DISCUSSION

This study showed that in the normal epidermis, in contrast to the dermis, Ia- and OK T6 antigens are expressed mainly by the same dendritic cells. It also showed that in those MF lesions where no epidermal dendritic cells expressing Ia antigens could be detected, OK T6 antibody reactive dendritic cells were still unaffected. One possible explanation for the epidermal intercellular Ia pattern seen in these patients and in other MF cases described before (11, 14) could be that Ia antigens are shedded from Langerhans or other dendritic cells as a part of the disease process. Otherwise the Ia antigens could be shedded from activated T lymphocytes deposited in the skin, since an increased number of anti-Ia-reactive circulating T cells have been noticed in many patients with MF (14).

Another possibility is that, on certain stimuli, keratinocytes might start to synthesize Ia-like antigens (13). The fact that neither extensive washing of the sections before the incubation of the anti-Ia antibodies nor dilution of the antibodies eventually replaced the intercellular pattern with that of dendritic cells might favor this explanation. So might also the earlier findings in single epidermal cell suspensions of Ia-like antigens on cells suggestive of keratinocytes (14).

Besides, in some cases MF (11, 14), we have earlier observed the epidermal intercellular Ia pattern also in acute lichen planus (12), while others have noted anti-Ia-reactive keratinocytes in graft-versus-host disease (2, 3) in the rat. Interestingly, lichen planus-like lesions indistinguishable from the idiopathic form can be seen as the skin manifestations of graft-versus-host disease in man (9).

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Thiol Levels in Normal and Psoriatic Corneocytes

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Abstract. A technique is described for obtaining small samples of corneocytes suitable for chemical analysis. Thiol and protein levels have been measured in water- and detergent-extracts of corneocytes from healthy controls,

psoriatic uninvolved skin, and psoriatic lesions; no differences in thiol: protein ratios were found. This observation does not support earlier views regarding the chemical defect in psoriatic keratin.

Key words: Thiol; Psoriasis; Corneocyte

Although sellotape stripping is an established method in experimental pathology, the use of the adherent corneocytes for chemical analysis of the skin surface has remained unexplored. During an investigation along these lines we have measured thiol (sulphydryl) and protein levels in extracts of corneocytes from normal and psoriatic epidermis. Our findings, which do not support earlier concepts, are presented below.

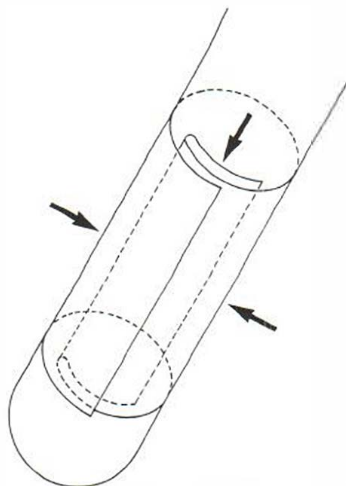

Fig. 1. Method of mounting sellotape on the test-tube. Arrows indicate glued surface.

MATERIALS AND METHODS

Materials

Monobromobimane (Thiolyte ™MB) was obtained from Calbiochem-Behring Corp., La Jolla, Calif., USA. All other chemicals were of analytical quality and were purchased from Merck (Darmstadt, Germany). The sellotape employed for stripping was 'Tesafilm' (2.5 cm wide), and was obtained from Beiersdorf B. V., Aalsmeer, Netherlands. Fluorescence was measured using the 'Fluorispek', Baird Atomic Europe, B. V., The Hague, Netherlands.

Patients with psoriasis had stable, chronic lesions which had been left untreated for at least 2 weeks prior to the investigation. Scales were removed by gentle scraping, and were stored when necessary in an air-tight container at −20°C. Controls were members of the staff of this Department.

Preparation of samples

Weighed samples of scales (1–2 mg) were de-fatted for 30 min each in chloroform, 2 portions of *n*-hexane and ether. After air-drying, the specimens were homogenized in 1 ml water using an all-glass Potter-type homogenizer.

Corneocytes were obtained by sellotape stripping. Sellotape was wrapped around the outside of a Pyrex test-tube (10 mm external diameter) with the glued surface outward as shown in Fig. 1. The tape-covered portion of the tube was rolled, slowly and with a firm pressure, over the selected area of skin. The test-tube was then placed inside a glass centrifuge tube (12 mm inside diameter) containing 1 ml chloroform; a gentle 'pumping' action was sufficient to dissolve the glue and dislodge all adherent cells. After removing the inner test-tube, 2 ml of *n*-hexane was added and the cells pelletted by centrifugation. The pellet was washed by centrifugation with 2×2 ml portions of *n*-hexane followed by 2 ml ether, and was air-dried. The dry corneocytes were finally re-suspended in 1 ml water and homogenized as above.

