after topical application of TA, with groups of only three animals per treatment regimen. No statistically significant differences were seen in the erythema index of irradiated skin as a result of the TA treatment. Since 6-8 animals are usually required to attain significant differences in erythema index increment, and since we have previously shown that this treatment is effective in reducing erythema (Trevithick *et al.*, 1992), this suggests that larger numbers of animals may be required.

The trend to a large increase in rolling cells after UVB was consistent with an induction of leucocyte chemoattractants as a result of the UVB irradiation and increase in free radicals reported to occur (Norins, 1962; Pathak and Stratton, 1968; Fuchs *et al.*, 1989), as is the case in some other inflammatory responses. These would be expected to be precursors of the cells invading the irradiated skin and resulting in the inflammation and erythema. This finding is consistent with TA treatment lowering the concentration of lymphocyte chemoattractants in the irradiated treated skin. For instance, in the antibacterial cellular response free radical production as a result of leucocyte myeloperoxidase occurs and this appears to result in stimulation of the migration of more lymphocytes and macrophages to the same area. Associated with the inflammatory response are found skin edema and swelling, observed ultrastructurally (above). These are responsible for the visible edema or "elephant skin" appearance noted above after exposure to UVB.

We have previously shown that magnetic resonance imaging (MRI) can quantify the increase in skin thickness associated with the inflammatory response and edema (Trevithick *et al.*, 1992). Leading from the observation that the line delineating sunburned or tanned areas of skin is usually quite sharp if the clothing has been tight-fitting, and Cole *et al.* (1983)'s observation of sharp delineation of the area of visible edema in the skh-1 mice exposed to UVB irradiation, one question which might be asked is whether the change in thickness of one area of the skin is independent of the adjacent area receiving a different treatment and/or irradiation exposure? The experiment which divided a mouse back into two contralateral halves which were exposed or protected, indicated that each side responded independently of the other. This approach could then be used as appropriate to provide either a positive or a negative control using the contralateral halves of the mouse back as independent areas for sampling. Using this approach reduced the number of mice required to obtain a measurement, from groups of 6-8 mice down to half the back of one mouse. We evaluated the effect of delay of application of TA after UVB irradiation. The finding of effectiveness for delayed application of TA at intervals after UVB exposure is of great interest since it points to possible clinical applications. It may be possible using this strategy to decrease the magnitude of the erythema and the inflammatory response even if the application of the topical TA is delayed significantly, for a period of up to 8 hours. Analysis of the progression of the damage after application of TA using statistical linear regression analysis also did not indicate a large decrease in sunburn damage because of the delay in application of the TA.

The effect of diluting the TA on the MRI skin thickness increase also indicated that the 5% TA in Myritol 318 was effective in reducing the skin thickening. By regression analysis, a significant concentration dependence was indicated for this effect.

Taken together, these results indicate that the topical application of TA appears to increase the concentration of free tocopherol in the skin. If UVB irradiation preceded the TA treatment, leucocyte adherence to venule walls was also decreased, and fewer leucocytes entered the irradiated skin. Erythema as determined by the associated skin thickening were decreased as a result of this. The effect may have a threshold since low concentrations (5% TA diluted into Myritol 318) are still effective, and delays after UVB irradiation of up to 8 hours before topical application are tolerated without significant diminution of the protective effect observed after 24 and 48 hours from the original UVB exposure. The threshold may be related to the necessity of maintaining the free radical level below some critical concentration in the skin after UVB exposure.

Acknowledgements

The authors acknowledge with thanks the financial support of the Ontario Government University Research Incentive Fund; Henkel Corporation, Fine Chemicals Division; and Ciba-Geigy Canada Ltd., self-medication Division. SJK received a Career Scientist Award from the Sterling Winthrop Imaging Research Institute.

References

Bissett DL, Chatterjee R, Hannon DP (1990) Photoprotective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse. Photodermatol Photoimmunol Photomed **7**, 56-62.

