PREFACE

The Oslo´ Symposium of skin Care and Function. Environmental and Occupational Influences´ was distinguished by the high quality of the many excellent speakers and the interest and the enthusiasm expressed by the participants.The symposium covered a vast field of interesting skin research including areas such as skin function and its measurement, skin care, immunology and the occupational, allergic and toxic influences on skin. The intention of bridging the interests shared by clinical dermatologists with those of basic researchers within the field of skin function and dermatologic cosmetology was fulfilled. It was also intended originally to publish the lectures presented at the symposium. Since this has been delayed until now, several papers have appeared in other dermatological journals, but many other papers of high quality deserve publication. The present volume contains the bulk of the remaining papers and I express my gratitude to the authors for their patience. I don´t think the delay has diminished in any way the relevance and value of their contents.

Per Thune

CONTENTS

QUANTITATIVE X-RAY MICROANALYSIS OF SKIN 1
Bo Forslind

AN ORIGINAL PREDICTIVE METHOD FOR IN VIVO PERCUTANEOUS ABSORPTION STUDIES 9
D. Dupuis, A. Rougier, C. Lotte, R. Roguet

THE ADVERSE REACTIONS OF VEHICLES AND EXTERNALLY APPLIED DRUGS 22
A. Zesch

COMPARISON OF TWO PHOTOELECTRIC TECHNIQUES FOR QUANTITATIVE MEASUREMENTS OF SKIN SURFACE LIPIDS 30
P. Thune, T. Gustavsen

A NEW METHOD FOR MEASURING THE SKIN CLEANING EFFECT OF SOAPS AND DETERGENTS 33
G. Weber

DESQUAMATION OF STRATUM CORNEUM: KINETICS FOLLOWING UV INDUCED INJURY 35
P. Corcuff, F. Chatenay, J.L. Leveque

REPLICATIVE AND REPAIR DNA SYNTHESIS AFTER SOLAR DAMAGE 40
A. Born, W. Born

THE SIGNIFICANCE OF INTERFERON IN PHOTOIMMUNOLOGY 43
J.K. Livden, J.R. Bjelke, M. Degre, H.-K. Krogh, R. Matre

INFLUENCE OF TOPICALLY APPLIED ORGANIC SOLVENTS ON UVB-MINIMAL ERYTHEMA DOSE (MED) 47
J. Barth and H. Kadner

ABNORMAL LYMPHOCYTIC RESPONSE TO UV-RADIATION AND X-RAY IN MULTIPLE SKIN CANCER 49
A. Wærstedt, B. Munch-Petersen, G. Frentz

FULL SPECTRUM SOLAR RADIATION AS A CAUSE OF DERMAL PHOTODAMAGE: UVB TO INFRARED 53
L. Kligman

MOLECULAR ASPECTS OF ALLERGIC CONTACT DERMATITIS 62
C. Benezra

HAIR BULB ACCUMULATION OF LANGERHANS CELLS IN ALLERGIC PATCH TESTS. OBSERVATIONS WITH 2,3-EPOXY-PROPYL TRIMETHYL AMMONIUM CHLORIDE 64
L. Kanerva, R. Jolanki, T. Estlander

CONTENTS

COMPOSITAE AND FRULLANIA PHYTODERMATITIS 69
J.C. Mitchell

ALLERGY TO COSMETICS 77
Etain Cronin

DETECTION OF ALLERGIC DERMATITIS TO FRAGRANCES 83
W.G. Larsen

ALLERGY TO LICHEN AND COMPOSITAE COMPOUNDS IN PERFUMES. INVESTIGATIONS ON THE SENSITIZING, TOXIC AND MUTAGENIC POTENTIAL 87
P. Thune, M. Sandberg

OCCUPATIONAL CONTACT DERMATITIS AND CONTACT URTICARIA CAUSED BY EPOXY RESINS 90
R. Jolanki, L. Kanerva, T. Estlander

ORGANIC PIGMENTS IN PLASTICS CAN CAUSE ALLERGIC CONTACT DERMATITIS 95
R. Jolanki, L. Kanerva, T. Estlander

CONTACT URTICARIA FROM RUBBER GLOVES: A DETAILED DESCRIPTION OF FOUR CASES 98
T. Estlander, R. Jolanki, L. Kanerva

TOPICAL SUBSTANCES WHICH PREVENT POISON IVY ALLERGIC CONTACT DERMATITIS 103
S.M. Orchard, J. Fellman , F. J . Storrs

A NEW SUBSTANCE TO REDUCE ROUGHNESS OF HUMAN SKIN 107
G. Weber

Acta Derm Venereol Stockholm 1987; suppl 134 :1-8

QUANTITATIVE X-RAY MICROANALYSIS OF SKIN

Particle probe evaluation of the skin barrier function

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INTRODUCTION

The basic function of the epidermis is to provide a barrier which will separate the interior of the organism from the environment. The reason for this separation is of course the rigid demands for homeostasis in the tissues of the body and that mere fluctuations in the water content of the body would put an immense strain on such homeostasis feed-back systems. Thus the skin provides a barrier which helps to control the continuous loss of water at the time as the barrier prevents foreign substances and materials to gain entrance into the body and body circulation. It is obviously essential that such a barrier does not become dysfunctional due to wear and tear. Hence, one may say that the main task for the epidermis is to provide continously a fresh barrier.

The physiology of the epidermis is extremely hard to study in vivo since the thickness of the entire tissue is roughly 120 µm (Fig. 1).

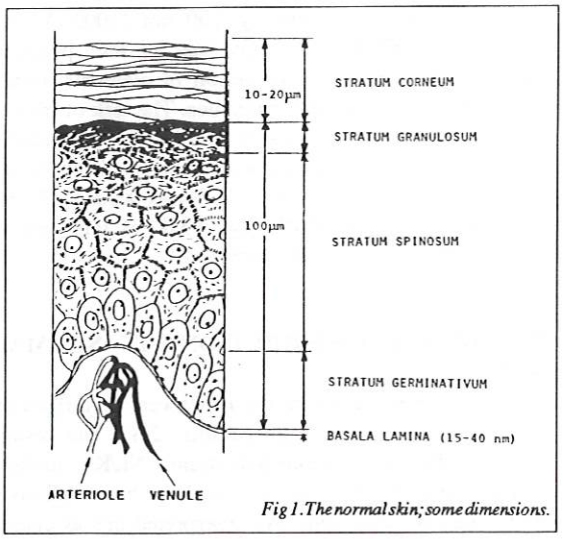


Fig 1. The normal skin; some dimensions.

The barrier proper is considered to reside in the first few cell layers above the stratum granulosum and occupy only a minor part of to total thickness of the stratum corneum which is roughly 10-20 µm thick. With these dimensions in mind it becomes clear that localization of physiological microprobes such as ion-sensitive microprobes in a specified cell in a specified cell layer is a formidable, if not impossible task. Not until the event of the particle microprobes has it been possible to analyse the elemental content of cells and cellular layers in the epidermis under conditions which can be said to represent true physiological conditions. However, rigid requirements on the preparation procedures are necessary if the analytical results are to correspond to the living state of the tissue. Most likely this is the reason for the

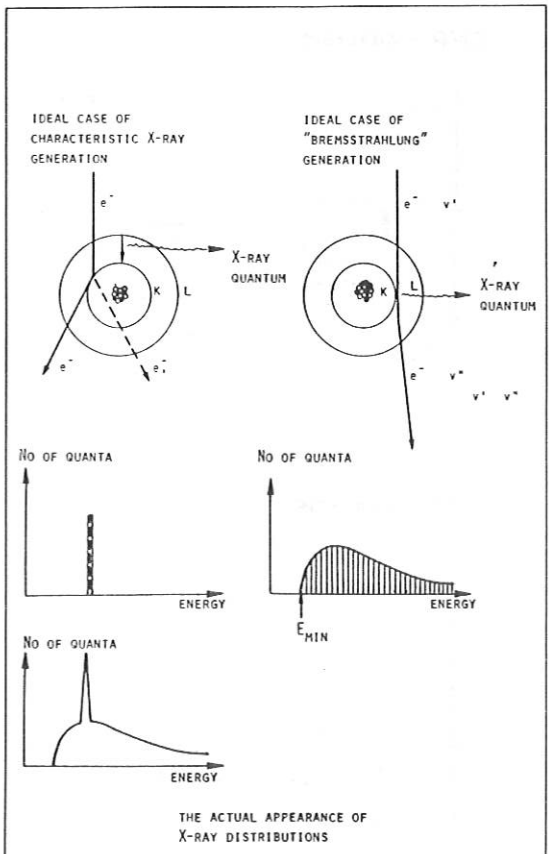


Fig. 2. The principle of X-ray quanta generation at particle probe analysis. The upper right panel ("Bremsstrahlung") is valid for the EMP care only when the primary particle is a light particle, eg an electron. The lower left curve represents the actual spectrum composed of the characteristic signals overlayering the continuum (the background).

few publications in the field up till to-day. In this presentation I will briefly present the physics of the

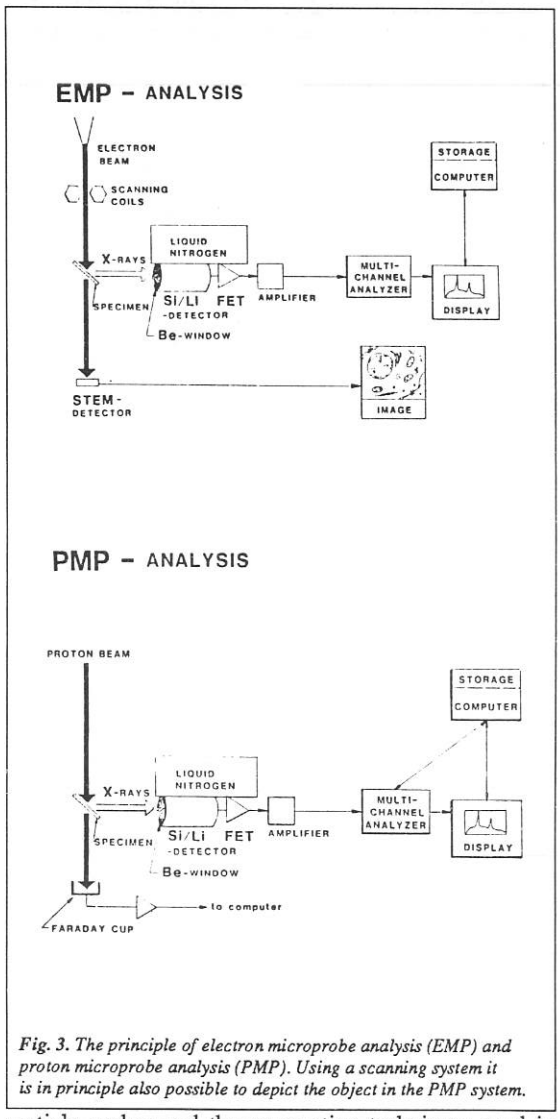


Fig. 3. The principle of electron microprobe analysis (EMP) and proton microprobe analysis (PMP). Using a scanning system it is in principle also possible to depict the object in the PMP system.

particle probes and the preparation techniques used in the analysis of skin. Recent results on the elemental distributions in normal and pathologocal skin will be presented as well as preliminary experiments on the penetration of nickel and chromate ions through the skin.

THE PARTICLE PROBES

When charged particles such as electrons or protons impinge on a target (the object under analysis) they collide with inner orbital electrons which, when ejected at the collision, leave a vacancy behind (Fig. 2). Such a vacancy in an inner electron shell of an atom is immediately filled by an outer shell electron the surplus energy of which is emitted as a characteristic X-ray quantum.

All X-ray quanta produced are typically detected by energy dispersive detector systems which are sensitive to elements down to sodium (Z=11) for electron microprobes (EMP) and aluminum (Z=13) for proton microprobes (PMP) (Fig. 3).

The characteristic feature of the energy dispersive detector system is that the recording of the elements present in the area of analysis is a simultaneous recording and thus it is possible to denote quotients of elements present, eg. the Na/K quotient which give valuable information of the condition of the cell/cell layer/under investigation. The light particles, eg electrons are, however, also retarded by the object in a non-specific way. The energy loss caused by this retardation gives rise to X-ray quanta which are not related to specific elements but represent a spectrum of energies. This spectrum appears in the recording as a substantial background which prevents detection of elements present in trace amounts. The lower limit for the sentitivity of EMP is thus rougly 200 ppm. In contrast, the heavy protons cause no background of this kind. The background present in the PMP is caused by the ejected secundary electrons which may contribute to the total spectrum by a very small continuum. The PMP thus allows detection down to concentrations of 10 ppm. Hence trace elements such as zinc (Zn:30), iron (Fe:26) and calcium (Ca:20) may be readily detected by the PMP. Recently we have shown that the EMP and PMP methods are indeed complementary (1.).

With very thin specimens eg 100 nm (1000 Å) the resolution of EMP is roughly that of the specimen thickness and this allow detection of elements within volumens as small as cell organelles. The task of freeze-sectioning at these dimension is however formidable and most of our work has been confined lately to so called bulk specimens with a thickness around 12 µm. The resolution with EMP and PMP at this section dimension is that of a cell layer (1A).

ELEMENTAL DISTRIBUTIONS IN NORMAL SKIN

Our first experiments with the EMP were performed on guinea-pig epidermis thin sections (2,3). The results showed that the sodiom/potassium (Na/K) quotient drastically changed in moving from the basal cell layer to the stratum spinosum. We interpreted this as caused by dysfunctional Na/K-pumps in the cell membrane of the stratum spinosum cells which for this reason no longer can be regarded as living cells in the meaning that

they are competent to go through mitosis. Obviously this is one of the means to confine cell divisions to the basal cell layer. We also noted a high phosphorus content of

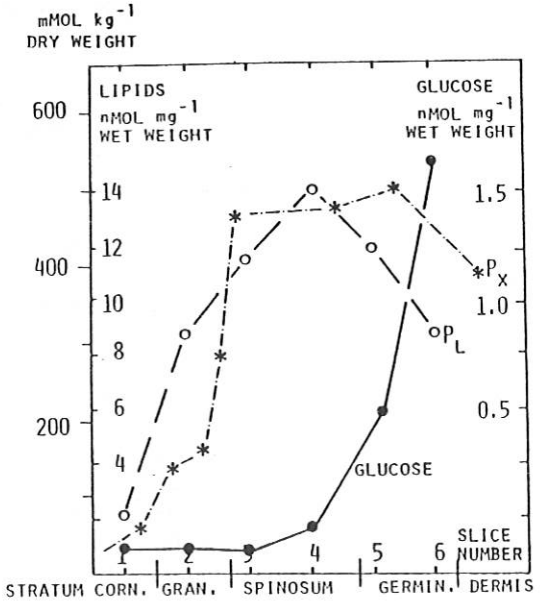


Fig. 4. The distribution of phosphorus (P_x) as analysed by X-ray microanalysis, plotted in relation to data on phospholipid (P_L) distribution and glucose distribution according to Long (4).

the basal cell layer and the phosphorus distribution declined moving up towards the stratum corneum in a way that was conspicuously like that shown for phospholipids by Long (4) (Fig.4).

Phospholipids are known to be replaced by ceramides during the differentiation process and it has been postulated that they provide energy for the anabolic and katabolic processes in the higher layers of the epidermis (5).

Investigating the feasibility of using the proton microprobe for investigation of the elemental distributions in normal human skin showed that not only were the results of parallel analysis with the electron microprobe (EMP) in close agreement witn PMP data (6) but also trace elements such as zinc (Zn:30) and iron (Fe:26) readily detected and revealed the highest concentrations to be confined to the basal cell and lowest stratum spinosum region (Fig.5).

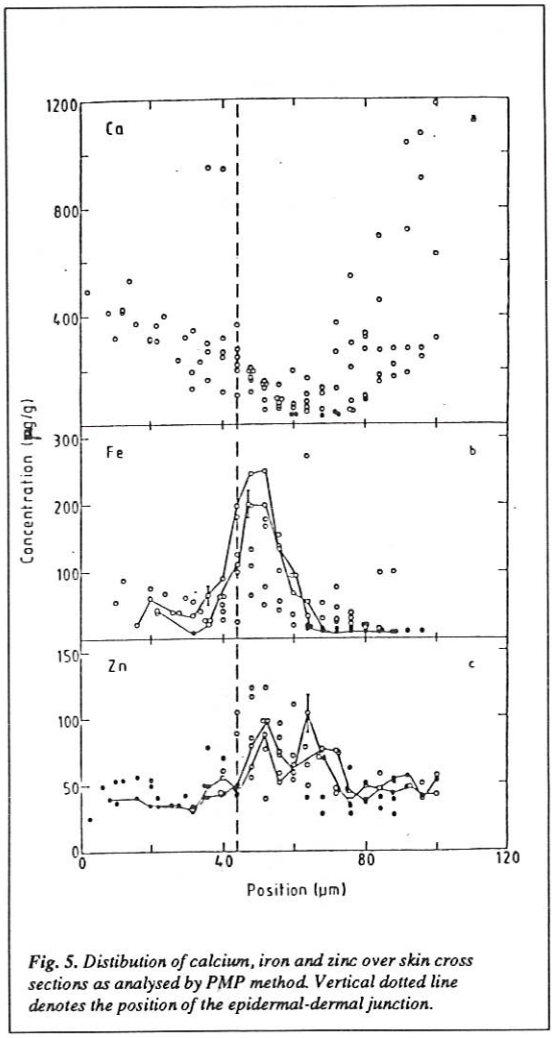


Fig. 5. Distibution of calcium, iron and zinc over skin cross sections as analysed by PMP method. Vertical dotted line denotes the position of the epidermal-dermal junction.

Also a calcium profile was shown which peaked close to the stratum corneum (7). This is in nice congruence with recent data from epidermal cell cultures which differentiate when the calcium content of the medium is increased over 1 mM (8,9,10) as well as data from ultrastructural localization of ionic calcium by ion-capture cytochemistry (11).

It was suggested from our first EMP studies of guinea-pig skin that the phosphorus profile was to a great extent corresponding to the phospholipid distribution over the epidermis. To test this notion we have an investigation underway where human skin sections inertly prepared for particle probe analysis have been extracted with

petroleum ether. Our preliminary data show indeed that a substantial part of the epidermal phosphorus is extracted by this procedure which thus confirms the above contention. Obviously the particle probes, eg EMP and PMP allow a quantitative analysis of epidermis in quench frozen specimens inertly processed for analysis in a way which has not hitherto been possible.

ELEMENTAL DISTRIBUTIONS IN PATHOLOGICAL SKIN

Our interest in metal allergies prompted us to investigate the quantitative changes in the epidermis at exposure to nickel and chromate solutions. Guinea-pigs were injected with 0.05% $K_2Cr_2O_7$ or 1% $NiSO_4$ in isotonic saline. Bulk specimens were analysed with the EMP and our results showed that the two metal ion solutions had different effects on the epidermal tissue in vivo. Chromate is a potent oxidate agent and the cell damage caused reveales itself in decreased potassium and phosphorus levels and increased sodium and chlorine levels. This is in agreement with the results of a study of DNCB effects on epidermis in vivo (12). With nickel it is of interest to note the conspicuous increase in sodium, magnesium, phosphorus and chlorine suggesting a stimulation of the germinative cell population (13). (Fig. 6).

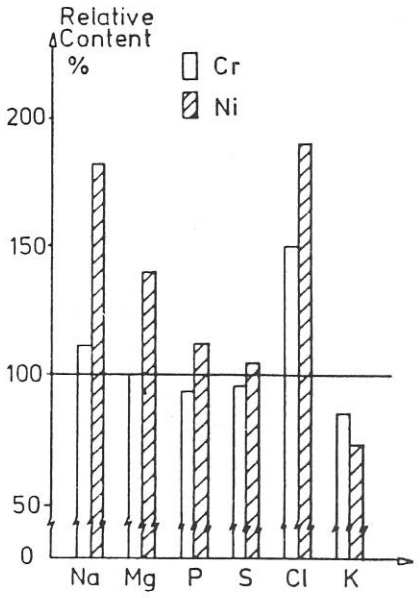


Fig. 6. Comparison of the relative elemental content in stratum germinativum of the keratinocytes after exposure to a 0.05% $K_2Cr_2O_7$ and a 1% $NiSO_4$ solution for 24 hours.

In a first attempt to study elemental distributions in psoriasis regarding this disease as a model for hyperproliferative conditions we have been able to show that the involved skin had higher concentrations of magnesium, phosphorus and potassium than had the uninvolved skin (14). PMP analysis of paralesconal psoriatic skin reveals high levels of zinc and iron in the upper strata in contrast to the distribution in normal skin (Forslind B., Lindberg M., Malmquist K., Roomans G.M. and Werner Y. unpublished results)(Fig. 7).

Penetration of the barrier by different substances is an important aspect of skin function. We have initiated studies of nickel and chromate penetration through the epidermal barrier. Our first experiments have been performed on cadaverous human skin obtained at autopsy not later than 24 hours post mortem. The whole-skin preparation was mounted so as to separate two vessels. The vessel facing the stratum corneum contained the metal ion aquous solution. Diffusion was allowed to take place during 18 hours at 4°C to minimize the effect of autolytic processes. The preparation procedure was our usual inert one producing bulk specimens (section thickness 12 μm). The penetration of nickel was less efficient than that of the chromate solution. The concentrations of the metal ions were much too low for an EMP analysis and the advantage of the PMP analysis was thus clearly demonstrated (15)
(Fig. 8).(Page 6)

FUTURE APPLICATIONS OF THE PARTICLE PROBES

We have convincingly shown the feasibility of particle probe analysis of skin under normal and pathological conditions. The methodological problems are essentially solved and the methods may now be applied to a broader spectrum of experimental approaches. In this report the existing elemental distributions of the skin have been investigated but also the introduction of foreign elements can be traced by the particle probes, eg the PMP in the case of nickel and chromium. It should be possible to study the barrier penetration even of organic molecules can they be "tagged" with elements usually not present in the epidermis. Thus in the particle probes we have at hand powerful tools for the study of skin penetration and its effects on the viable cells of the skin.

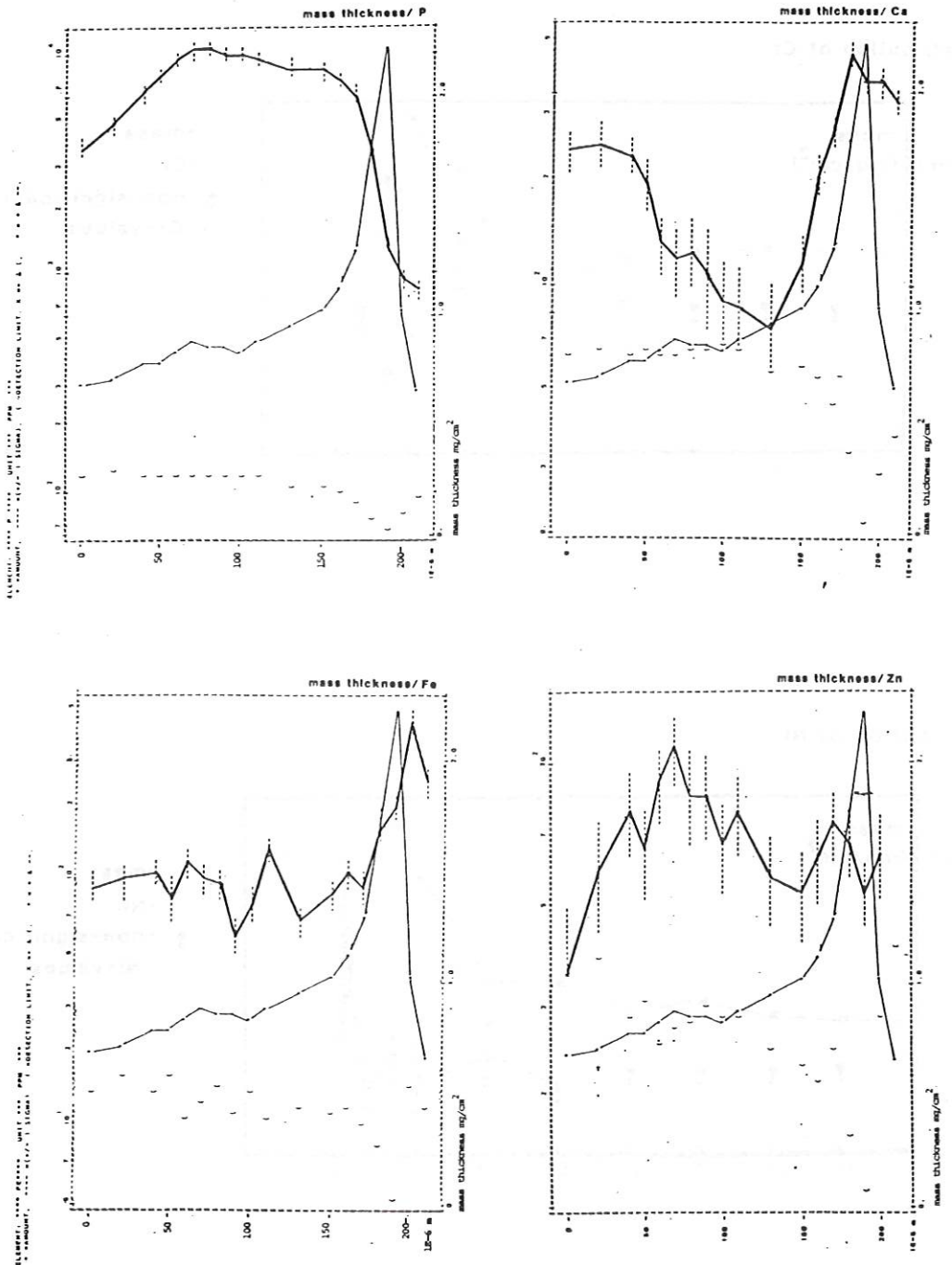


Fig. 7. PMP analysis of paralesional psoriatic skin. Note that the iron (Fe)destribution shows high values in the stratum corneum region in contrast to the distribution in normal skin (cf Fig. 5). Also the zinc (Zn) distribution deviates from that of the normal.
(From: Forslind,B:, Lindberg, M., Roomans G.M., and Werner, Y. Unpublished results).

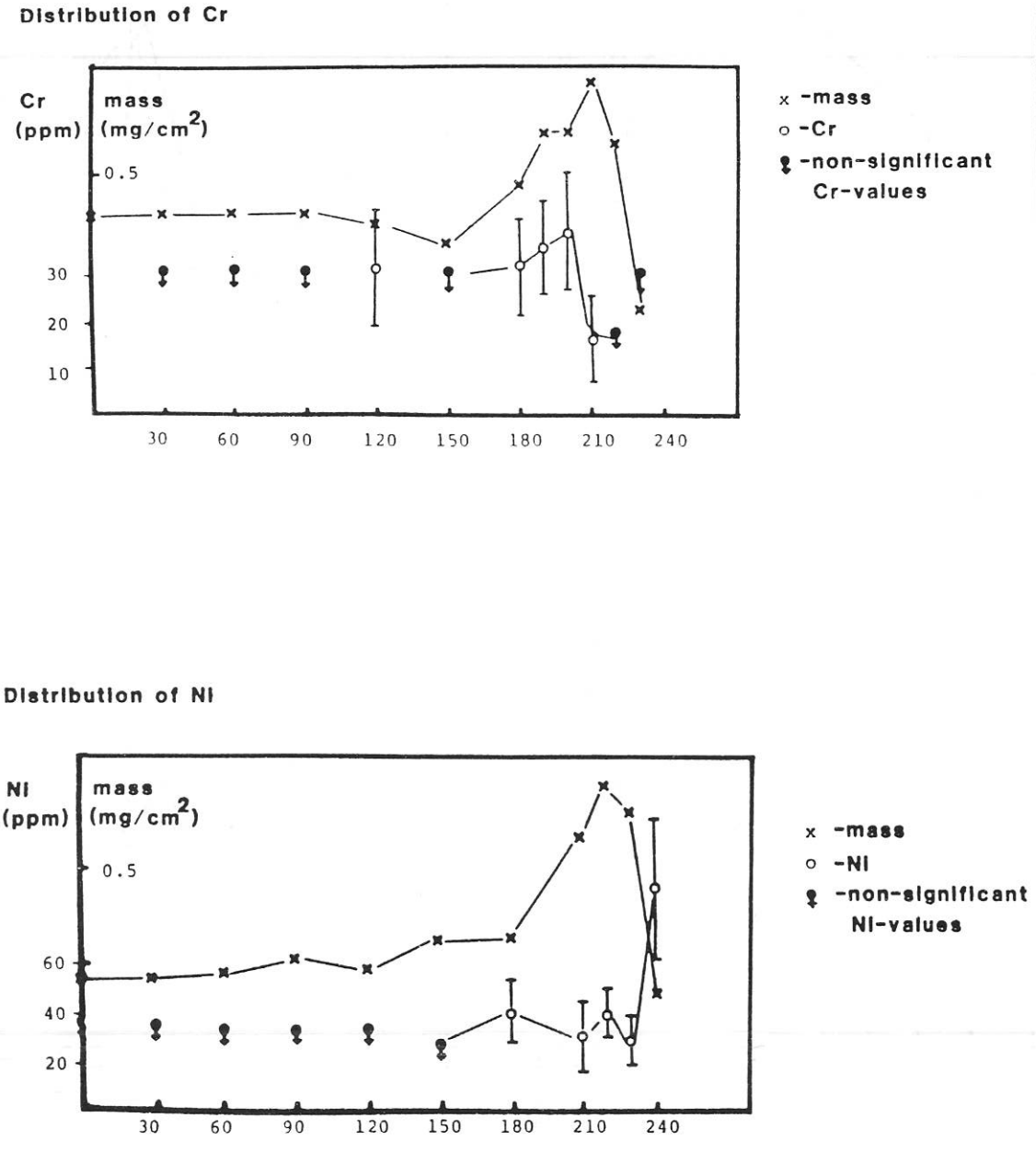


Fig. 8. Distributions of chromium (Cr, upper panel) and nickel (Ni, lower panel) after exposure of cadaverous skin at 4°C for 18 hours in in vitro experiments.

Acknowledgements

The studies summarized here have ben possible by the enthusiastic and able support of my co-workers which is greatfully acknowledged. Financial support was provided by the Swedish Work Environmental Funds (ASF 79/92, 82/80, 85/0175).

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AN ORIGINAL PREDICTIVE METHOD FOR IN VIVO PERCUTANEOUS ABSORPTION STUDIES.

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There is considerable evidence to suggest that the most important barrier to drug penetration through the skin is the stratum corneum (1-4), which also acts as a reservoir for molecules applied on the skin (5-7). These function seem closely related (6-9).
The results obtained from various methods used for measuring the in vivo penetration, for a given product, are rarely comparable. This is due essentially to the diversity of techniques used, animal species (10,11), anatomical location (1,12), concentration of the molecules tested (13,14), vehicle used (10,15), and duration of the experiment (10). Furthermore, experience has shown that it is very difficult to extrapolate to humans the results obtained with animals (16-18).
Considering these facts, we wondered whether there was a relationship between the stratum corneum reservoir function and the penetration of molecules in a laboratory animal, the hairless rat, and then in human.
In a third and a fourth part of this work, the principle of this method will be improved in rats, by testing its independence facing both the dose administered and duration of application.

MATERIALS AND METHODS

1. Experimentation in rats
We chose to test, under defined experimental condions, molecules having very different physichochemical properies and belonging to different chemical classes.

a. Materials
Ten radiolabeled molecules form The Radiochemical Centre Amersham (U.K.) were studied: (1,2-^{3}H) dexamethasone, (1,2,6,7-^{3}H) hydrocortisone, (1,2-^{3}H) dehydroepiandrosterone, (^{14}C) testosterone, (carboxyl-^{14}C) acetylsalicylic acid, (carboxyl-^{14}C) sodium salicylate, (8-^{3}H) caffeine, (ring-^{14}C) benzoic acid, D-(1-^{14}C) mannitol, (^{14}C) thiourea.
For each product, a group of 12 female hairless Sprague-Dawley (OFA) rats, aged 12 weeks, weighing 240 ± 10 g was used.

b. Application conditions
The molecules were applied on 1 cm^{2} of dorsal skin; the animals had previously been anesthetized by 0.5ml/kg i.p. injection of -butyrolactone. The surface of application was delimited by an open circular cell (S=1 cm^{2}) fixed by silicone glue (Tecsil) in order to prevent spreading. The vehicle, ethanol-water 95/5 (v/v) was chosen assording to the solubility of the products with the exception of D-mannitol which was dissolved in ethanol-water 25/75 (v/v) and of sodium salicylate dissolved in ethanol-water 50/50 (v/v).
The standard dose applied was 200 nmol.cm^{-2}, with the exception of benzoic acid which was applied at 200 and 450 nmol.cm^{-2}. The vehicle volatility limits the application period to 30 mins. After this time, the excess product on the applied area was rapidly removed by 2 washings (300ul) with the vehicle solvent, followed by 2 rinsings (300 ul) with distilled water and light drying with cotton wool.

c. Conditions for the percutaneous absorption measurements
At the end of the application and washing, the 12 animals were divided into 2 equal groups. The stratum corneum of the application area of the animals from the first group was removed by six strippings using "3M" invisible adhesive tape. Previous histologic studies have shown that this technique removes most of the stratum corneum, the horny layer of the hairless rat being composed of 7-8 cellular layers (thickness=10-15 μm).
The amount of substance contained in the stratum corneum after a 30 min application was determined as follows. The radioactivity on each strip was measured after complete digestion of the keratinic material in Soluene 350 (Packard), addition of Dimilume 30 (Packard) and liquid scintillation counting (Packard 460 C).
The animals of the second group, equipped with collars to prevent licking, were placed individually in metabolism cages for 4 days. During this time, food (Extra Labo) and tap water were provided ad libitum. Urinary

excretion was established by daily sampling of the urine and liquid scintillation counting. The feces were collected daily, pooled, and counted by liquid scintillation after lyopholosation, homogenization and combustion of the samples with an Oxidizer 306 (Packard). Four days later, the animals were sacrified by cervical dislocation. Series of 6 strippings were performed on the treated area in order to determine the amount of product not passed through the stratum corneum barrier within 96 h. The remaining skin of the treated area (epidermis + dermis) was sampled and counted by liquid scintillation after digestion in Soluene 350 (Packard). The animals were lyophilized, homogenized, and the samples were counted in liquid scintillation after combustion. For the tritiated molecules, radioactivity in the water of lyophilisation was counted to determine for each case the tritium exchange.
The total amount of substance penetrating in 96 h was determined by adding the amounts found in the excreta (urine + feces), in the epidermis and dermis of the application area and in the whole animal body.

2. Experimentation in human
In order to compare results, the same experimentation was performed in the two species, rat and human.

a. Materials
Four doses of (ring ^{14}C) benzoic acid supplied by The Radiochemical Centre Amersham (U.K.) were applied in 4 groups of 12 female hairless rats, Sprague Dawley (OFA) 12 weeks old and weighing 240 ± 10 g and in 4 groups of 5-7 male caucasian patients 25 ± 0.8 years old and weighing 74 ± 1.6 kg.

b. Conditions of application
Doses 125, 250, 500 and 1000 nmol. of benzoic acid with a specific activity of 0.1 µCi/nmol were applied in 20 ul of a vehicle constituted by ethylene glycol to which 10% Triton X-100 was added as surfactive agent. Prior to administration of the compound, the animals were anesthetized by a 0.5 ml/kg i.p. injection of -butyrolactone. Benzoic acid was applied onto the backs of the rats and to the external side of the arm of the volunteers on an area delineated by a 1 cm^2 open cell fixed with silicone glue (Tecsil). After 30 min of application the excess substance on the treated area was quickly washed twice (2x300 µl) with ethanol:water mixture (95:5) followed by 2 rinsings (2x300 µl) with distilled water and light drying with cotton wool.

c. Measurement of the percutaneous absorption
At the end of the application time, the 12 animals in each treated group were equally divided. The first group was used to measure the total amounts of benzoic acid penetrated within 4 days. These were calculated as the sum of the amount found in the following fractions: feces and urine, epidermis and dermis of the treated area (stratum corneum being stripped to descard the non penetrated benzoic acid), and in the whole body. In the second group, a series of 6 strippings (invisible tape "3M") was carried out on the treated area to determine the amounts of benzoic acid present in the reservoir of the stratum corneum at the end of application.
The methods used for measuring radioactivity were the same as in the rat experimentation.
In the human subjects, each group was treated with a different dose of benzoic acid on the external side of the right arm. Considering both the data in the literature on the urinary excretion of benzoic acid administered by several routes in human and in rat (19,20) and our own results on rat (21,22), the total percutaneous absorption within 4 days for benzoic acid applied on human was deduced from the radioactivity recovered in the urine within 48h after the application. In order to measure the reservoir effect of the stratum corneum, each patient received on the 7th day after the first application (on the right arm), the same dose of benzoic acid on the external side of the left arm. At the end of the application time (30 min), 15 strippings were carried out on the treated area and the radioactivity in each stripping measured as previously described in the rat experimentation.

3. Experimentation concerning the dose applied

a. Materials
The study was performed on 4 radiolabeled molecules from The Radiochemical Centre Amersham (U.K.): (8-^{14}C) theophylline, (carboxyl-^{14}C) acetyl salicylic acid, (carboxyl ^{14}C) nicotinic acid, and (ring-^{14}C) benzoic acid. For each product and each dose, a group of hairless Sprague-Dawley (OFA) rats, aged 12 weeks, weighing 240 ± 20 g was used.

b. Conditions of application
On previously anesthesized animals (i.p. injection of 0.5 mn/kg -butyrolactone, 125,250,500 and 1000 nmoles of each product dissolved in 20 µl of ethylene glycol: Triton X100 (90:10 v/v) were applied onto 1 cm^2 area of the back of the animals. The surface of application was delimited by an open circular cell fixed by silicone glue.

After 30 min. of application, the excess of product on the treated area was quickly washed twice (2x300 µl) with ethanol:water (95:5 v/v), then rinsed twice with distilled water (2x300 µl) and dried off with cotton wool.

c. Conditions for the percutaneous absorption measurements

For each compound and each dose applied, the total amount penetrating in 4 days was determined by adding the amounts found in excreta (urine + feces), in the epidermis and dermis of the treated area (the stratum corneum being stripped to take into account the non penetrated product), and in the whole animal body.

d. Conditions for the stratum corneum reservoir function measurements

The reservoir function of the horny layer for each compound and each dose applied was assessed by 6 successive strippings (invisible tape "3M") on the treated area of groups of 5 animals after a set application time of 30 min. The methods used for the measurements of radioactivity have been described in full details in the previous paragraph relative to the experimentation in rats.

4. Experimantation concerning the duration of application

a. Materials

The study was performed in 4 radiolabeled molecules (The Radiochemical Centre Amersham U.K.): (8-^{14}C) theophylline, (carboxyl ^{14}C) nicotinic acid, (carboxyl-^{14}C) acetyl salicylic acid and (ring-^{14}C) benzoic acid.

For each product and each duration of contact, a group of 10 female hairless Sprague-Dawley rats, 12 weeks old, weighing 220 ± 20 g was used.

b. Conditions of application

On previously anesthetized animals (i.p. injection of 0.5 ml/kg -butyrolactone), 20 µl of a solution containing 1000 nmoles of each substance was applied onto a 1 cm^2 area on the animal back. The vehicle used was constituted with ethylene glycol to which 10% Triton x100 was added as surfactive agent. The area was delimited by an open circular cell fixed by silicone glue.

The application times were 0.5, 2, 4, and 6h, chosen in order to cover most of the usage conditions of compound topically applied. At the end of application, the treated area was washed twice (2x300 µl) with ethanol:water (95:5), then rinsed twice with 300 ul of distilled water and dried off with cotton wool.

c. Conditions for the total percutaneous absorption measurements

For each compound and each duration of application, the total amount penetration in 4 days was determined by adding the amounts found in the excreta (urine + feces), in the epidermis and dermis, of the treated area (the stratum corneum being stripped to take into account the nonpenetrated product), and in the whole animal body.

d. Condition for the stratum corneum reservoir function measurements

The reservoir function of the horny layer for each compound was assessed by 6 successive strippings (invisible tape "3M") on the treated areas of 5 animals after a set application time of 30 min. The methods used for the measurements of radioactivity have been previously described in full details in the paragraph relative to the experimentation in rats.