All homogenates (scale or corneocytes) were centrifuged (20000 *g*, 10 min) and the supernatants transferred to clean tubes. The pellet was re-suspended in 200 μl of 10% aqueous sodium dodecyl sulphate, and the tube incubated for 1 h at 40°C. Following incubation, the sample was diluted to 1 ml with water and centrifuged ('detergent extract').

Chemical assays

Thiol was measured after reaction with the fluorometric reagent, monobromobimane, using a technique modified from Fahey et al. (1981). A stock solution of 10 mmol/l monobromobimane in acetonitrile was diluted to 100 μmol/l in 200 mmol/l borate buffer, pH 9.5, and a 50 μl aliquot of this reagent added to a 500 μl aliguot of the sample (water- or detergent-extract). After 30 min incubation at 37°C the fluorescence was measured (λ_{Ex}= 392 nm, λ_{Em}=484 nm). Appropriate blanks were subtracted, and the thiol content of the sample calculated by reference to a standard of 5 μmol/l glutathione. The tubes for thiol assay were protected from light at all times.

The protein contents of water- and detergent-extracts were determined by direct fluorescence (λ_{Ex}=280 nm, λ_{Em}=360 nm) using bovine serum albumin (100 μg/ml) as reference standard.

RESULTS

Table I shows the absolute amounts of thiol and protein which are solubilized by successive extraction of psoriatic scale with water and detergent. It is seen that the detergent removed a quantity of protein comparable to that extracted into water, the total soluble protein averaging about 85 μg per mg scale, i.e. 8–9% of the starting material. It is striking, however, that the thiol content of the detergent-extracted protein (col. 4) was nearly double that of the water-soluble material.

The thiol/protein ratios of extracts from 'stripped' corneocytes are listed in Table II. There is no significant difference (either water- or detergent-extract) between the values for healthy controls, psoriatic uninvolved skin, or psoriatic lesion, nor is there any difference between these values and the ratio calculated for psoriatic scales (Wilcoxon rank test).

Table II. *Corneocytes: thiol/protein ratios (nmol thiol per mg protein) for water- and detergent-extracts (numbers of specimens are given in parentheses)*

Extract	Healthy controls (6)	Psoriatic uninvolved (7)	Psoriatic lesion (6)
Water	42±18	39±23	34±14
Detergent	72±25	72±34	68±13

DISCUSSION

Our experiments using sellotape-adherent corneocytes indicate that this is a rapid, convenient and reproducible means of obtaining samples of the skin surface for chemical analysis. Two limitations must be recognized, however. First, the amount of material obtained (about 200 μg) is so small that rather sensitive analytical techniques, such as fluorometry, are mandatory. Secondly, it is difficult to obtain precise weights for the pelletted corneocytes; analytical parameters are therefore better expressed as internal ratios. Our observation that thiol/protein ratios of corneocytes stripped from psoriatic lesions are identical with those from the scale confirms that artifacts such as contamination with glue from the sellotape may be excluded.

The data of Table II do not support existing concepts. Several early workers (Zingsheim, 1952; van Scott and Flesch, 1954; Magnus, 1956) reported that the thiol level of psoriatic scale, expressed in terms of the weight of starting material, increases appreciably in comparison with healthy stratum corneum. The high thiol content has been interpreted as the result of incomplete oxidation to disulphide, the failure of the oxidation process being regarded as "an expression of an increased rate of keratinization" (van Scott & Flesch, 1954). Since disulphide bridges are probably important in maintaining the physical and chemical stability of mature keratin, the poor coherence of psoriatic stratum corneum is readily explained. Our present findings are thus at variance with the whole fabric of this hypothesis.

The conflict arises, in fact, not from any discrepancy in the actual data, but from the choice of reference variable. All previous investigators have used the assay described by Flesch & Kun (1950). In this technique the homogenate is shaken with Bennett's reagent (a solution of 1-(4-chloromercuriphenylazo)-naphthol-2) and centrifuged prior to the measurement of optical density. Clearly, therefore, only thiols which have entered solution can contribute to the colour. Nevertheless van Scott & Flesch (1954) comment that "the reagent was added directly to this suspension and therefore the values represent both water-soluble and protein-bound sulphydryl". Unfortunately this view ignores the large bulk of insoluble protein removed by centrifugation, and leads these authors to speak mistakenly of the thiol 'content' of the scale.