Black HS (1974) Effects of dietary antioxidants on actinic tumor production. Res Commun Chem Pathol Pharmacol **7**, 783-786.

Black HS, Chan JT (1975) Suppression of ultraviolet light-induced tumor formation by dietary antioxidants. J Invest Dermatol **65**, 412-414.

Burton GW, Ingold KU (1989) Vitamin E as *in vitro* and *in vivo* antioxidant. Ann NY Acad Sci **570**, 1-22.

Chou PP, Joynes PK, Bailey JL (1985) Determination of Vitamin E in microsamples of serum by liquid chromatography with electrochemical detection. Clin Chem **31**, 880-882.

Cole CA, Davies RE, Forbes PD, D'Aloisio LC (1983) Comparison of action spectra for acute cutaneous responses to ultraviolet radiation: Man and albino hairless mouse. Photochem Photobiol **37**, 623-631.

De Rios G, Chan JT, Black HS, Rudolph AH, Knox JM (1978) Systemic protection by antioxidants against UVL-induced erythema. J Invest Dermatol **70**, 123-125.

Diffey BL, Oliver RJ, Farr PM (1984) A portable instrument for quantifying erythema induced by ultraviolet radiation. Brit J Dermatol **111**, 663-672.

Fuchs J, Huflejt ME, Rothfuss LM, Wilson DS, Carlans G, Packer L (1989) Acute effects of near ultraviolet and visible light on the cutaneous antioxidant defense system. Photochem Photobiol **50**, 739-744.

Gensler HL, Magdaleno M (1991) Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by ultraviolet radiation. Nutr Cancer **15**, 97-106.

Hayat MA (1981) Staining. Chapter 4, in: Principles and Techniques of Electron Microscopy. Biological Applications. Second Edition, Vol. 1. Aspen Publishers, Aspen, CO, USA. pp. 313-340.

Kamimura M, Matsuzawa T (1968) Percutaneous absorption of α-Tocopherol acetate. J Vitaminol **14**, 150-159.

Karlik SJ, Wong C, Gilbert JJ, Noseworthy C, (1989) NMR studies in the relapsing experimental allergic encephalomyelitis (EAE) model of multiple sclerosis in the strain 13 guinea pig. Magnet Reson Imaging **7**, 463-473.

Khettab N, Amory MC, Briand G, Bousquet B, Combre A, Forlot P, Barey M (1988) Photoprotective effect of vitamins A and E on polyamine and oxygenated free radical metabolism in hairless mouse epidermis. Biochimie **70**, 709-1713.

Kligman LH, Kligman AM (1986) The nature of photoaging: Its prevention and repair. Photodermatol **3**, 215-227.

Kligman LH, Akin FJ, Kligman AM (1982) Prevention of ultraviolet damage to the dermis of hairless mice by sunscreens. J Invest Dermatol **78**, 206-214.

LeCak G, cited on pp. 29-30 of Djerassi D, Machlin LE, Nocka C (1986) Vitamin E: biochemical function and role in cosmetics. Drug and Cosmetic Industry (D&CI). pp. 29-34, 78.

Lever WF (1990) Histology of the skin. Chap. 2, in: Histopathology of the Skin. Lipincott, Philadelphia. pp. 9-43.

Lowe NM (1981) Ultraviolet light and epidermal polyamines. J Invest Dermatol **77**, 141-153.

Luger TA (1986) UVL and epidermal cell cytokine production. Photodermatol **3**, 123-124.

Miyachi Y, Horio T, Imamura S (1983) Sunburn cell formation is prevented by scavenging oxygen intermediates. Clin Exper Dermatol **8**, 305-310.

Moeller H, Potokar M, Wallat S (1987) Vitamin E als Kosmetischer Wirkstoff (Vitamin E as a cosmetic material). Parfuemerie und Kosmetik **68**, 688-694.

Moeller H, Ansmann A, Wallat S (1989) The effects of vitamin E on the skin in topical applications. Fett-Wissenschaft Technologie **8**, 295-315.