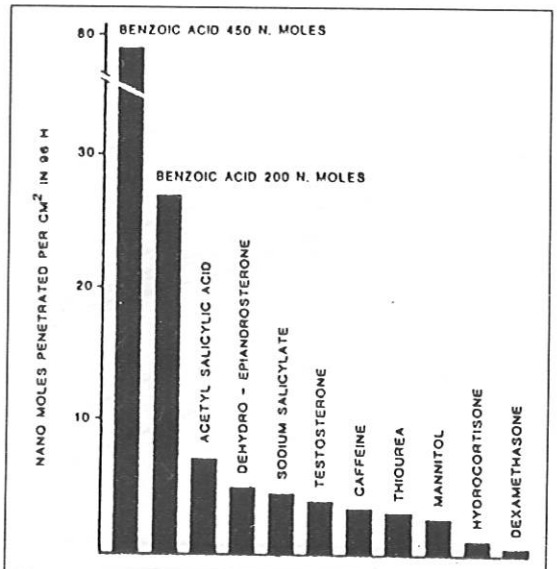


Fig. 1 Total percutaneous absorption levels (nmol.cm^{-2}) of the products tested 96 h after their topical application on the hairless rat.

RESULTS AND DISCUSSION

1. Experimentation in rats

Table I shows the distribution of the molecules tested in urinary and fecal excretions as well as their amount in the whole body; the penetration rate (illustrated by Fig. 1) of the molecules 96h after topical application; and the amounts of products present in the stratum corneum at the end of the application period (30 min).

Fig. 2 illustrates the distribution of the compounds within the stratum corneum of the treated area 30 min after their application. Fig. 3 represents the linear relationship between the penetration of the substances after 4 days and their level in the stratum corneum at the end of the 30 min application period.

The substances tested were chosen because of the wide

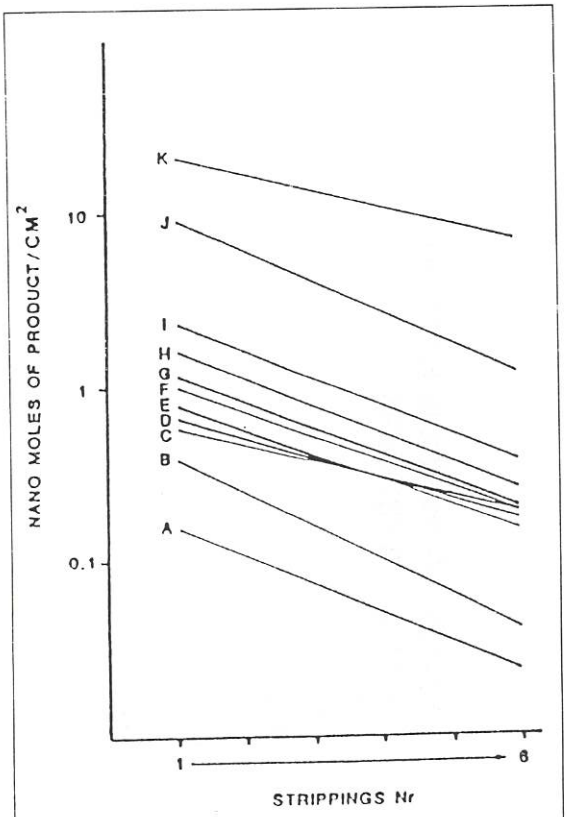


Fig. 2 Distribution of the products in the stratum corneum of the treated area 30 min after their application.

(A) - Dexamethasone (B) - Hydrocortisone
(C) - Dehydroepiandrosterone (D) - Testosterone (E) - Mannitol
(F) - Thiourea (G) - Caffeine (H) - Sodium Salicylate
(I) - Acetyl Salicylic Acid (J) - Benzoic Acid 200 nmoles.cm⁻²
(K) - Benzoic Acid 450 nmoles.cm⁻²

variety in their structure and physicochemical properties, and because their penetration ability (Table I) covers a large range (1-50). It is important to comment on how the elimination of the substances, particularly by urinary and fecal excretion (Table I). Except for the steroids, the substances tested are eliminated 80-95% by the urinary pathway, although their physicochemical properties differ.

The percutaneous absorption results in agreemant with those obtained in the literature, show that after 96h there are large differences in the amounts of substances that have penetrated through the skin (Table I,Fig.1). The rank order established in humans by Feldmann et al (23) in the level of steroid penetration (dexamethasone, hydrocortisone, testosterone, and dehydroepiandrosterone) is seen again in our results in rat. Furthermore, hydrocortisone penetrates 5 times less than testosterone and dehydroepiandrosterone which have identical penetration properties. This agreement with the literature is also verified for acetyl salicylic acid and benzoic acid, the latter known to be very well absorbed (24). Likewise, it is known thath acetyl salicylic acid and salicylic acid have similar penetration properties (24), where as their sodium salts exhibit diminished penetration (25) and, indeed, we observed that sodium salicylate penetrates less than acetylsalicylic acid.

It has been estblished that the penetration of a compound increases with the amount applied (13,14). This phenomenon has been observed in the case of benzoic acid when applied at 200 and 450 nmol. cm^{-2}.

Despite good agreement of the percutaneous absorption

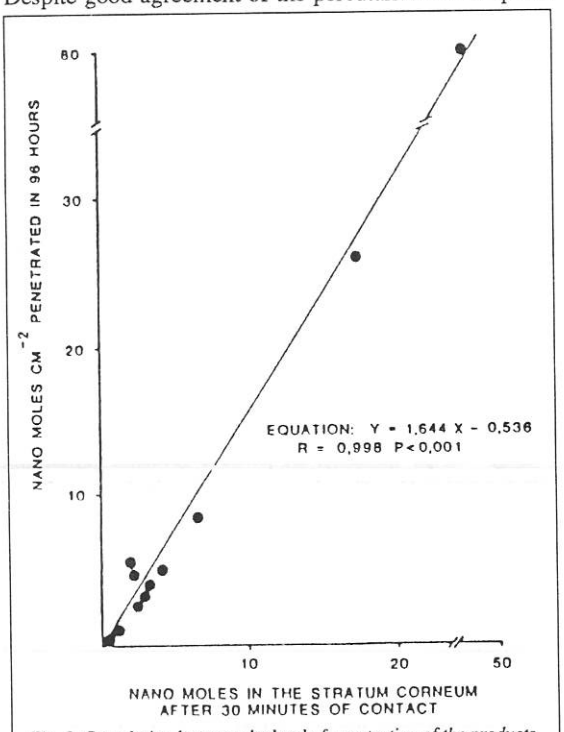


Fig. 3 Correlation between the level of penetration of the products after 96 h and their concentration in the stratum corneum after 30 min of application (decimal scale).

Molecules	Total amounts found 96 hours after topical application					Amounts in stratum corneum treated area 30 min after application
	Urine	Feces	Epidermis + dermis area treated	Animal body	Total penetration	
Benzoic acid						
450 nmol·cm^{-2}	71.20[a]	6.25	1.70	0.16	79.30	48.10
	(2.90)[b]	(1.65)	(0.40)	(0.02)	(3.60)	(5.10)
200 nmol·cm^{-2}	24.80	1.32	0.50	0.01	26.60	17.60
	(0.50)	(0.30)	(0.05)	(0.004)	(0.70)	(1.50)
Acetylsalicylic acid	5.40	0.43	0.85	0	6.70	5.15
	(0.70)	(0.15)	(0.20)		(0.70)	(0.64)
Dehydroepiandrosterone	1.67	3.00	0.19	0.43	5.28	1.95
	(0.15)	(0.30)	(0.03)	(0.08)	(0.20)	(0.40)
Sodium salicylate	3.50	0.48	1.00	0	4.98	3.85
	(0.50)	(0.10)	(0.17)		(0.71)	(0.29)
Testosterone	1.72	2.05	0.24	0.33	4.32	2.06
	(0.43)	(0.60)	(0.05)	(0.15)	(0.46)	(0.23)
Caffeine	2.52	0.42	0.15	0.65	3.73	2.76
	(0.25)	(0.07)	(0.01)	(0.06)	(0.30)	(0.34)
Thiourea	2.10	0.76	0.20	0.20	3.23	2.63
	(0.40)	(0.04)	(0.02)	(0.04)	(0.48)	(0.33)
D-Mannitol	1.60	0.42	0.30	0.30	2.63	2.22
	(0.20)	(0.15)	(0.10)	(0.10)	(0.17)	(0.37)
Hydrocortisone	0.46	0.33	0.04	0.03	0.86	0.93
	(0.07)	(0.07)	(0.01)	(0.01)	(0.08)	(0.15)
Dexamethasone	0.16	0.30	0.10	0.04	0.60	0.41
	(0.02)	(0.05)	(0.02)	(0.01)	(0.06)	(0.02)

[a] Expressed in nmol·cm^{-2} application area.
[b] SD (n = 6).

Parameters of percutaneous absorption of molecules Table I

ranking of some substances in animals with the ranking established in human, extrapolations in absolute values remain problematic. In fact, there are many examples of substances, which, penetrating weakly in animal, can be carried in large amounts through the human skin and vice versa. As an example, in humans, caffeine penetrates approximatively 50 times more than thiourea and has almost the same absorption rate as benzoic acid (24). In the rat, our results have shown that penetration of caffeine, testosterone, and thiourea are similar, i.e., 7 times less than that of benzoic acid. These findings clearly illustrate species differences.

The total amount of substances present at the end of application (30 min) in the reservoir constituted by the stratum corneum has been determined by adding the amounts in each strip (Table I). The formation of significant substance reservoir within the horny layer may be due to the existence of the vehicles in which the molecules are dissolved (7,10), to the particular affinity that a compound may have for the horny layer (26,27), or to the combined action of these different factors.

As already known from in vitro studies, (28) Fig. 2 clearly shows that, in vivo, the distribution of the compounds inside the horny layer decreases according to an exponential relation. Considering the diversity of the molecules tested, it seems that this fact may be considered as one of the factors governing skin absorption.

The most important feature of this study is that there is a linear relationship between "the horny layer reservoir effect" at the end of the application period (30 min) (x) and the total amounts of substances penetrating within 96h (y), independently of the nature of the molecules tested. The linear relationship between the amount of substance penetrating and the amount of substance in the horny layer is of y=axx + b type, where a=1,644 and b= -0.536 (y and x being expressed in nmol.cm^{-2}).These two variables are correlated with a coefficient of 0,998 and a $p<0.001$ (Fig. 3).

Benzoic acid applied (nmol/cm²)		Total amounts found 4 days after topical administration					Amounts in stratum corneum of treated area 30 min after application
		Urine[a]	Feces	Epidermis + dermis treated area	Animal body	Total penetration in 4 days[b]	
125	Rat n = 5	2.52[c] (0.43)[d]	0.14 (0.03)	0.05 (0.008)	0	2.71 (0.44)	1.69 (0.18)
	Human n = 5	0.92 (0.06)	—	—	—	1.14 (0.07)	0.81 (0.04)
250	Rat n = 5	5.23 (0.92)	0.48 (0.15)	0.15 (0.06)	0.03 (0.03)	5.89 (1.06)	3.88 (0.69)
	Human n = 6	1.84 (0.22)	—	—	—	2.30 (0.28)	1.61 (0.18)
500	Rat n = 5	8.24 (0.72)	1.06 (0.37)	0.22 (0.05)	0.05 (0.04)	9.57 (0.74)	5.47 (0.64)
	Human n = 5	3.65 (1.20)	—	—	—	4.56 (1.49)	2.64 (0.36)
1000	Rat n = 5	16.25 (2.38)	2.03 (0.27)	0.40 (0.13)	0.06 (0.02)	18.75 (2.57)	10.46 (0.98)
	Human n = 7	7.12 (2.10)	—	—	—	8.90 (2.63)	5.01 (1.06)

[a] For human: amounts found in the first 48 h.
[b] For human: corrected values from (a).
[c] Expressed in nmol/cm² application area.
[d] SD.

Parameters of percutaneous absorption of benzoic acid Table II

This correlation means, that measuring the amount of a substance within the stratum corneum at the end of the application time (30 min.) gives a good prediction assessment of the total amount penetrating in 4 days. This relationship, verified in a single animal species (rat), should now be tested in humans.

2. Experimentation in human

Table II shows, for the rat, the distribution of benzoic acid in the urine, feces, and in the rest of the body; the amount of benzoic acid penetrated 4 days after administration (illustrated by Fig. 4); and the amounts of benzoic acid present in the stratum corneum 30 min after application. For the human, Table II shows the amount of benzoic acid excreted in the urine within 48h after the administration; the amount of benzoic acid penetrated within 4 days (Fig. 4), extrapolated from the amount excreted in the 48h urines; and the amounts of benzoic acid present in the stratum corneum 30 min after the application.

Fig. 5 shows, for human and rat, and for the various doses of benzoic acid administered, the linear relation-

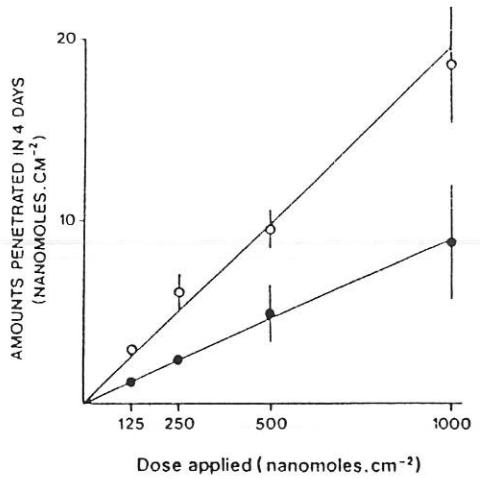


Fig. 4 Relationship between dose applied and penetration rate of benzoic acid in rat (o—o) and human (•—•).

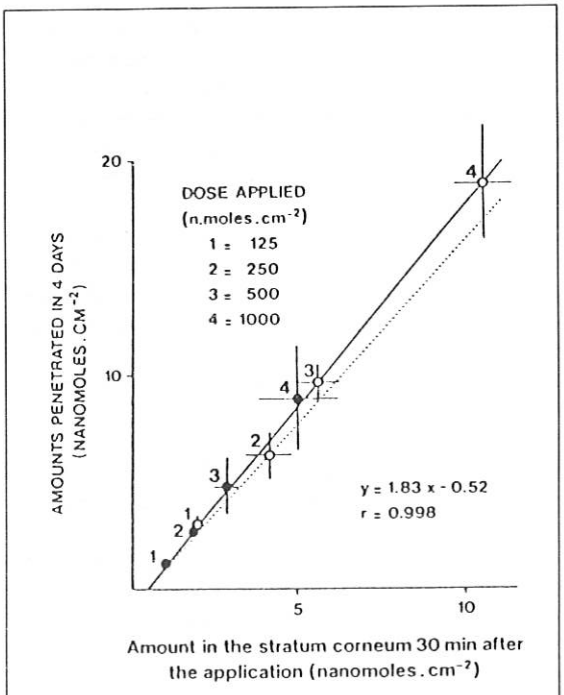


Fig. 5 Correlation between the level of penetration of the different doses of benzoic acid after 4 days and their level in the stratum corneum after 30 min of application in rat (o—o), in human (•—•), and in rat with 10 radiolabeled molecules (.....).

ship between the total amount penetrated in 4 days (y), and the amounts present in the stratum corneum reservoir at the end of application (30 min) (x).

Due to its lack of toxicity (29,30), benzoic acid was chosen. Moreover, it is a well-penetrating substance (24,29) nearly totally excreted in the urine as hippuric acid (19,20). Effectively, as it has been previously shown (24), the kinetics of the urinary excretion of ben zoic acid is extremely rapid. Thus, in both rat and human (20), it has ben demonstated that benzoic acid, administered orally was totally recovered as hippuric acid in the 24h urines.

Furthermore, our findings in the rat (21,22) show that the amount of benzoic acid found in 48 h urines following topical application represent about 80% of the total amount penetrated in 4 days.

This recovery is in agreement with that obtained in this species when benzoic acid was injected i.v. (19). With these data, it is possible to calculate the total amount of benzoic acid that penetrated in human in 4 days by measuring the excretion in the 48 h urine, considering it represents 80% of the total amount absorbed. The findings on percutaneous absorption of benzoic acid both in human and rat (Table II, Fig. 4) show that the amount absorbed is directly proportional to the applied dose. These results are in agreement with those obtained with other substances, in other species (13,14).

It is common knowledge, that percutaneous absorption varies greatly within a species according to the anatomic

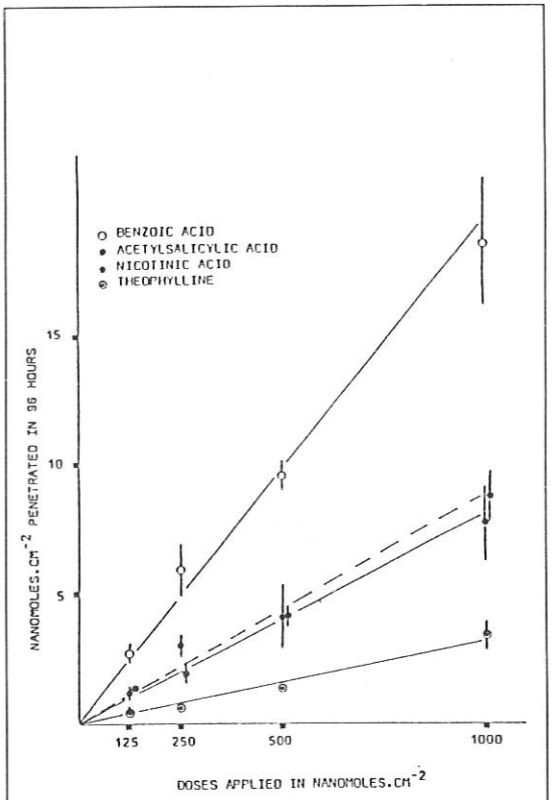


Fig. 6 Relationship between absoption levels of the molecules tested and the doses applied on the hairless rat.

area selected (1,31). Bearing this in mind, our results show that the skin of the back of the hairless rat is twice as permeable as the skin of the human arm, whatever the dose of benzoic acid applied (Table II, Fig. 4). For each dose of benzoic acid applied, the total amounts present at the end of application (30 min) in the reservoir of the stratum corneum were determined by adding the

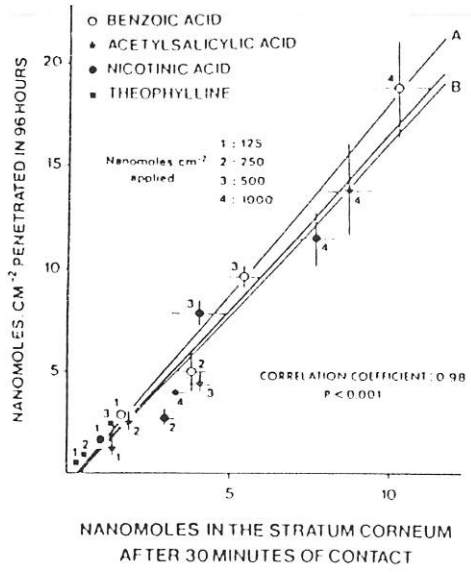


Fig. 7 Correlation between the level of penetration for different doses of products after 96 hours and their concentration in the stratum corneum after 30 min of application.

Curve A: Correlation obtained in man with different doses of benzoic acid
Curve B: Correlation obtained in rat with 10 molecules

amounts found on each stripping both in human and rat (Table II). It may be observed (Fig. 5), that, if the stratum corneum reservoir function in rat is twice that of human, the stratum corneum barrier effect is half as much (percutaneous data in rat being twice those in human) (Table II).

This statement illustrates the directly invers relationship existing between these two cutaneous functions. Fig. 5 shows, for human and rat, and for the various doses of benzoic acid administered, the linear relationship existing between (x), the amounts present in the stratum corneum reservoir at the end of application (30 min), and (y), the total amount penetrated in 4 days. It is worth noting that the data obtained for both species belong to the same straight line described by $y=ax + b$, where $a=1.83$ and $b=-0.52$, x and y being expressed in $nmol.cm^{-2}$. ($r=0.998$, $p<0.001$). It should also be mentioned that this curve is nearly similar to that established in the previous study in rat (Fig. 3) with 10 radiolabeled molecules having very different physicochemical properties. For numerous obvious reasons, it would have been difficult to carry out such a study on humans. Since for rat, the correlation curve obtained with 10 molecules is similar to that obtained with increasing doses of benzoic acid, the correlation curve established in human with increasing doses of benzoic acid should be valid for all types of molecules.

These data, show that in human and rat, the total amount of a drug susceptible to penetrate in 4 days may be predicted from the amounts present in the stratum corneum 30 min after the application of the drug. Since the linear relationship between these two parameters is the same for human and rat, it could also be a rule for other spieces.

Comparing the results obtained in rats with 10 different molecules and in human and rat (Table I;II) it clearly appears that this method of measurement of total skin absorption is not only independent of the nature of the compound applied and of the vehicle used (on one hand for example benzoic acid being solubilized in ethanolic solution on the other hand in ethylene glycol Trition X100 mixtures), but also of the species tested.

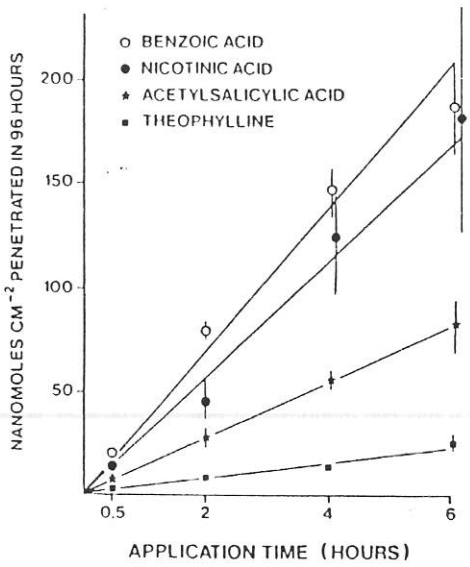


Fig. 8 Relation between the penetration levels ($nmol.cm^{-2}$) of the molecules and the application time (h) on the hairless rat (amount applied 1000 $nmolcm^{-2}$).

TABLE III Parameters of percutaneous absorption of molecules according to the dose applied on hairless rat

MOLECULES	TOTAL AMOUNTS FOUND 96 HOURS AFTER TOPICAL APPLICATION					AMOUNTS IN STRATUM CORNEUM OF TREATED AREA 30 MINUTES AFTER APPLICATION
	URINE	FAECES	EPIDEMIS DERMIS AREA TREATED	ANIMAL BODY	TOTAL PENETRATION	
THEOPHYLLINE						
.125 n.moles.cm^{-2}	0,32a (0,04)b	0,10 (0,02)	0,01 (0,003)	0	0,43 (0,05)	0,25 (0,01)
.250 n.moles.cm^{-2}	0,44 (0,04)	0,19 (0,03)	0,05 (0,02)	0	0,58 (0,04)	0,51 (0,07)
.500 n.moles.cm^{-2}	1,50 (0,26)	0,51 (0,12)	0,12 (0,06)	0	2,23 (0,36)	1,34 (0,08)
.1000 n.moles.cm^{-2}	2,85 (0,33)	0,80 (0,09)	0,21 (0,06)	0	3,86 (0,33)	3,43 (0,48)
NICOTINIC ACID						
.125 n.moles.cm^{-2}	0,48 (0,14)	0,22 (0,09)	0,31 (0,12)	0,50 (0,10)	1,51 (0,23)	1,16 (0,15)
.250 n.moles.cm^{-2}	0,77 (0,23)	0,29 (0,08)	0,67 (0,22)	0,83 (0,16)	2,58 (0,37)	3,05 (0,31)
.500 n.moles.cm^{-2}	2,36 (0,53)	0,57 (0,25)	2,56 (1,02)	2,36 (0,58)	7,84 (1,23)	4,08 (0,52)
.1000 n.moles.cm^{-2}	2,52 (0,46)	0,76 (0,34)	3,71 (1,07)	4,43 (0,93)	11,42 (1,47)	7,70 (0,70)
ACETYL SALICYLIC ACID						
.125 n.moles.cm^{-2}	1,03 (0,34)	0,05 (0,02)	0,12 (0,07)	0,09 (0,04)	1,30 (0,33)	1,39 (0,23)
.250 n.moles.cm^{-2}	2,16 (0,54)	0,14 (0,04)	0,17 (0,07)	0,20 (0,03)	2,68 (0,53)	1,91 (0,39)
.500 n.moles.cm^{-2}	3,57 (0,50)	0,20 (0,04)	0,24 (0,08)	0,42 (0,07)	4,44 (0,47)	4,14 (0,38)
.1000 n.moles.cm^{-2}	10,46 (1,73)	2,17 (0,62)	0,40 (0,08)	0,76 (0,10)	13,81 (2,48)	8,76 (1,01)
BENZOIC ACID						
.125 n.moles.cm^{-2}	2,52 (0,43)	0,14 (0,03)	0,05 (0,008)	0	2,71 (0,44)	1,68 (0,18)
.250 n.moles.cm^{-2}	5,23 (0,92)	0,48 (0,15)	0,15 (0,06)	0,03 (0,03)	5,89 (1,06)	3,86 (0,69)
.500 n.moles.cm^{-2}	8,24 (0,72)	1,06 (0,37)	0,22 (0,05)	0,05 (0,04)	9,57 (0,74)	5,47 (0,64)
.1000 n.moles.cm^{-2}	16,25 (2,38)	2,03 (0,27)	0,40 (0,13)	0,06 (0,02)	18,75 (2,57)	10,46 (0,96)

a: expressed in nanomoles. cm^{-2} application area
b: 5.0. (n=5)

Table III

3. Experimentation concerning the dose applied

Table III shows for each dose applied the distribution of each compound in the urine, feces and in the rest of the body; the amounts of products penetrated 4 days after administration (illustrated by Fig. 6); and the amounts of these molecules present in the stratum corneum 30 min after application. Fig. 7 shows for each compound, and for the various doses administered, the linear relationship between (x), the amounts present in the stratum corneum reservoir at the end of application (30 min), and (y), the total amounts penetrated in 4 days.

One of the most important parameters acting on percutaneous absorption is the dose applied. It is generally admitted that penetration increases with the dose administered (13,14). Though in the two previous paragraphs dealing with penetration of benzoic acid in rat and human this general statement was showed again, it was however important to verify the relation between stratum corneum reservoir function and penetration in the case of various compounds applied at different doses. The examination of results presented in Table III and Fig. 6 clearly shows, for the 4 compounds tested, that at least up to 1000 nmoles, penetration is directly linked to the dose applied. Considering the different structures of these molecules, it seems that this fact might be considered as a general law, at the very least for a range of concentrations.

The most important point is the correlation obtained (Fig. 7) between stratum corneum reservoir function measured after 30 min of application and the total pene-

Compounds (application time (h)	Total amounts found 96 h after topical application					Amounts in stratum corneum of treated area 30 min after application
	Urine	Feces	Epidermis + dermis area treated	Animal body	Total penetration	
Theophylline						
0.5	1.61[a]	0.20	0.04	0	1.85	2.53
	(0.11)[b]	(0.03)	(0.09)		(0.11)	(0.30)
2	5.78	0.77	0.35	0.08	6.98	
	(1.22)	(0.08)	(0.17)	(0.05)	(1.36)	
4	10.79	1.14	0.25	0.14	12.31	
	(1.12)	(0.23)	(0.07)	(0.05)	(1.18)	
6	21.18	2.93	0.57	0.42	25.12	
	(4.31)	(0.62)	(0.13)	(0.19)	(4.78)	
Acetyl salicylic acid						
0.5	6.86	0.80	0.42	0	8.09	5.15
	(1.16)	(0.53)	(0.11)		(1.67)	(0.60)
2	21.29	2.53	1.72	0.15	25.69	
	(0.14)	(0.45)	(0.41)	(0.05)	(1.90)	
4	45.28	4.57	3.71	0.01	53.57	
	(2.64)	(1.89)	(0.77)	(0.006)	(2.39)	
6	72.21	8.02	2.40	0.01	82.65	
	(13.90)	(1.14)	(1.32)	(0.01)	(13.99)	
Nicotinic acid						
0.5	6.67	1.12	0.71	7.20	15.71	9.72
	(1.35)	(0.22)	(0.06)	(1.01)	(2.46)	(0.86)
2	16.30	6.11	3.06	19.31	44.75	
	(5.39)	(1.14)	(0.23)	(5.81)	(11.67)	
4	48.02	6.62	4.87	63.94	123.45	
	(11.35)	(1.23)	(0.49)	(13.90)	(25.50)	
6	79.02	7.40	8.71	84.89	180.02	
	(26.74)	(2.75)	(1.45)	(33.83)	(61.47)	
Benzoic acid						
0.5	17.43	0.85	0.40	0.06	18.75	10.46
	(2.38)	(0.27)	(0.13)	(0.02)	(2.57)	(0.98)
2	73.10	4.65	0.63	0.21	78.60	
	(3.06)	(2.85)	(0.15)	(0.01)	(5.18)	
4	135.58	7.77	1.73	0.23	145.32	
	(13.53)	(1.67)	(0.39)	(0.07)	(12.69)	
6	173.20	9.90	1.81	0.25	185.20	
	(22.72)	(3.12)	(0.36)	(0.11)	(22.55)	

[a] Expressed in nmol·cm^{-2} application area.
[b] SD (n = 5).

Parameters of percutaneous absorption of the molecules according to their application time on the hairless rat Table IV

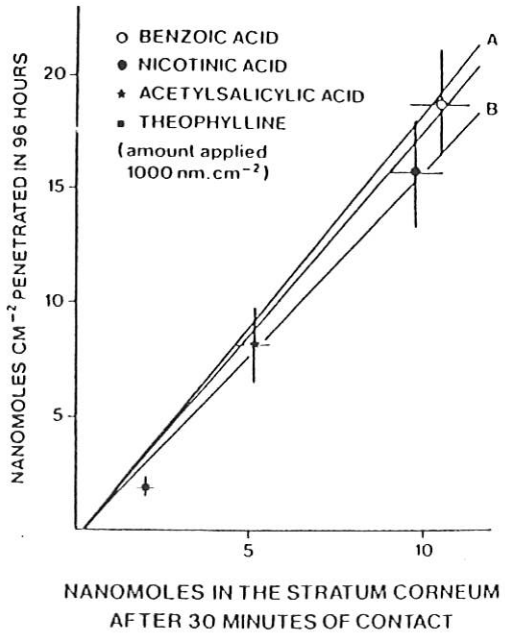


Fig. 9 Correlation between the penetration levels of the molecules within 96 h and their concentration in the stratum corneum after 30 min application in the hairless rat.

Curve A: Correlation obtained in human with increasing doses of benzoic acid
Curve B: Correlation obtained in rat with 10 molecules

tration in 4 days of these compounds applied at various doses.

Furthermore, it is worth noting that this correlation is similar to those obtained either in human with different doses of benzoic acid (Fig. 7 curve A) or in rat with 10 different molecules (curve B).

From the totality of the results obtained, one can ascertain that this method is independent not only of the nature of the compound applied, of the vehicle used, and of the species tested but also of the dose administered.

4. Experimentation concerning the duration of application

In Table IV are reported the percutaneous parameters of the compounds according to their application time: recovery in the urinary and fecal routes, body retention, and total absorption levels (illustrated by Fig. 8) 4 days after application and the amounts of drugs present in the stratum corneum 30 min after application.

Fig. 9 shows, in the case of a 30 min application, the linear relation existing between (x), the amounts of molecules present in the stratum corneum at 30 min and (y), the total amounts penetrated in 4 days.

The totality of the experiments discussed up to now in this paper has been related to a 30 min application time. Since the duration of contact of a compound may be closely linked to its field of use, it was interesting to verify in which way, this parameter could influence the relationship mentioned between stratum corneum reservoir function and the total penetration.

It is obvious that the intensity of the penetration of a molecule depends on its time of application. No doubt it is the reason why the evaluation of this parameter in vivo has not been given the attention it deserves.

Numerous in vitro experiments on excised skin (32,33) have outlined, following a lag time depending on the drug applied, the linear relation between the total amounts of product recovered in the subdermal medium and the duration of the experiment. Our data (Table IV, Fig. 8) clearly show that the in vivo penetration of various molecules is strictly proportional to the time of application (in the limits of our experimental conditions: time ranging from 0.5-6 h). This type of correlation having been found on 4 molecules with different physicochemical properties, it is reasonable to assume that it may constitute one of the laws of in vivo percutaneous absorption phenomena. Moreover, from a theoretical viewpoint, this relation provides evidence that a constant flux of penetration does really exist in vivo.

The fact that, in vivo, such a flux is reached at a short time (30 min), suggests that the delay time of penetration is extremely short.

Table IV and Fig. 9 show that in the case of a 30 min application, the total amounts of molecules recovered within the stratum corneum reservoir are strictly correlated with the amounts that penetrated in a 4 day period. The total percutaneous absorption of a compound being directly linked to the duration of its application (Table IV and Fig. 8) it is therefore evident that the single knowledge of the reservoir effect of the stratum corneum for a molecule applied for 30 min permits the prediction of its total penetration within 4 days for various times of appliction. Moreover, this method, when applied to human, offers the advantage to detain subjects for only a short period.

CONCLUSIONS

In summary, taking into acount all the data obtained, three main conclusions may be drawn.

First: Barrier and reservoir functions of the horny layer may be closely related.

Second: The linear relationship existing between the reservoir function of the horny layer and penetration may be outlined as one of the interspecies laws.

Third: The simple measurement of the amount of a substance within the stratum corneum at the end of 30 min, gives a good prediction of its total penetration in 4 days whatever the compound and the species tested (human or rat), the dose applied, and the time of application.

For technical reasons, this mildly invasive method was carried out by using radiolabeled compounds. Nevertheless, the relatively large amounts of substances present within the stratum corneum reservoir at the end of application time (30 min) should allow penetration studies by sensitive but non radioactive techniques. This possibility enhances the feasability of perctuaneous absorption studies either in laboratory animals or in humans, even with radioactive material due to the less important amount of radioactivity required by this method.

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THE ADVERSE REACTIONS OF VEHICLES AND EXTERNALLY APPLIED DRUGS

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The primary function of the skin is not absorption; but rather the protection of the body. This protection is provided mainly by the uppermost layer of the epidermis, the horny layer, which consists of upper, loosely packed layers of horny cells (strata disjunctum) and lower densely packed compact layers of these same cells. The cells themselves are dead, dried epidermis cells. During the natural growth of the epidermis, the horny cells are constantly shed.

All ingredients of the applied drug enter the loosely packed layers of the horny layer. The active substance as well as all other ingredients may concentrate in this layer. All pharmacologically active substances as well as most inert ingredients penetrate into the compact layers (1,2). This process occurs quickly — much more quickly than new horny cells are produced. Because of these facts, absorption should be at great concern in the evaluation of topically applied products.

Pharmacologically inert auxiliary agents usually penetrate into the deep layers of the horny layer. From here they penetrate to various depths of the epidermis and dermis just as do the active substances. Because of this, side effects can be expected from all substances which are applied to the skin (3,4)(Fig. 1-6).

We expect creams, ointments, lotions and emulsions to have greater physical effects in the structure of the horny layer — moistening and softening — than the active substances, antioxide agents and preservatives. These physical effects often results from the vehicles as a whole rather than from its individual components.

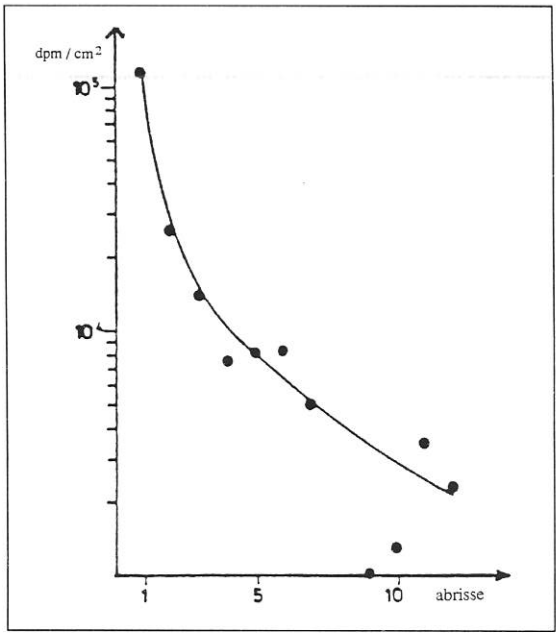


Fig.1
In der Hornschicht wiedergefundene Aktivitätsmenge in dpm/cm2 der einzelnen Tesafilmabrisse von radiomarkiertem PEG 4000 in PEG 4000 als Vehikel für die Penetrationszeit 30 min.*

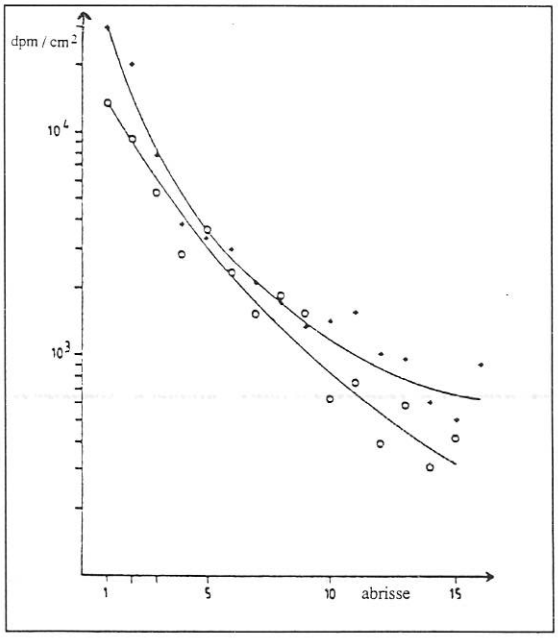


Fig.2
Verteilung der in der Hornschicht wiedergefundenen Aktivitätsmenge von radiomarkiertem Octadecan in dem Vehikel wasserhaltige hydrophile Salbe für die Penetrationszeiten 30 min (+—+): 100 min (o——o).

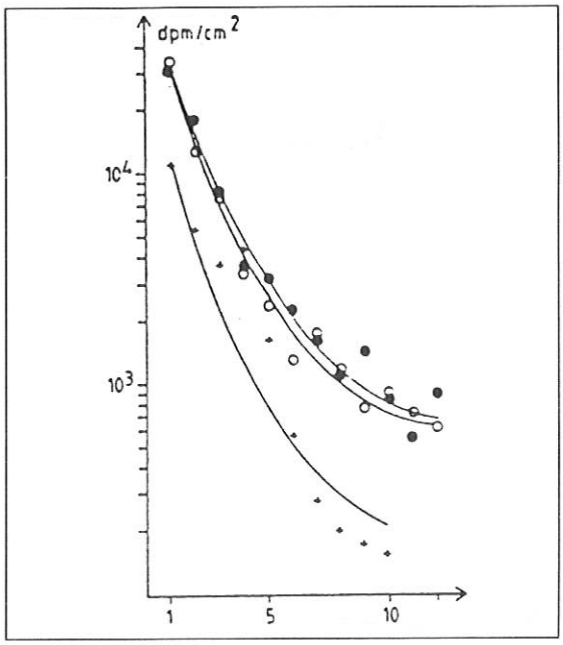


Fig.3 Verteilung der in der Hornschicht wiedergefundenen Aktivitätsmenge (dpm cm2) von radiomarkiertem Cetylalkohol im Vehikel wassehaltige hydrophile Salbe (DAB 8) für die Penetrationszeiten 30 min (+——+): 100 min (o——o): 1000 min (•——•).