Magnus (1956) recognizes this problem and is careful to use the term 'available thiol'. He comments that certain of his observations are incompatible with 'the classical conception of keratinization', and regards the higher thiol levels extracted from psoriatic material as a difference of 'availability' rather than content. Since we demonstrate here that the thiol content of the extracted protein (i.e. the molar percentage of cysteine residues) is unchanged in psoriasis, we may support Magnus's view and conclude simply that more protein is solubilized.

These observations re-open the question of the chemical defect in psoriatic keratin, and indicate that further work in this field is called for.

Table I. *Psoriatic scale: absolute values for thiol and protein extracted into water and detergent*
Figures are means ± SD for 6 specimens

Extract	Thiol (nmol/mg scale)	Protein (μg/mg scale)	Ratio (nmol thiol/ mg protein)
Water	1.40±0.21	36.6±7.0	38±8
Detergent	3.12±0.53	48.3±7.5	65±6

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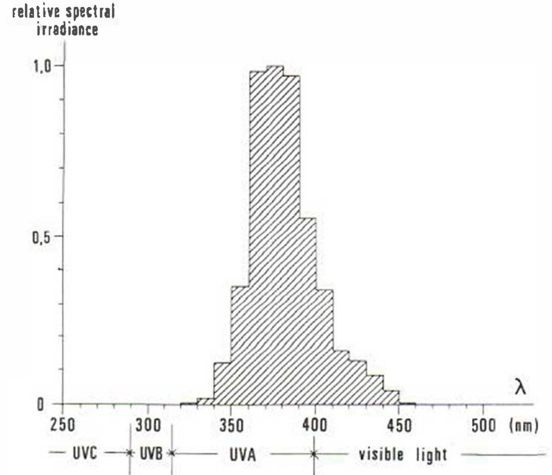

Fig. 1. Spectral distribution of the test lamp.

Phototoxic Reactions from Some Common Drugs Provoked by a High-intensity UVA Lamp

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Abstract. Human volunteers were tested for phototoxic reactions with high UVA doses after intake of four different drugs. Four out of ten became more sensitive to UVA light after intake of nalidixic acid, two out of ten for hydrochlorothiazide, one out of ten for doxycycline but no one of the ten taking promethazine reacted with a higher sensitivity to UVA radiation. One test person reacted unexpectedly with a polymorphous light eruption for 48 J/cm² UVA even without drug intake. The safety of giving high UVA doses for cosmetic purposes in connection with drug intake is discussed.

Key words: Hydrochlorothiazide; Doxycycline; Nalidixic acid; Promethazine; UVA-radiation phototoxicity

Textbooks on dermatology often contain long lists of drugs that can cause phototoxic reactions. These lists are compilations from case reports in the literature and give us practically no information on how often one can expect such reactions to occur. Magnus (3) has however tried to relate the frequency of reported phototoxic and photoallergic reactions to the overall prescribing of the drugs. The mere distinction between phototoxic and photoallergic reactions is rather difficult (4). Systematic studies have not been made in the human but have been made on microorganisms and laboratory animals (1, 2).

As ultraviolet therapy is becoming very common for skin diseases and cosmetic purposes, it is necessary to gather better information on the risk of adverse reactions arising from the use of common drugs in connection with UV light exposure.

High-intensity UVA sources to be used for cosmetic purposes have been developed. These light sources are practically free of UVB irradiation and are therefore very suitable for experiments on phototoxic reactions in the human.

We have tested some commonly used drugs that are reported in the literature as being photosensitizing. After drug ingestion the skin was exposed to UVA light. The short-term use of the drug before exposure to light and the fact that each individual was tested for only one drug minimizes the risk of involving immunological reactions. On this basis we feel that it is relevant to characterize the reactions as phototoxic if they have a morphology which does not indicate a delayed-type immunological reaction.

MATERIALS AND METHODS

The four drugs tested were hydrochlorothiazide (Esidrex-K forte, Ciba, in a dose of 50 mg o.d. for 3 days), doxycycline (Idocycline, Ferrosan, first day 100 mg b.i.d. and the following 2 days 1 tablet of 100 mg), nalidixic acid (Negram, Winthrop, 1 g q.i.d. in 3 days) and promethazine (Lergigan, Recip, 25 mg 4 hours prior to light exposure).

Forty human healthy volunteers (17 males, 23 females) took part in the study. They were medicial students and