Norins AL (1962) Free radical formation in the skin following exposure to ultraviolet light. J Invest Dermatol **39**, 445-448.

Norusis, M.J. (1988) SPSS/PC V. 2.0, Base Manual. SPSS Inc., Chicago, IL. pp. 180-181.

Ohzawa S, Arai M, Takeda Y (1984) Vitamin E in topical preparations. Nippon Koshin Kakakkaishi (BJJAEV) **8**, 18-27.

Pascoe GA, Reed DJ (1987) Vitamin E protection against chemical-induced injury. II: Evidence for a threshold effect of cellular α-tocopherol in prevention of adriamycin toxicity. Arch Biochem Biophys **256**, 159-166.

Pathak MA, Stratton K (1968) Free radicals in human skin before and after exposure to light. Arch Biochem Biophys **123**, 468-476.

Penny RHC, Muirhead MR (1986) Skin. Chap. 5 in: Diseases of Swine. Lenman A, Straw B, Glock RD, Mengelny WL, Penny RHC, Schoel E (eds.). Iowa University Press, Ames, Iowa. pp. 82-84

Potapenko AJ, Piszov MJ, Abijev GA, Pliquett F (1983) α-Tocopherol, ein Inhibitor von durch UV-Strahlung induzierten Veraenderungen mechanoelektrisher Hauteigenschaften (α-Tocopherol, an inhibitor of changes induced in the mechanoelectrical properties of the skin by ultraviolet rays). Dermatol Monatsschr **169**, 300-304.

Potapenko AY, Abijev GA, Pistov MY, Roshchupkin DI, Pliquett F, Ermolayev AV, Sarycheva IK, Evstigneeva RP (1984) PUVA-Induced erythema and changes in mechanoelectrical properties of skin. Inhibition by tocopherols. Arch Dermatol Res **276**, 12-16.

Record IR, Dreosti IE, Konstantinopoulos M, Buckley RA (1991) The influence of topical and systemic vitamin E on ultraviolet light-induced skin damage in hairless mice. Nutr Cancer **162**, 219-225.

Roberts LK, Samlowski WE, Daynes RA (1986) Immunological consequences of ultraviolet radiation exposure. Photodermatol **3**, 284-298.

Roshchupkin DI, Piszov MY, Potapenko AY (1979) Inhibition of ultraviolet light-induced erythema by antioxidants. Arch Dermatol **266**, 91-94.

Saperstein H, Rapaport M, Rietschel RL (1984) Topical vitamin E as a cause of erythema multiforme-like eruption. Arch Dermatol **120**, 906-908.

Schmidt EE, MacDonald IC, Groom AC (1990) Interactions of leucocytes with vessel walls and with other blood cells, studied by high-resolution intravital videomicroscopy of spleen. Microvasc Res **40**, 99-117.

Traber MG, Cohn W, Muller DPR (1993) Absorption, transport and delivery to tissues. Chap. 3, in: Vitamin E in Health and Disease. Packer L, Fuchs J (eds.). Marcel Dekker, New York. pp. 35-42.

Trevithick JR, Xiong H, Lee S, Shum DT, Sanford SE, Karlik SJ, Norley C, Dilworth G (1992) Topical tocopherol acetate reduces post-UVB, sunburn-associated erythema, edema, and skin sensitivity in hairless mice. Arch Biochem Biophys **296**, 575-582.

Young AR (1987) The sunburn cell. Photodermatol **4**, 127-134.

Discussion with Reviewers

M. Lindberg: How many mice did you use for each measurement?
Authors: An average of seven or more individual measurements was obtained. Each measurement was made at a different location on each differently treated half of the back of one mouse, when only half of the back was treated; when the whole back was measured, the different measurements were made at different locations on the MRI cross section of the back. Although the pixel size is 0.217 mm, averaging in this way permitted a more precise skin thickness to be measured.