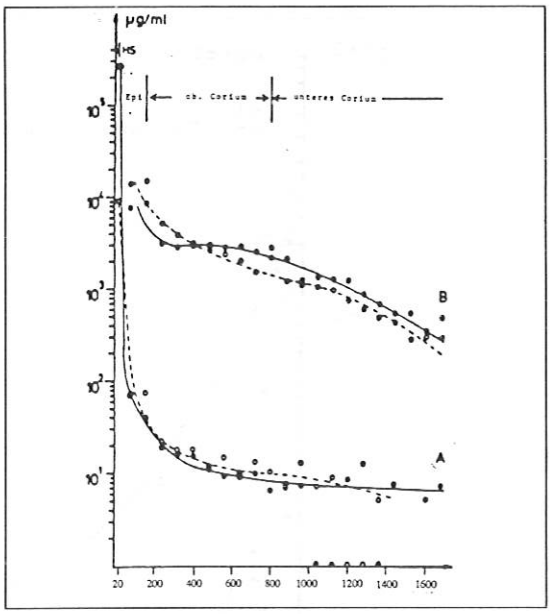


Fig. 4 Vergleichende Darstellung der wiedergefundenen Substanzmenge in ug/ml von radiomarkiertem PEG 4000 in PEG 4000 als Vehikel in den einzelnen Schichttiefen der Haut bei Untersuchungen an intakter Haut (A) und an Haut ohne Hornschicht (B) für die Penetrationszeiten 300 min (o——o) und 1000 min (•——•).*

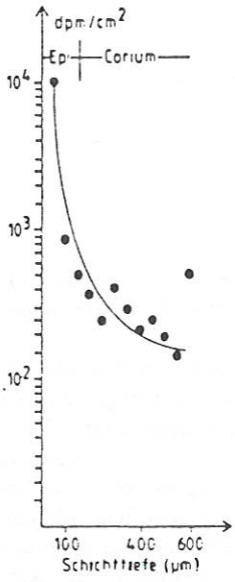


Fig. 5 In Epidermis (Epi) und Corium wiedergefundene Aktivitätsmenge (dpm cm2) von radiomarkiertem Cetylalkohol in wasserhaltiger Wollwachsalkoholsalbe (Schnittdicke 40 um). Penetrationszeit 1000 min.

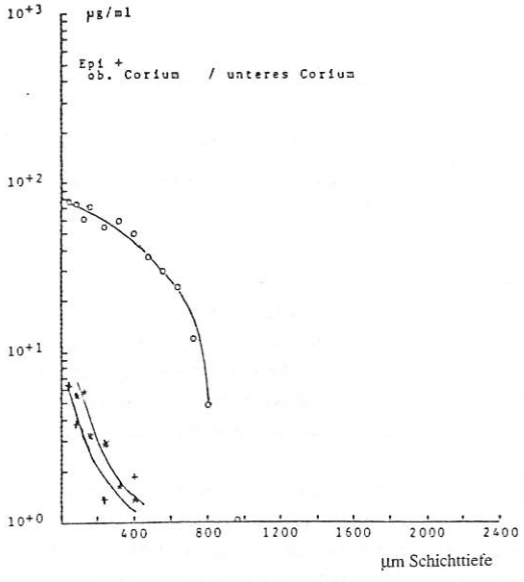


Fig. 6 Penetration von 4.4% Isopropylmyristat aus der W/O-Creme
(+ + +) = 100 min (* *) = 300 min*
(ooo) =1000 min

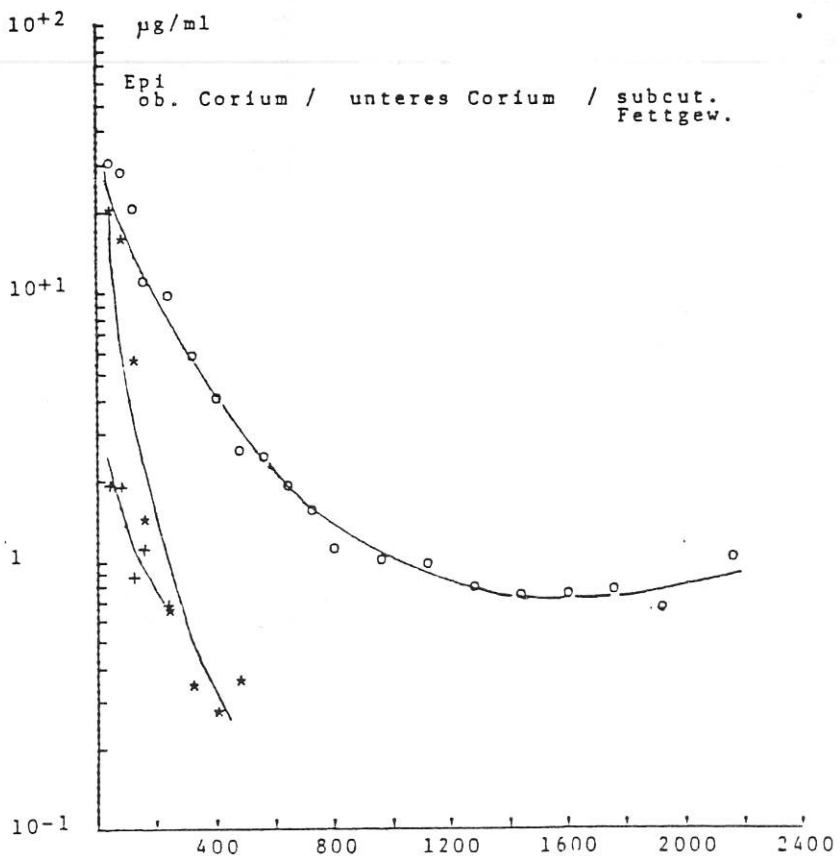


Fig. 7 Penetration von 2.1% Sorbitol aus der W/O Creme
(+ + +) = 100 min *(* * *) = 300 min*
(ooo) = 1000 min

I would like to mention that such physical effects of ointments, creams, powders, and other topical drugs often represent a large part of the total therapeutic effect of many products. One is repeatedly surprised at the small difference between the therapeutic effect of the vehicle with the active substance and the effect of the vehicle alone.

The penetration of the active substances and of the auxiliary substances are dependent on the vehicles (Fig. 7,8). Therefore, the contact allergic properties of cetyl alcohol, myristicyl alcohol and lanolin can be variously effective depending on the composition of the topical product (5). For the question of contact allergy, penetration is the determinant parameter. For skin irritancy, in contrast, the penetration value is unimportant. For this, the concentration in the vehicle and the presence of possible other physically acting agents is determinant.

Local toxic effects are usually minor and reversible. They are likely to begin with a drying out of the horny layer of the surface of the treated skin. This can be caused directly by the vehicle or its ingredients and indirectly by a pharmacological effect on the sebaceous glands. Usually, the beneficial effects of the vehicle last longer than the negative pharmacological effects, so that no acute irritation of the skin can be seen (6)(Tab. 1).

Acute local risks are usually easily detectable during the clinical investigation of new products. Risks can be increased, however, by the method of application (7,8), the condition of the treated skin, the location of the treated skin, as well as concurrent therapeutic measures and changes in the ingredients of the vehicle (Tab. 2,3,4). Vehicle ingredients can cause a change in the penetration process of the active substances and thus cause a drying effect on the surface of the horny layer. This can result in chronic damage. Hygroscopic substances such as PEG as well as for liquid parafins, lipid solvents such as alcohol and acetone emulsifiers and as propylene glycol or o/w emulsions with large proportions of vaporizable water can cause such chronic damage (9,10).

The drying out process begins with the removal of sebum and oils by emulsifiers as well as with the removal of natural moisturing substances from the horny layer by the ingredients of the vehicle. This drying out can then provoke irritation of the horny layer. Direct irritation of the epidermis cells is usually already detected at the early stages of animal investigations and thus seldomly occurs in clinical use.

Summations of local effects can become serious when drying out by an active substance, benzoyl peroxide, for example, is aggrevated by the vehicle to such an extent that irritation results. Topically active substances such as dithranol, Vitamin A acid and 5-flurouracil can cause

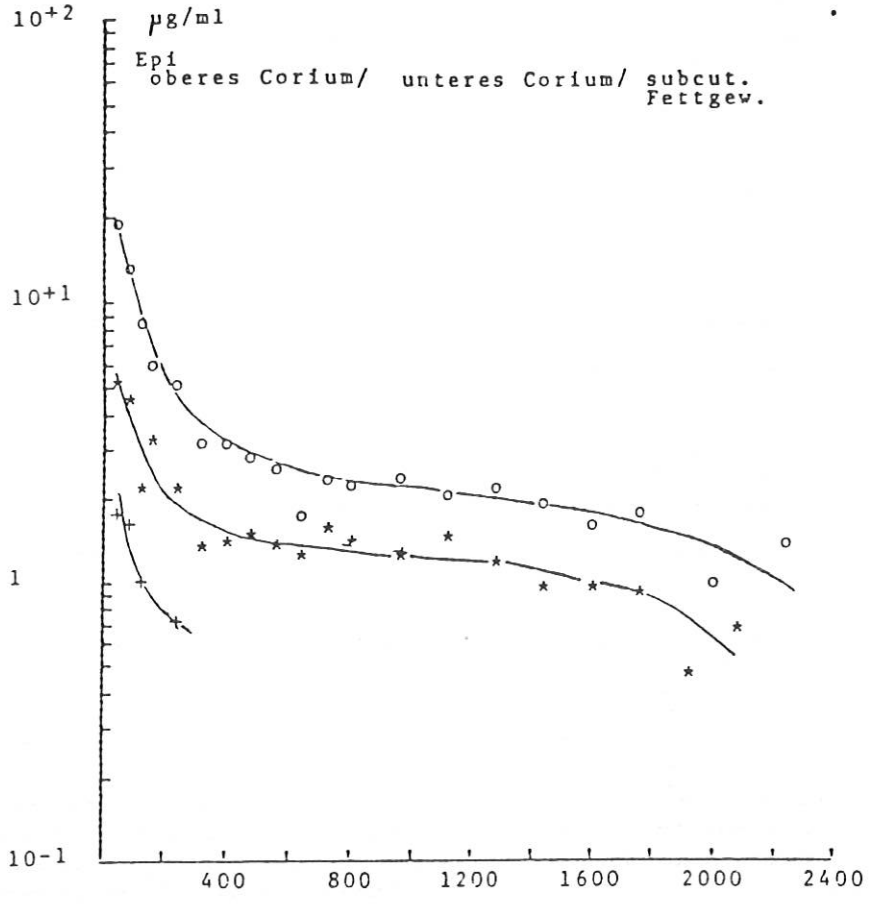


Fig. 8 Penetration von 2.1% Sorbitol aus der O/W-Creme
(+ + +) = 100 min (* *) = 300 min*
(ooo) =1000 min

Table 1 The comparison between salt cream and placebo cream on dryness and scaling in asteatosis. For the statistical analyses (Fischer's test), the improvement of 1 and 2 steps were taken together and compared with the group of no improvement.

steps of improvement	3	2	1	0	n	P
Dryness						
Salt cream	-	6	17	3	26	<<0.001
Placebo cream	-	-	8	18	26	
Scaling						
Salt cream	-	5	18	3	26	<<0.001
Placebo cream	-	-	8	18	26	

Table 2 Additional therapeutic measures

Stimulation of blood circulation:
- intermal medication
- hot baths
- infrared- resp. thermal radiation

UV-light (sun, artificial)

Horny-layer-"softening":
- baths, saltwater baths
- occlusive dressing
- disposable napkins

Table 3 Lindane concentration in the skin (µg / ml tissue) after local application in relation to the penetration period and nature and time of washing of skin with intact stratum corneum

Washed with	washed after (h)	Total penetration period	Concentration (µ / ml tissue) in: stratum corneum	Epidermis	Dermis upper	Dermis lower	Subcutaneous fat
		10	745	0.461	0.155	0.098	0.017
water soap	3	10	258	1.21	0.679	0.689	0.162
water soap	10	24	178	4.67	0.893	0.67	0.0386
water	10	24	378	3.27	1.52	0.657	0.345

Table 4 Mean maximal and minimal values (ng ml) of curves representing kinetics in 2 volunteers each using the same mode of washing (Applications at 1st. 24th. and 48th h).

Group	Removal by washing with	time after application (h)	Mean maximal value	Mean minimal value	Initial value	Final value after 143h	Difference
I	Water and soap	3	22.1	4.8	2.7	6.2	3.5
II	Water and soap	10	6.4	2.3	2.8	2.6	0
III	Water only	10	13.3	3.7	3.2	4.6	1.4

an acute inflammation at the beginning of treatment. This is often accepted as unavoidable. It is not always clear whether inflammation is accompanied by irritation by the vehicle.

The risk of side effects caused by active ingredients can be decreased by the reduction of the concentration used (Tab. 5). Although this can, of course, lead to a loss of effectiveness, experience has shown that a markedly longer treatment with a less-irritating, less-concentrated preparation can often be just as therapeutically effective, for example, topical therapy with a 0.1% dithranol vaseline or a 2-3% benzoyl peroxide preparation can be just as effective as the usual 0.5% to 1% dithranol ointments or the 5% to 10% benzoyl peroxide preparations. The lower concentrations result in fewer side effects.

Table 5
Concentration - dependent risks, also depending on the individuum and on the vehicle

Active ingredient	from ... % onward skin irritation
Benzoylperoxide	3
Tar	10
Podophyllin	10
5-Flourouracil	1
Dithranol	0.5
Acid. Tannic.	5
Acid. Lactic.	2
Chrysarobin	15
Phenol	5
Resorcin	10
Sulphur	5

Serious local damage can also occur directly at, or very near the treated skin. Usually such damage is seen only after long periods of time. The topical treatment may already have been discontinued when the damage is observed, so that often no direct connection is made between the damage and the previous treatment. The topical side effects of corticosteriods were discovered only after 15 years of mass use (Tab. 6). When such effective active substances are prepared with special penetration-promoting vehicles, then risks become even greater.

At present, there is a trend towards the return to old, proven therapies. Although the use of preparations containing mercury or lead salts is not being used, there is an intensification of the use of the old tar therapy. Although extensive studies have given no specific proof of a direct relationship between general topic tar therapy and skin cancer in man, we do have proof of such a relationship between treatment of certain areas of the skin with tar and cancer and between tar therapy in combination with utraviolet light and cancer. For example, an increased rate of occurence of carcinomas after longterm treatment of scrotal eczema with tar has been found, although other causal factors could not be excluded (11).

The penetration of locally applied substances is much higher in intertriginous areas as well as some areas of the eyelid and the vulva or scrotum and thus the risk of cancer in these areas is increased.

Even when the treatment of such especially endangered skin areas is avoided, the benefit-risk evaluation for potentially carcinogenic substances should emphasize the safety side (12)(Tab. 7).

Risk of malign variations of dermal cells after long-term use of Vitamin A acid in connection with ultraviolet light has been suspected. Malign variations are also suspected to occur after UV light exposure during treatment with tumor-promoting substances such as benzoyl peroxide and dithranol. Animal experiments can be helpful in testing whether such morphological variations can be provoked. However, since the results of experiments on the non-pigmented and hairy animal skin are not generally applicable to human skin, such experiments can only serve to direct our attention to possible risks and their prevention (Tab. 8).

These difficulties are even greater when the toxic effects have no relationship to the treated skin, but are rather the result of percutaneous absorption of the active substances. Thus, it is important to consider the kinetics of topically applied substances since they are quite different from those of the same substance applied by other routes. Low substance concentration

Table 6
Undesirable consequences of corticosteroid therapy for the human skin

Disease	Effects	
	morphological	functional
Atrophy - epidermis	Reduction in the mitosis rate	Inhibition of DNA synthesis
- corium	Collagen atrophy, rarefaction of the ground substance	Inhibition of muco-polysaccharide synthesis
- vessels	Vascular purpura, perivascular loss of substance, telangiectases	Catabolism vasco-activity (blanching "vasoconstriction")
- fatty tissues		
Striae	Rarefaction of the elastica	
Steroid acne	Folliculitis (no increase in the formation of sebum)	Infection (candida, bacteria)

Table 7 Absorption studies

Polynuclear Aromatic Hydrocarbons of 2 % Crude Coal Tar in Petrolatum thought to be a carcinogen, cocarcinogen or tumor initiator	Ointment Analysis-level PPM µg/ml	Blood Levels before application (on intact skin) PPB ng/ml	Blood Levels after application (on intact skin) PPB ng/ml
Phenantrene	650	ø /0.5/1.4/1.7/3.1	11.0/0.4/1.2/3.1/6.3
Anthracene	190	ø / ø / ø / ø / ø	4.7/0.08/ø /0.5/1.6
Fluoranthene	400	ø / ø / ø / ø / ø	3.3/0.1/ ø /0.5/1.7
Pyrene	330	ø / ø / ø / ø / ø	ø / ø / ø /0.2/1.1
Benzothiophene and alkylated derivates	18	ø / ø / ø / ø / ø	1.3/ ø / ø /13.0/21.7

Table 8
Potential risks of some topical drugs.

Active ingredientsof topical drugs	Suspicion arising from animal experiments
tretionin	teratogenic potential
benzoylperoxide	+ UV-light: tumor-promoting activity increased
8-MOP (PUVA)	
dithranol	
acyclovir	the drug has a mutagenic potential
idoxuridin	the drug has a carcinogenic and mutagenic potential
tar	known human carcinogen (location!)
tromantadin	the drug has a mutagenic potential
5-fluouracil	the drug has a carcinogenic and mutagenic potential
podophyllin	
metronidazol	the drug has a carcinogenic and mutagenic potential
salicylic acid	the drug has a teratogenic potential, exhibited in animals at high doses
pyoktanin	the drug has a mutagenic potential (high concentrations)

peaks in the serum are followed by prolonged diffusion periods with very low serum concentrations. These diffusion periods can last several days since the large take up capacity of the horny layer produces a reservoir effect and the very low transport capability of this layer produces a barrier effect.

Acute damage from active substances is therefore seldom. Furthermore since the acute toxic effects of the active substances are usually known, they are most easily detectable. However, the lack of a firstpass effect of locally applied substances is often overlooked. This is especially true for substances (which affect the central nervous system) such as salicylid acid, hexachlorophene and caffein. Some of these salicylic acid and coffein, for example, which are relatively nontoxic when applied enterally, are sometimes toxic when applied topically (13,14).

Auxiliary substances such as sodium lauryl sulfate, tweens, spans, and propylene glycol can also indirectly increase the risks of potentially toxic substances by increasing the penetration even on undamaged skin. Pharmacokinetic characteristics of topically applied products which often result in very low serum concentration levels make the evaluation of chronic systemic toxicity very difficult. The evaluation of the results of animal experiments on embryotoxicity, teratogenicity, mutagenicity and cancerogenicity is almost impossible. Many substances known to be cancerogenic or teratogenic when applied internally are used externally. In order to allow reasonably reliably conclusions on chronic toxicity, studies must be carried out percutaneously on animal skin. Such studies are not usually done routinely. The conclusion that a substance is safe because it does not penetrate the skin, or if it does only in small amounts, cannot always be accepted since the term of treatment and the effects of concurrent measures are often overlooked. Because of the low serum concentration peaks, low percutaneous absorption, unknown threshhold levels and the general lems connected with the applicability of animal experiprobments to man, we cannot, in good conscience, at present, say there is a "no effect level".

Every health professional who prescribes a potentially carcinogenic substance — in any concentration, with

whatever penetration characteristics or by whatever application method — should be aware of these dangers, especially since the public is increasingly skeptical about drug therapies. Fortunately, since adverse effects of topically applied drugs usually affect the area of the skin treated, they are usually easily detectable. Thus in spite of the dangers, topical application is usually safer than all other routes of application.

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Acta Derm Venereol Stockholm 1987; Suppl. 134:30 - 32

Comparison of two photoelectric techniques for quantitative measurements of skin surface lipids.

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A method for quantitative assessment of skin surface lipid by means of extraction with solvents, was first developed by Kligman & Shelley (1). Later Strauss & Pochi (2) developed the gravimetric method using lipid absorbing cigarette paper. This method has since been modified by Cunliffe & Shuster (3). A further refinement in technique was developed by Schafer & Kuhn-Bussius (4) utilising a spectrophotometric principle with lipid absorbing ground glass plates. This technique was modified by Saint-Leger & Leveque (5) and later by Cunliffe, Kearney & Simpson (6). The modified gravimetric method has been proven to be both accurate and reproducible but it is laborious and time consuming. It was therefore most encouraging when it was shown that comparable values could be obtained with the modified photometric technique (5,6,7). In the present study we have compared two spectrophotometric techniques with each other while determining the casual level of skin surface lipids.

Methods and material

The casual level (5,6) was measured by means of two different photometric techniques, the sebumeter SM420 (Schwartzhaupt) and the lipometer (l´Oreal). The lipometer of l´Oreal was used as described elsewhere (5,6). Briefly, skin surface lipids were sampled using a sapphire plate which was permanently attached to an applicator. Sampling was performed by the application of the plate to the skin surface for 15 seconds and with a constant pressure of 0.55 kg cm^{-2}. To measure the lipid collected the plate and the applicator were immediately inserted into the machine and the read-off recorded in the four positions indicated. The mean of these four readings were taken as the lipid content of the plate. Sampling was performed four times on the same site and the summation of the mean values was taken as the lipid level. Before each sampling the sapphire plate was cleaned with acetone and the indicator was electronically ´zeroed´ by a microprocessor in the machine.

The photometrical principle of the SM420 is based upon measurement of light transmittance through a transparent plastic folium which is pressed (6 N) against the skin for 30 seconds allowing adherence of skin lipids. The plastic folium was changed before each measurement and controlled in the indicator by a zero reading. Only one sampling and one reading was necessary. The readings were converted into $micgcm^{-2}$ by means of a formula.
The measurements were performed on symmetrical sites on the right and left side of the forehead in 12 male subject aged 20 to 48 year. Only one of the subjects was hyperseborrhoeic. Comparisons were made between the two methods which were randomly adjusted to each side. Other studies have shown that there exists a good correlation between symmetrical left and right sides (6,7).

RESULTS AND DISCUSSION

In the present study a strong correlation was found between the data obtained with the two different photometric techniques and also between the casual levels on the right and left side of the forehead (Tables I and II).

The sebumeter SM420 is easy to use but it should be tested on a larger patient group of hyperserborrhoic subjects and the results together with the sebum excretion rate determinations could then be compared with the gravimetric technique which is the most reliable.

Table I. Results of skin surface measurements with the two types of meters.

	micgcm^{-2}	
Pat. no.	lipometer l´oreal	SM420
1.	72	73
2.	108.5	135
3.	176	125
4.	197	184
5.	29	15
6.	35.5	33
7.	31	53
8.	29	67
9.	131	129
10.	16	22
11.	143	121
12.	264	320

Table II. Analysis of the obtained values showing a high degree of correlation; r=0.94 ($p<<0.001$)

	Lipometer l´Òreal	SM420	Difference
n patients	12	12	12
Mean + SEM	102.7 + 23.3	106.4 + 24.6	3.7 + 8.3
Median med	90.3	97.0	-0.5
95% conf. int.	(31 - 143)	(33 - 129)	(- 13 - 22)
Range	16 - 264	15 - 320	- 51 - 56
p - value			p2 = 0.75

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Acta Derm Venereol Stockholm 1987; Suppl. 134: 33-34

A NEW METHOD FOR MEASURING THE SKIN CLEANING EFFECT OF SOAPS AND DETERGENTS.

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Abstract

Following the method of Schrader we used a special skin washing mashine to investigate the skin cleaning effect. Synthetic detergents and various soaps were applied to the skin of the forearm in two groups of healthy controls and patients suffering from various dermatoses.

We could observe the different effects of lavation, depending on the kind of soap or synthetic bar used, on the sebum-secretion-type of the skin and on different skin diseases.

Evaluating the result, we found that the so-called mild effect of some soaps depends on their lavation activity.

The use of detergent bars has been increasing rapidly in FRG during the past 15 years. The major product among these soap free detergents is named SEBA MED.

The commercial success of this compound is based on

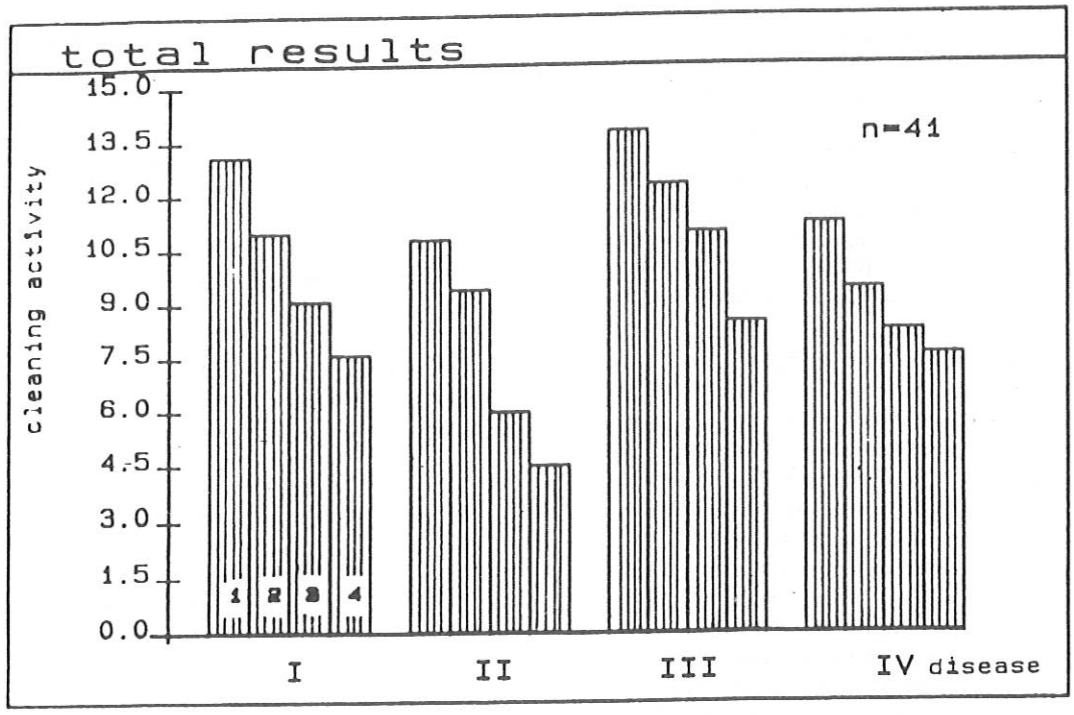


Fig. 1. *The cleaning effect of four different products on the forearm in subjects with skin diseases and with normal skin.*

I = psoriasis (n=8)
II = atopic dermatitis (n=5)
III = controls with skin diseases (n=16)
IV = normal controls (n=12)

1 = Sebamed
2 = Minon
3 = Neutrogena
4 = Lux

its chemical properties: a high degree of cleaning effect combined with applicability and nonallergy in healthy and pathologically altered skin. Because of its pH (5,5) it also maintains the acid mantle of the skin, and can be used for the treatment of soap-or alkali-eczemas and for wound-cleaning of burn-patients (1-4, 6-11).

In this context we have investigated the cleaning effects of 4 soaps and detergents on normal and pathological skin: LUX, NEUTROGENA, MINON and SEBA MED.

The cleaning activity was determined by means of a skin washing machine, which cleans under standard conditions the artificially stained skin to the forearm (5). Four brushes of the same weight wash the stained skin in limited areas. The cleaning effect was assessed by a photometer, which determined the difference of skin colour before and after the procedure of washing. The result is the socalled wash number. A high number represents a high cleaning effect and vice versa (5).

Because of a possible influence of the skin cleaning substances on the pathologically altered skin, we investigated four different groups of volunteers: 8 patients suffering from psoriasis, 5 from atopic dermatitis, 16 controls with various skin diseases but free of skin lesions in the area of the forearm and 12 normal symptomfree controls. We could observe, that under normal skin conditions SEBA MED had the highest cleaning effect, followed by the product MINON, NEUTROGENA and by LUX (Fig. 1). Similar results could be seen in the cases of atopic dermatitis (FIg. 1), but a reduced cleaning effect for NEUTROGENA and LUX was noted. This could have been caused by the dryer skin seen in atopic dermatitis and therefore by the firmer staining of the altered skin. Skin diseases at localizations other than the test area did not influence the activity of detergents and soaps (Fig. 1).

In psoriasis we observed a higher cleaning effect than in normal controls (Fig. 1). We assume that the detergents and to a lesser degree also the soaps remove the psoriatic scales together with the colour. If we compare the effect of the various cleansing substances and the various skin conditions it can be stated the SEBA MED has the greatest cleaning effect in all groups tested. The lowest cleaning effect was registered by the soap LUX in atopic dermatitis patients (Fig. 1).

In summary, the cleaning effect of soaps and detergents depend on their content of wash lotion and, to a lesser degree, on the skin condition itself. The characterisation of a soap or detergent as "mild" would also appear to indicate a lower cleaning effect.

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DESQUAMATION OF THE STRATUM CORNEUM : KINETICS FOLLOWING U.V. INDUCED INJURY

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The visible consequences and their chronology, following sunburn, are well known to the holidaymaker after his first stay on beach: erythema, occasionally oedema, then, a few days later, peeling of the skin. Cosmetology could play an important part in each step in this chronology of events, in preventing or minimizing such uncomfortable consequences. A recent aim of cosmetologists concerns precisely the peeled aspect of the skin surface.

In this field of investigation, they basically do have a sufficient knowledge and understanding of the phenomenon and of its kinetics, before claiming the efficacy of their products. Surprisingly, very few studies recording the desquamation of the stratum corneum following U.V. exposure have been conducted in the past years. Thus, in the present study, a cascade of fully standardized methods have been selected which allow one to describe and measure the damage caused by U.V. radiation on the skin.

MATERIAL AND METHODS

Fourteen healthy subjects, male and female, were selected according to their skin type. Their backs were exposed to U.V. irradiation, using the methodology of PESSIS et al. (1982) (1). The solar simulator consisted of four OSRAM ULTRA VITALUX lamps 70 cm from the skin surface. The back was overlaid with a thick tissue through which regularly spaced square windows, 2 by 2 cm, were punched out, so that exposure was limited to small skin areas.

The Minimal Erythema Dose (MED) was first determined on the upper portion of each back, two days before the experiment. Then, the different U.V. doses applied to the various areas were controlled by opening the windows for the corresponding times. They received 1, 2 and 3 MED as a single dose. The desquamation of the stratum corneum was assessed by using the wool-turbine machine previously described (2). It consists of a stainless steel disc covered with a sheet of wool, which causes a friction at the skin surface by rotation of the disc with an electric motor. The rotating system was enclosed in a plastic chamber into which 0.5 ml of 0.1% triton X 100/phosphate buffer ph 7.9 solution was injected during the time of friction, allowing collection of the corneocytes. The addition of 10 µl of cristal violet/basic fuchsin mixture to the suspension of corneocytes allowed automatic measurements by image analysis. The number of corneocytes per square centimetre of skin and their mean projected areas were recorded from up to 500 cells. The diameter of the chamber (10 mm) authorized four samplings per irradiated square as shown in Fig. 1. Reference measurements were obtained from the adjacent non-exposed areas. Sampling was performed from day 1 to day 26 according to the calendar in Fig. 2. Each sample of the irradiated area had its corresponding reference and during the experiment, 638 samples were taken.

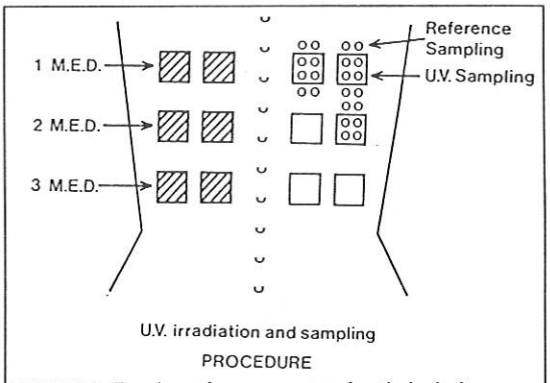


FIGURE 1 : Experimental arrangement used on the back of subjects, showing the irradiated squares and the sampling procedure

RESULTS

The rate of desquamation

The number of corneocytes which were released during the experiment showed large daily and individual variations. The variability of this parameter is shown in Fig.3. From 50,000 to 170,000 corneocytes were harvested on the reference sites. Furthermore, the non-homogeneity of subpopulations (i.e. day 4 and 5) was obvious. With these conditions the best expression of

SAMPLING PROCEDURE

	DAYS AFTER U.V. exposure	1	4	5	8	9	10	11	12	15	21	25	26
NUMBER OF SUBJECTS	1 M.E.D.	13	7	6	13	6	7	7	7	13	14	7	7
	2 M.E.D.	14	7	6	14	6	6	7	7	14	13	7	6
	3 M.E.D.	13	7	6	13	6	7	7	6	14	13	7	6
SUB-TOTAL		40	21	18	40	18	20	21	20	41	40	21	19
TOTAL		319 x 2 = 638 Samples											

└─┘ pooled for statistics on area measurements

***FIGURE 2** : The calendar of the experiment*

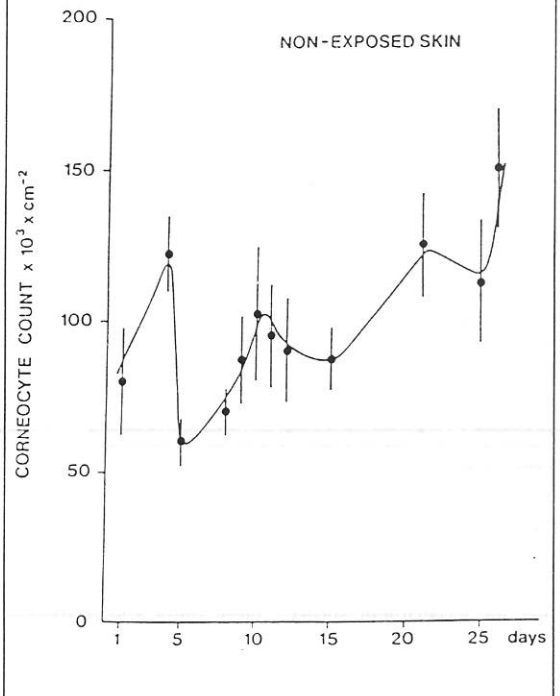


***FIGURE 3** : Plots of the mean corneocyte count ± S.E. of the non exposed areas versus the number of days following the U.V.irradiation*

results was defined by the following rate of desquamation :

$$R = \frac{Ne - Nr}{Nr} \times 100$$

Ne being the corneocyte count of the irradiated skin and Nr the corneocyte count of its adjacent reference zone. Increases or decreases of more than 20% were considered to be significant.

The three curves of the level of desquamation during the 26 days following the U.V. irradiation are summarized in Fig. 4. For 1 MED, a slighty increase was observed at day 9 and remained until day 15. With 2 MED, a sharp pead of +120% was recorded at day 10 and it was followed by a plateau of + 50% from day 12 to day 25. The 3 MED curve showed the same increase of over + 160% at day 10, but the plateau was not observed.

The size of corneocytes (Fig. 5).

The projected area of corneocytes of the reference zones showed a remarkable stability during the month of the experiment. A mean value of 990 μm^2 was recorded and variations were considered significant above 1020 μm^2 and 960 μm^2. The 1 MED curve showed the same stability as the reference curve. With 2 MED, a progressive decrease in size started at day 10 and reached a minimum value of 840 μm^2 at day 20, then increased afterwards to a value lower than that of the reference up to day 25. Quite different kinetics were observed for 3 MED: the fall in size started at day 9 or 10, but reached a minimun of 910 μm^2 as early as day 12. Nevertheless, values lower that of the reference were maintained up to day 25.

DISCUSSION

A short induced injury can provoke either short or long-term modifications, depending on the U.V. dose applied. An example of short-term modifications is illustrated by the peaks of desquamation at day 10. The sudden loss of the stratum corneum barrier would be quite complete in a period of 3 days for 3 MED. Thus, the epidermis must protect the surface of the skin by synthetizing a transitional barrier (3), the strong cohesiveness of which would not allow further corneocyte release when subjected to the wool-turbine. We noticed that the day-ten peak had an amplitude related to the

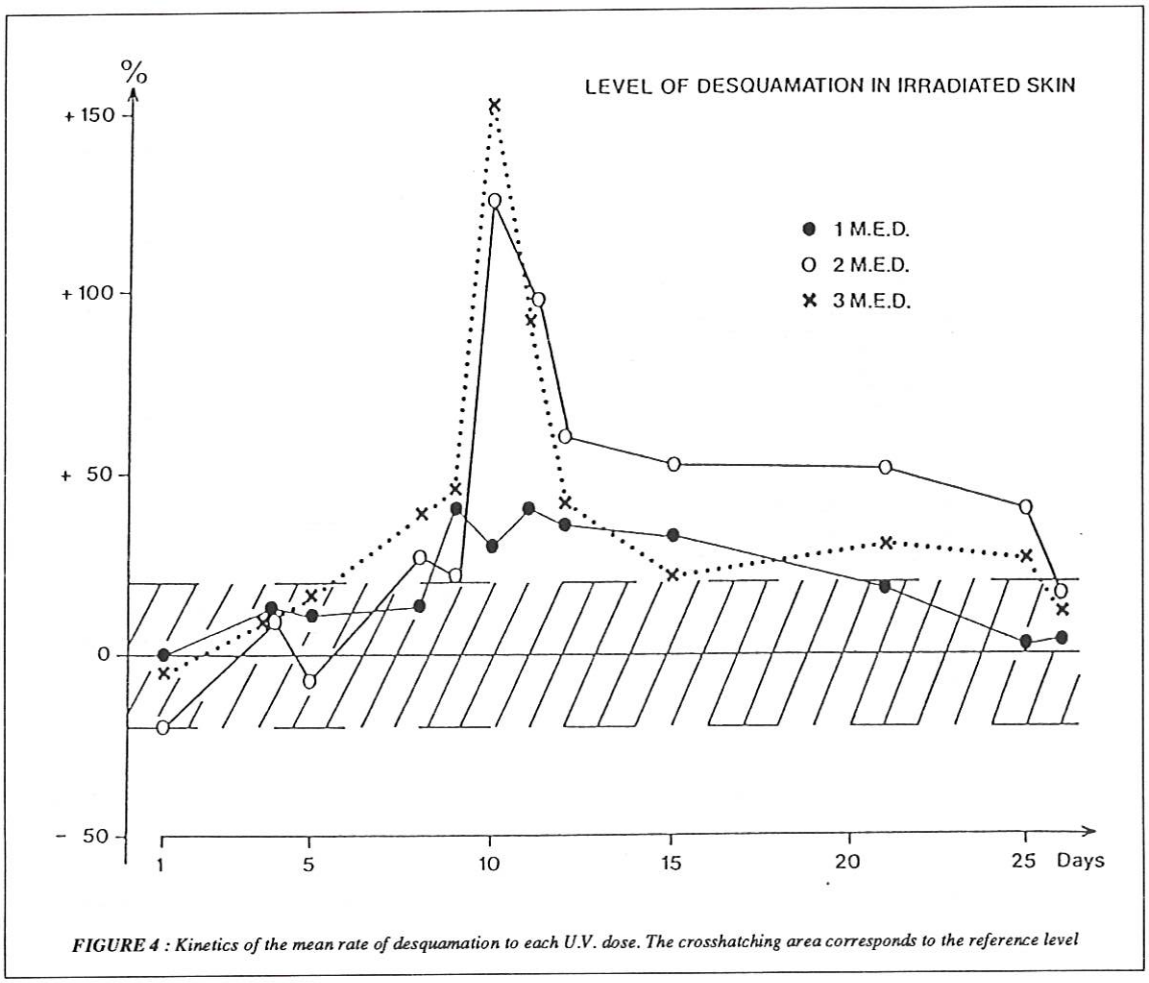


FIGURE 4 : Kinetics of the mean rate of desquamation to each U.V. dose. The crosshatching area corresponds to the reference level

that the day-ten peak had an amplitude related to the MED. All other results suggest that different responses occurred as a function of the depth of injury leading to several different scenario. The superficial damages caused by 1 MED involved only the resident stratum corneum. The response of the skin was a slight loss in cohesiveness of the horny layers and did not need any intervention of the epidermis. Indeed, it is currently well accepted (4) that a decrease in the size of corneocyte means an accelerated turn-over time of the epidermal cells. No change in corneocyte size was observed with 1 MED. The decrease in size recorded for 2 MED was in perfect agreement with the literature (5) which reported a transit time of about twenty days for the epidermis in healthy skin. This corresponds precisely to the minimum corneocyte area measured in this experiment. At day 20, the wool-turbine harvested most of the newly synthetized corneocytes. The possibility that the apparatus might extract a mixture of old and new corneocytes at different rates, may partly explain the surprising kinetics of corneocyte size observed for 3 MED. A further experiment, conducted under the same experimental conditions, gave similar kinetics and an equivalent amplitude.

