An endocrinological study of 15 psychiatric patients with primary cutis verticis gyrata (CVG) and 7 control patients was carried out. The investigation of the pituitary-gonadal axis, pituitary-adrenal axis, pituitary-thyroid axis, prolactin and human growth hormone (basal values and circadian bioharmless) did not show any significant difference between the CVG and the control patients. Only levels of free testosterone were significantly lower in patients with CVG than in the control group \( (p < 0.05) \), probably reflecting an increased peripheral use of testosterone. \textit{Key words: Mental retardation; Schizophrenia; Testosterone; Pituitary-gonadal axis.}

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Cutis verticis gyrata (CVG) is a rare skin pathology, characterized by the thickening of the scalp which appears circumvolute, gyrate and raised in ridges. Primary or idiopathic forms can be noted for which no specific causes have been identified, and also secondary forms caused by various illnesses which affect the scalp either directly (inflammatory diseases, naevi, skin disorders) or indirectly (endocrine or paracostatic disorders etc.) \((1)\). The observation of secondary, hormone-induced forms, regressing after the normalization of hormone secretion, and associated with certain clinical aspects of the idiopathic affection (such as the occurrence after puberty, the prevalence in male subjects and the disappearance after castration) leads to the supposition that the pathogenesis of primary CVG could have an endocrinological basis \((2-6)\).

To our knowledge, few endocrinological studies of patients with CVG have been carried out and for the most part with limited case material \((2, 5, 7)\). Having a sufficient number of cases at our disposal, we carried out an endocrinological screening on psychiatric patients with primary CVG.

**METHODS**

**Hormonal study**

Fifteen ml samples of venous blood were taken from both the 15 patients with CVG and the 7 control patients at 7.00, 11.00 and 15.00. The blood was subdivided into two tubes: 5 ml for human adrenocorticotropic (ACTH) determination and 10 ml for determination of the other hormones. In order to avoid degradation of ACTH the samples were carried out “cold” (syringes kept in refrigerator at 4°C until the sample was taken, and the test-tube placed in a container with water and ice) and 0.5 ml of Trasylol was added to the test-tube containing the 1 ml of EDTA.

The serum and/or plasma samples were analysed in one session. Human growth hormone (hGH) and dehydroepiandrosterone-sulfate (DHEA-S) were assayed with a Lio phase kit RIA Schlo; 4-androstenedione, testosterone, free testosterone (Free Ts), sex hormone-binding protein (SHBG) and 17α-hydroxy-progesterone (17αOHPG) with RIO kit in solid phase from Medical Systems; ACTH and prolactin (PRL) with RIA kit and IRMA kit from SORIM in solid phase respectively; luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from Immucor (Cortisol) by LIA method and thyroid stimulating hormone (TSH) by ILMA method of the AMERLITE system from AMERSHAM; and finally, Cortisol with the RIA kit in solid phase from EQUIPAR.

**RESULTS**

The results of the study are summarized in Table I. No differences were observed between CVG and control patients with regard to most of the hormones investigated. Only levels of Free Ts were significantly lower in the CVG group than in control subjects \((24.1 \pm 7.4 \text{pg/ml vs } 30.0 \pm 5.1 \text{ pg/ml, means } \pm \text{ S.D., } p < 0.05)\). It is worthy of note that in both groups levels of Free T tended to values below the norm, whereas for total testosterone and FSH there was a tendency towards values above the norm.

**DISCUSSION**

In 1964, Åkesson \((2)\) hypothesized an endocrinological etiopathogenesis of primary CVG following the observation that certain patients excreted small amounts of urinary gonadotropins; and in 1965 the same author \((3, 4)\) described a case of CVG associated with thyroid aplasia. Paolo et al. \((7)\), in 1970, found elevated levels of urinary gonadotropins in 2 patients with CVG, while the adrenal function examined by the Thorn test in 3 patients and with corticosteroid stimulation in 7 patients proved normal. The authors came to the conclusion that it was not possible to identify any obvious pituitary or adrenal alteration \((7)\). In 1971 Lundberg & Walinder \((5)\) studied subjects with CVG secondary to acromegaly, triplo-X and myotonic dystrophy with no success in distinguishing significant differences between hormone secretion in these patients compared to those without CVG.
Table I. Plasma hormone levels (means ± S.D.) in patients with CVG and in the control group