M. Lindberg: Why did you choose MRI for measurements of skin thickness and not, for example, skin fold thickness measurements with a caliper?
Authors: We had performed some preliminary experiments with a spring-loaded pocket thickness gage manufactured by Mitutoyo (#7309). This caliper had been reported previously to have been used for measuring skin fold thickness. We decided not to continue for the following reasons: (**1**) Repeating of the individual measurements for 8 individual measurements which could be averaged for statistical purposes resulted in a mildly invasive procedure because the calipers were spring-loaded to increase the reproducibility of the measurement. (**2**) The reproducibility of the measurements was not good. (**3**) Instead of measurements at various points on the skin, the result obtained for the calipers was an average over a much larger area of skin. In contrast to this, MRI permitted truly non-invasive independent measurements of skin thickness at 7 or more different locations, which did not result in damage to or pressure on the skin. MRI also has an advantage over color-based erythema measurements which cannot be used to estimate erythema after application of any colored topical vehicle.

B. Forslind: The actual site of processing of TA in the skin is not discussed in your paper. For instance, the skin barrier of mice is not identical to that of man with respect to the composition of lipids. What is your opinion about this?
Authors: Tocopherol acetate (TA) is an oil at room temperature, and as an oil is soluble in the lipid of the cell membrane. Kamimura and Matsuzawa (1968) indicated that there are two routes for tocopherol acetate to be absorbed by the skin: (**1**) from the stratum corneum into the epidermis and then the dermis, and (**2**) through the hair follicles by way of the pilosebaceous canal and into the outer root sheaths and eventually into dermal tissue, the connective tissue sheaths. At the cellular level, as Pascoe and Reed (1987) speculated, tocopherol and its esters may differ in their pathways of transport within cells. Free tocopherol may be absorbed and because of the lipid solubility remain within the cell membrane, while esters may pass through the membrane and be hydrolysed within the cell cytoplasm or cellular organelles, releasing free tocopherol at some critical intracellular site such as the mitochondria. Thus, as Pascoe and Reed (1987) showed, tocopherol esters may be more effective than the free vitamin.

Although esterases hydrolysing tocopherol esters have been reported in the small intestine by Burton and Ingold (1989), no similar esterases have ever been shown in skin: their presence can be inferred from the increase of free tocopherol following topical application of the ester which we have reported here.

B. Forslind: It is a known fact that treatment of a specified area on the skin of experimental animals has some carry-over effects to untreated areas. This is one of the major problems in using the same animal for experiment and control.
Authors: When UVB exposure of an area of skin occurs, the area of erythema resulting is sharply limited to the irradiated area of the skin, as we showed in Figure 1 and Table 1A, and the response is similar for animals in which the whole back is irradiated, or just half is irradiated and the other half is shielded. When TA is applied topically to a particular area of the skin, it is possible that, after being taken up by the skin, it may be transported by blood or lymphatic circulation to other areas of the body. This may occur before or after hydrolysis to free tocopherol. The extent of this transport is currently under investigation using radioactive tracers. In the experiments reported here, TA was applied to two halves of one mouse back at 1 and 2 hours post UVB, respectively, and for a second mouse, at 4 and 8 hours post UVB, respectively. In all these cases, the erythema was reduced significantly.

In another experiment in which another antioxidant was applied to half of a mouse back, of which both halves had received UVB, the antioxidant did not significantly decreased the erythema-associated skin thickening measured for the untreated half of the back. If significant migration of the antioxidant from the treated to the untreated half had occurred, such a reduction would have been expected.

H. Black: What parameter did the application of TA at 8 hours reduce significantly?
Authors: In previous experiments we have shown that skin sensitivity, erythema, and skin thickening are all correlated to UVB exposure, and reduced by antioxidant treatment. In the experiments we described here, we measured only the MRI skin thickness increase. With the caveat that transport of tocopherol and TA may have occurred from the opposite half back, which was treated at 4 hours post UVB, this result suggests that TA treatment may be effective even if delayed up to 8 hours after UVB exposure.