This study shows that U.V. irradiation, delivered as a single dose, constitutes a powerful experimental model for the study of the chronology of various events occuring at the skin surface. These events closely overlapped others such as early erythema, oedema, temporary modifications of the skin microrelief recently reported (6), late tanning and so on. For a better understanding of these phenomena, comparative studies which include a variety of experimental approaches are required: repeated doses of U.V.A and/or U.V.B, natu-

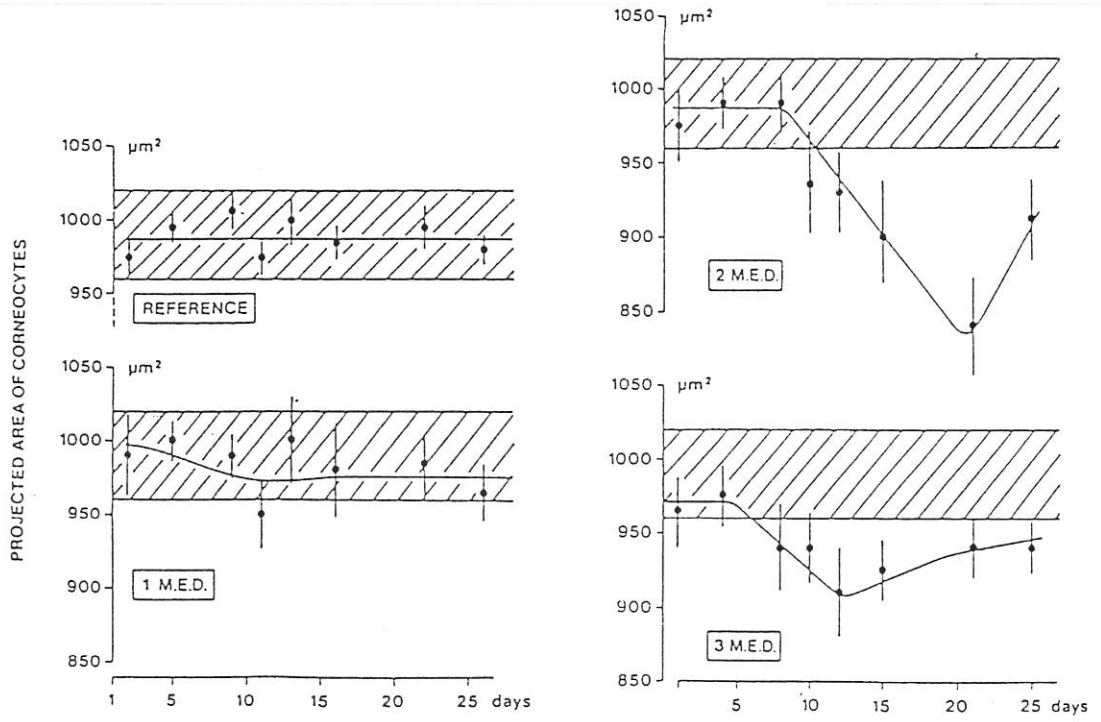


FIGURE 5 : Kinetics of the mean projected area of corneocytes ± S.E. Crosshatching areas correspond to non irradiated skin values

ral exposure to sunshine. In this last example, we reported (7) that consequences of a long period of exposure to sunshine in summer was a two fold increase in the spontaneous desquamation (+ 100%) and a decrease in the size of corneocytes from 960 μm^2 to 810 μm^2. These results were remarkably similar in amplitude to thos obtained in the present study. In natural conditions however, the difficulty consists in making an evaluation with respect other environmental factors such as temperature, humidity, wind, individual habits, bathing.....etc.....

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REPLICATIVE AND REPAIR DNA SYNTHESIS AFTER SOLAR DAMAGE

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Introduction

Several environmental influences may cause actual changes in the replicative or scheduled semiconservative DNA synthesis (SDS) in human skin (4). Even minor irritations, like a slight friction of skin, are followed by an acceleration of the S phase in the cell cycle and of the epidermal replicative cell turnover. Both changes have been confirmed using tritium thymidin autoradiography techniques (3).

Even simple occlusion of skin with plastic band-aids, induces the epidermal basal cells to react with a slower DNA synthesis and a long turnover time (3), whilst in case of sunlight erythema, the cells stop their DNA synthesis already during the irradiation period, hours before the erythema becomes visible. The signs of a phototoxic depression of both DNA synthesis duration as well as replicative cell turnover time, are followed within 24 hours by a longer period of enhanced activity of the scheduled DNA synthesis. Similar effects can be shown in the skin after other types of irradiation. For example, photochemotoxic reactions and soft X-ray reactions will induce for several days and 1 week respectively scheduled DNA synthesis depression, before the onset of rebound activity (3).

On the other hand, in the protected skin close to the irradiated areas, epidermal cells start with an enhanced replicative DNA synthesis immediately after the irradiation without any previous depression (2).

This sensitive response of the SDS system raises the question as to whether a continuous unscheduled DNA repair synthesis (UDS) in many cells of the epidermis could somehow influence the actual scheduled replicative DNA synthesis in the germinative compartment of the epidermis (6). This is of major interest when using combined replicative and repair models, because it seems to be impossible to separate typical repair labels in the basal cell layer from weak replicative labels by tritium thymidine autoradiography. Therefore, repair label counts could turn out unreliable, if the UDS itself was one of the many possible factors influencing the course and extent of the SDS.

Investigations

Immediately after a one minute (13) close up ultraviolet irradiation (with a high pressure Xenon quartz lamp) of 21 small skin areas (1 cm in diameter) on the upper back of 7 volunteers, skin biopsies (2 mm Ø) were taken from each test site, and incubated for 1/2 hr with tritium thymidine for subsequent autoradiography. The results were then compared with specimens from 9 non-irradiated skin sites.

No signs of DNA repair activity could be found in the latter, but 64 of 2000 counted basal cells, that is 2,46%, were labelled in the S phase, representing only one third of the total number seen in non-irradiated skin.

Seven of the 21 irradiated test areas were topically pretreated for 48 hrs under occlusive tape with an ointment (oil in water cream) containing 10% Repair complex CLR (an extract from inactivated bifidus bacteria cultures), in order to enhance the natural and individual repair capacity (10) of human skin. This effective method was first described in 1982 (7), but it is not yet generally known.

Whilst the UDS repair label of the pretreated skin areas was 40% enhanced, there was no evidence of a change in SDS index (19 out of 2260 counted basal cells were labelled, that is 0,84%), compared with 7 untreated and another 7 placebo treated specimens (31 out of 3800 counted basal cells were labelled=0,82%). It would appear that even a marked additional repair activity did not influence the UVR-depressed number of epidermal cells presenting themselves in the S phase of the cell cycle.

Comments

There are several possible artefacts and errors discussed in the literature concerning DNA repair. Among others, as to whether it necessary to establish a method of safe and active radiation protection (7,8). The use of hydroxyurea is not recommended in order to inhibit the replicative or semiconservative DNA synthesis in repair studies (7,8,9). It has been suggested by Vijg (16), that hydroxyurea migh influence the DNA repair. Another point of interest is the sodium butyrate effect on repair counts, as discussed by Williams and Friedberg (17). Sodium butyrate may decrease the intracellular thymidine pool, and in that way increase the radioactive thymidine uptake. We have therefore excluded the presence of butyrates in the Repair Complex CLR by

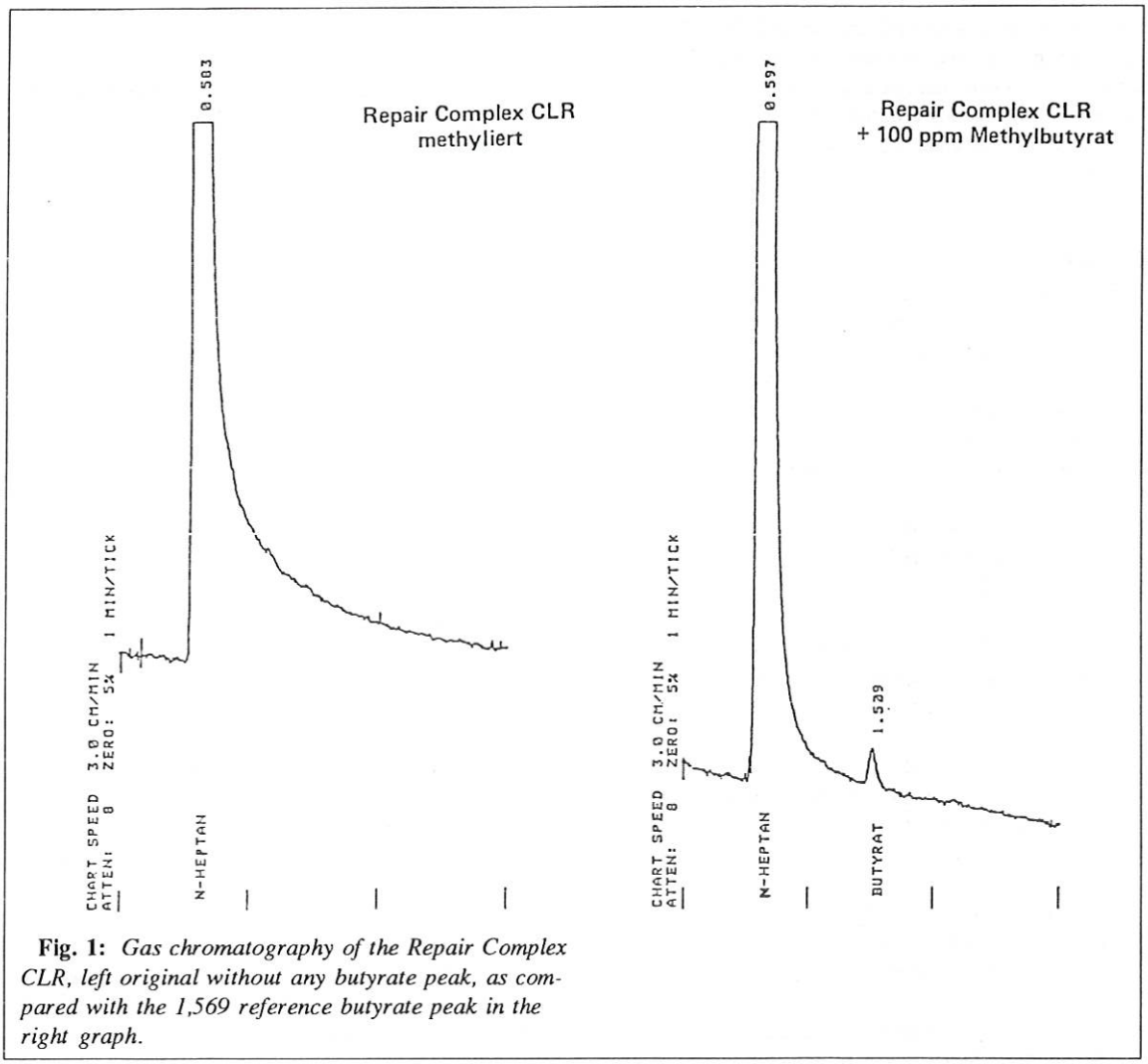


Fig. 1: *Gas chromatography of the Repair Complex CLR, left original without any butyrate peak, as compared with the 1,569 reference butyrate peak in the right graph.*

means of gas chromatography at a level of 0,01% (Fig. 1). In addition the bifidus bacteria used do not produce any butyrates in their metabolism (12,14).

In any topical treatment with drugs, the release of the drug from the vehicle and its penetration into the skin, are of major importance consequently. The efficacy of the Repair Complex CLR may vary with the use of different compounds: We have obtained our best results with the already mentioned oil in water emulsion as well as with a gel, both containing only stabilizing additives. We did not achieve better results with for example an aqueous solution, even in a higher than 10% concentration.

Analogous observations with the Repair Complex CLR have been noted (unpublished) with the completely different method of nucleoid sedimentation (15).

Summary and Conclusion

It is possible to enhance the individually maximal spontaneous extent of unscheduled DNA synthesis after ultraviolet light damage in human skin by 40% using topical pretreatment with bifidus fraction compounds (7,8,9). This new and biological method of active radiation protection (4,5,7), has been shown to be unaffected by any artificially produced findings due to hydroxyurea, butyrates of misintererpreted thymidine

labels from replicative SDS and repair UDS. The original experimental results could be reproduced also by others, even using completely different test methods. Obviously, the results are dependent on an appropriate topical compound allowing sufficient release and penetration.

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THE SIGNIFICANCE OF INTERFERON IN PHOTOIMMUNOLOGY

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UV radiation has many effects on human skin. UV radiation alone or in combination with photosensitizer like in PUVA therapy has gained increasing popularity in the therapy of several inflammatory skin disorders, in particular psoriasis. The mechanisms behind the beneficial effect of UV radiation are unknown, but could be related to an immuno-modulation. Recently, we found increased levels of interferon (IFN) both in serum and skin blister fluid from patients with psoriasis receiving Goeckerman therapy (UVB + coal tar)(Livden et al., in press). Since this could imply that IFN is a mediator in photoimmunology, we have also studied the effect of UV radiation on IFN serum levels in healthy volunteers.

MATERIALS AND METHODS

Sera from 22 healty individuals were examined before, during and after 20 treatments with UVB. Sera and suction blister fluids were examined from 35 patients with psoriasis vulgaris before Goeckerman therapy and from 22 patiens after Goeckerman therapy. Local treatment was interrupted at least one week before treatment. None of the patients received systemic treatment, and the patients received an average of 24 treatments.

A Waldman UV-1000 treatment cabin equipped with 26 Sylvania UV6 fluorescent sunlamps was used as UVB source, and the dose was gradually increased from 50 mJoule/cm^2 to maximun 1200 mJoule/cm^2, substaining a mild erythema.

Sera were collected from the healthy individuals before, after 10 and after 20 UVB treatments as well as 4 weeks after treatment. In the patients with psoriasis suction blisteres were produced both in lesional and unaffected skin using the method described by Kiistala (1968). Serum samples and suction blister fluid were collected at the same time.

IFN activity was tested by an infectivity inhibition micromethod employing human embryonic lung fibroblast cells and vesicular stomatitis virus (VSV) as the challenge virus. Twofold dilutions of the samples were added to the cell cultures in triplicate, incubated overnight at 37°C and challenged with 10 infectious units of VSV. After 3 days of incubation the virus caused marked cytopathogenic effect on the cells. Interferon treated cells were protected. The antiviral activity expressed in IFN units/ml was calculated as the resiprocal of the highest dilution preventing development of cytopathogenic effect in 50% of the cells at the time when control cells were completely destroyed. This concentration contains one unit of IFN in our assay. Only sera and blister fluids containing antiviral activity of 16 units/ml or higher were considered positive (Degre et al., 1976).

To characterize the antiviral activity sera and blister fluids were tested on heterologous cells and for heat sensitivity. Sera and blister fluids were also treated at pH2, tested with specific anti human IFN-α antiserum and tested on a bovine kidney cell line (MDBK) only sensitive to IFN-α. A reduction of the IFN activity in the pH2 treated sera and blister fluids of 50% or more was considered significant.

The results are given as median levels as variables were not normal distributed. The median levels were compared by the method of Wilcoxon and Mann-Witney. P values ≤ 0.05 were considered to be significant.

RESULTS

IFN was detected in 8 of 22 (36%) sera before treatment and in 17 of 22 (77%) sera after 20 treatments in the UVB treated group of healthy individuals. The increase in the IFN levels during treatment was statistically significant ($p<0.05$). The median IFN levels before, during and after treatment are given in Table 1. A statistically significant increase in the median IFN levels appeared during the 10 first treatments. During the last 10 treatments there was no statistically significant increase. Four weeks after treatment the median IFN levels was condsiderably reduced.

Table 1. Median IFN levels (units/ml) before, during and after UVB treatment of healthy individuals.

Before treatment	12.67
After 10 treatments	15.83
After 20 treatments	16.67
4 weeks after treatment	14.33

In patients with psoriasis IFN was detected in 27 of 35 (77%) of the blister fluids from lesional skin, in 7 of 21 (33%) of the blister fluids form unaffected skin and in 19 of 34 (56%) sera before UVB treatment (Table 2). The median IFN level in blister fluids from lesional skin was significantly higher than the median IFN level in unaffected skin ($p<0.05$), whereas there was no significant difference as compared with the corresponding sera ($p=0.24$) (Table 2). After Goeckerman therapy increased IFN levels were found both in blister fluids from lesional skin, unaffected skin, as well as in sera. IFN activity was found in 18 of 20 (90%) blister fluids from lesional skin, in 13 of 18 (72%) blister fluids from unaffected skin and in 20 of 22 (91%) sera. The median IFN levels in blister fluids from both unaffected skin and in sera were significantly higher in the patients receiving Goeckerman therapy than in untreated patients ($p=0.01$ and $p=0.0001$ respectively). There was no statisically significant difference in the median levels of IFN in blister fluids from lesional skin before and after Goeckerman therapy ($p=0.15$). Furthermore, after Goeckerman therapy the IFN levels in sera were significantly higher than the IFN levels in blister fluids from unaffected skin ($p=0.001$) and the IFN levels in blister fluids from affected skin ($p=0.05$) (Table 2).

The antiviral activity in blister fluids and sera was characterized as IFN by its species specificity, by its heat sensitivity and its lack of direct neutralization of the virus. Treatment at pH2 and IFN-αantiserum reduced the antiviral activity in 7 sera examined in the non psoriatics. Treatment at pH2 reduced the antiviral activity in 8 of 13 sera and in 7 of 10 blister fluids form patients with psoriasis. Treatment with the IFN-α antiserum reduced IFN activity in 4 of 10 sera and 6 of 11 blister fluids from patients with psoriasis. The results indicate the presence of both acid stable and acid labile IFN-αas well as IFN-γ (Table 3).

	Lesional skin	Unaffected skin	Serum
Before Goeckerman therapy			
Number examined	35	21	34
Number positive	27	7	19
Median IFN units/ml	35	10	25
After Goeckerman therapy			
Number examined	20	18	22
Number positive	18	13	20
Median IFN units/ml	50.5	26.5	89

Table 2. Number of patients and IFN levels given as median units/ml before and after Goeckerman therapy

Treatment	Samples	Number examined	Reduced	Unchanged	Increased
Healthy individuals:					
pH2	Sera	7	0	0	7
Anti-α IFN	Sera	7	0	0	7
Psoriasis:					
pH2	Sera	13	4	1	8
	Blister fluids	10	2	1	7
Anti-α IFN	Sera	10	6	0	4
	Blister fluids	11	5	0	6

Table 3. The effect of incubation of pH2 and of anti IFN-α antibody in IFN activity in sera from healthy individuals and sera and blisterfluids from patients with psoriasis.

DISCUSSION

The results showed that UVB increased the serum level of IFN in healthy individuals. In patients with psoriasis there was an even higher IFN increase after Goeckerman therapy. Since UVB radiation is the most effective part of the Goeckerman regimen (Belsito et al., 1982) the IFN increase is probably mainly due to UVB.

The results indicate the IFN is produced locally in psoriatic lesions. The data also indicate that this local production is stimulated by UVB therapy. UVB penetrates to the upper part of the dermis and might stimulate IFN production by affecting both epidermal and dermal cells. Recently, it has been demonstrated that epidermal cells have the capacity to produce IFN-α after viral infection (Schipper et al., 1984). In addition, some data indicating the IFN-γ production by epidermal cells were presented (Aubock et al., 1985): It is also possible that UVB radiation stimulates IFN production by T lymphocytes in mononuclear cell infitrates in the dermis of lesional skin or scanning normal skin. Further investigation is necessary to determine whether the UVB induced IFN production in psoriatics actually differs in quality or quantity from that in healthy individuals.

Until now, the described immunologic effects of UV light on human skin are suppressive affecting either Langerhans cells (Friedman, 1981) or suppressor T lymphocytes (Hersey et al., 1983). Our findings indicate a more complex effect of UV light.

Both IFN-γ as well as acid stable and acid labile IFN-α were detected before and after UVB treatment. IFN-γ has both immunostimulatory and suppressive effects, depending on the concentration. Acid labile IFN-α is a relatively recent discovered type of IFN, whose function is unclear. The presence of acid labile IFN-α mainly in connection with autoimmun disorders (Hooks et al., 1982) and AIDS (Eyster et al., 1983), has led to the assumption that it may suppress normal immune response and aggravate certain immune disorders (Ytterberg & Schnitzer, 1982).

IFN can enhance the expression of HLA-DR antigens and Fcγ-receptors (Trinchien & Perussia, 1985). Both HLA-DR antigen and Fcγ-receptors are important immuno regulatory molecules, and are found on different cell types, both in normal and inflamed skin (Bjerke et al., 1982, Bjerke & Matre, 1983). Production of IFN in the skin following a "physiological" stimulus like UV exposure or psoriatic inflamation is therefore of

importance in the skin's function as an organ of immunity. Thus, the role of IFN in photoimmunology and dermatology is an interesting area which merits further investigation.

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INFLUENCE OF TOPICALLY APPLIED ORGANIC SOLVENTS ON UVB-MINIMAL ERYTHEMA DOSE (MED)

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Our knowledge on the basic pharmacological mechanisms of UV-erythema is now becoming more clear.
In UVB-erythema formation Prostaglandin E_2, D_2 and I_2 should be of importance, among the lipoxygenase products 12-Hete but not Leukotriene B_4 are elevated in skin after UVE-irradiation [1]. The involvement of other mediators besides arachidonic acid products is very likely. Our question was, whether the formation of UVB-erythema is influenced by removable substances on skin surface.

MATERIAL AND METHODS:

We have studied the UVB-erythema development on upper back and inner forearms in groups of 50 healthy volunteers and in 19 psoriatics after cleaning of skin with different organic and non organic solvents. For this purpose we used a pattern with three rows of 10 holes of 1 cm^2 area each. Skin under outer rows was alternatively cleaned by one of the following solvents: diethylether, petrolether, 80% ethanol, water or aqueous tensids respectively. The middle row served as control. Cleaning was carried out 4 times with cotton either before or after irradiation. For UV-radiation an Osram-Ultravitalux lamp was used, which mainly emmits in the UVB and UVA-region. The average minimal erythema time in a distance of 50 cm is 5-6 minutes. The irradiation time of the 10 areas varied within 1 and 10 minutes. After 24 hours we determinded the minimal erythema dose as the first sharply edged area by direct observation under daylight conditions.

RESULTS

Pretreatment of upper back and inner forearms with diethylether causes a significant increase in MED compared to controls. Cleaning of skin just after irradiation has no any influence.
The differences are statistically significant. The average MED on inner forearms is significantly higher compared to those on upper back, a well known phenomenon, which also could be influenced by the surface lipid content, because upper back is unlike inner forearms a seborrhoic area.
Also with petrolether, a mixture of saturated chain hydrocarbons, the pretreatment of the areas on upper back and inner forearms just before irradiation leads to significant increase in MED, while cleaning after irradiation does not cause any increase. The same happens by use of 80% ethanol. In contradiction to this, the pretreatment of skin with water or aqueous tensids, has no any effect.
In further studies we tried to confirm this finding in psoriatic skin. In 19 psoriatics with mixed form of the disease (plaque type combined with exanthematic psoriasis) we found the same increase in MED after pretreatment with diethylether.

DISCUSSION

As well in normal as psoriatic skin the pretreatment with diethylether leads to a significant suppression in UVB-erythema development. The same happens in normal skin with petrolether and ethanol. There exist at least two possibilities in which way ether and ethanol pretreatment can influence the erythema development. Cleaning of skin could remove ether and ethanol soluble substances which are directly or indirectly involved in the mechanism of erythema formation. These substances could be lipids especially phospholipids, which are precursors of arachidonic acid metabolites. This mechanism should act very fast, because cleaning of skin just after irradiation does not show any effect. At present we try to confirm this hypothesis by analyses of the skin eluate and by further investigations on this matter. A second explanation is, that application of ether and ethanol before irradiation changes the optical properties of the horny layer in that way, that diminished eryhema development results. The first explanation is supported by studies of Tronnier et. al [3], who found that horny layer has not only a light protective function but probably maintains mediators, substrats of mediators or other influencing factors of erythema formation too. Stripping of horny layer does not result in a significant increase in UVB-sensitivity. In further studies Tronnier [4] could produce a small inflammation by application of UV irradiated horny layer material on epidermis with removed horny layer. Roshchupkin et. al [2] were able partly to suppress the UVB-erythema.

For practical purposes our observation could become useful in so far as it could open an other possibility to diminish the limiting erythema reaction in phototherapy. We are now doing some research work to prove the practical value of these findings in psoriatics.

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ABNORMAL LYMPHOCYTIC RESPONSE TO UV-RADIATION AND X-RAY IN MULTIPLE SKIN CANCER.

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We have had the opportunity to study a large population of patients with multiple skin cancers of basal cell and squamous cell types. The main aim of the studies has been to detect general cellular defects which could explain the development of multiple skin cancers.

In these studies we have used the lymphocyte as a readily available cell model to investigate presumed cellular abnormalities and predispositions and report our results using the following three methods: 1. UVC-induced DNA syntheses (="DNA repair synthesis"), 2. In vitro UVC-tolerance (= UVC-dose necessary for 50% reduction in phytohemagglutinin stimulateed lymphocyte proliferation), 3. X-ray-induced DNA strand break repair. These tests are well standarized and documented with regard to inter-and itraindividual varations (1,2,3).

In all three tests the blood samples were taken in the morning during the winter-season, to avoid diurnal or seasonal variations. The lymphocytes were isolated using the Ficoll-Paque gradient technique and washed. The cell number was determined by Coulter counting and the viability was controlled by the trypan blue ex-

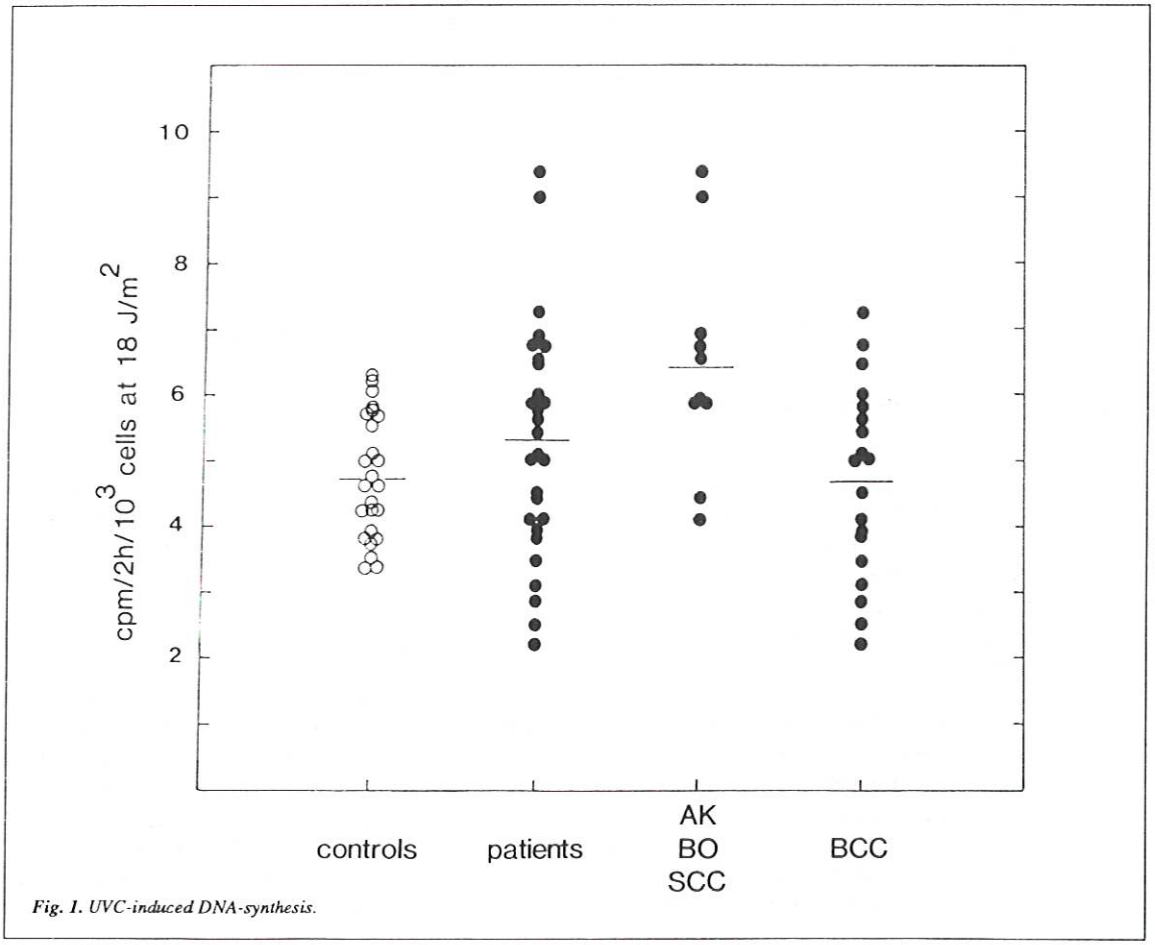


Fig. 1. UVC-induced DNA-synthesis.

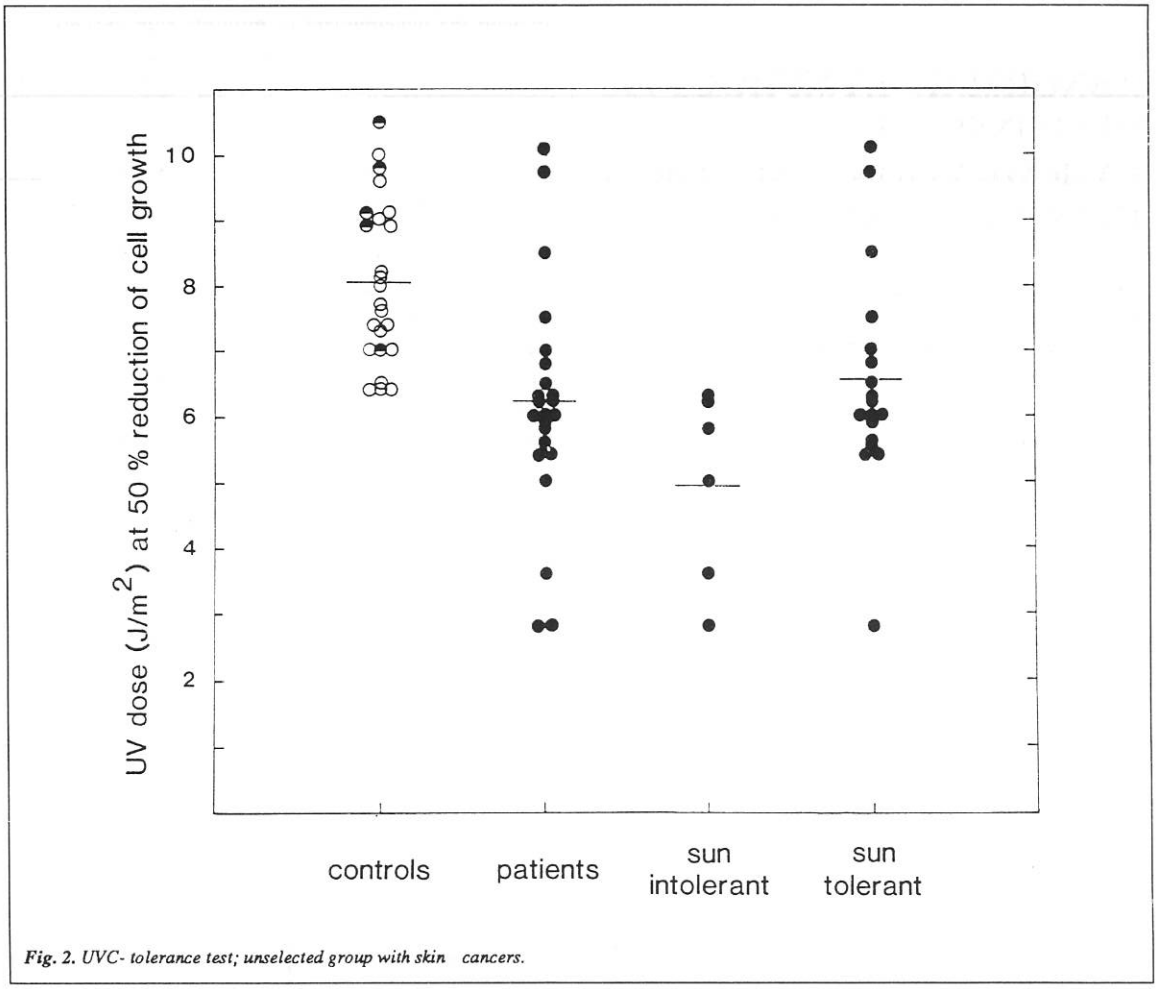


Fig. 2. UVC- tolerance test; unselected group with skin cancers.

clusion test.

In tests 1 and 2 the lymphocytes were irradiated with graded doses of UVC 254 nm. In test 1 we studied the amount of incorporated thymidine after 2 hours incubation in phosphate buffered saline. A low UVC-induced DNA synthesis suggests that these patients have a reduced ability to repair the chromosomal damage following irradiation.

In test 2 we measured the cells ability to proliferate 7 days after irradiation, stimulated by phytohemagglutinin. As growth and proliferation of cells are a far more complex process than the mechanisms involving thymidine incorporation measured in test 1, we regard the UVC-tolerance test as a more comprehensive assessment of the cells´ ability to function normally following irradiation. It also seems to be a more sensitive test.

In test 3, the x-ray induced strand break repair was measured by fluorometric analysis of DNA alkaline unwinding following radiation with 200 kV, 0,7 GY, 16 mA, and subsequent incubation.

One study involved 29 patient with multiple skin cancers (Fig. 1). This group was unselected with regard to carcinogenesis. Anamnestically known carcinogens differed, but included excessive sun exposure and UVB-treatment, tar, arsenic and x-ray treatment. A minority of the patient-group had psoriasis and there were 25 healthy controls. Lymphocytes from these subjects were only tested with regard to in vitro UVC-tolerance and UVC induced DNA synthesis.

Lymphocytes from patients with squamous cell tumours had a higher UVC-induced DNA synthesis com-

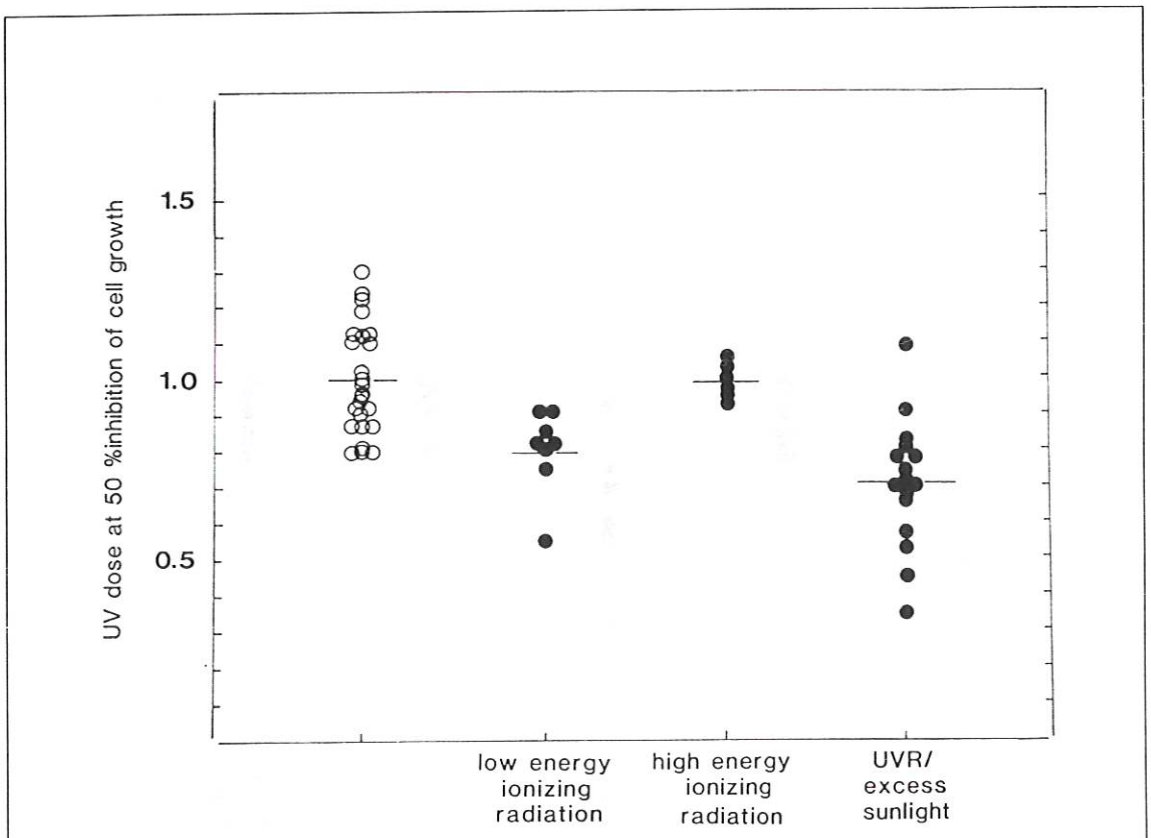


Fig. 3. UVC-tolerance test; selected group with multiple skin cancers (n=30).

pared to lymphocytes from patients with multiple basal cell carcinomas only (p=0.005). This would appear to imply that there has to be a more serious, or different cellular defect in order to induce an epidermal tumor with spinocellular morphology (Fig. 1). The mean value of the patients did not differ significantly from the controls, although there is a significantly larger scatter of the values. The results are illustrated in Figs. 1-4.

Fig.2 shows the results of the in vitro UVC tolerance test. There is a significantly lower tolerance in lymphocytes from patients with multiple carcinomas than in those from normal controls. The lowest lymphocytic UVC-intolerance occurred in patients with clinical sun intolerance (skin type 1 and 2)(p=0.05). We did not, however, find low in vitro UVC-tolerance in lymphocytes from normal persons of skin type 1 or 2, as illustrated by semi-circles.

Thirty patients with multiple carcinomas and with a clearly defined carcinogenesis were investigated in order to single out a specific factor which could be correlated to low in vitro UVC-tolerance. There were 24 age and sex matched controls. In this study we performed the x-ray induced strand break repair test on the first 22 patients in the group (Fig. 4).

