HSLC was performed by means of a Waters Ass. Model ALC 202 liquid chromatograph with M6000 pumping system and detected with a Waters Ass. Model R401 refractive index detector. 2 Shodex GPC columns (A801 × 1, A802 × 3, porous polymer of styrene divinylbenzene) was used. For analysis of trail pheromone, the methylation of the fatty acids (fraction 2 in HSLC) was carried out using diazomethane and then analyzed by gas chromatography-mass spectrometer (GC-MS)[6]. The instrument used consists of JGC-20KP gas chromatography (JEOL Co. Ltd, Japan) equipped with the column which was coupled to a JMS-D100 mass spectrometer (JEOL Co. Ltd, Japan) having electron impact (EI) and chemical ionization (CI) combination sources and measured the folowing conditions: column, 10%-DEGS Gaschrom Q (100–200 mesh) glass column (3 m × 3 mm); column temperature programmed 150° to 200 °C (5 °C/min); injection temperature 300 °C; carrier gas He 2.1 kg/cm^2; ionization voltage 23eV. When the CI source was used, iso-butane was used for the reactant gas. The individual components showing alarm response of fraction 3 in HSLC were isolated by preparative gas chromatography (GC), and then identified by IR-spectra on comparison with those of authentic specimens. The S-assay described by Hangartner[7] and O-assay by Tumlinson[3] were employed to check the biological activity of the trail pheromone. While the alarm activity was tested by the Blum's method[8].

Results and discussion. The trail pheronome[9]. The biological activity of the trail pheromone was detected in fraction 2 which was obtained from preparative HSLC. The threshold of detection of the trail pheromone was 10^{-4} to 10^{-7} g/ml. The IR-spectrum of the fraction showed the absorption band at 1700 cm^{-1} due to carbonyl group (showing the presence of the acid). The acid fraction was reacted with diazomethane and submitted to GC-MS analysis. Some of the methyl ester of the fatty acids showed no molecular ion (e.g. $C_{20:5}$) in MS using EI source, so the CI source was used instead of EI in case of $C_{20:5}$ acid. The 9 fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:4}$, $C_{20:5}$) were identified by GC-MS as the components of the trail pheromene. The individual fatty acids were further identified by GC. The isolation and identification of trail and alarm pheromones are shown in the table.

Alarm pheromone. The ants were irritated and then they secreted the alarm pheromone in test tube. After extracting the ants with n-pentane, an odorous oil was obtained. The gas chromatogram of the oil showed 4 peaks which were very similar to those of the fraction 3 in HSLC. The individual components were further isolated by preparative GC and identified by IR-spectra on the comparison with those of authentic specimens. Further identification was performed by GC. The main constituents of alarm pheromone were β-pinene together with limone, α-pinene and camphene. It is of interest that the monoterpene hydrocarbons were detected as the alarm pheromone in P. pungens as well as Nasutitermes exitiosus (Hill)[11].

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The effect of ultraviolet light (UVL) on the lysosomes of hairless mouse epidermis

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Summary. An increased release of acid phosphatase from the lysosomes of UVL irradiated hairless mouse epidermis is demonstrated. The results indicate that lysosomal membrane stability is decreased when the hairless mouse is exposed to either acute or chronic UVL.

The lysosomes are single membrane, subcellular organelles which contain many acid hydrolases[2,3]. Ultraviolet light (UVL) is capable of lysing isolated rat liver lysosomes[4–6] causing release of their catalytic enzymes. Johnson[7] using mice, and Fand[8] using hairless mice, rats and human foreskin found that the acid phosphatase activity in skin exposed to 10 times the minimal erythema dose (MED) of UVL was lower than the activity from skin irradiated through window glass[7] or when the skin was protected with a sunscreen[8]. Histochemical studies of human skin, by Johnson and Daniels[9], indicated that the lysosomes of human skin exposed to 10 times the MED of UVL began significant lysis after 1 h post irradiation and increased to a peak at 6–8 h post exposure. Little effect of UVL was observed on mitochondrial 'marker' enzymes[7–9], indicating an accelerated leakage of acid phosphatase from the lysosomes. No attempt was made to determine the ultimate fate of the enzyme.

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Effect of acute UVL exposure in vivo and in vitro on the acid phosphatase activity from hairless mouse epidermis

UVL exposure	Acid phosphatase activity Epidermis	Isolated lysosomes
None	64.6	61.9
5 times MED	74.9	58.1
10 times MED	47.0	56.7

Irradiation administered as follows: 5 times MED = 0.62 Joules/cm², 10 times MED = 1.24 Joules/cm².