In the patients who had received large doses of UVB treatment or excessive sunlight as the main known carcinogenic factor, we found as previously noted, a low in vitro UVC-tolerance (Fig. 3). We do not know if this is induced by the exposures alone or is due to preexisting factors. We hope, however, that this test will identify those patients who should not receive large dose of UVB treatment for psoriasis etc. The patients who developed cancers after low energy ionizing radiation ("Bucky" or "Grenzrays", 12-25 kV) had low in vitro UVC tolerance. These patients had additionally an increased lymphocyte-sensitivity to in vitro x-ray

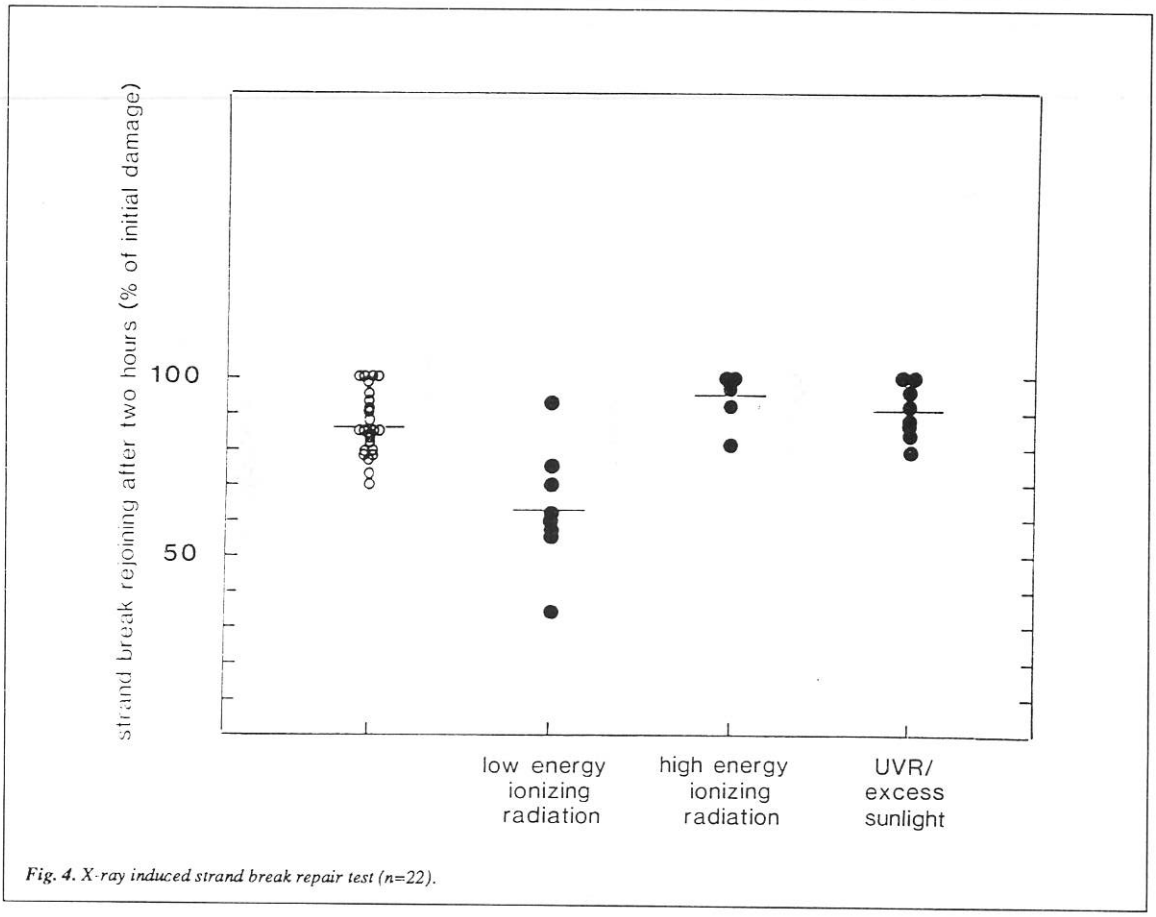


Fig. 4. X-ray induced strand break repair test (n=22).

(p= 0.001)(Fig. 4). Lymphocytes from patients who had developed cancers following exposure to high energy ionizing radiation showed no such sensitivity. This indicates a different biological effect of low and high energy ionizing radiation. Cellular cross-sensitivity to UVC-irradiation and x-ray has not previously been demonstrated.

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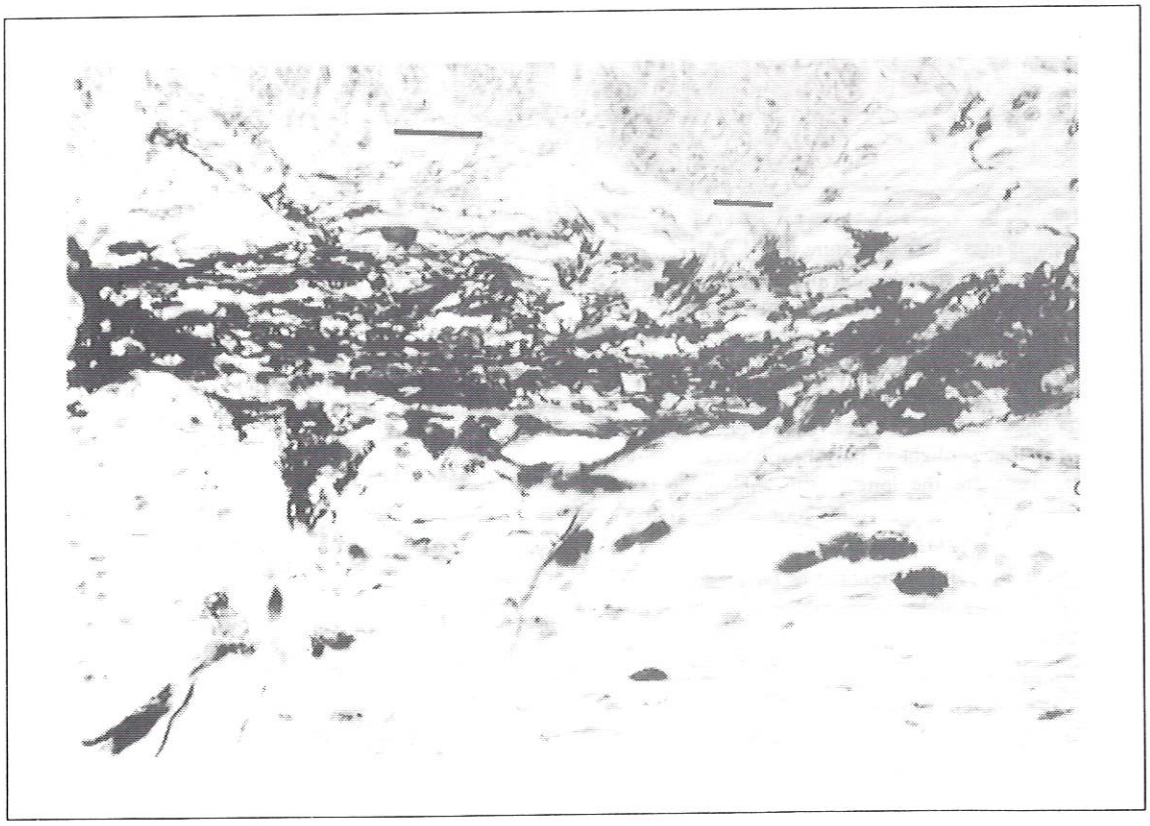
FULL SPECTRUM SOLAR RADIATION AS A CAUSE OF DERMAL PHOTODAMAGE: UVB TO INFRARED

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The gross and microscopic cutaneous changes that are a consequence of chronic solar radiation comprise the condition described as photoaging. The visible manifestations include a plethora of wrinkles, a yellowed, redundant, dry, leathery surface with a variety of benign, pre-malignant and malignant neoplasms. It is often believed that these striking changes in the exposed skin, notably that of the face and back of the hands, are merely an exaggeration of the characteristics of normal, instrinsic aging. However, evidence is accumulation that photoaging is not simply an acceleration of the inevitable age-dependant alterations. Photoaging is an entity into itself with features that are unique and distinct from those of normal aging (1). True enough, sunworshippers look prematurely aged, but this is the sole basis for the common misconception. The fact is, aged protected skin can be smooth and unblemished, showing mainly some thinning and loss of elasticity.
Underlying most of the visible aspects of photoaged skin is severe damage to dermal connective tissue. This includes massive quantities of thickened, tangled, degraded elastic fibers (2), which finally degenerate into

Fig. 1. UVB-induced elastosis: hairless mouse. Dense accretions of thickened elastotic fibers concentrated in the uppermost dermis. Dermal-epidermal junction (—). Luna's stain. (x370).



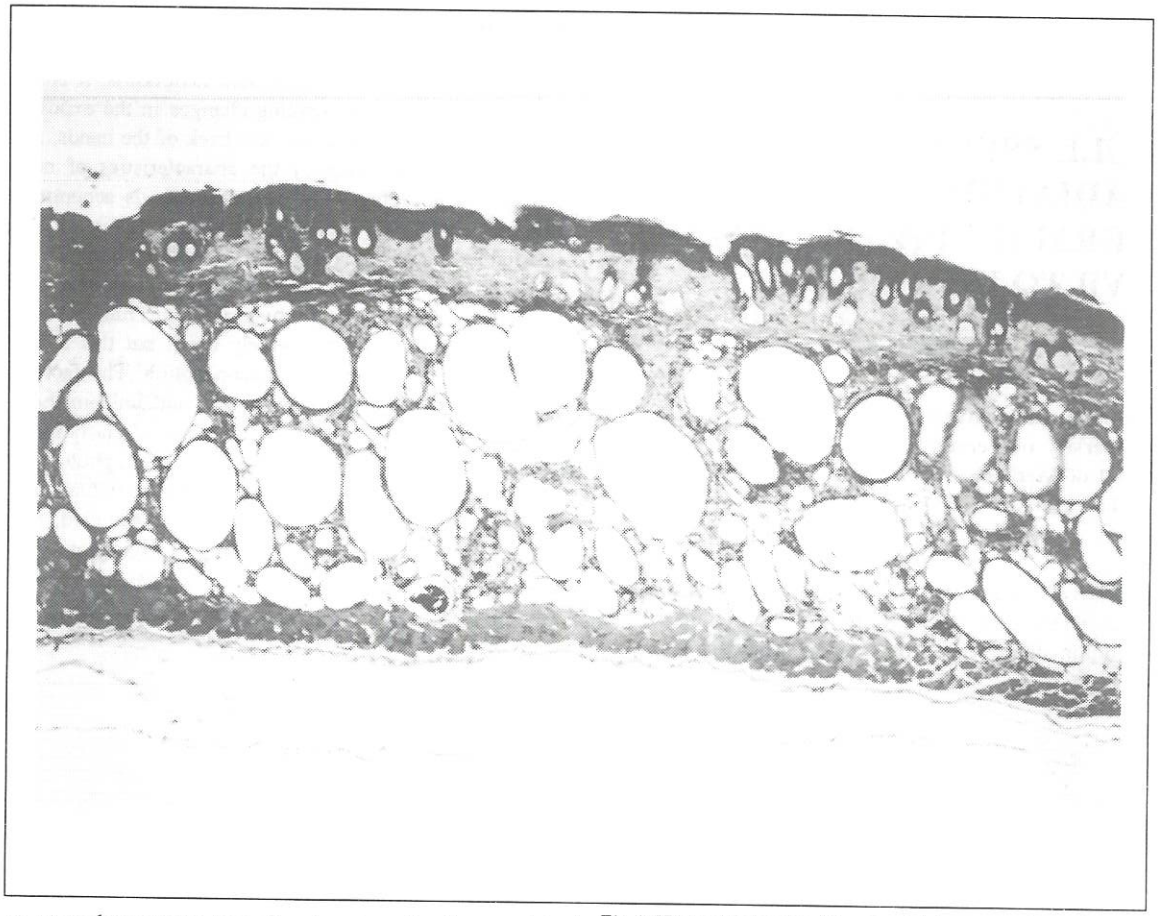


Fig. 2. UVB-induced cyst proliferation: hairless mouse. Rows of dermal cysts have increased from 1-2 in the unirradiated mouse to 4-5. H & E (x37).

an amorphous mass, loss of mature type I collagen with a compensatory overproduction of reticulin fibers (3), and a large increase in the proteoglycans and glycosaminoglycans comprising the ground substance (3). Past accounts have focused on UVB radiation (280-315nm) as that portion of terrestrial sunlight responsible for these dermal changes. However, it must be emphasized that sunlight is polychromatic. In addition to UVB, there are the longer wavelengths of UVA (315-400nm), visible light (400-700nm), infrared radiation (700-1x10^6nm) and ultimatately, radiowaves. The later can probably be ignored as far as skin is concerned, but visible light, seemingly innocuous cannot. Deleterious effects ranging from photoxic reactions (4) to tumor enchancement (5) have been reported. Although the effects of visible light on the dermis is unknown, recent work shows that concern must be extended to both UVA and infrared with regard to connective tissue photodamage.

Abbrevations:
MED; minimal erythema dose
J/cm2; Joules/cm2
SSR; solar simulation radiation
SPF; sun protection facter.

Action Spectrum of Photoaging

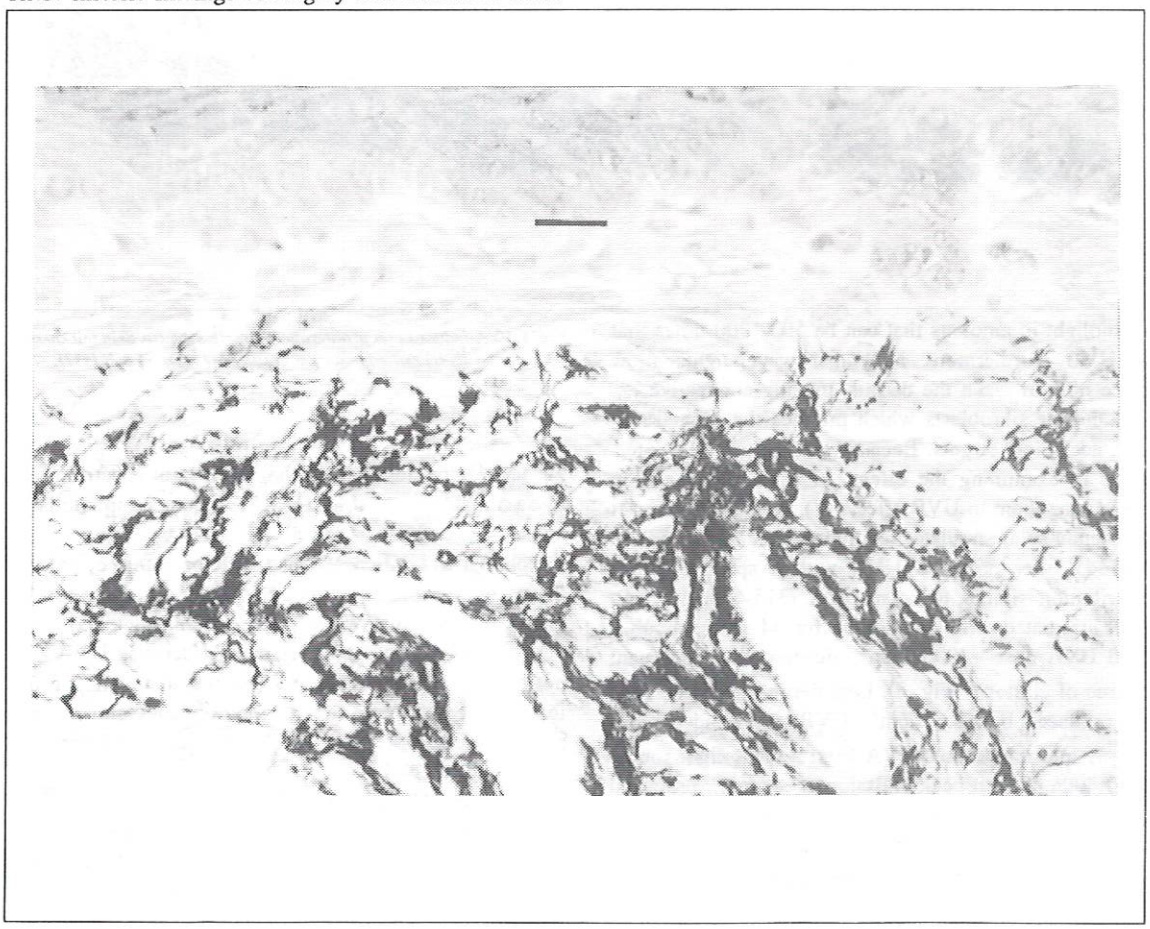
UVB Effects: Because of its clear implication in the prodution of erythema and in carcinogenesis, UVB was the obvious candidate as the causative agent in human dermal photodamage. Actual proof awaited the appropriate animal model. Early studies by Sams et al (6), using severely damaging doses of UVB and UVC (30-50 MED) in haired mice, showed a modest degree of elastic fiber hyperplasia. With the development of the hairless mouse, an animal extremely sensitive to UV

radiation, it has been possible to produce extensive connective tissue damage with far lower doses. Normally these animals have little elastic tissue, but severe elastosis (Fig. 1) was produced with UVB delivered as only 2 MED per exposure thrice weekly for ~30 weeks (7). The UV source was a bank of 8 Westinghous FS-20 "sunlamps" filtered with a cellulose triacetate film to attenuate UVC. In irradiated animals, fibroblasts became very numerous with ultrastructural features characteristic of high metabolic activity. The dermis thickened as a result of increased deposition of new collagen, while a decreased affinity for Van Gieson´s stain suggested severe damage to preexisting mature collagen. Reticulin fibers, normally limited to the basement membrane zones, were prominent throughout the upper dermis, indicating new collagen synthesis (8). Finally, the ground substance was greatly increased compared to unirradiated tissue.

These historic findings are highly reminiscent of those found in human photodamaged skin, making the hairless mouse a relevant model for photoaging. As in human photoaging, the general response to chronic UV radiation is one of hypertrophy, This was especially notable in the keratinizing cysts, typical of this animal, which lie in the lower half of the dermis. Normally present in 1 or 2 rows, after chronic UVB radiation, the cysts increased and occupied 4 or 5 rows (Fig. 2).

UVA Effect: Recent work has shown that acute exposure to UVA (315-400nm) can, like UVB, produce erythema (9) and damage to blood vessels (10). Since such effects required doses that were approximately 1,000 times greater than UVB, the danger from UVA was thought to be negligible. However, UVA is present

Fig. 3. UVA-induced elastosis: hairless mouse. Elastic fibers extend deeply into dermis. Dermal-epidermal junction (-). Luna´s stain. (x370).



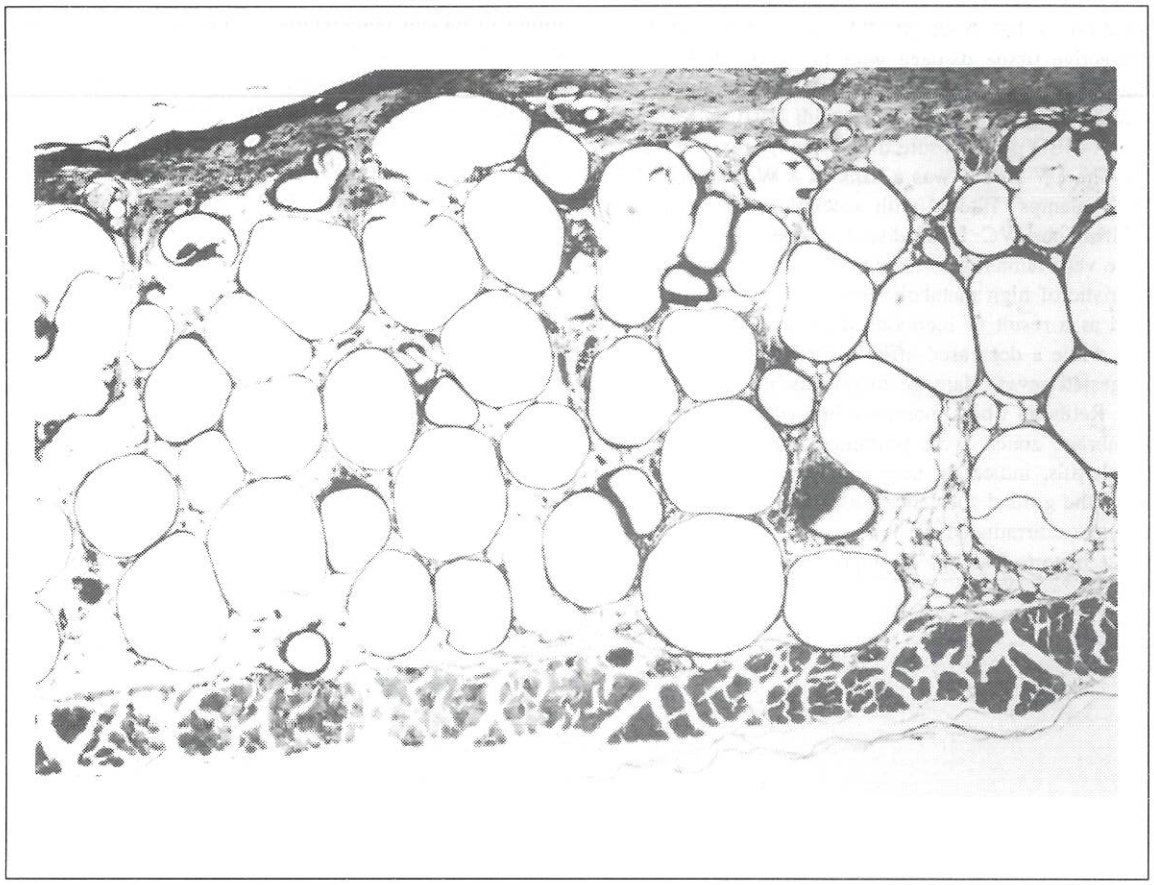


Fig. 4. UVA-induced cyst proliferation: hairless mouse. Skin thickness is vastly increased and cysts occupy 6-7 rows. H & E (x37).

in sunlight in amounts that can be 500-1000 times that of UVB. Furthermore, its longer wavelenghts allow more of it to reach the dermis, making connective tissue and the fibroblasts which produce the dermal matrix, accessible targets. Because of this, we were interested in examining the effects of UVA alone and in comparing them to UVB effects (7). A 5000W compact arc xenon solar simulator, equipped with a 2mm Schott WG345 filter provided UVA with a spectral power distribution similar to solar UVA (315-400nm). Animals irradiated with this source for 34 weeks, with a total UVA dose ~3000 J/cm^2, developed a significant degree of elastosis (Fig. 3). Less dense than the elastosis produced by ~5.6 J/cm^2 UVB; it extended more deeply into the dermis. UVA from a black light source (350-400nm) with peak emission at ~365nm, produced only mild elastic fiber hyperplasia despite a total dose of ~13,000 J/cm^2. This strongly suggests that the UVA wavelengths of 315-400nm, abundant in solar UVA, but lacking or deficient in the black light spectrum, are those most responsible for UVA-induced photodamage.

In contrast to UVB, UVA had little or no effect on collagen. A possible explanation for this divergent results lies in the fact that while UVB radiation evoked a severe inflammatory reaction in the dermis, UVA did not. There is conciderable evidence that cells of the inflammatory repertoire (i.e. macrophages and neutrophils) secrete proteolytic enzymes capabel of hydrolyzing collagen (11,12).

Like UVB, UVA radiation caused an increase in ground substance as visualized with Mowry´s stain. The distribution, however, was different. While UVB produced granular blue staining material localized in

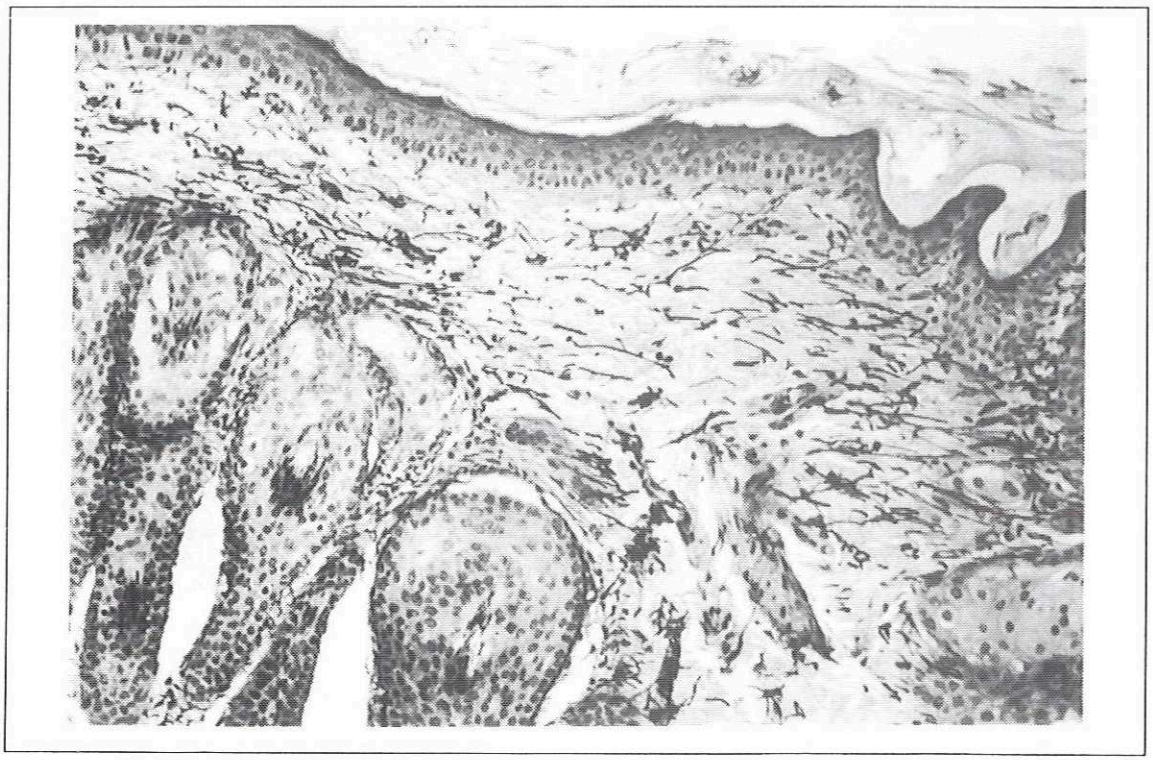
the uppermost dermis, UVA-induced ground substance was deposited throughout the dermis imparting to it a blue color. Additionally, densely stained material was deposited at the dermal-epidermal junction. One of the most striking results was proliferation of the keratinizing dermal cysts in response to solar simulating UVA. Attaining 6 to 7 rows (Fig. 4), it exceeded the proliferation induced by UVB.

These findings provide compelling evidence that UVA radiation, especially solar simulation UVA (315-400nm) is capable of inducing profound photodamage to dermal connective tissue. Furthermore, the dose used in these experiments was realistic in terms of human esposure. For example, a one hour exposure at midday in June, at 40°N latitude will provide ~10 J/cm^2 UVA. Within 4 years, summertime exposure alone could result in the accumulation of 3,000 J/cm^2. In the more northern latitudes, where summer days are longer, there are even greater amounts of UVA.

Solar Simulating Radiation Effects:

While the effects of UVA alone are interesting from a scientific point of view, UVA is inseparable form solar UVB. Thus, the cumulative effects of the entire UV spectrum are of utmost concern. These were studied by irradiation a group of animals with the 5000W xenon arc fitted with a 1 mm Schott WG320 filter, providing solar simulating radiation (SSR). The total UVB dose, delivered during the 34 week experiment, was ~5.6 J/cm^2 with a hundred-fold more solar simulating UVA. In some respects, the photodamage reflected the influence of both wavebands, while in others, it was like UVB alone. Elastosis was severe; more dense than that produced by UVA alone, it nevertheless extended deeply into the dermis. Collagen was damaged to an extent comparable to UVB alone. The increase in ground substance was equal to or slightly increased compared to UVB irradiated tissue but had the darkly stained material at the dermal-epidermal junction characteristic of UVA alone. Cyst proliferation was greater than with UVB alone again showing the additive effect of UVA.

Fig. 5. UVB- induced elastic fiber hyperplasia: guinea pig. A moderate thickening and increase in fiber content compared to unirradiated controls. Luna's stain (x110).



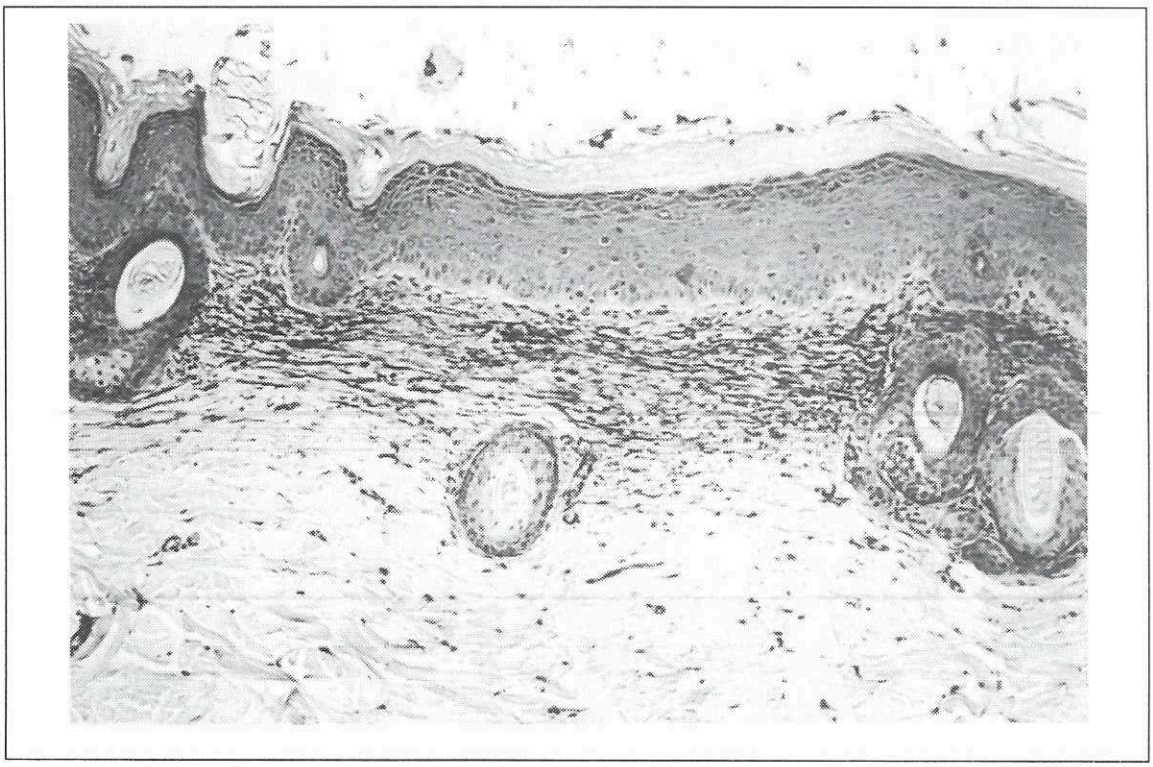
Infrared Radiation Effects:

Like UVA, infrared radiation (IR) is inseparable form sunlight. The harmful effect of heat is ancient knowledge (13). Human skin chronically exposed to IR radiation often develops the condition erythema ab igne, with its characteristic mottled pigmentation resulting from damaged, leaking blood vessels. Severe elastic fiber hyperplasia, extending deeply into the dermis is often present, along with thermal keratoses which are almost identical to those produced by UV radiation. Despite abundant clinical reporting on the effects of IR, little experimental work has been done. It was of interest to determine if heat, in the physiologic range (~40°C) could contribute to UV-induced photoaging. Because it was expected that many months of irradiation might be necessary, the guinea pig, a longer lived animal than the mouse, and one not prone to UV-tumorigenesis was chosen (14). Groups of albino animals, epilated weekly, were irradiated either with UVB or IR radiation, or the two wavebands combined for a total of 45 weeks. Animals irradiated with UVB alone showed a modest increase in the quantity and thickness of elastic fibers (Fig. 5). IR alone produced moderately dense skeins of fine parallel elastic fibers (Fig. 6). The combined irradiations resulted in the deposition of dense mats of elastosis consisting of both thickened and fine fibers (Fig. 7). Thus, combined with the epidemiologic and clinical observations, the experimental data strongly implicate IR radiation as a contributing factor in photoaging (13), adding to the cumulative insults of UVB and UVA.

Prevention of Dermal Photodamage with Broad Spectrum Sunscreens

In the hairless mouse model, broad spectrum sunscreens, with a sun protection factor (SPF) of 15, provided effective protection against SSR-induced connective tissue damage. Elastosis was prevented but elastic fibers were slightly increased in quantity. Collagen appeared undamaged, and although dermal cellularity was increased, massive inflammation did not occur. Ground substance was slightly, but not remarkably increased compared to unirradiated controls. In these experiments, sunscreen application began at the time of

Fig. 6. Infrared-induced elastosis: guinea pig. Deposits of fine parallel elastic fibers. Luna's stain (x70).



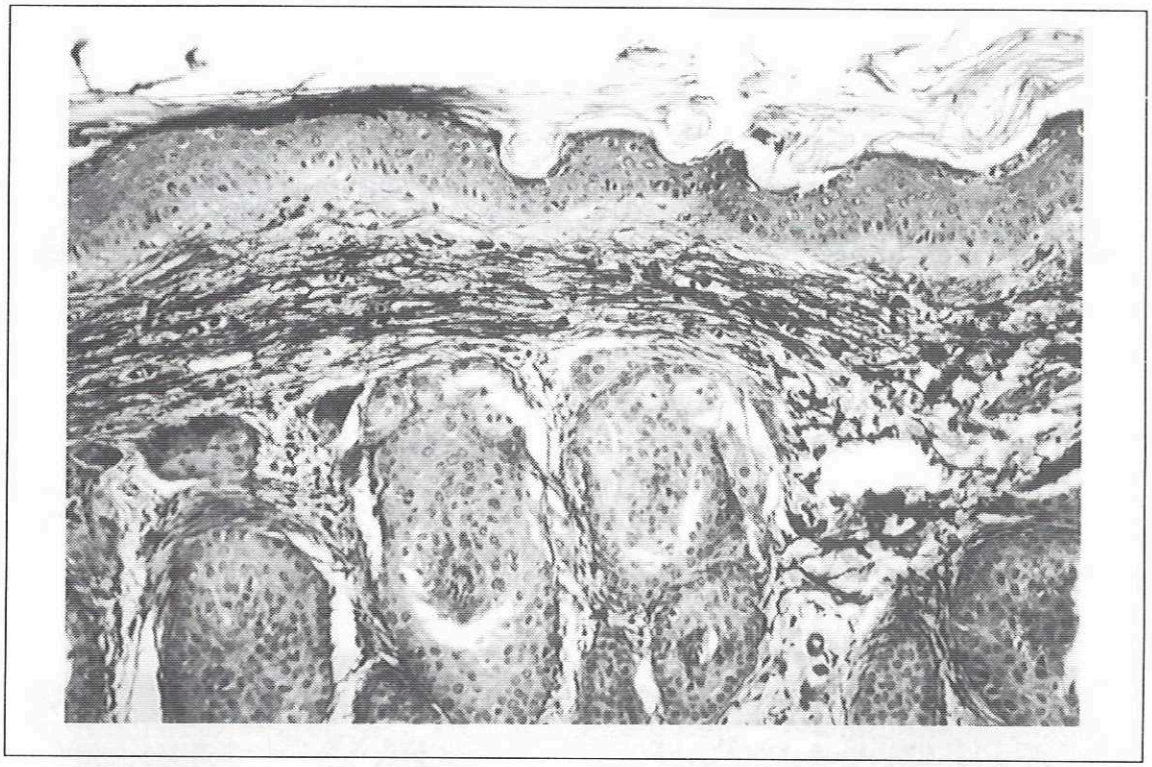


Fig. 7. Elastosis produced by UV + IR: guinea pig. A summation effect. Dense mats of elastosis comprised of thickened elastic fibers and fine parallel fibers. Luna's stain (x110).

the first exposure. This does not conform to human practice where protection is usually not adopted until some degree of photodamage is clinically visible (i.e., the first wrinkle). By that time, considerable connective tissue photodamage has been acquired. Previously, it was believed that this damage was irreversible. In our early studies with hairless mice (8) it was discovered that substantial repair could occur within a few months, even after extensive photodamage, if the UV exposures were stopped. Broad regions of new normal collagen were deposited in the sub-epidermal dermis, pushing downward, the old elastotic material (Fig. 8). It was of interest to determine if the skin could repair itself in a like manner under conditions that simulated human experience (15). Groups of animals were irradiated for a total of 30 weeks with UVB (~3 MED/exposure). Sunscreens of SPF 6 and 15 were applied to separate groups after 10 or 20 weeks of irradiation. Control animals irradiated without sunscreen protection for the full 30 weeks sustained, as expected, severe connective tissue damage which included extensive elastosis, loss of mature collagen and greatly increased ground substance. In the other hand, protected animals had only the degree of damage that reflected the amount of irradiation before sunscreens were applied. For example, mice that were protected after 10 weeks suffered only mild elastic fiber hyperplasia at the end of the 30 week irradiation period; those receiving sunscreens after 20 weeks developed a more severe elastic fiber hyperplasia. In these latter animals, collagen was also severely damaged during the first 20 weeks. However, during the final 10 weeks of irradiation, with sunscreen protection, a repair zone of new normal sub-epidemal collagen was deposited. These experiments demonstrated that further damage could be prevented and repair could occur, even in the face of continuing radiation, once sunscreens were applied. In general, earlier application and higher SPF provided the greatest degree of protection against photoaging.

Concluding Comments

It should be noted that currently available broad spectum sunscreens are highly effective against the acute effects of sun exposure, namely sunburn. However, ab-



Fig. 8. Repair zone: A region of new collagen deposition sub-epidermally. Compressed elastosis, formerly in the uppermost dermis forms the lower boundary (→). Mast cells (*) and fine new elastic fibers are present in the new dermis. Luna's stain (x370).

sorption is effective only up to ~330 nm, providing little protection against a substantial portion of UVA and none against IR radiation. Clearly, new molecules are needed to extend the filtering range. The findings that photodamage is irreversible to a significant degree make it all the more desirable for protection to be as broad as possible. It appears that destruction and repair go on simultaneously under continued assault by actinic radiation. The balance is shifted toward repair when the radiation stress is relieved. Although early protection from sunlight, before severe photodamage occurs, is most desirable, it is deemed advisable to council even older persons with photoaged skin to adopt protective measures, thereby allowing repair processes to occur.

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MOLECULAR ASPECTS OF ALLERGIC CONTACT DERMATITIS

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CHEMICAL STRUCTURE OF CONTACT SENSITIZERS

A) Haptens with electrophilic properties

The majority of skin sensitizers have electrophilic properties, i.e. they are able to accept electron doublets from nucleophiles such as amino(NH_2) and thiol (SH) groups, present in cutaneous proteins. The bond thus formed is a strong covalent bond and the modified, initially "self" protein is now perceived as an intruder, an antigen, by the organism.

Among natural skin allergens with these electrophilic properties let us quote: sesquiterpene lactones (such as frullanolide, the agent responsible of allegic contact dermatitis or ACD in lumberjacks), quinones (such as deoxylapachol, the main allergen of teak, another occupational sensitizer), α-methylene γ-butyrolactones (such as tulipalin A from tulip bulbs), aldehydes (such as citral, used in the perfume industry). Other ocupational skin sensitizers include acrylates (in the printing industry), metal allergens such as Ni salts, bichromates, etc...

B) Prohaptens

Not all skin sensitizers have electrophilic properies. For many of them, an in vivo transformation is required before they become electrophilic and then truely haptenic. For example, paraphenylenediamine (PPD) can be transformed in benzoquinone, as hydroquinone or azo dyes. Ethylenediamine is probably transformed into glyoxal, an excellent electrophile.

C) Other haptens

Some compounds need the presence of light to be true haptens. They are photosensitizers. In the presence of light, they are transformed into one-electron species called radicals. These can couple to appropriate functional groups of proteins: again the complete allergen (i.e. the modified protein) is formed, causing ACD. Examples include tetrachlorosalicylanilide (TCSA), tribromosalicylanilide, etc...

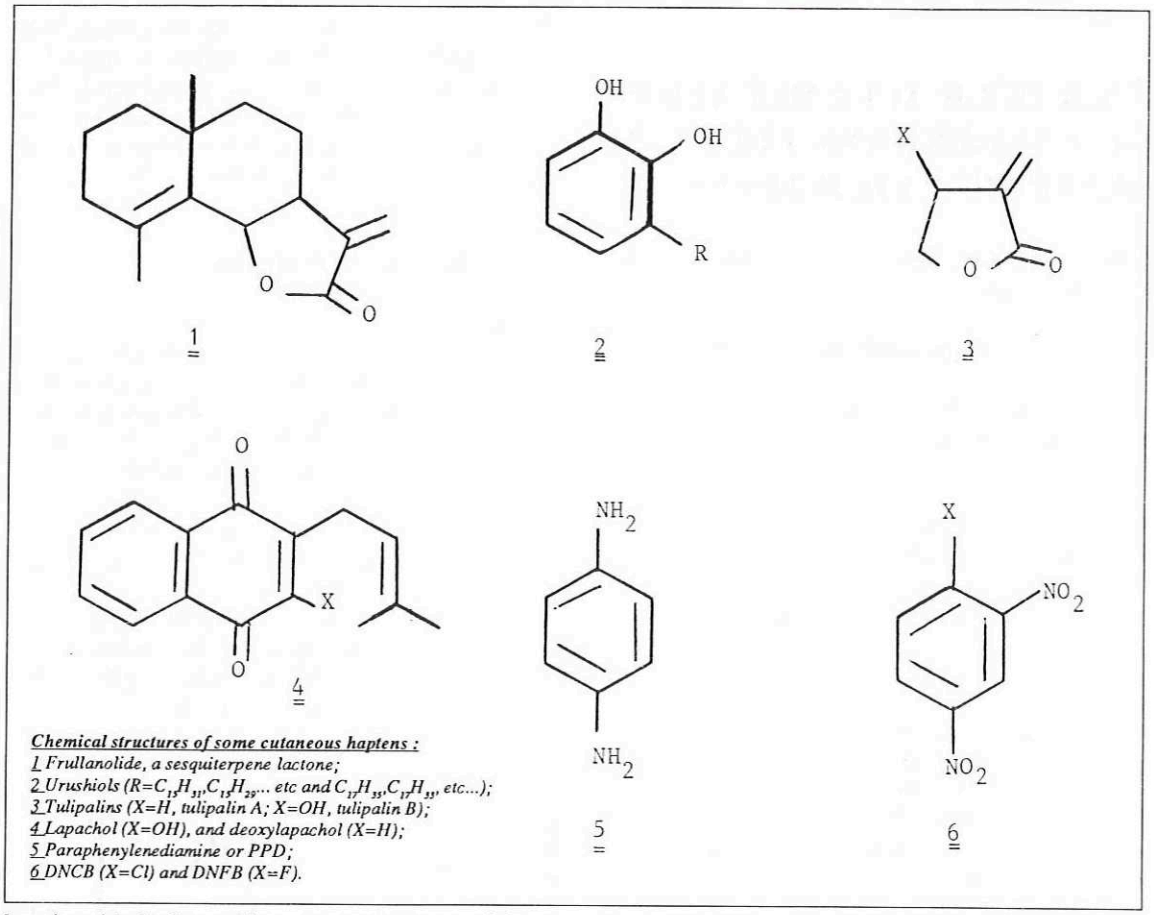
Another complete different group of substances are excellent skin sensitizers: pyrocatechols with long hydrocarbon chain (such as in poison ivy and poison oak), long hydrocarbon chain phenols (ginkgolic acid, cashew nut deratives, etc...), aliphatic long chain alcohols (such as in lanolin) etc... The mecanisms seems different: probably inclusion (or "anchoring") into cell membranes, forming hydrophobous bonds (less strong than covalent bonds but still strong).

CROSS-SENSITIZATION

Until recently, it was thought that sharing the same functional groups was sufficient to observe cross-reactions. It seems that this is not quite true. At the molcular level, cross-sensitivity can be rationalized if:

- the same hapten metabolite is formed (thus PPD and p-aminoazobenzene both can give benzoquinone)
- similar hapten metabolite is formed (PPD gives benzoquinone, p-toluenesiamine or PTD gives methylbenzoquinone)
- the same hapten-protein complex is formed: DNFB (2,4-dinitrofluorobenzene) and DNCB (2,4-dinitrochlorobenzene) both give dinitrophenylated or DNP-proteins
- structures are similar: cross-reaction between Frullania and Laurel.

However very recently several results, especially in Strasbourg, have shown that ACD was enantiospecific i.e. only one enantiomer is recognized. Thus (+)-Frullanolide sensitized guinea pigs did not respond to its mirror-image or enantiomer, -(-)- frullanolide. Both enantiomers exist in nature, in two different species of the liverwort Frullania. Other examples of enantiospecific ACD include usnic acid (in d-usnic acid sensitized lumberjacks, only the d-form is active but in licken pickers collecting species containing both d- and



Chemical structures of some cutaneous haptens :
1 Frullanolide, a sesquiterpene lactone;
2 Urushiols (R=$C_{15}H_{31}$,$C_{15}H_{29}$... etc and $C_{17}H_{33}$,$C_{17}H_{31}$, etc...);
3 Tulipalins (X=H, tulipalin A; X=OH, tulipalin B);
4 Lapachol (X=OH), and deoxylapachol (X=H);
5 Paraphenylenediamine or PPD;
6 DNCB (X=Cl) and DNFB (X=F).

l-usnic acids, both enantiomers react as expected forms a polysensiitization) and some dalbergiones (for review see Benezra). Tulip bulbs contain two glucosides, tuliposides A and B which can yield, through enzymatic or acidic hydrolysis, tulipalines A and B respectively. Only tulipalins B can exist in two enantiomeric forms of which, only one, (-)-tulipalin B is natural. We have syntesized both enantiomers and sensitized two groups of guinea pigs to each of them: no cross-reaction was observed, proving ACD was indeed enantiospecific (Papageorgiou and Benezra, unpublished results).

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HAIR BULB ACCUMULATION OF LANGERHANS CELLS IN ALLERGIC PATCH TESTS.

Observations with 2,3-epoxypropyl trimethyl ammonium cloride.

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Key words: *Occupational skin diseases - allergic contact dermatitis - glycidyl trimethyl ammonium chloride - epoxy resin - immunohistochemistry - electron microscopy - Langerhans cell - hair follicle*

SUMMARY

The occurrence of ocupational allergic contact dermatitis due to 2,3-epoxypropyl trimethyl ammonium chloride (EPTMAC) is reported and supplemented with immunohistochemical and electron microscopic observations. Four young workers developed hand dermatitis at a factory in which modified, cationic starch is manufactured. EPTMAC, a quaternary ammonium compound used as a cationizing chemical in the process, produced allergic reactions in all four patients in epicutaneous testing. The patients had only been in contact wiht EPTMAC for a short time (one to three months) before developing allergic eczema, which indicates that EPTMAC is a strong sensitizer. Immunohistochemistry showed that dendritic OKT6+ cells (Langerhans cells) increase in the hair follicles and the peribulbar infiltrate during the allergic patch test indicating that hair follicles might actively be involved in delayed type allergic reactions, possibly as a shunt way for allergens. Using electron microscopy, mitotic immunocompetent cells were found in the epidermis during the allergic patch test.

1 INTRODUCTION

We have recently reported allergic contact dermatitis to 2,3-epoxypropyl trimethyl ammonium chloride (EPTMAC)(1). In the present paper the clinical findings are briefly reviewed, and emphasis is directed to morphological observations of the allergic patch tests. New observations of the kinetics of immunocompetent cells in hair follicles in allergic patch test as well as the presence of mitotic immunocompetent cells in the epidermis are reported.

2 MATERIAL AND METHODS

2.1 Report of cases

In a factory where cationic starch is manufactured, four of a total eight workers developed dermatitis a short time after commencing their work. The dermatitis was suspected to be due to EPTMAC (Fig. 1). None of the patients had had atopic dermatitis or other atopic symptoms. An analysis of our cases is seen in table 1; three out of four patients were process men and the fourth was a female laboratory technician. The clinical details have been published elsewhere (1).

Epicutaneous tests included the ICDRG (International Contact Dermatitis Research Group) standard series (Epikon Oy, Helsinki, Finland), a series of plastics and glues (Epikon Oy), glove material as such, and

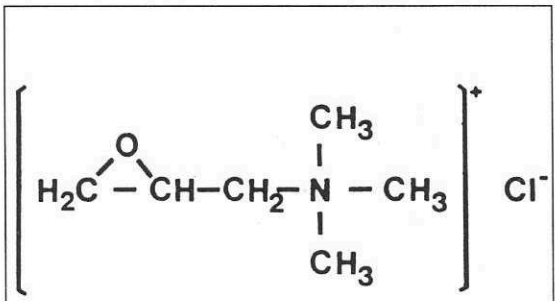


Fig. 1 *Structure of 2,3-epoxypropyl trimethyl ammonium chloride (EPTMAC).*

EPTMAC cationizing chemical 0.05 - 1% (w/w) in petrolatum. In one case the end product, cationic starch, was also tested as such. The patch tests were made on the patients´ back using the Finn Chamber method with a 24 h application time. The tests were read on removal, as well as 24, 48 and 96 h after removal.

The results of the patch test with EPTMAC cationizing chemical calculated as concentrations of EPTMAC in pet. are seen in table 1. Tests with 0.2% (w/w in pet.) and with a dilution series of the compound gave posi-

tive allergic reactions in all four patients. One of them even reacted to 0.05% (w/w) concentration of the compound. All control tests were negative. They were performed on 20 persons with 0.2% (w/w) concentration of the compound.

2.2 Immunohistochemistry and electron microscopy

Biopsies for immunohistochemistry and electron microscopy were taken in two cases from uninvolved skin and at 24, 48 and 96 h from the positive 0.2% and 0.05% patch tests. Half of the 4 mm punch biopsies were immediately frozen in liquid nitrogen and the other half fixed in phosphate-buffered glutaraldehyde with postosmification as previously described (5). The immunocompetent cells were characterized with the following antibodies (Ortho Pharmaceutical Corporation): OKT11 (pan-T-lymphocyte), OKT4 (helper/inducer T-lymphocyte), OKT8 (suppressor/cytotoxic T-lymphocyte), OKT6 and OKIal (Langerhans cell). The avidin-bitin-peroxidase method was used as earlier described (2,3). A semiquantitative estimation (2) of the immunocompetent cells was performed.

Table 1. *Analysis of 4 patients*

Case	1	2	3	4
Age (years)	19	23	20	23
Gender (m=man; f=female)	m	m	f	m
Occupation	process man	process man	lab. techn.	process man
Exposure before onset of symptoms (months)	3	3	1.5	1
Location of eczema	hands, feet	hands, arms, face	hands	hands, arms, face
Use of protective gloves with cationizing chemical	daily	daily	occas.	never
Patch test results				
EPTMAC 1 % in pet.	++	+++	+++	nt
" 0.5 % in pet.	nt	nt	nt	++
" 0.2 % in pet.	++	+++	+++	++
" 0.1 % in pet.	nt	nt	nt	++
" 0.05 % in pet.	nt	+	nt	nt
Standard series	-	-	ni (++) Co (++) PPD(++)	-
Series of plastics and glues	-	-	-	-

nt = not tested

3 RESULTS

3.1 Immunohistochemistry

The immunohistochemical study of the allergic patch tests showed that most inflammatory cells were OKT11+. The majority were OKT4+ and the minority OKT8+, the ratio being 2:1. The ratio was the same in stronger and weaker patch tests and did not change during the observation period. The number of OKT6+ cells remained constant in the epidermis, while OKIal positive dendritic cells decreased in the epidermis, suggesting that part of the Langerhans cells (LCs) lost their Ia surface antigen during the allergic patch test. OKT6+ cells increased in the dermis during the study. Special attention was paid to the kinetics of immunocompetent cells in the hair follicles.

In the control samples, a moderate amount of OKT6+ cells were observed in the infundibular area of the hair follicles, their amount decreasing below the level of the sebaceous glands. Below the bulge (attachment of the arretor pili muscles), OKT6+ cells were missing. During the allergic patch test small to moderate amounts of OKT6+ cells were observed below the bulge and in the peribulbar infiltrate (Fig. 2A). The number of OKIal+ cells decreased during the course of the patch test in the follicular epithelium (Fig. 2B). Most inflammatory cells in the dermis were OKIal+.

3.2 Electron microscopy

A distinct eczematous reaction was observed in the epidermis consisting of widened intercellular spaces containing a fine granular substance, a moderate number of exocytic small lymphocytes, and some macrophages. Gaps in the basal lamina and basal cell herniation (4) was observed. Primaty attention was paid to the epidermis and Langerhans cells (LCs). A normal number of LCs was observed. They looked active with a large cytoplasm, many Golgi areas, coated vesicles, widened rough endoplasmic reticulum containing fine granular sub-

Fig. 2 24 h allergic patch test to 0.05 % EPTMAC.

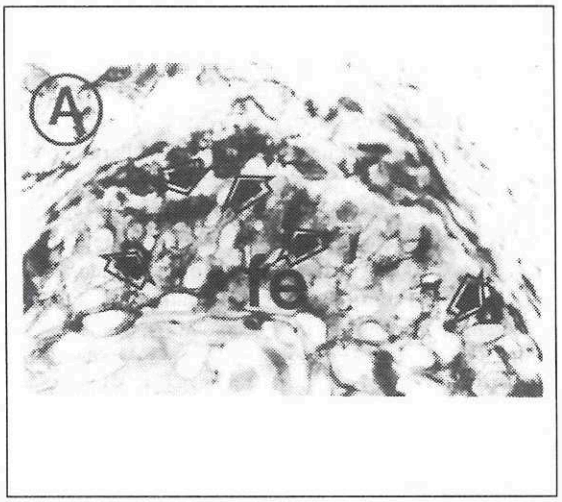


Fig. 2A *OKT6+ cells (arrow) in the follicular epithelium (fe).*

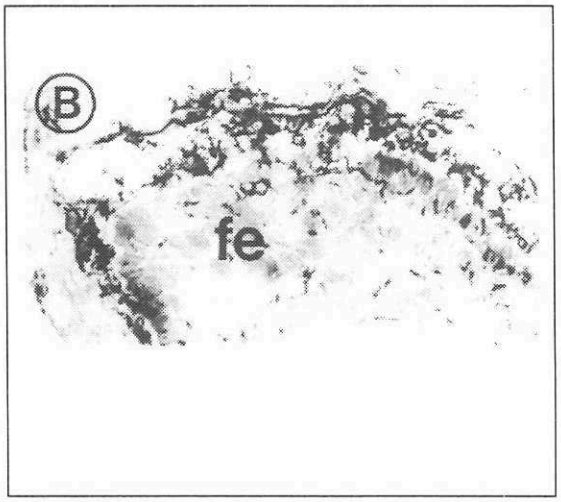


Fig. 2B *OKIa1+ cell around the follicular epithelium (fe). Note the different localization of OKT6+ and OKIa1+ cells.450.*

stance, many mitochondria and lysosomes (Fig. 3A). Necrotic LCs were not observed. Interestingly, we found one intraepidermal macrophage (24 h patch test) and one indeterminate cell (96 h patch test) in mitosis (Fig. 3B). In the 96 h patch test, some of the keratinocytes showed several vacuoles and some LCs had a light cytoplasm, possibly indicating swelling, but containing the organelles needed for active protein synthesis as above. LC-mononuclear cell contacts (2,5) were not observed in the present study.

4 DISCUSSION

EPTMAC is a low molecular weight substance which has a skin irritating effect like other quaternary ammonium compounds. Thus an irritant patch test could be misinterpreted as being allergic. In our cases the allergic nature of positive patch test reactions to EPTMAC was proved by a dilution series and negative controls. No allergy to standard epoxy resin or to epichlorohydrine was shown.

The immunohistochemical and electron microscopic findings corresponded to our previous observations of allergic patch tests (2,4,5,6), indicating that the reaction pattern in allergic contact dermatitis is idential for various allergens (7). LC-mononuclear cell contacts were not observed. This might have been due to the relatively low number of mononuclear exocytic cells in the epidermis in our samples. LCs are considered the antigen presenting cells in the epidermis (8), but there is no general agreement on their kinetics in the epidermis during allergic patch tests. Both a decrease (9), and an increase (10) has been reported (see 7). Both our previous studies and this study indicate that LCs persist in the epidermis during allergic patch tests, although they partially loose their Ia-surface staining (11). Since LCs persist in the epidermis and increase in the dermis, it is obvious that LCs present in the dermis during later phases of allergic patch tests are mainly derived from lymph nodes and/or blood.

The bulbar accumulation of dendritic OKT6+ cells has also been reported in alopecia areata (12) and it might reflect a general epidermal inflammation pattern analogous to the OKT4+ cell dominance in dermal infiltrates of most inflammatory dermatoses, but it could also indicate that hair follicles might actively be involved in delayed type allergic reactions, possibly as a shunt pathway for allergens. The mitotic immunocompetent cells in the epidermis are of further interest. A mitotic LC in the epidermis in an allergic patch test has been reported once before (5).

In conclusion, 2,3-epoxypropyl trimethyl ammonium chloride is obviously a strong human sensitizer. Immu-

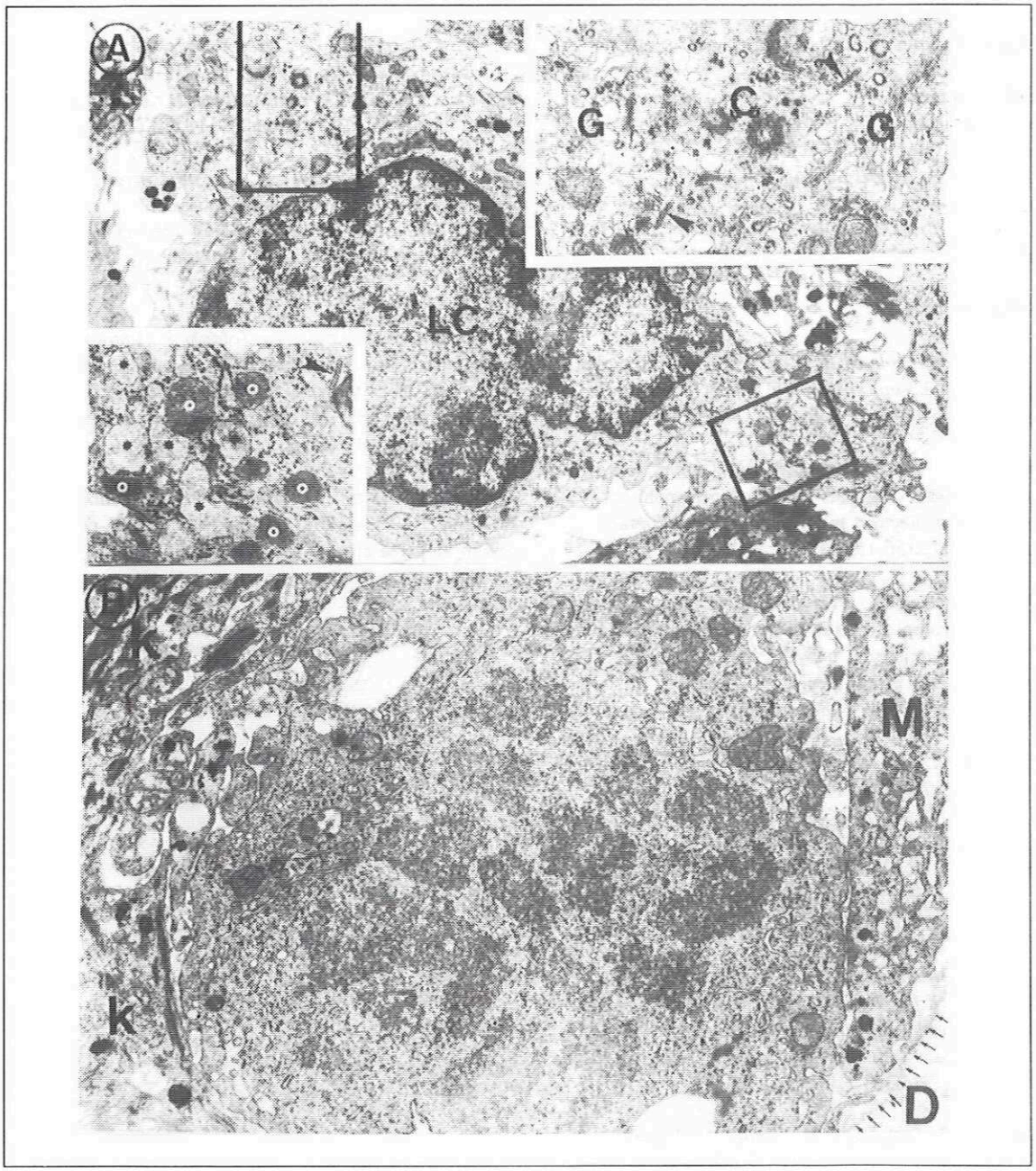


Fig. 3A *24 h allergic patch test to 0.2% EPTMAC. Active looking Langerhans cell (LC) with abundant cytoplasm. Details of the cytoplasm are seen in insets. x 10 000. Inset, left. Widened sacs of rough endoplasmic reticulum containing fine granular substance (*). o = lysosomes; arrowhead = LC-granule. x 25 000. Inset, right. Centriole (C); well developed Golgi apparatus (G) and LC granules (arrowhead) are observed. x 25 000.*

Fig. 3B *96 h allergic patch test to 0.2% EPTMAC. Mitotic immunocompetent cell is observed in epidermis. D = upper dermis; arrows point to basal lamina, M = melanocyte, k = keratinocyte. x 13 200.*

nohistochemistry and electron microscopy showed the features of allergic patch test with standard allergens.

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COMPOSITAE AND FRULLANIA PHYTODERMATITIS

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Abstract
Clinical features, botany, phytochemistry, patch testing and ecology of Compositae and Frullania (liverwort) allergic contact phytodermatitis are discussed. Allergic contact dermatitis from Compositae continues to attract world-wide attention owing to stread of noxious weeds in India, Europe, Japan and Australia.

1. Compositae phytodermatitis

Plant dermatitis due to Compositae affects primarily the exposed surfaces, not usually covered by clothing, of adult male farmers and rural workers. Allergic contact dermatitis of this type can resemble photodermatitis. Ragweed (Ambrosia), and other plants which are responsible, belong to the Compositae family of plants. The chemicals responsible for the contact allergy are sesquiterpene lactones (1).

Other patterns of dermatitis result from exposure to salad plants - chicory (Chicorium), lettuce (Lactuca) -: horticultural plants - chamomile (Anthemis, Matricaria), Arnica -: plants of perfumery - costus (Saussurea), champaca (Michelia) -: herbal plants - tansy (Tanacetum) and by cross-sensitivity with plants of other families from a content of related lactones: Laurel (Laurus); Magnolia, liverwort (Frullania).

Clinical features

Compositae dermatitis affects primarily the hands, forearms, neck and face. Exacerbations occur during the growth of the plants but, with continued exposure, the dermatitis becomes perennial and can become disseminated. Acute vesicular reactions can occur but characteristically weed dermatitis is chronic and lichenified. The skin of the upper eyelids, the retroauricular and submental areas that are spared in photodermatitis, are involved in weed dermatitis. Women and prepubertal children are rarely affected. The eruption can become generalized and produce a universal erythroderma that is not responsive to systemic corticosteroids and can be fatal. The only effective treatment has been to remove the patient from the area in which the weed is growing.

Botany

Most botanical family names end in ´-aceae´. The Chrysanthemum family is correctly named Asteraceae. The name Compositae, also used for this family, is an old one which is still preferred and used by many botanists. The flower head of plants of the Compositae (Chrysanthemum) family is a composite community of flowers which performs the same function as a single flower in other plants. Weeds of this family are aggresssive pioneer plants taking over vacant ground, competitive, prolific, and of high reproductive capacity.

Ragweed (Ambrosi) is the principal North American agressor that fluorishes in disturbed prairie soil and has since spread widely in the Central States and Canada. Ragweeds are reported from Assam, Japan, Hawaii, Australia, Britain, France, Germany, Belgium, Holland, Switzerland, Austria and Italy (Mitcell & Rook 1979). Feverfew (Parthenium) was introduced to India where it has caused thousands of cases of allergic contact dermatitis (31,39). Many other Compositae plants can cause weed dermatitis: tansy (Tanacetum), marshelder (Iva), sneez-weed (Helenium), cocklbur (Xanthium), pyrethrum (Chrysanthemum), etc. Some Compositae weeds in pastureland are harmful to cattle, contaminate milk, and interfere with crops and agricultural production (30).

Phytochemistry

Oleoresin is an ill-defined term for an oily resin which contains some of the sensitizing chemicals identified from the plants.

Sesquiterpene lactones, over 1,000 in number, have been isolated from Compositae family weeds and horticultural plants, e.g. Chrysanthemum (42,23). The chemical requisite of allergenic sesquiterpene lactones is a methylene group attached to the lactone ring, but bulky oxygenated (hydroxyl or acetate) substitutions adjacent to the lactone tend to lack reactivity (7).

Many species of the tribes Anthemidae and Heliantheae of the Compositae family have been reported to cause dermatitis (21,22). These tribes are rich in species that yield sesquiterpene lactones. Lactones are not

a feature of some tribes, e.g. Senecioneae, few species of which are reported to be allergenic.

Patch testing with weeds

In most cases, patch testing with the following Compositae plant extracts will produce one or more positive reactions in weed-sensitive patients:

Short ragweed	(Ambrosia)
Marshelder	(Iva)
Feverfew	(Parthenium)
Sneezeweed	(Helenium)

Owing to regional differences in clinical exposure and sensitivity, other extracts may be used if the diagnosis is strongly suspect and the above tests are negative. The following extracts are recommended:

Tansy	(Tanacetum)
Sagebrush	(Arthemisia)
Cocklebur	(Xanthium)
Chrysanthemum	(Chrysanthemum)

The plant extracts can be purchased from Hollister-Stier Laboratories, Box 3145 T.A., Spokane, WA 99220, U.S.A. The extracts should be applied to the skin and allowed to dry. Patches should then be applied to the sites. Since severe and multiple positive reactions can occur in highly sensitized patients, warning should be given to tested patients to remove the patches early, if itching is marked. The extracts, notably cocklebur, can produce irritant reactions in some patients at some times. The extracts are recommended for aimed patch testing in suspected cases. Cross-sensitivity with liverwort (Frullania) occurs (35).

Before patch testing, the patient should be cleared of dermatitis with withdrawal from weed exposure, often by hospitalization, and by topical and systemic therapy, often with corticosteroids. Patch tests with the extracts carried out on patients who have active dermatitis can, and often do, produce false positive reactions (angry back). Open tests as used by Shelmire (33,34) can be used instead of closed tests. Since active sensitization can occur, patch testing is best carried out only in those patients who are strongly suspect of weed dermatitis and screening tests are generally inadvisable for risk of active sensitization. Some current aspects of Compositae dermatitis are discussed below:

Pollen:

The pollen of sunflower (Helianthus annuus) was reported to be allergenic (25). Concerning Compositae, Shelmire (33) stated that " the pollens of many weeds in this series [the major and minor sensitizing weeds of the southwest U.S.A.] seem much less antigenic than other portions of the plant. One gram of the specific antigenic pollen blown directly on the moistened face of weed-sensitive persons from a distance of 18 inches failed to cause a dermatitis". Inclusion of ´pollen´ and antigenic´ in the same phrase has led to much subsequent confusion.

American ragweed (Ambrosia) dermatitis is not confined to the ragweed pollen season but corresponds to the growing season of the plant, that is, from early spring to the first frost that kills the weed (14,33). Contact of sensitized persons directly with the plant or with contaminated intermediary agents is followed by dermatitis (14,33). Such agents include hay, cotton, grain, flour, udders, boots, hat bands and bedclothes (17,33). Clinical observations by these authors support the concept that direct or indirect contact with ragweed is required to produce ragweed contact dermatitis and that pollen is not involved.

Undefatted ragweed pollens obtained from Hollister-Stier Laboratories, Box 3145 T.A., Spokane, WA 99220, U.S.A. were used to patch test ragweed contact-sensitive patients and patients who had been sensitized to sesquiterpene lactones by exposure to Frullania and Costus oil (Saussurea). The pollens produced negative patch test reactions. We were unable to detect sesquiterpene lactones in the pollen samples (Mitchell & Towers 1975, unpublished observations) and concluded that the pollen samples tested were not allergenic clinically and from lack of the lactones which are the principal sensitizers of ragweed. The notion that such ragweed pollen is allergenic by way of lactones for Type IV hypersensitivity can be abandoned. Further investigation is required, however, since a modified pseudoguainolide, named ambrosic acid, has been isolated from pollen of the ragweed, Ambrosia artemisiifolia, which was introduced to Japan from North America a century ago (15). Consideration of the structure of the molecule suggests that ambrosic acid might be a potential allergen.

Dermatitis at a distance from ragweed

The notion that airborne plant parts can cause dermati-

tis at a distance has not received scientific scrutiny. When the plants wither and die at the end of the growing season, they can be trampled by humans and animals; a fine dust can thus be produced and is readily blown about (Mitchell, unpublished observations). Whether such airborne dust could contaminate, for example, clothing hanging in a line is dabatable. The trichomes (plant hairs) of the plant are readily detached, especially when the plant is dried. These fine hairs can also be blown about and they contain allergenic sesquiterpene lactones (32). It can be restated, however, that if there is any airborne component to Compositae sesquiterpene lactone dermatitis, it is not due to pollen.

Ecology

The relative and increasing scarcity of Parthenium dermatitis in Texas is in contrast to its epidemic widespread occurrence following introduction of the weed to India (8). Howell (14) believes that dermatitis in Texas is uncommon because large numbers of United States residents rarely contact sensitizing weeds since agriculture is so mechanized in that country. Certainly there is much closer contact of Indian residents, both rural and urban, with the weed because of its ubiquitous spread and their life-style (19).

Little is known of the risk of sensitization and of dermatitis from exposure to Compositae in terms of numbers of persons at risk/versus number sensitized/versus number who develop dermatitis. In the case of Parthenium hysterophorus in India, 56% of adult males exposed to the plant became sensitized as shown by patch testing but only 4% of them acquired Parthenium dermatitis (8,36).

It can be assumed that from a low level of sensitivity, or lack of further exposure, not all who become sensitized will actually demontrate dermatitis.

Sex

The sparing of women and children from Compositae weed dermatitis has not been adequately explained. Howell (14) believes that it is a matter of exposure, more men than women being repeatedly exposed to sensitizing plants. A few cases of weed dermatitis were reported in women (33), perhaps reflecting a greater involvement of women in outdoor farmwork in years past (14). However, in India, women frequently work in the fields but are rarely affected and of 20,000 children employed in pulling the plant, none were known to be sensitized (Lonkar 1975, personal communication). Owing to the manner of dress, the feet and ankles of Indian women are generously exposed to the plant.

Atopic dermatitis and Compositae dermatitis

Patient who have atopic dermatitis may be more susceptible to irritant contact dermatitis but are at lower risk for allergic contact dermatitis than individuals who do not have atopic dermatitis (14). He had not seen one case in which atopic dermatitis and weed dermatitis coexisted and quoted Dr. Shelmire (pers. comm. to Howell 1978) for a similar experience.

Type I hypersensitivity (allergic rhinitis) from Compositae weeds is caused by water-soluble proteinaceous chemicals of the plant pollens. These two types of hypersensitivity to weeds are immunologically unrelated and rarely coexist in a patient.

Cross-sensitivity

Compositae and Frullania cross-react because of a content of related lactones. Similary, Compositae-sensitive patients often cross-react to plants which are not present in their environment and are thus of immunological but not of ecological relevance.

Colophony and trupentine, derived form Pinaceae species sometimes cross-react. The latter is a marginal irritant and we have found that positive reaction to it observed by concomitant patch testing can prove negative by sequential patch testing (angry back).

For true positive reactions, the finding of delta-3-carene, the principal allergen of turpentine (26) in Ambrosia will direct attention to Compositae terpenoids.

Abietic acid and its derivatives (28) may also repay study in respect to balsams and wood tars which can cross-react with Compositae.

Photosensitivity and Compositae dermatitis

The distribution pattern of Compositae weed dermatitis, affecting primarily the exposed skin surfaces not ordinarily covered by clothing, resembles photodermatitis. In the later there is charcteristic sparing of the upper eyelids, submental and retroauricular skin. In contrast, contamination of these sites by direct contact with the plant or by contaminated intermediary agents, e.g. the fingers, leads to their involvement by weed dermatitis. A question arises as to whether photosensitivity plays any part in the clinical picture of Compositae dermatitis. Against this possibility is the fact that afflicted patients usually recover when they are re-

moved from contact with the weeds although they are still exposed to sunlight (19). For this possibility is the finding that afflicted patients can show abnormal responses to various wavelengths. A patient who had Compositae dermatitis presented a lowered minimal erythema dose for UVB, 10 sec (average normal 25 sec) and positive reaction to UVA by exposure for 30, 20 and 10 min to uninvolved skin of the lower back. The patient by history and scratch tests was also probably atopic and it was concluded that dermatitis of the exposed skin surfaces might have a complex and varied etiology (29).
Rao has been looking for photosensitivity in Parthenium dermatitis for a long time and has finally found a patient who reacted negatively to Parthenium by patch and photopatch tests but reacted to Parthenium extract by photopatch test (2). This sems to be an authentic exception to the ´rule´ that Lonkar´s patients, as he says, get better when they get away from the weed even though they are still in the sunshine on the coast. However, lately he has begun to suspect photosensitivity in some cases.

More recently, patients suspected, in some cases for years, of various types of photodermatitis including actinic reticuloid, have been found to be patch test positive to extracts of Compositae weeds and this finding has led to a revision of the diagnosis from photodermatitis to allergic contact dermatitis (9,10,11,12,13). In some cases, photosensitivity was also present.
Chronic persistent photodermatitis almost exclusively seen in middle-aged or elderly males results in its severest form, in the development of thickened plaques in light-exposed skin with reticulotic histopathological features - hence the term ´actinic reticuloid´ (11,16). The role of actinic radiation in the pathogenesis of this disorder is supported by the consistent finding of abnormal responses to UVA, often extending to visible light. The possibility that allergic contact sensitivity might play a part in at least some of these cases was raised by the development of chronic photosensitivity in patients with allergic contact dermatitis from tetrachlorsalicylanilide and related compounds (41). That the association between allergic contact dermatitis and chronic persistent photodermatitis is a close one has been shown by Frain-Bell & Johnson (10) who found that of 55 patients with chronic photosensitivity dermatitis including actinic reticuloid, 47 produced positive closed patch test reactions to plant extracts. The relationship between allergic contact sensitivity, photoallergy or phototoxicity, and chronic photodermatitis is not yet clear but Frain-Bell et al. (10,12) in a study of five patients with allergic contact sensitivity to Chrysanthemum and chronic actinic dermatitis, found that action-spectrum studies with the monochromator demonstrated increased reactivity to short and long wavelength UV in all patients, extending into the visible spectrum in two. In only one of these was there evidence of a photocontact reaction; UV irradiation of the patch test site after application of the allergen caused a slightly amplified patch test reaction at 1 and 10% allergen concentrations. Pre-irradiation of skin before patch testing caused a modest enhancement of the reactions to the undiluted Chrysanthemum extract and to the 50% dilution in two patients, but this effect could not be reproduced in the others. In two subjects pre-irradiation of the Chrysanthemum extract before closed patch testing produced a slightly greater response than did unirradiated extract but pre-irradiation had no influence on the reaction in others. In vitro tests for phototoxicity with Chrysanthemum oleoresin and human red blood cells were positive, but fractionation of the extract by thin layer chromatography revealed that the fractions causeing in vitro phototoxicity and positive patch tests were different.

Although an association between allergic contact dermatitis to plants and chronic photodermatitis has clearly been established in some patients, the precise nature of this relationship remains uncertain. Their findings neither confirm nor exclude phototoxic or photoallergic mechanisms. The inconclusiveness of their results may possibly be due to the use of limited wavelengths of ultraviolet; the patients themselves clinically reacted to a wider range of the solar spectrum (6).

Nature of the photoactive agent

Numerous sesquiterpene lactones of Compositae were found to have no phototoxic activity for Candida albicans by Daniels´ (5) method (Mitchell & Tower 1976, unpublished observations). Frain-Bell et al. (12) found that alantolactone did not evoke phototoxicity for human red blood cells by their method. The fractions responsible for the contact allergic sensitivity in some patients were found to differ from those shown to produce a phototoxic action in vitro. In a study of Chrysanthemum sensitivity, they were unable to demonstrate photocontact reactions or the presence of a re-

sponsible photoproduct. There was some evidence, however, that pre-irradiation of the skin might have enhanced the severity of the contact allergic response to the Chrysanthemum extract (12). Following our negative findings, we looked for photoactive compounds other than the lactones which produce contact sensitivity and found that Compositae abound with polyacetylenics, many of which were antibiotic and some phototoxic for Candida; a thiophene derivative, alpha-terthienyl, evoked a biphasic phototoxic response for man (3,40).

Of 65 sesquiterpene lactones, only one evoked phototoxicity, namely glaucolide G. The Compositae examined were largely North, Central and South American species with the inclusion of some Australian and Malaysian species.

Though the position of glaucolide G is disparate and awaiting confirmation, it seems that sesquiterpene lactones in general are not presently likely to add photoactivity to their role as allergic contact sensitizers. Nor is alpha-terthienyl a likely candidate for clinical Compositae photoreactivity since Frain-Bell et al. (12) found that whatever the photoactive substance was, it was not alpha-terthienyl, which moves almost with the solvent front in the thin layer chromatography system they used and, if present at all in their chrysanthemum extract, was at a low concentration. Also, alpha-terthienyl was not detected in their chromatography system nor in their chrysantemum extract used for testing patients.

Although photosensitivity has been observed in some patients who have eczema (27), it is not a recognized feature of allergic contact dermatitis in general.

Accordingly, it can be postulated that some Compositae plants yield photo alergens which have not yet been identified.

2. Frullania (liverwort) phytodermatitis

Phytodermatitis from Frullania (liverwort) has been observed in 140 British Columbian forest-workers (20,24).

All patients observed have been male ranging in age from 18 years to middle life. The incubation period, that is the time between starting work in the forest and developing dermatitis from exposure to liverwort (Frullania), has varied from a few months to 15 years. The history has been similar in sensitized patients.

Dermatitis of the exposed skin surfaces not usually covered by clothing appears within a few days of working in the forest. Since gloves are regulary worn, especially in wet weather, the eruption has often started around the wrists, spread up the forearms and appeared on the face. When the hands have become wet inside the gloves, the dermatitis has affected the hands, particularly the finger-webs and sides of the fingers. Where other skin sites have become wet by rain, notably the lower limbs, dermatitis has appeared there, and occasionally disseminated. Following leaving work in the forest, the eruption has cleared in 2-3 weeks, sometimes longer when complicated by secondary infection. The workers involved have been those most exposed to vegetation, particularly the bark of trees. Fallers (fellers) who fell the trees have been the workers most often affected. Hook-tenders, those who encircle the trunks with chains in order to drag the trees from the forest, have also had a high incidence. Others are buckers, toppers, choker-setters, scalers and timber cruisers. All those affected have been workers in the forest products industry working in forest areas with the exception of one male patient, a geologist. He made frequent trips to forest areas and also picked up rocks during the course of his occupation.

A major geographical source area of dermatitis has been the west coast of Vancouver Island. In this area there is a higher rainfall and liverworts are more common than elsewhere. In the interior of the province in the colder drier areas with fewer liverworts, fewer problems have arisen for workers and some have been able to work there when they could not work on the coast. No cases have been seen from the secondary forestry industries, that is from mills. In such mills the bark of the tree is usually removed by machinery and there is no exposure from that source to liverworts. Cases of dermatitis in the mills have been found to be due to other causes and tests with liverworts have been negative. Therefore this type of dermatitis seems to be confined to forest workers.