That lysosomes may be involved in the etiology of carcinogenesis is implied by studies with chemicals known to cause lysosomal enzyme release in vitro. Croton oil[10] and phorbol myristate acetate (PMA)[11] increases the permeability of isolated lysosomes in vitro[10] and the release of β-glucurosidase from human polymorphonuclear leukocytes[11]. Both croton oil and PMA are widely used chemmical promoters of carcinogenesis and have been used as a promoter with UVL as the initiator[12]. Vitamin A, which increases the permeability of the lysosomal membrane in vitro[13], increased the yield of tumors on rabbit skin[14] and in the hamster cheek pouch[15,16] when applied simultaneously with dimethylbenzo(α)-anthracene (DMBA). The results in this paper indicate that both acute and chronic UVL exposure affect the lysosomal membrane of hairless mouse epidermis.

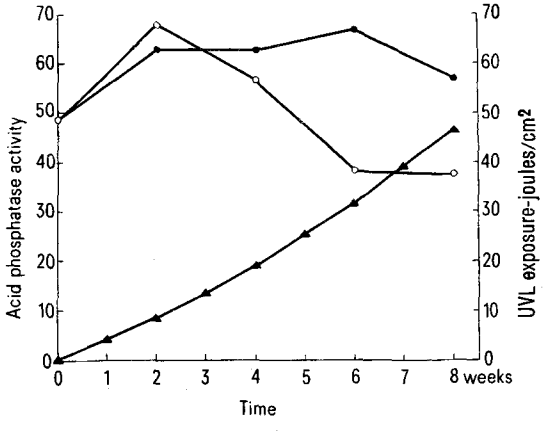
Materials and methods. In all experiments the epidermis was isolated from a flap of dorsal skin[17] by scraping[18]. For the in vivo experiments, the epidermis was isolated by scraping from the dermis after UVL exposure and homogenized in 0.25 M sucrose containing 1 mM EDTA. Complete lysing of the lysosomes was assured by making the homogenate 0.2% in Triton X-100 and the 20,000 × g supernatant was assayed for acid phosphatase. When the effect of UVL on the acid phosphatase activity in vitro was to be determined, the epidermis was homogenized in 0.25 mM EDTA, centrifuged at 20,000 × g for 20 min, portions irradiated, and the acid phosphatase activity determined. Acid phosphatase activity is reported as the μg *p*-nitrophenol liberated from *p*-nitrophenylphosphate per min per mg protein in the 20,000 × g supernatant.

To determine the effect of acute UVL exposure, 5 times and 10 times the minimal erythema dose (5 MED and 10 MED, respectively) of UVL was administered from a Westinghouse FS-20 lamp. Mice were sacrificed approximately 30 min post exposure and the epidermal acid phosphatase activity determined. Controls were non-irradiated animals.

The direct effect of UVL on the epidermal acid phosphatase was determined by isolating the lysosomal enzymes and administering UVL from a Westinghouse FS-20 lamp at the same energy levels as was administered to the mice in vivo. To determine the effect of chronic ultraviolet light (UVL) exposure, female hairless mice (hrhr) were given suberythemic irradiation 5 days per week for 8 weeks. This regimen of UVL is known to cause skin tumors if continued for 16 weeks[17]. Mice were sacrificed at 2-week-intervals and the epidermal acid phosphatase activity determined.

Results and discussion. The acid phosphatase activity from the skin of hairless mice given 10 MED is considerably less than the control (table) which agrees with the results of Johnson[7] and Fand[8]. When only 5 MED was given, however, an increase in the activity occurred. No difference in activity occurred when the isolated enzymes were irradiated in vitro, indicating that loss of enzyme, not inactivation, occurred in vivo. One possible explanation is that at the 5 MED level of irradiation the permeability of the lysosomal membrane is increased, allowing a slow, continued release of enzyme. The longer, 10 MED, exposure may cause lysis of the lysosomal membrane, releasing most of the enzyme, possible ultimately to the blood.

That chronic, suberythemic, UVL irradiation causes skin cancer in hairless mice is well established[19]. When these mice were subjected to the same regimen of UVL, the acid phosphatase activity stays about equal to the non-irradiated controls for 2 weeks and then begins to gradually decline to a maximum at 8 weeks (figure). After an initial increase at 2 weeks, the activity of the epidermal acid phosphatase from the non-irradiated controls remained equal throughout the 8-week-experiment. Although the daily chronic irradiation was considerably less than the acute, the decrease in acid phosphatase activity which occurs after 2 weeks of irradiation probably notes a decrease in the stability of the lysosomal membrane in the epidermis of the irradiated mice.



Effects of chronic UVL exposure on the acid phosphatase activity in hairless mouse epidermis. ●—, control; ○—, chronic irradiation; ▲—, total accumulated energy (Joules/cm²) received through the respective week of the experiment.

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