Patch test to Frullania (as is) have been positive in all cases, negative in controls. Most of those who were so tested have shown positive reactions to alantolactone (0.1% petrolatum). Patients were tested to d-usnic acid (1% petrolatum) with positive results in three-quarters of the cases; some reacted to atranorin, avernic and perlatolic acids. Exposure to lichens is as ubiquitous as esposure to liverwort in the industry but the lichen chemicals seem to have a lower sensitizing capacity than Frullania. All patients who have been so tested, about 70, have shown multiple positive patch test reactions to sesquiterpene lactones derived from Compositae species. This finding results from the fact that the

lactones of Frullania are chemically related to the Compositae lactones which were tested. The finding is immunologically relevant but usually ecologically irrelevant.
Garden chrysanthemums and tansy (Tanacetum) have produced recurrences in a few forest workers who were also hobby gardeners, and sage-brush (Artemisia) in a few who hunted. Many Crysanthemum contact-sensitive patients, hobbyists and industrial plant growers, have also been observed to react to Frullania by patch test but the Frullania sensitivity has not been ecologically relevant to them.
In view of reports of photodermatitis induced by lichen chemicals (37,38), special attention has been paid to the possibility of photosensitivity in taking the histories. We have not been able to detect any evidence of such photosensitivity and the industrial problem has remained, in this area, confined to forest workers occuring when they are at work in the forest. If aroma chemicals (fragrances and flavourings) made from Compositae or lichens (4) were to produce recurrences in these patients, we probably would have seen them, but have not done so.
Some workers who are not strongly sensitized have been able to continue at work with recurrent mild dermatitis. The great majority, however, seem to have been more strongly sensitized and they have been obliged to give up work in the forest. Since a faller has a high rate of pay, there has been a substantial loss of income for many of the workers due to a necessity to change to lower paid work. They have been able to continue in forest work by driving a truck or in other occupations where there is no exposure to wet vegetation. The great majority of fallers who have become sensitized have been obliged to quit falling.
This problem which was described in France (18) was reported to account for 3% of cases of contact dermatitis in general seen in the Bordeaux area. We have not been able to count the numbers of persons at risk in order to obtain a percentage of sensitized persons. Since screening has not been carried out, we do not know the proportion of sensitized workes, workers who actually develop dermatitis. Workers may also present after experiencing years of partial disability. Cases have been reported from the neighboring state of Oregon (35).

Until the problem was defined, many seriously disabled workers were not compensated. Because of the risk of active sensitization, we do not recommend screening with Frullania but aimed testing is justifiable. Liverworts are common in moist areas and it is probable that allergic contact dermatitis from liverwort will yet be found to be more widely distributed than is at present recognized in France, the Pacific Northwest, Spain and Czechoslovakia.

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ALLERGY TO COSMETICS

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Cosmetics flood over our markets and in Western countries their popularity among men is increasing. They usually cause little trouble but they do cause some and these adverse reactions may be overlooked by the doctor and repudiated by the patient.

Cosmetics can be irritants and sensitisers. It is difficult for dermatologists to assess their level of irritancy because responsible cosmetics are usually identified by the users and discarded. Complaints may be made to the manufacturers but only in exceptional circumstances is the customer seen by a dermatologist. The best assessment of these mild irritant reactions are the complaint files of cosmetic companies. In contrast for sensitisers the dermatologist is extremely well informed whereas cosmetic manufacturers and their suppliers are often surprisingly unaware.

Perfumes and preservatives are the commonest allergens in cosmetics and the incidence of sensitisation is increasing. A much smaller number of patients continue to develop dermatitis from nail varnish, hair preparations, lanolin or lanolin derivatives and cosmetic colours.

Larsen's (1) study of perfume sensitive patients and identification of the fragrance compounds to which they reacted led to the formulation of a perfume-mix which is now included in most standard patch test series. The introduction and use of this patch test material was quickly followed by recognition of the frequency and importance of perfume sensitivity. This standard perfume-mix contains the 8 ingredients cinnamic alcohol, cinnamic aldehyde, alpha amylcinnamic aldehyde, eugenol, isoeugenol, oakmoss absolute, hydroxycitronellal and geraniol. To avoid irritant patch test reactions the initial concentrations have in the last few months been reduced from 2% to 1% of each component giving a total of 8% in the mixture. It is estimated by Larsen (1) that this mixture detects 70-80% of perfume sensitive subjects. The remainder react only to their own perfumes, indicating how essential it is to use them for patch testing.

The ranking order of reactions ot these perfume compounds is interesting because the variation from country to country presumably reflects difference in preference for the various fragrances. The results from North America (2), two studies in the Netherlands (3,4) and St. John's in London for 1979-1980 (5) and for 1984 are listed below. The most striking and unexplained difference is the consistantly high incidences of sensitisation to cinnamic aldehyde in London compared with North America and the Netherlands.

TABLE 1

USA 1977-80		Netherlands 1984	
Compound	No + PTs		% + among cosmetic pts
Cinnamic alcohol	14	Eugenol 8%	11.1
Isoeugenol	10	Hydroxycintronellal 10%	10.5
Hydroxycitronellal	9	Cinnamic alcohol 5%	10.5
Cinnamic aldehyd	5	Cinnamic ald. 0.5%	3.7
Geraniol	5	Geraniol 1%	1.6
Eugenol	4		
Amylcinnamald.	2		
Oakmoss	1	Netherlands 1985	No + pts among 179 ? cosmetic pts
		Isoeugenol (8%)	36
		Oakmoss (10%)	21
		Geraniol (10%)	11
		Amylcinnamic alcohol (10%)	7

TABLE 2

	St. John's					
	1979-80 M + F		1984 M		1984 F	
Total tested	2461		803		1033	
% + Perf.mix or ingred	6%		6.6%		12%	
Ingred perf-mix	No + Pts	Ranking order	No + Pts	Ranking order	No + Pts	Ranking order
Cinnamic aldehyde	94	(1)	23	(1)	38	(1)
Cinnamic alcohol	61	(2)	10	(4)	12	(5)
Isoeugenol	48	(3)	12	(3)	19	(3)
Hydroxycitronallal	36	(4)	7	(5)	13	(4)
Oakmoss	29	(5)	14	(2)	34	(2)
Eugenol	25	(6)	10	(4)	12	(5)
Geraniol	7	(7)	4	(6)	6	(6)
Amylcinnamic ald.	5	(8)	NT		NT	

Concentration each 2% in petrolatum

The problem with perfume sensitivity is no longer that of diagnosis but of persuasion of the patient of its relevance to their eczema. Perfumes are pleasant and ubiquitous being in cosmetics, toiletries, domestic cleaners, antiseptics, insecticides and even some medicaments, and as flavours they are in foods, sweets and toothpastes. Fragrances are not easy to avoid and without conviction of their importance few patients will make the effort. Even worse, the

embargo on contact should apply not only to the patient but also to their immediate household. Continual exposure to perfumes is probably a significant factor in the chronicity of many eczemas but it is difficult to prove.
It is particularly important to know which preservatives are currently used in cosmetics because these chemicals are constantly changing and sensitisation is frequently unexpected. The sources of contact are definitive and with perseverance they can be identified and the allergen removed from the patients´s environment often resulting in a cure of the eczema. An accurate assessment of the frequency of sensitisation can only be obtained by including the preservatives in the standard series. The reported incidences of sensitisation vary considerably from country to country.
The parabens group consists of methyl, ethyl, propyl, butyl and benzyl esters of benzoic acid. They have been used in cosmetics, including the hypoallergenic ranges for many years. Patients with cosmetic dermatitis have been studied in France (6) where the parabens incidence of sensitisation was 4.4% of 46 patients, in Spain it was 8% of 460 cosmetic patients (7), in the Netherlands it was 0.6% of 179 patients suspected of cosmetic dermatitis (4), and in North America of 149 patients patch tested with cosmetic ingredients 15 (10%) reacted to parabens (2).
At. St. John´s parabens sensitivity is uncommon. In the 1970´s the incidence was 0.7% of all those attending the Contact Clinic and in 1984 it was 0.4% (Table).

Year	Tested	+	%	Males + %	Females + %
1971-74	9983	72	0.7	0.8	0.7
1984	1836	8	0.4	0.4	0.5

TABLE 3 Incidence of parabens sensitivity St. John´s 1971-74 and 1984

In 1984, eight patients reacted to parabens, 5 women and 3 men. The face was affected in all 5 women but 4 had in addition a constitutional eczema and other sites were involved. In each woman the parabens was considered a significant cosmetic allergen but it was the entire cause of the dermatitis only in the patient with eczema confined to her face. Among the three men the parabens allergy was considered as contributing to their eczema in 2 but unexplained in the third.
For patch testing 3% of each of the five esters is combined in petrolatum to give a final 15% concentration. To detect sensitisation the mix of esters is needed because no one ester will screen for the rest.

	Parabens Esters				
Patient	Propyl	Butyl	Methyl	Butyl	Ethyl
1	-	-	+	-	+
2	+	?	?	-	?
3	-	-	-	-	+
4	+	?	?	-	+
5	?	?	+	-	-
6	+	+	?	-	+

? = weak reaction - = no reaction

TABLE 4 Patch test reactions in 6 patients tested to the components of the parabens-mix (St. John´s).

The importance of Dowicil 200 (Quaternium - 15: chloroallyl hexaminium chloride) as a sensitiser seems to vary from country to country presumably depending upon local exposure in the community. There are few reports in the literature of incidences of sensitisation. In North America (2) adverse reactions to cosmetics were studied from 1977-1980 and of 149 patients patch tested with cosmetic ingredients 32 (20%) were sensitised to Quaternium 15 making it the second most frequent cosmetic allergen superceded only by a fragrance in 39 (26%) of these patients. In the Netherlands of 179 patients suspected of a cosmetic allergy 5 patients (2.8%) were sensitised to Dowicil 200 (4). A high incidence does not occur in France where only 0.8% reacted of 465 patients suspected of having a contact dermatitis from cosmetics, medicaments, clothing or industrial products (8).
This preservative is a most significant allergen among the patients seen at St. John´s and since 1981 has been included in the standard series. The incidence of sensitisation has been 3-4% of all the women attending the clinic and 1% of the men (Table).

	Females		Males	
	Tested	+%	Tested	+%
1981	1115	3	802	1
1982	1117	3	867	1
1983	1004	4	820	1
1984	1033	4	803	1

TABLE 5 Incidence of sensitisation to Dowicil 200 in women an men (St. John´s 1981-1984)

In 1984, 50 patients, 41 women and 9 men, were found to be sensitised and in 36 of these patients 29 women and 7 men it was the only cosmetic allergen. The features of this group of 36 patients have been studied. In 11 allergy to Dowicil 200 was the entire cause of their eczema in 25 it was an aggravating factor. The sites involves are listed below showing that Dowicil 200 is a significant allergen in eczema of the face (18/36 patients) and hands (16/36 patients).

	Dowicil sensitivity	
Sites of eczema	Entire cause of eczema	Aggravating factor
	11	25
Face	10	6
Scalp	1	0
Hands		13
Hands + face		2
Hands + feet		1
Generalised		2
Leg		1

TABLE 6 Sites of eczema in 36 patients in whom Dowicil 200 was the only cosmetic allergen.

The concentration of Dowicil 200 used for patch testing is 1% in petrolatum. It is a formaldehyde releaser and between 1980 and 1984 of those sensitised, 60% reacted to formaldehyde but the concentration of formaldehyde used for patch testing was then 2% in water and some of these responses may have been irritant.

Germall 115 (Imidazolidinyl urea), another cosmetics preservative, seems to be only a moderate sensitiser. In the North American cosmetic series (2) it had the same incidence as parabens sensitising 15 of 149 (10%) of the patients. In the French report their incidence was 1.2% (8).

At St. John´s in 1984 only selected patients were tested with Germall 115 but it is present in a battery of allergens for patients with facial eczema. During that year 9 patients, 8 women and 1 man were found to be sensitised. Three of the women had a dermatitis of the face and other sites which was attributed solely to sensitisation to Germall 115. A fourth patient had an allergic contact dermatitis from Germall 115, nail varnish and perfume. Two light sensitive patients may have been sensitised by sun creams and in 3 patients no source or relevance for the allergy was found. The patch test concentration is Germall 115 1% in petrolatum. Among the patients seen at St. John´s none reacted to formaldehyde.

Sensitisation to Bronopol (bromo-nitropropane-diol) was investigated in detail by Storrs and Bell (9) and they stressed the difficulties of patch testing with Bronopol because of its irritancy. In the USA the most common source of exposure is the emollient Eucerin ^{R} which contains 0.05% bronopol. From the Mayo Clinic (10) a valid 12.5% incidence was reported among eczematous patients, and by stopping the routine use of Eucerin ^{R} their level of sensitisation dropped to 2.3%. In the French series (8) of patients suspected of a contact dermatitis 4.7% reacted to Bronopol. However, it was the cosmetic allergen in only 8 of the 149 (5%) patients in the North American study (2) and in only one of 179 ? cosmetic patients patch tested in the Netherlands (4).

At St. John´s Bronopol is an infrequent sensitiser and has been added to the standard series only in the past few months.

For patch testing Storrs and Bell (10) recommended 0.5% and 1% Bronopol in petrolatum.

The isothiazolones (Proxels, I.C.I.: Kathons, Rhom and Haas) are a group of biocides used for many years in industry to preserve aqueous solutions, they are well known allergens and cases of sensitisation are reported sporadically.

Kathon CG R (cosmetic grade), one of this group, is soluble in water and effective against bacteria and fungi. It is a mixture of a chlorinated isothiazolone (3 parts) and a non chlorinated isothiazolone (1 part)

5-chloro-2-methyl-4-isothiazolone-3-one

2-methyl-4-isothiazolone-3-one

with magnesium chloride and nitrate added as stabilisers. This biocide is used in wash off products such as shampoos, hair preparations and bath additives and also in stay on cosmetics including creams, lotions and eye cosmetics. The Rohm and Haas data sheet recommends concentrations varying from 0.035% by weight in a shampoo to 0.15% in a product containing protein.

Kathol CG is an allergen in guinea pigs (11) and man but how frequently the low concentration in toiletries and cosmetics sensitise has yet to be definitely assessed. Judging by the scarcity of reports in the literature the incidence appears to be low. In the Netherlands (12) 2 patients with an allergic contact dermatitis of the face were sensitised by the same brand of moisture cream containing 0.08% Kathon CG. A third patient was actively sensitised by a patch test with Kathon 1% in petrolatum and reacted to a challenge patch test of 0.67% in petrolatum.

At St. John´s since 1983, Kathon CG 100 ppm aqueous has been included in the standard series. During 1984, 1836 patients were patch tested, of the 803 men none reacted and of the 1033 women 5 reacted (0.5%). In two patients the source was traced, one patient had used a night cream containing 0.1% Kathon CG and an emollient lotion containing 0.05%. The second patient hac used an eye gel and eye balm both containing 0.6% Kathon CG. In the third and fourth patient the source was not traced and in the fifth patient it was not sought.

For patch testing too high a concentration will cause active sensitisation, a dilution of 100 ppm in water is used by the International Contact Dermatitis Group. Even this concentration occasionally causes slight irritant reactions but these responses are not reproducible and can be ignored.

Other cosmetic allergens continue to sensitize but less frequently than perfumes and preservatives. The standard series will screen for hair dye dermatitis from paraphenylene deamine and sensitisation to wool alcohols in emol-

lients. Patch testing with nail varnish resin is selective but important, in1984, a total of 15 women were found to be sensitive. In 7 nail varnish was the complete cause of their dermatitis, in 4 nail varnish was the partial cause of an allergic dermatitis, 2 women had a nail varnish dermatitis complicating an endogenous eczema and in 2 women the reaction was of past relevance only.
Occasionally, the antioxidants, butyl hydroxyanisole and tertiary butylhydroquinone sensitise in facial cosmetics (13).

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DETECTION OF ALLERGIC DERMATITIS TO FRAGRANCES

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BACKGROUND

The North American Contact Dermatitis Group recently completed a 5-year study of cosmetic reactions and found that the fragrance component was the most common cause of allergic contact dermatitis (1). This was followed closely by allergy to preservatives (Tables I and II).

Table I
TYPES OF INGREDIENTS MOST OFTEN
RECORDED AS CAUSING CUTANEOUS REACTIONS

Ingredients	Number of Cutaneous Reactions
Fragrance and ingredients	161
Preservatives	149
p-Phenylenediamine	41
Lanolin and derivatives	29
Glyceryl monothioglycolate	25
Propylene glycol	25
Toluenesulfonamide/formaldehyde resin	23
Sunscreens and other UV absorbers	20
Acrylate or Methacrylate	9
Others (< 3)	56
Total	536

THE FRAGRANCE MIXTURE

Beginning ten years ago the compounds of various perfumes were examined to determine which chemicals were allergenic (2). It became apparent that a handful of chemicals was responsible for most reactions. A fragrance mixture was developed which consisited of eight different fragrance materials at a concentration of 2% each in petrolatum (Table III). This fragrance mixture detected approximately 70-80% of fragrance allergies (3)(Table IV). One problem with the mixture was that it was a marginal irritant and reactions had to be interpreted with great care. Investigators in Europe came out with an 8% perfume mixture with each component present at 1%. However, this is not satisfactory because it will miss some reactions such as oakmoss and geraniol which may actually be too low at even 2% consentration. A compromise might be to reduce cinnamic aldehyde to 1% and retain the remainder at 2%.

Table II
FRAGRANCE INGREDIENTS CAUSING CUTANEOUS REACTIONS

Ingredients	Number of Cutaneous Reactions
Fragrance, unspecified	67
Cinnamic alcohol	17
Hydroxycitronellal	11
Musk ambrette	11
Isoeugenol	10
Geraniol	8
Cinnamal (cinnamic aldehyde)	6
Coumarin	4
Eugenol	4
Others (<3)	23
Total	161

Table III
FRAGRANCE MIXTURE

Cinnamic alcohol	2%
Cinnamic aldehyde	2%
Isoeugenol	2%
Hydroxycitronellal	2%
Oakmoss	2%
Geraniol	2%
Alpha amyl cinnamic alcohol	2%
Eugenol	2%
Total	16%

THE PERFUME SCREENING SERIES

Last year the North American Contact Dermatitis Group stopped using the fragrance mixture and substituted a screening series of fourteen individual fragrance materials at nonirritating concentrations (Tabel V). This perfume screening series adds 14 extra tests, but it is warranted because of the importance of detectiong fragrance allergy. When combined with the routine series of 20, we have a total of 34 tests, which is not cumbersome to apply when the Finn Chamber tecnique is used.

All the fragrance materials are pure chemicals except oakmoss and sandalwood oil. It is always desirable to use a pure chemical because natural materials can vary considerably. Testing with atranorin might be suitable as a substitue for oakmoss. Santol could possibly be used as a substitute for sandalwood oil.

Table IV
PERFUME MIXTURE RESULTS

January 1979 - March 1980	Tested	Positive	Percent
Perfume mixture	2,461	172	7
Cinnamic aldehyde		94	4
Cinnamic alcohol		61	2
Isoeugenol		48	2
Oakmoss		29	1.2
Eugenol		25	1
Geraniol		7	0.2
Hydroxycitronellal		36	1.4
Alph amyl cinnamic alcohol		5	0.2

Table V
PERFUME SCREENING SERIES

	Common Fragrance Allergens *(can be used for routine screening)*	
1.	Cinnamic alcohol	5% petrolatum
2.	Cinnamic aldehyde	1%
3.	Hydroxycitronellal	4%
4.	Isoeugenol	5%
6.	Oakmoss absolute	5%
	Less Common Fragrance Allergens	
7.	Alpha amyl cinnamic alcohol	5%
8.	Geraniol	5%
9.	Benzyl salicylate	2%
10.	Sandalwood oil	2%
11.	Anisyl alcohol	5%
12.	Benzyl alcohol	5%
13.	Coumarin	5%
	Photoallergen	
14.	Musk Ambrette (also a contact allergen)	5%

THE FUTURE

The International Fragrance Association (IFRA) makes recomendations regarding the upper limits of certain fragrance materials in perfumes and also those that should be completely deleted. The International Fragrance Association (IFRA) follows the incidence of reactions to fragrance materials, and also takes into account the maximization testing done by the perfume industry.

As some fragrance allergens are eliminated and other substitutes are added we must look for newer allergens. Perfume companies are increasingly cooperative by supplying coded breakdowns or partitions of fragrances so that we can identify the specific offending chemical, and yet maintain the secrecy of the formula.

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ALLERGY TO LICHEN AND COMPOSITAE COMPOUNDS IN PERFUMES. INVESTIGATIONS ON THE SENSITIZING, TOXIC AND MUTAGENIC POTENTIAL.

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Several plant extracts exhibit anti-tumor, antimicrobial and cytotoxic activity as well as powerful mutagenicity that can cause chromosomal aberrations. In addition some compounds are also capable of inducing allergic contact dermatitis, while others possess a phototoxic potential and are well-known photosensitizers.

Many substances derived from lichens and Compositae plants have received conciderable attention because of these properties (1-4). Probably the most important source of contact with lichens and Compositae compounds is cosmetics, which frequently contain fragrances such as oak moss and Compositae extracts (5,6) and in one provious study we observed that 1/3 of those who were sensitive to lichen extracts also reacted to different Compositae-oleoresins, i.e. sesquiterpenes (7). The frequent occurrence of contact-allergic sensitivity to Compositae extracts and lichen compounds in patients with persistent light sensitivity has been underlined (8) and in a recent report contact allergy to lichens was found to be twice as common compared to Compositae(9).

The present report constitutes a preliminary study in which in vivo and in vitro effects of some isolated lichen compounds and Compositae extracts were examined.

MATERIALS AND METHODS

In vivo. Sensitivity to different brands of perfumes, deodorants, after-shave lotions etc. in 55 of our patients was verified by repeated questioning, and/or testing. Sixteen patients had a definite history of a contact allergic reaction to plants, following direct contact and 19 had a history of photosensitivity. Patch and photopatch testing was performed in the 55 patients (20 males and 35 females) according to the SPDRG protocol (10) with purified lichen extracts in 0.1 conc. and with the same conc. of purified isolated lichen substances to which the patient had reacted. Dilutions of isolated compounds, atranorin, physodalic acid and cetraric acid 0.1%, 0.01%, 0.001%, respectively, were also tested by irradiation (5J UVA) on 5 patients. The same tests were performed with different Compositae extracts. The purified, isolated lichen substances and Compositae extracts as listed in Tables I and II were obtained from Y. Solberg NLH, 1432 Ås, Norway (Tables I and II).

Tests in 5 Duncan-Hartley guinea pigs were performed as described by Ichikawa, Armstrong & Harber (11). The application time was 1 hour and the test conc. were 10%, 1% and 0.1% of atranorin, evernin, ceraric acid and physodalic acid.

Table I. Test series with aromatic lichen acids.

1. Atranorin. 2. Lecanoric acid. 3. Gyrophoric acid.
4. Psoromic acid. 5. Evernic acid. 6. Barbatic acid.
7. Strictic acid. 8. Lobaric acid. 9. Salazinic acid.
10. Norstictic acid. 11. Diffractaic acid.
12. Physodic- and physodalic acid. 13. Usnic acid.
14. Alectoric acid. 15. Protolichesteric acid.
16. Lichesteric acid. 17. Thamnolic acid.
18. Pulvinic acid. 19. Vulpinic acid.
20. Fumarprotocetraric acid.

Table II. Test series with Compositae extracts.

Achillea millefolium. Achillea ptarmica. Arctium lappa. Anthemis tinctoria. Artemis vulgaris. Aster tripolium. Centaurea cyanus. Centaurea scabiosa. Chrysanthemum leucanthemum. Erigeron acre. Hieracium vulgatum. Hypochoeris maculata. Leontodon autumnalis. Matricaria indora. Petasites frigidus. Senecio vulgaris. Solidago virgaurea. Tanacetum vulgare. Taraxicum officinale. Tragopogon pratensis.

+ Test conc. 2% in petrolatum.

In vitro. Candida albicans phototoxicity test (12) was

performed with 20 isolated lichen compounds and 6 different Compositae extracts (Artemisia vulgaris, Arctium Lappa, Matricaria inodora, Senecio jacobaea, Chrysanthemum leucanthemum, Anthemis tinctoria, in conc. 0.1%). Cytotoxicity tests were performed with Reuber H4-II-E rat hepatom cells in culture (E Dybing, SIFF, Oslo). Cell protein was determined as an expression of growth using Dulbecco's MEM medium with 10% calf serum, 10% foetal calf serum and 60 mic/ml mycostatin; cell conc. $2.5x10^5$ cells/dish. After 24 hours growth in the medium atranorin, evernin and alantolactone in DMSO were added at a final conc. of 1, 10, and 100 micM. Cultures with the addition of DMSO only served as controls. Mutagenic studies: Atranorin, evernin and allantolactone in amounts of 1, 10, 100, and 1000 micg were tested on Salmonella typhimurium TA 98 and TA100 with supernatant protein from Aroclor 1254-pretreated rat and NADPH (E Dybing, SIFF, Oslo.

RESULTS AND COMMENTS

By patch testing we were able to induce only contact allergic reactions with the compounds shown in Table III. All 55 patients had positive patch test reactions to the lichen mixture containing Hypogymnia tubulosa, H. Physodes, Parmelia sulcata, Psevdovernia furfuracea and Physcia tenella extracts in conc. of 1%. Thirtyfive were sensitive to the fragrance oak moss (mousse de chène) used in perfumes while 27 reacted to Cetraria Islandica and 14 to Compositae extracts (Table III). Several of the lichen allergens remain unidentified since patch tests with oak moss and also isolated lichen acids were negative in some patients who reacted to the lichen mixture. In addition we also observed 5 patients who reacted to oak moss and not to the lichen mixture.

In the photopatch testing photoallergic reactions were induced by atranorin and evernic acid at 0.1% and 0.01% conc. in 2 patients. In the remaining patients there were either equal reactions on irradiated and non-irradiated sites or a +1 stronger reacton followint irradiation. None of the Compositae extracts induced clear-cut photosensitivity.

Using the guinea pig test, only contact allergy could be induced by cetraric acid and physodalic acid and no clear-cut photoallergy could be observed. Only a minimal growth inhibition (0.5-2mm) was observed in the Candida albicans phototoxity test, with physodalic acid, barbatic acid, salazinic acid, diffractaic acid and evernin. Lobaric acid, cetraric acid and fumarprotocetraric acid were also active in the dark. The Compositae extracts of Matricaria indora and Chrysanthemum leucanthemum showed moderate growth inhibition (2-3mm). In the cytotoxicity test atranorin showed total growth inhibition at 10 micmol and alantilactone at 100 micmol conc. Everin could not be tested because or poor dissolution. None of the substances showed mutagenic activity in the absence of radiation.

The Compostiae substances which are involved in phototoxic reactions are presumably not the oleoresins but polyacetylenes and thiophene derivatives (4,8). Alphaterthienyl and phenylheptatriyne among other biomolecules, can damage structural membrane proteins in vivo as well as in vitro (1,2,3) but the main substances responsible for photosensitive reactions in patients are to a great extent unknown. The aromatic lichen acids are UV-absorbers and can induce photohemolysis (13). It has also been demonstrated that they

Table III. Positive patch tests to plant extracts and isolated compounds in 55 patients with verified contact allergy to cosmetics.

	n reactions
Lichen mixture (in petr.)	55
Oak moss (mousse de chène) abs. 2%	35
Cetraria Islandica extr. 2% in petr.	27
Isolated lichen acids (in total 28 reactions)	
Atranorin	21
Usnin	9
Evernic acid	12
Physodes and physodalic acid	6
Vulpinic acid	3
Lecanoric acid	3
Thamnolic acid	2
Barbatic acid	2
Psoromic acid	1
Fumarprotocetraric	1
Gyrophoric acid	1
Different Compositae extr.	14 (total)
Anthemis tinctoria	12
Tanacetum vulgaris	7
Chrysanthemum leucanthemum	4
Senecio vulgaris	4
Artemisia vulgaris	3
Erigeron acre	2
Achillea millefolium	2
Solidago virgaurea	2
Centaurea cyaneus	1

can act as photosensitizers, although weakly in humans (6,7).
In the present study we have been able to show that plant extract particularly from lichens but also from Compositae, are important allergenic constituents in cosmetics. They also possess cytotoxic potential presumably through membraneous damage.

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OCCUPATIONAL CONTACT DERMATITIS AND CONTACT URTICARIA CAUSED BY EPOXY RESINS

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ABSTRACT

Over a ten-year period (1974-1983) 542 cases of allergic occupational contact dermatitis were diagnosed in our clinic. Epoxy resins caused 71 cases (13.1%) of occupational allergic contact dermatitis (CD), and three cases (0.6%) of irritant CD. One contact urticaria was caused by an epoxy resin hardener, methyl hexahydrophthalic anhydride. Epicutaneous testing with standard epoxy resin 1% in pet. (mainly oligomer of molecular weight 340) produced allergic reactions in all the patients with allergic CD with the exception of three. These three patients had allergic reactions only to their own resins. Testing the 71 patients of allergic CD with epoxy hardeners produced allergic reactions in 19 (26.8%). None of the patients had separate delayed contact allergy to epoxy hardeners without simultaneous contact allergy to standard epoxy resin.

Key words: occupational allergy - occupational skin diseases - exposure - diglycidylether bisphenol A - molecular weight - solid epoxy resins - high molecular weight epoxy resins - methyl hexahydrophthalic anhydride - open test- epicutaneous test.

Six of the allergic CDs and all three irritant CDs caused by epoxy resins were confirmed to be due to diglycidylether bisphenol A (DGEBA) epoxy resins with an average MW above 700 (HAMW epoxy resins). The causative products contained from 6% to 100% of DGEBA epoxy resins, and the amount of oligomer of MW 340 was betwen 0.2% and 15.0%. None of the nine patients had handled epoxy resins with an average MW below 700. All six patients with allergic CD had allergic reactions when tested with standard epoxy resin. Thus the patients had become sensitized to the oligomer of MW 340, and the sensitivity was due to contact with HAMW epoxy resins dissolved in organic solvents. The solvents probably promoted sensitization to oligomer of MW 340 via skin penetration, even though the amount of oligomer was low. All three patients with irritant CD had handled solid epoxy resin powder of HAMW. High average molecular weight epoxy resins should also be regarded as potential sensitizers, and they should be marked with labels specifying the amount of oligomer with an MW of 340, contrary to the EEC directive.

1. INTRODUCTION

Over 90% of epoxy resins are made by the reaction of epichlorohydrin and bisphenol A and they are called diglycidylether bisphenol A (DGEBA) epoxy resins (1). DGEBA epoxy resins belong to the most common causes of occupational allergic contact dermatitis. The capacity of DGEBA epoxy resins to cause sensitization depends on molecular weight (MW). The oligomer of MW 340 is a strong allergen (2). Epoxy hardeners, especially aliphatic amines, and reactive diluents in epoxy products are moderate to strong sensitizers (3,4).

In the present report we have analyzed the cases of contact dermatitis and contact urticaria caused by epoxy products during a ten-year period. Special attention was paid to find out whether resins of high average MW can be considered safe from the allergenic point of view as recently stated by the Association of Plastics Manufacturers in Europe (APME) Epoxy Resin Committee according to the EEC Directive (5).

2. MATERIALS AND METHODS

2.1. General

The occupational epoxy dermatoses were found amongst 2484 cases of skin diseases that were investigated and diagnosed at our clinic during a ten-year period (1974-1983). 1082 were occupational, and 1402 were non-occupational. Among the occupational cases there were 542 cases (50.1%) of allergic contact dermatitis, 510 cases (47.1%) of irritant contact dermatitis, 12 cases of contact urticaria, and 18 cases of other skin diseases. The details are published elsewhere (6)

2.2. Epicutaneous testing

Every patient with contact dermatitis has undergone epicutaneous testing using at least the European standard series including epoxy resin 1% in petrolatum. From 1974 to 1981 the test substances were bought from Trolab, Denmark, and thereafter from Epikon Oy, Finland.

Patients who had been exposed to epoxy products or to other plastic materials were tested also with our series of plastics and glues including three epoxy hardeners, ethylene diamine (1% pet.), triethylene tetramine (0.5% pet.), and 4,4´-diaminodiphenylmethane (0.5% pet.) (1974-1980 from Trolab, 1981-1983 from Epikon Oy). Bisphenol A (1% pet.) and epichlorohydrin (1% pet.) were also included in the series of plastics and glues from Epikon Oy. Reactive diluents were not tested separately.

Testing with patient´s own products was also performed. These substances were diluted in petrolatum, water, ethanol, or acetone. The initial test concentration was usually 1-10%, but dilution series were used, if irritant reactions were suspected.

Positive allergic non-standardized test substances were patch tested with the same concentration on 10-20 controls.

The patch tests were made by the Finn-chamber method (7) with an occlusion time of 24 hours. The tests were applied on the back and read on removal and 24h, 48h, and 96-120h after removal (at least three readings). All readings were made by a dermatologist.

2.3. Prick tests

Prick tests were done with 20 environmental allergens (prepared by Dome, England, Bencard, England, and Allergologisk Laboratorium A/S, Denmark).

2.4. Test performed to detect contact urticaria

Open tests were performed with a mixture of epoxy resin, hardener, accelerator and colorant, with the mixture without hardener, and separately with all components of the mixture. The test substances were undiluted and applied on the antecubital area (0.5cm x 1.0cm) of the arms. The tests were read after 20 min. The open test with the undiluted hardener was also performed on 12 control persons.

2.5. Determination of oligomer of MW 340 in epoxy resins

Amounts of oligomer of MW 340 in one epicutaneous test substance batch of epoxy resin from Epikon Oy, and patients´ epoxy products (table 2) were analyzed in Turku Regional Institute of Occupational Health using a high-performance liquid chromatography (HPLC) method (8,9).

3. RESULTS

3.1. General

During the ten-year period 74 cases (55 men, and 19 women) of occupational contact dermatitis, and one case of contact urticaria (woman) caused by epoxy products were diagnosed. 71 cases were allergic and three irritant contact dermatitis. Thus epoxy products caused 13.1% (71 out of 542) of occupational allergic contact dermatitis during the decade, 0.6% (3 out of 510) of irritant contact dermatitis, and 8.3% (1 out of 12) of occupational contact urticaria cases.

3.2. Contact dermatitis

3.2.1. Patch test results

3.2.1.1. Allergic contact dermatitis

Among the 1082 cases of occupational skin diseases, standard epoxy resin (mainly oligomer of MW 340*) produced allergic reactions in 83 (7.7%). In 68 cases the epoxy allergy was relevant to the occupational exposure and dermatitis, and in 15 cases the primary causes were other than epoxy resins (table 1). Three had relevant allergic reactions only to their own epoxy resin products. One of these products was a heterocyclic dimethylhydantoin-based epoxy resin (1% pet.), one was an insulating tape containing a novolac epoxy resin (undiluted), and one was an epoxy primer (1% pet.) of which we could not get any specific information. We did not analyze the resins in these commercial products.

19 of 71 cases with allergic epoxy dermatitis were allergic to epoxy hardeners: one to ethylenediamine, three to triethylenetetramine, six to 4,4´-diaminodiphenylmethane, and nine to patients´ own hardeners

TABLE 1 Cases of occupational skin diseases with allergic patch test reactions to epoxy resin (1% pet.). Primary diagnosis other than epoxy dermatosis. Cases diagnosed during 1974-1983.

Diagnosis	Main cause	Number of cases
Eczema allergicum	rubber chemicals	6
-"-	chromium	5
-"-	glues	1
-"-	formaldehyde	1
-"-	plastic gloves	1
Exzema toxicum	wet and dirty work	1

only. No cases with separate allergic contact dermatitis to epoxy hardeners without simultaneous contact allergy to standard epoxy resin were diagnosed.

Patch tests with bisphenol A and epichlorohydrin were negative in all the 71 cases of allergic epoxy dermatitis.

3.2.1.2. Irritant contact dermatitis

In three patients with contact dermatitis all patch tests, i.e. the standard series, the series of plastics and glues, and the epoxy products used at their work, were negative. The diagnosis of occupational irritant contact dermatitis due to epoxy products was made based on clinical findings (contact dermatitis of the hands) and anamnestic data (see below).

3.2.2. Causative agents

The causative agents in the 74 cases of epoxy contact dermatitis were: paints and their raw materials (25 cases), electrical insulation (16 cases), glues (13 cases), manufacture of epoxy objects (9 cases), surface coatings (paint excluded)(6 cases), and construction applications, e.g. materials for repairing cracks in concrete (5 cases).

3.2.3. Exposure time

The exposure time was less than one month in 15.5% (11 out of 71) and 1-12 months in 29.6% (21 out of 71) and more than one year in 54.9%. When the exposure time was more than one year, the patients often had a few days before the first appearance of skin symptoms been accidentally heavily exposed to epoxy products e.g. when the airline of a spray gun had exploded.

Two of the three patients with irritant contact dermatitis had handled epoxy powders for three months and the third for two years before the onset of symptoms.

3.2.4. Contact dermatitis caused by epoxy resins of high average molecular weight (HAMW)

According to the information given by the manufacturers six of the allergic dermatitis cases caused by epoxy resins were caused by HAMW epoxy resins (average molecular weight, MW, above 700). The causative products contained from 6% to 100% of diglycidylether bisphenol A (DGEBA) epoxy resins, and the amount of oligomer of MW 340 was between 0.2% to 15% (table 2).

In all six cases of allergic contact dermatitis, sensitivity was caused by contact with HAMW epoxy resins dissolved in organic solvents. To our knowledge none of these patients had handled epoxy resins with average MW below 700.

All three patients with irritant contact dermatitis had handled epoxy resin powders of HAMW.

3.3. Contact urticaria caused by epoxy hardener.

One case of contact was diagnosed during the ten-year period. The patient was a worker in an electrical factory, who had to fill condensers with a slightly warmed uncured mixture of an epoxy resin, a hardener, an accelerator and a colourant. Within two months she developed urticarial eruptions on her face, neck, breast and arms. Two weeks after changing her job the skin eruptions disappeared.

Open tests with the filling mixture, and the hardener tested separately were positive (small urticas on test area), but tests with all the other components, both mixed and separately applied, were negative. Prick tests were negative, too. Tests with the undiluted hardener in 12 control patients were all negative.
The epicutaneous tests with standard series, series of plastics and glues, and the patient´s own epoxy resin (1, 0.5, and 0.1% pet.), hardener (1% pet.), accelerator

* *One test substance batch of standard epoxy resin (1% pet.) analyzed by HPLC contained 0.55% of oligomer of MW 340.*

Table 2. Analysis of patients with contact dermatitis caused by epoxy resins of high average molecular weight (HAMW).

Case	Gender	Diagnosis	Occupation	Causative products with epoxy resin of HAMW		
				product	epoxy resin (%) 1)	oligomer MW 340 (%) 2)
1	man	e. allergicum	spray painter	two-component paint No. 1	19	1.8
				" 2	16	1.8
				" 3	30	2.8
				" 4	19	1.8
2	man	"	"	" 5	15	0.2
3	man	"	"	" 6	25	1.9
				" 7	25	1.9
4	man	"	process man	solid epoxy resin No. 1	100	15.0
				" 2	100	1.1
				" 3	100	11.9
5	woman	"	painter	two-component paint No. 1	19	1.8
				" 3	30	2.8
				" 8	6	0.6
6	man	"	spray painter	" 6	25	1.9
7, 8	man	e. toxicum	process man	solid epoxy resin (powder) No. 1	100	15.0
				" 4	100	3.2
				" 5	100	5.1
				" 6	100	2.9
9	man	"	spray painter	" 7	100	0.3

1) According to the material safety data sheet
2) Determined by HPLC in Turku Regional Institute of Occupational Health, Finland

(0.5% pet.), and colourant (1% pet.) were all negative.

The causative hardener, according to the material safety data sheet, was methyl hexahydrophthalic anhydride.

4. DISCUSSION

Epoxy resins were found to be one of the most frequent causes (13.1%) of occupational allergic contact dermatitis. Only chromium (19.9%9 and rubber chemicals (18.8%) are more frequent causes of the cases at our institute (6).

All the cases (1082) of occupational skin diseases were tested with epoxy resin 1% in petrolatum. The incidence of sensitivity was high, 7.7% reflecting the selection of patient material at our clinic.

The contact allergy usually developed within a few months. In several cases allergic epoxy dermatitis developed after accidental contact with epoxy resin. Therefore it is very important to take care of even small wounds and abrasions of the skin and immediately to change clothes which have become contaminated with (liquid) epoxy products.

The most frequent causative agents for epoxy dermatitis were paints and the raw materials of paints. This reflects the fact that most (80-90%) of the epoxy resins imported to Finland are used in paint factories.

Among our cases of allergic contact dermatitis due to epoxy products we did not detect any cases with isolated contact allergy to epoxy hardeners or reactive diluents. All were sensitized to the resin component. One reason for this is that we had only three hardener components and no reactive diluents in our series of plastics and glues. Nine patients reacted only to their own hardener components, but none of them to the three hardeners in the series. However, testing only with patients' own products is not completely reliable, because the allergens may become too much diluted, producing negative patch tests. In 1984 we changed our series of plastics and glues, and since then diethylenetriamine (DETA)(1% pet.) is included in the series. We have already had one case of allergic contact dermatitis from an epoxy paint with an isolated contact allergy to DETA without sensitivity to epoxy resin. Recently reactive diluents were also included in the series of plastics and glues.

A few cases of allergic contact dermatitis due to HAMW epoxy resins have already been reported (10,11). In our material six patients who had not handled epoxy resins with average molecular weight below 700 had allergic reactions when tested with standard epoxy resin. The patients had obviously become sensitized to the oligomer of MW 340. The sensitivity was due to contact with HAMW epoxy resins dissolved in organic solvents. The solvents probably promoted sensitization to oligomer of MW 340 by increasing skin penetration, even though the amount of oligomer was low. High average molecular weight epoxy resins should also be regarded as potential sensitizers, and they should be marked with labels specifying the amount of oligomer with an MW of 340, contrary to the EEC directive (5).

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ORGANIC PIGMENTS IN PLASTICS CAN CAUSE ALLERGIC CONTACT DERMATITIS

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ABSTRACT
A short review on organic pigments in plastics as a cause of allergic contact dermatitis is presented. Previously, organic pigments have been reported as provoking allergic pigmented contact dermatitis when used in cosmetics. Here we present the case of a patient who developed allergic contact dermatitis from an organic pigment (Irgalite Orange F2G) in a plastic glove. This shows that organic pigments in plastics can also cause allergic contact dermatitis. The potential sensitizing capacity of organic pigments should be noted.

Key words: organic pigment - plastic - colourant - gloves - Pigment Orange 34 - Irgalite Orange F2G - additives in plastics - occupational dermatosis - allergic contact dermatitis - occupational allergy

1. INTRODUCTION

Pigments are inert, stable colourants, and unlike dyes, insoluble in the medium in which they are used. Both inorganic and organic pigments are used in plastics. Compared to the inoranic, organic pigments generally have superior brilliance and tinctorial strength, smaller particle size, but lower resistance to heat and light (1).

Organic pigments are used not only in plastics but also in coatings, e.g. paints, and printing inks, in cosmetics, e.g. lipsticks, and in the colouring of rubber. In the textile industry, organic pigments are used in pigment printing using a resin binder, or in certain pastes used in textile printing (2).

2. CONTACT ALLERGY CAUSED BY ORGANIC PIGMENTS IN PLASTICS

An organic pigment (C.I. Pigment Red, C.I. 15800) in cosmetics has been reported to provoke allergic pigmented contact dermatitis (3,4). We have not found any other reports of allergic contact dermatitis due to organic pigments in plastics apart from our own which is reported more fully elsewhere (5).
Our patient was a female cleaner, who had previously been sensitized to her rubber gloves. Becaused of rubber sensitivity the patient had used unlined plastic gloves, and within a year a new phase of typical sharpbordered glove eczema was found on her hands and arms. She had worn the gloves without inner gloves.

A patch test with the patient´s polyvinyl chloride (PVC) plastic glove produced a weak allergic reaction. Testing with the individual components of the glove´s material gave no allergic reactions, except for the pigment which showed two plus (2+) allergic reactions at concentrations of 2% and 0.5% in petrolatum.

The sensitizing pigment in our case (5) was Irgalite Orange F2G manufactured by Ciba-Geigy. It is also sold under many other trade names (table 1). The generic name of the pigment is C.I. Pigment Orange 34, and the constitution number is C.I. 21115. The chemical structure is seen in Fig. 1.

Pigment Orange 34 is based on 3,3´-dichlorobenzidine, and the coupling agent is phenyl methyl pyrazolone. It belongs to the disazo pigments, and is also known as a pyrazolone orange (1).

Pyrazolone oranges are low in cost and have good strength, but poor lightfastness and bleed resistance (7). Bleeding of the pigment and the poor state of the skin after contact dermatitis obviously promoted the development of sensitivity in this case.

Pyrazolone oranges can also be used in plastics other than PVC, e.g. cellulosic, polyethylene, polystyrene, phenolic, and polyester plastics (1). These disazo pigments are also widely used in the manufacture of printing inks and in the mass colouration of rubber(2).

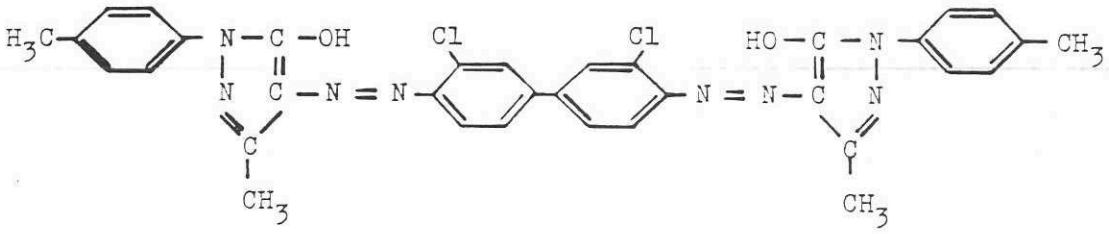


Fig. 1: Chemical structure of Irgalite Orange F2G (C.I. Pigments Orange 34, C.I. 21115) (6)

Table 1.

Trade names of C.I. Pigment Orange 34 (C.I. 21115) (6)
(The names of manufacturers are listed in Corour Index)

Aquarine Orange ONW
Benzidine Fast Orange 600
Diarylide Orange BO-55, BO-55D
Fanchon Orange YB-5834, YB-5835
Helio Fast Orange GR
Iragon Orange P-4S
Irgalite Orange F2G, F2GX
Irgaphor Orange R2G
Isol Diaryl Orange GT 7592, GX 7591
Light Orange JFG
Majestic ORange X-3082
Novopeim Orange HL
Orange B, BS, BY
PV-Orange RL
Roma Pigment Orange B, BS, By
Romasperse Orange BA, BSA
Sanyo Pigment Orange FL
Sico Fast Orange D2850, K2850
Tinolite Orange MG
Unisperse Orange G-P

3. DISCUSSION

Plastics consist of large molecules, polymers (usually synthetic), and other ingredients, such as fillers, colourants and plasticizers. Plastics normally only produce allergic contact dermatitis when monomers or other starting materials, or semicured products are handled. In general completely cured synthetic polymers are not allergenic. Exceptions are urea formaldehyde polymers which give off formaldehyde. Plastics, however, can contain leachable additives causing contact allergy (8). This report shows that organic pigments are among these potentially sensitizing leachable additives in plastics.

The same organic pigments can be used in both plastics and rubbers (2). Organic pigments could be one explanation for those cases of plastic and rubber glove eczema in which only the materials of the paricular glove concerned have given positive patch test reactions (9,10).

A patient who had allergic reactions in epicutaneous testing with a dye used in plastics, produced negative patch test reactions with finished plastic products, obsiously owing to the fact that the dye was completely embedded in the plastic material (11). Thus only organic pigments (and dyes) which are completely fixed in the plastic materials should be used. If possible, the colourants should also be included in the patch test series whenever positive reactions to any plastic materials have been obtained.

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CONTACT URTICARIA FROM RUBBER GLOVES: A DETAILED DESCRIPTION OF FOUR CASES

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ABSTRACT

Four cases of contact urticaria from rubber gloves are presented in detail. Three of the four patients, including two atopics had present or previous hand dermatitis. The causative gloves were made of natural rubber. Interestingly, one patient also reacted to her synthetic nitrile rubber gloves which the distributor had sold her as plastic gloves, and explanation for this case is discussed. Contact urticaria should be taken into consideration when there is recalcitrant hand dermatitis in spite of regular use of polymeric protective gloves. Manufacturers should give more accurate and reliable information on the composition of the glove materials to distributors and consumers.

Key words: *natural latex - synthetic nitrile rubber - hand dermatitis - additives - provocation test - latex prick - coca solution*

1. INTRODUCTION

Urticaria caused by rubber products has been known since at least 1927, when a case of chronic urticaria from a rubber prosthesis was described (see ref. 1). Rubber has also been presented in some cases as a cause for Quincke´s oedema and asthma (see ref. 1). But it was not until 1979 that the first case on contact urticaria due to rubber gloves was reported (2). Since then further reports have been published (3-10).

Contact urticaria from rubber gloves is an immediate reaction provoked by close skin contact with the gloves. It may be an immunological reaction, and involves whealing and flare of the skin contact area in 10-30 min. Sometimes the skin reaction is followed by other symptoms including eye irritation, rhinitis, asthma, and even anaphylactic reactions. Recently two patients who suffered from anaphylactic reactions: generalized urticaria, bronchial obstruction, and hypotonia to surgeons gloves used during their own operations, have been described (8).

Here we present a detailed description of four cases of contact urticaria from rubber gloves. Two of these cases are included in our recent report on occupational contact dermatoses due to rubber and plastic gloves during a ten year period 1974-1983 (10).

2. MATERIALS AND METHODS

2.1 Report on cases

The dermatological analysis, occupations, use of causative gloves and information on glove materials are given in Table 1. All the four patients were women. Three of them suspected their protective gloves as a primary cause or a cause for worsening for their hand dermatitis. The fourth had no suspicions of the gloves. Two of the patients cases (1 and 3) had always had the same job where protective gloves are necessary every day. The two others had earlier had jobs in which protective gloves were not needed.

Case 1, an industrial worker had itching, urticarial eruption in her hands only when she used these household natural rubber gloves at work. At the same time she had had occasional rhinitis. For some months she was able to work with the mild eruption when she used separate inner gloves made of cotton. However, gradual worsening of the skin symptoms obliged her to stop using the gloves. She had neither personal atopy nor atopy in her family. The skin of the hands was healthy at the time of examinations.

Case 2, a construction worker, had had hand dermatitis periodically for six years. She also suffered from periodic dermatitis on other parts of the body and extremities. Her hands became swollen, and itchy only when

she used the gloves without inner gloves. She had atopic rhinitis, but had never had atopic dermatitis. Her mother was allergic to animal epithelia. The patient had dermatitis on the backs of her hands and on shoulders, neck, upper trunk and legs, corresponding to clothing contact areas.

Case 3, a nurse in a surgical ward, had had periodic hand dermatitis for about 20 years. She had always used different types of surgical disposable gloves at work but was sure that a particular type of gamma sterilized glove was the cause of recent worsening of her hand dermatitis. The dermatitis almost disappeared when she stopped using the gloves. She had had atopic dermatisis in childhood. Her three brothers had atopic rhinitis or asthma. At the time of investigations the skin of the hands was merely dry and rough.

Case 4, an industrial cleaner, had neither atopy herself nor atopy in her family. She commenced using protective gloves after suffering from dermatitis of the hands. The first gloves were household natural rubber ones used without separate inner gloves. Because of permanent hand dermatitis, which persisited in spite of regular use of rubber gloves, the gloves were changed. The second gloves were industrial nitrile gloves which the distributor had sold as plastic gloves. The condition of the hands still worsened although the patient wore separate cotton inner gloves. She had dermatitis of the fingers and hands when she came for investigations. The dermatitis was thick and scaly with rhagades on the back of the hands.

2.2 Investigations

Investigations included, apart from anamnesis, inspection of the skin, provocation and skin tests. Provocation, epicutaneous and prick tests with common environmental allergens were performed on all patients. Specific prick and scratch chamber tests were made only on the fourth patient. No scratch chamber tests with rubber additives were made. Testing was carried out during repeated visits to our department, depending on the state of the skin and amount of tests to be done.

Provocations tests included wearing of the gloves with a little water inside to promote release of allergens to the skin. The exposure lasted 30 min.

Prick tests were made with 24 standard allergens (Allergologisk Laboratorium A/S, Denmark). In case 4 a prick test was made with an antigen prepared from 60% Malaysian natural rubber latex (NR).The white milky latex liquid was obtained from a Finnish rubber factory. The latex antigen solution was prepared with the routine cell prick method at Allergologisk Laboratorium (Alk) in Denmark. A prick test was also performed with an antigen made by ourselves using coca solution to dilute latex liquid (11). Coca solution contains 0.4% (w/w) phenol. In addition, scratch chamber tests with pieces of the causative gloves (natural and synthetic nitrile) were performed. The tests were applied on a superficial scratch on the patient´s back testing the inside and outside of the glove separately, and read after 30 min.

Epicutaneous test contained the ICDRG (International Contact Dermatitis Research Group) standard series (Epikon Oy, Helsinki, Finland), an ICDRG rubber series and an ICDRG series for plastics and glues (Epikon Oy or Chemitechnique Diagnostics Ab, Malmö, Sweden). The series of plastics and glues was supplemented with textile resins (Trolab, Denmark) in case 3. Pieces of the materials of the causative gloves were tested as such. Other epicutaneous tests were made, depending on anamnesis, and these included a series of antimicrobials (Epikon Oy). In case 4 a commercial detergent solution used every day at work (5-2% w/w) and all components of the solution (1-10% w/w) were tested in petrolatum, with exception of the carbamide component, which was mixed in water. The preservative, chemically 2-chloracetamide, was tested in dilution series 0.33, 0.1, 0.033% w/w. The epicutaneous tests were applied on the patients´ back using the Finn Chamber method and 24 h occlusion time (12). Glove materials were left on the skin for 48 h. The tests were read 24 - 48 - and 96 - 120 h after the application of the tests.

4. RESULTS

The results of provocation and skin tests are seen in table 2. All provocation tests with suspected natural rubber gloves, and in case 4 also with synthetic nitrile gloves were positive. After 10 min. the patients reported itching, and after 30 min. in case 1 redness and separate urticas, and in cases 2 and 4 worsening of dermatitis with redness and urticas could be seen, while in case 3 there was only redness of the skin. In case 4 a similar reaction was also provoked by the synthetic nitrile gloves (sold as plastic gloves). Provocation tests

with synthetic neoprene gloves were negative in every patient, as were those with PVC gloves in case 4. Thirty minutes after the end of exposure, patients no. 1 and 4 developed rhinitis accompanied by sneezing and eye irritation. An otorhinolaryngologist immediately inspected patient no. 4 and found subjective and objective obstruction of the left nasal duct. Nasal smear showed eosinophilia as a feature of an allergic reaction.

In case 4, scratch chamber tests with pieces of suspected natural household rubber and synthetic nitrile rubber gloves gave positive histamine size reactions. The prick test with natural latex antigen (ALK) also gave a positive histamine size reaction. The test with antigen dilution solution was negative. A prick test with our own latex antigen was also positive. Unfortunately the coca solution (used to dilute the antigen) also gave a positive histamine size reaction. Control scratch chamber tests with glove materials and latex prick tests (Alk) were negative on 10-20 unexposed patients.

Standard prick test were negative in all but one case. Patient no. 2 reacted to some pollens, animal epithelia, and house dust. She had atopic rhinitis and atopy in the family.

Epicutaneous test with causative gloves were negative. In case 3 these were not tested because the patient withdrew from further investigations. Rubber series and series for plastics and glues were negative in all cases tested. Patient no. 2 reacted with positive 2+ allergic reaction to etherfied methylol melamine (10% pet) and also to formaldehyde (1% aq) in standard series. These reactions are probably associated with the textile finishes in her own clothing. She had dermatitis on textile contact areas. She also reacted to 2-chloroacetamide (0.2 w/w) which might be associated with materials used previously at work.

Patient no. 4 reacted with 2+ reaction to a detergent solution she had used daily at work. The causative agent was the preservative, 2-chloroacetamide. Tests with the preservative (obtained from the manufacturer) in dilution series and with 2-chloroacetamide gave positive 2+ reactions.

5. DISCUSSION

The development of delayed and immediate reactions to rubber gloves depends on many factors including the quality of the rubbers and the amount and type of additives in the rubber mixtures, the duration of contact with the skin and the health state of skin. Additional factors are profuse persipiration and high temperature, which might promote the release of allergenic materials from the gloves.

Table 1. CONTACT URTICARIA FROM RUBBER GLOVES
An analysis of four cases

CASE	1	2	3	4
Year of diagnosis	1982	1983	1985	1985
Age (years)	33	37	50	49
Sex	female	female	female	female
Occupation	industrial worker	construction worker	nurse cleaner	industrial
Duration of occupation (years)	13	3	25	3
Own/family atopy	-/-	+/+	+/+	-/-
Hand eruption	permanent (10 months)	periodical (6 years)	periodical (20 years)	permanent (18 months)
GLOVES				
Use of gloves	daily (10 months)	daily (3 years)	daily (20 months)	glove 1 daily (1 year) / glove 2 daily (6 months)
Material	natural rubber	natural rubber	natural rubber	natural rubber / nitrile rubber
Type	unlined household	unlined household	disposable surgical	unlined household / unlined industrial
Colour	pale green	yellow	colourless	yellow / bright green
Country of manufactor	?	Australia	Australia	France / Spain

Table 2. RESULTS OF PROVOCATION AND SKIN TESTS

CASE	1	2	3	4
PROVOCATION TESTS				
causative gloves (table 1)	positive	positive	positive	positive (both)
Other gloves industrial neoprene	negative	not done	negative	negative
PVC household	not done	not done	not done	negative
PATCH TESTS				
materials of causative gloves	negative	negative	not done	negative
rubber series	negative	negative	negative	negative
series of plastics and glues	negative	etherified methylol melamine ++	not done	negative
standard series	negative	formaldehyde ++	negative	negative
antimicrobials	negative	2-chloro-acetamide ++	negative	2-chloro-acetamide ++
other	not done	not done	not done	detergent solution ++
				preservative in detergent ++
PRICK TESTS				
standard series	negative	positive to some pollens, animal epithelia, house dust	negative	negative
latex prick (ALK)	not done	not done	not done	positiv

During 1974-1983, 2484 patients, suspected of having an occupational skin diseas were investigated in our clinic (13). Of these only two had contact urticaria due to rubber, which shows that it is a rare disease. Since then we have had two more cases this year (1985).

In our cases the causative gloves were made of natural rubber which corresponds well to earlier reports. Most cases have been attributed to the natural latex component in the glove materials. It has been suggested that protein materials in natural latex are responsible for the reaction (2-6,8). Accordingly, provocation tests were positive with suspected natural rubber gloves but negative with synthetic neoprene rubber gloves, in case 4 with PVC gloves, too. In one positive case (no. 3) only redness of the skin could be seen. Possibly the exposure was too short, compared with real working conditions, to provoke urticas. Two of the patients also developed symptoms of rhinitis during exposure, and this is also in accordance with previous reports (2, 4, 6, 8). Interestingly, one patient (case no. 4) also reacted to her synthetic nitrile gloves with a similar urticaria reaction. Confirming the results of the provocation tests with gloves, scratch chamber tests made with both glove materials and latex prick (ALK) were positive. An attempt to use coca solution to prepare prick test antigen from latex liquid was unsuccesful because the coca solution also gave a histamine size positive reaction. An explanation of the reaction caused by the synthetic nitrile rubber gloves could be that the material contains natural latex as well as syntetic rubber. The causative agent may also be one of the additives used in the rubber mixture. Recently additives have been listed as possible causes for urticaria reaction (9). Tests to demonstrate immediate reactions to rubber additives were not performed because no answer to our inquiry on the composition of these nitrile gloves has as yet been given by the Spanish manufacturer.

Contact urticaria from rubber gloves has been described both in atopics and among those with allergic dermatitis of the hands (4-7). Consequently three of our patients, including two atopics, had present or previous hand dermatitis. In epicutaneous tests none of the patients reacted to the glove materials, nor to rubber additives. Relevant allergic reactions could be found to formaldehyde, etherified methylolmelamine and, in two cases, to 2-chloroacteamide. Allergic reactions from 2-chloroacetamide in ointments, body lotions, glues and paints have been reported (14-15). Three of

the patients had begun to use gloves after they developed dermatitis. Inner cotton gloves did not completely prevent the skin reaction, which is understandable because the material of cotton gloves in usually thin.

Contact urticaria from rubber gloves can be a primary eruption or secondary reaction to hand dermatitis among atopics or among those with no history of atopy. It can be a cause for unexplained rhinitis and sneezing. The possibility of this glove reaction should be taken into consideration in cases where there is recalcitrant dermatitis although the hands are carefully protected with rubber of "plastic" gloves and although separate inner gloves are used. Manufacturers should give more accurate and reliable information on glove materials to distributors and consumers. A provocation test with suspected gloves is probably the easiest and most reliable way to demonstrate contact urticaria from gloves. The investigation should be completed with scratch tests with glove materials, and prick tests with specific latex antigens when possible.

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TOPICAL SUBSTANCES WHICH PREVENT POISON IVY ALLERGIC CONTACT DERMATITS

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Poison ivy and poison oak are the major cause of allergic contact dermatitis in the United States. People at particular risk include workers in logging, agriculture, forest fire fighting, construction, and utility maintenance, as well as outdoor enthusiasts. The allergens in poison ivy and poison oak have been identified as a series of penta-and heptadecyl catachols which vary in the number of double bonds they possess. Our research was directed at developing a means of preventing this dermatitis using a topical preparation. This is similar to how sunscreens are currently used to prevent sun damage.

We have succeeded in developing a closed patch test system for the evaluation of potential protectants. This system has been used to evaluate 156 different preparations which were based on 22 different chemicals. From these evaluations we have identified a family of chemicals consisting of different polyamine salts of a linoleic acid dimer which totally prevent a dermatitis in 50-73% of subjects exposed to a poison ivy extract for 48 hours. In addition, we have demonstrated that 75-100% of subjects tested are protected, if the skin is washed 8-12 hours after the sequential application of the protectant and the poison ivy extract.

Our testing procedure consisted of the application of 15 different potential protectants to designated areas of the back. The protectants had been coded by numbers so as to blind the evaluator. While the protectants were drying, an individually-determined amount of a poison ivy extract was placed on filter paper discs. The amount of catechol equivalents in the extract had been determined previously by Hollister-Stier. The amount of extract to be used in the testing panels had been determined by previous testing of each individual subject. This was done because individuals vary greatly in their sensitivity to the poison ivy antigens, and we wanted to cause a reproducible degree of dermatitis without causing an excessive reaction. Once the protectants were dry, the antigen-containing discs were taped to the centers of each protected area. One disc was taped to an unprotected area in order to act as an antigen control. The tape was removed at 48 hours and the reactions were graded at 48 hours and on Day 7 according to the following criteria. A Grade 1 reaction consisted of macular erythema, a Grade 2 reaction of papules or vesicles, and a Grade 3 reaction showed spreading of the dermatitis.

In most cases the control site reacted vigorously and a number of the other sites reacted as vigorously as the control, suggesting that the potential protectants placed at these sites were not effective. A number of the sites, however, showed a marked diminution of the reaction, and in some cases the dermatitis was totally prevented. This suggested that the substances placed at these sites did have protective value.

Using this method, we found that the most promising preparations we evaluated consisted of a variety of polyamine salts of a linoleic acid dimer. Linoleic acid dimer is the product which results when two linoleic acid chains combine. The polyamines we used are polyoxypropylene amines, which go by the trade name of "Jeffamins". They are designated by their approximate molecular weights. If a polyamine is added to the linoleic acid dimer, its amine groups can react with the carboxylic groups of the dimer, to form a very viscous salt, which is probably a linear polymer.

The results shown were all obtained using various polyamine salts of the linoleic acid dimer. Hereafter, these substances will be referred to as dimer salts.

FIG. 1 EFFECTS OF CONCENTRATION ON PROTECTION

Polyamine -Salts of Linoleic Acid Dimer in Petrolatum

	10% conc #P/T * %	35% conc "P/T %
Jeffamine D230	1/5 (20)	11/15 (73)
Jeffamine D400	1/11 (9)	11/15 (73)
Jeffamine T403	3/10 (30)	12/17 (71)

* *#P/T = number of subjects totally protected from a dermatitis/number of subjects tested*

Figure 1 shows the effect of concentration on the ability of various dimer salts to totally prevent poison ivy dermatitis. If you take the Jeffamine D400 dimer salt as an example, you can see that the percentage of protected subjects increases from 9 to 73% as the concentration of the dimer salt increases from 10 to 35%. Note that the 35% concentrations of these particular dimer salts are able to prevent a dermatitis in approximately 70% of subjects. Appropriate controls with the individual ingredients or vehicles did not prevent the dermatitis.

One might wonder if these preparations are effective because the amines react chemically with the poison ivy antigens. If so, one would expect that the protection would be concentration dependent. To investigate this, we made several preparations with increasing proportions of the polyamine. As you can see in Figure 2, the preparation with a six times greater amount of the amine is not consistently better than the other preparations. This suggests that the preparations are more likely acting as physical barriers, than as chemical detoxifiers.

FIG. 2 EFFECT OF AMINE CONCENTRATION ON PROTECTION

Polyamine Salts of Linoleic Acid Dimer

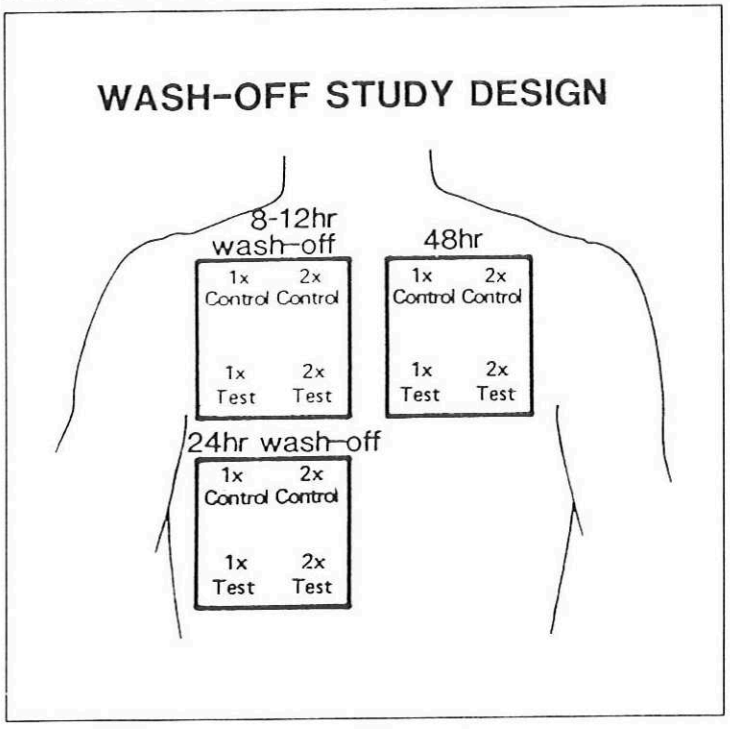
	10% conc #P/T * %	35% conc #P/T %
Jeffamine D400 (1:1) $	1/11 (9)	11/15 (73)
Jeffamine D400 (2:1)	3/10 (30)	5/10 (50)
Jeffamine D400 (6:1)	3/9 (33)	5/9 (56)

* *#P/T = number of subjects totally protected from a dermatitis/number of subjects tested*

$ *Amine: Linoleic Acid Dimer*

I would like to point out that these results were obtained under conditions which are biased against the effectiveness of a protectant. One of the biases is that since we are evaluating 16 different sites simultaneously on the back, a dermatitis at any one of these sites can enhance the tendency of the nonspecific irritability known as the "angry back" phenomenon. A 2nd bias is that the antigen is being held in contact with the skin for 48 hours, ...this is much longer than the usual clinical situation. A 3rd bias is that the test sites are occluded, which enhances penetration of the antigen.

In a 2nd phase of our study, the patch test procedure was modified to take into acount the fact that, in the usual clinical situation, the poison ivy antigen is generally washed off within 12 hours after exposure. This modification consisted of dividing the back up into 3 different regions, with each region containing a control site and a test site (see Figure 3). A control site and a test site in which the dose of the antigen was doubled was also included. This was done in order to see if the protection provided was easily overwhelmed by additional antigen. The protectant and the appropriate amount of poison ivy extract were taped to the back as before. However, one region was washed with soap and water 8-12 hours after application. And the remaining region had the tape removed at 48 hours; as had been done in the initial portion of the study. Figure 4 summarizes the results of a wash-off trial. With a 35% concentration of the Jeffamine D400 dimer salt in petrolatum, there is complete protection of 13 out of 14 subjects of the antigen and the protectant are washed-off within 8-12 hours. In all but one case the control sites had significant reactions, illustrating that the lack of dermatitis in the protected areas was not due simply to washing off the antigen. Note that the persentage of protected subjects drops significantly as the amount of time that the antigen remains in the skin increases. Note also, that a similar degree of protection was provided when the dose of the challenging extract was doubled.



The fact that the incidence of dermatitis increases as the protectant and antigen remain on the skin for longer time intervals also suggests that the protectant may be acting as a physical barrier rather than as a chemical detoxifier. To further investigate this idea, a mixing experiment was performed.

During this test, the protectant and the antigen were mixed together prior to placing them on the back. The ratio of the protectant and the antigen was varied, as was the time interval between mixing the substances and patch testing. Twenty-four hours prior to patch testing, the protectant (Jeffamine D230, 35% in ethanol) was mixed with the antigen in four different rations —15:1, 5:1, 1:1, and 1:5. The 1:1 ratio represented the amount of each agent normally used in the screening evaluations. This mixing was repeated at 2 hours prior to and at 15 minutess prior to patch testing. The mixtures were then placed on the back and readings were taken at 48 hours and 7 days, as usual. Figure 5 shows the results form this study, using 7-8 subjects. The numbers represent the percentage of patients totally protected from a dermatitis. Essentially all of the patients were protected with the highest ratio of protectant and no protection was provided at the lowest ratio of the difference. These results also seem most consistent with the protectant acting as a physical barrier, as had been previously suggested.

FIG. 4 PROTECTION DURING WASH-OFF TRIAL

35% Jeffamine D400 Salt of Linoleic Acid Dimer in Petrolatum

	Usual amount of extract		Twice the amount of extract	
	#P/T *	%	#P/T	%
8-12 hour wash-off	13/14	(93)	14/14	(100)
24 hour wash-off	9/14	(64)	8/14	(57)
48 hour	6/14	(43)	6/14	(43)

* *#P/T = number of subjects totally protected from a dermatitis/number of subjects tested*

FIG. 5 MIXING EXPERIMENT RESULTS*

	15 min	2 hr	24 hr
Protectant: Antigen			
15:1	88	100	100
5:1	71	86	57
1:1	37	25	50
1:5	0	14	0

* *% patients protected from dermatitis*

Finally, some very preliminaty tests have been performed to see if the protectant is effective in patients who are allergic to other allergens. We have evaluated one patient who is allergic to Neomycin and one who is allergic to paraphenylene diamine. As before, the severity of their allergy was evaluated prior to testing the protectant. In neither instance was any protection demonstrated, suggesting that, although the protectant appears to be acting primarily as a physical barrier, it is probably limited in the type of substances it will hold onto. Further testing with additional allergens obviously needs to be done.

In conclusion, we have demonstrated that the prophlactic use of several different polyamine salts of a linoleic acid dimer, applied just prior to a 48 hour exposure to a poison ivy extract...totally prevents the usual allergic contact dermatitis in 50-73% of subjects. In addition, it has been found that washing off the poison ivy extract and the protectant 8-12 hours after application, prevents a dermatitis in 75-100% of subjects.

Much more work obviously remains to be done on both the mechanism of action and the longterm efficacy and toxicity of these substances. However, we have found these preliminary results to be extremely encouraging.

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A NEW SUBSTANCE TO REDUCE ROUGHNESS OF HUMAN SKIN

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Abstract

The influence of a new chemical compound consisting of glucuronic acid and urea on skin roughness has been investigated. Using the method of Padberg we demonstrated a prolonged and significant reduction in skin roughness. In a forced prophetic test we observed no allergic reactions in our sample. The reduction in skin roughness has been detected in a double-blind-study by the examination doctors as well as by the patients themselves using different criteria. Three-year studies of the stability of the drug showed that in appropriate vehicles there is no deterioration. This compound both supplements the functions and provides excellent care for human skin.

The smoothness of the skin is dependent on the water content of the stratum corneum and of the cutis. The water binding capacity of the stratum corneum is further dependent on a compound of sebum, amino acids and the physical properties of the scales. The water binding of the cutis depends on its content of mucopolysaccharides. One of the most important substances is the glucuronic acid, which stabilizes the collagen and elastic fibers and the water content of the skin, and possesses antibacterial properties. Because of its natural origin and its non-allergic property we developed a

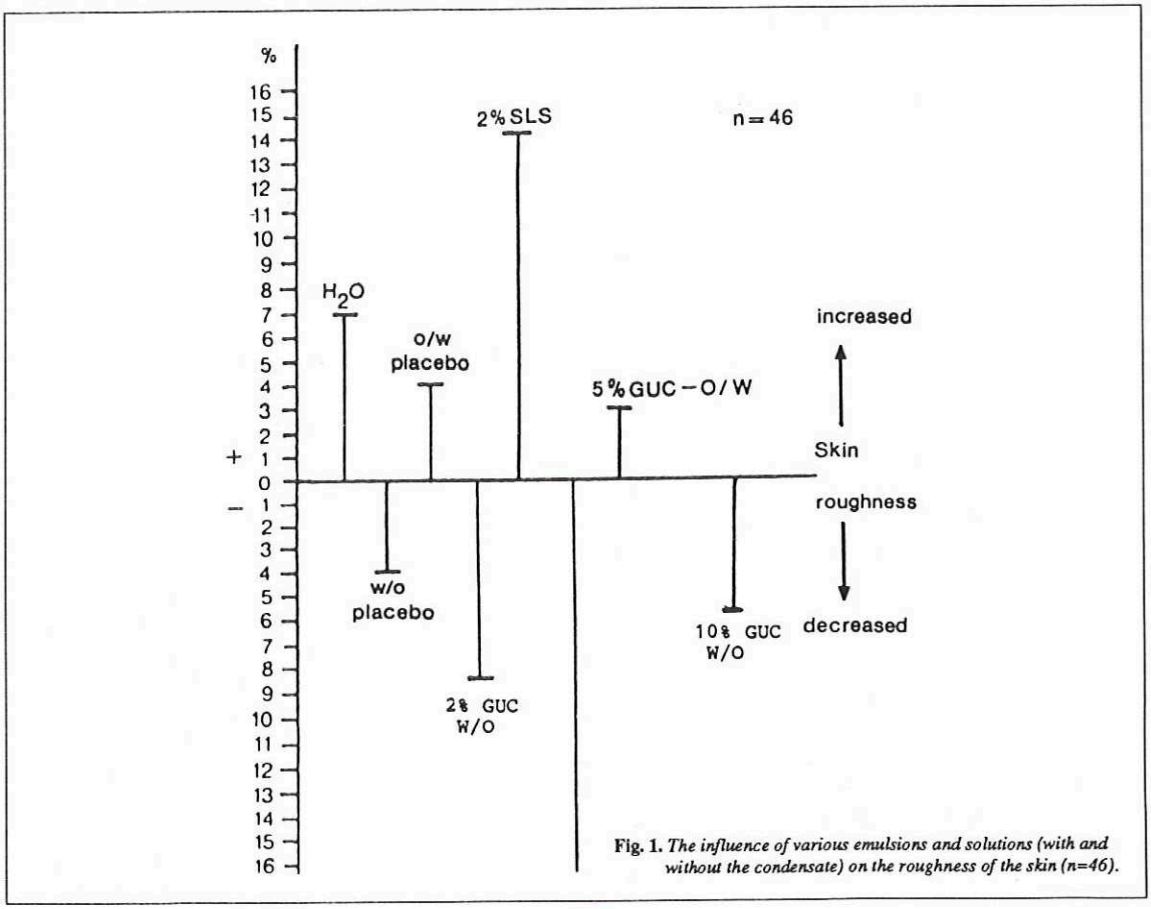


Fig. 1. *The influence of various emulsions and solutions (with and without the condensate) on the roughness of the skin (n=46).*

condensate consisting of glucuronic acid and urea. Urea is well known for its water binding capacity.

In the area of the forearm we investigated the roughness of the skin under the influence of various water in oil and oil in water emulsions containing different concentrations of glucuronic acid and urea, as well as the condensate and various cosmetic creams.

We measured the roughness of the skin using the method described by Padberg in 1969. The change in the roughness of the skin was evaluated by the methylene blue test. The concentration of methylene blue, retained by the stratum corneum and later eluted, is used as a measure of the skin roughness.The eluate was measured in a special photometer at 660nm.

We could observe a direct relationship between the increasing skin concentration of glucuronic acid and the decreasing skin roughness. The later effect was even more apparent when using the condensate. If the concentration of this compound was increased more than 5% we observed a reverse effect which caused irriation of the skin. The effect was visible and could be measured histologically. Fig. 1 shows the influence of various emulsions and solutions with and without the condensate on the roughness of the skin in a sample of 46 volunteers. When using 2% sodium Lauryl sulfate solution the roughness increased markedly: When using various emulsions we could observe a diminished roughness depending on the concentration of condensate. The optimum of smoothness was achieved by the application of an W/O-emulsion of 5% condensate.

In another experiment of 50 patients, including normal controls as well as patients suffering from eczemas, we performed repetitive patch-testing with various emulsions containing increasing concentrations of the condensate. The tests were carried out as a patch test in that the test substance was applied to the skin for 24 hours repeatedly for 3 weeks. Starting on a Monday, the reaction was read off 4 days later; the second test was applied to the skin on Thuesday and was read off 3 days later and the third test was applied on Wednesday and read off 2 days later. The same procedure was repeated in the second as well as in the third week in the same test area. No experimental sensibilisation could be found under these conditions.

Four model emulsions were handed out to 4 different groups each consisting of 17 volunteers, with healthy or impaired skin. Each was asked to apply the same quantity of the emulsion supplied, in the same way once or twice a day on the healty or impaired skin of left upper arm. The participants received questionaries

1. side effects:

1.1.	itching
1.2.	redness
1.3.	formation of knots
1.4.	formation of pimples
1.5.	rougness of the skin
1.6.	no changes caused by the application of the emulsion on the skin

2. appearance of the skin:

2.1.	smoother
2.2.	softer
2.3.	more elastic

3. emulsion on the skin:

3.1.	pleasant
3.2.	unpleasant

4. distribution of the emulsion on the skin:

4.1.	good
4.2.	bad
4.3.	can not judge

5. odour of the emulsion:

5.1.	pleasant
5.2.	unpleasant
5.3.	no opinion

6. influence on impaired skin:

6.1.	not better
6.2.	a litle better
6.3.	much better
6.4.	worse

7. interruption of the treatment on account of:

7.1.	allergic reaction
7.2.	incompatibility of various kind
7.3.	unpleasant odour

Questionary to be answered by the subjects. **Table 1**

(Table I) concerning side effects about the appearance of the emulsion, the distribution and odour of the emulsion on the skin and the influence on impaired skin. The test method was performed in such a way that the attention of the clients was diverted from the main intention, i.e. the reduction of the skin roughness, by asking them to judge other qualities of the model emulsions. All results obtained from the participants, who had used the model emultions containing the condensate, showed that 68% indicated that the skin was smoother, 27% indicated a softer skin, 18% indicated a more elastic skin, 8% were of the opinion that the

cream could easily be distributed, that the odour and the sensation on the skin were pleasant.
The accuracy with which the patients selected emulsion containing the condensate from the samples distributed was unlikely to be coincidental, either from a statistical point of view or from a clinical one, and rather confirms the effect of the glucuronic ace-urea-condensate on skin-roughness.

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