<table>
<thead>
<tr>
<th></th>
<th>CVG group</th>
<th>Control group</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free T₁</td>
<td>6.1±0.9</td>
<td>6.0±0.9</td>
<td>4.0–8.0 pmol/L</td>
</tr>
<tr>
<td>Free T₂</td>
<td>11.2±2.8</td>
<td>13.2±7.7</td>
<td>10–25 pmol/L</td>
</tr>
<tr>
<td>TSH</td>
<td>1.1±0.6</td>
<td>0.9±0.4</td>
<td>0.24–2.90 UI/ml</td>
</tr>
<tr>
<td>LH</td>
<td>10.8±2.6</td>
<td>12.2±2.6</td>
<td>1.5–14 UI/ml</td>
</tr>
<tr>
<td>FSH</td>
<td>12.8±6.3</td>
<td>12.7±4.0</td>
<td>1.0–10 UI/ml</td>
</tr>
<tr>
<td>Testosterone</td>
<td>9.5±4.2</td>
<td>9.4±3.3</td>
<td>3.6–9.0 ng/ml</td>
</tr>
<tr>
<td>Free Ts</td>
<td>24.1±7.4</td>
<td>30.0±5.1</td>
<td>17.2–27 pg/ml</td>
</tr>
<tr>
<td>SHBG</td>
<td>62.4±23.9</td>
<td>67.1±28.6</td>
<td>10–73 nmol/L</td>
</tr>
<tr>
<td>ACTH</td>
<td>7 h 36.7±41.7</td>
<td>37.0±41.6</td>
<td>20–80 pg/ml</td>
</tr>
<tr>
<td></td>
<td>11 h 20±0</td>
<td>20±0</td>
<td>0–40 pg/ml</td>
</tr>
<tr>
<td></td>
<td>15 h 20±0</td>
<td>20±0</td>
<td>0–40 pg/ml</td>
</tr>
<tr>
<td>Cortisol</td>
<td>7 h 183±43</td>
<td>192±36</td>
<td>60–230 ng/ml</td>
</tr>
<tr>
<td></td>
<td>11 h 142±50</td>
<td>145±56</td>
<td>60–230 ng/ml</td>
</tr>
<tr>
<td></td>
<td>15 h 113±31</td>
<td>85±49</td>
<td>10–85 ng/ml</td>
</tr>
<tr>
<td>4-andro-stenedion</td>
<td>3.6±2.4</td>
<td>3.8±1.9</td>
<td>1.1–4.5 ng/ml</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>1.4±0.8</td>
<td>1.6±1.1</td>
<td>0.7–5.3 ng/ml</td>
</tr>
<tr>
<td>17αOHPG</td>
<td>1.4±0.4</td>
<td>1.5±0.6</td>
<td>0.5–2.9 ng/ml</td>
</tr>
<tr>
<td>HGH</td>
<td>3.1±1.9</td>
<td>2.7±0.4</td>
<td>0–5 ng/ml</td>
</tr>
<tr>
<td>PRL</td>
<td>140±93</td>
<td>116±39</td>
<td>61–426 UI/ml</td>
</tr>
</tbody>
</table>

* p < 0.05

As far as we know, no other studies have investigated the endocrine function in patients with CVG. In the present study, we examined the pituitary-gonadal axis, the pituitary-adrenal axis, the pituitary-thyroid axis, prolactin and hGH – both relevant to basal values – and circadian biorhythms. The results do not indicate significant differences between patients with primary CVG and psychiatric control subjects. Only levels of Free Ts were found to be slightly lower in CVG patients compared to the control group. This reduction of Free Ts in patients with primary CVG could arise from an increased peripheral use of testosterone, which, in turn, would justify the histological aspect (hypertrophy of the piliferous follicle and sebaceous glands), the greater occurrence in the male sex and the disappearance of CVG after castration. We could also hypothesize the production of a hormonal substance with an activity similar to that of testosterone but incapable of interacting with the pituitary-gonadal axis, which might explain the appearance of CVG as a paraneoplastic disease within the group of secondary forms (11, 12). However, due to the limited number of observations, further data are required to confirm our data on Free Ts levels.

Another interesting finding of this study is the tendency towards higher values than the average of androgen hormones both in patients with CVG and in the psychiatric control group. With regard to this, the FSH tendency to values above the norm, common to the two groups, could be the expression of the ineffectiveness of the negative feedback relative to hyperandrogenism. These alterations in the pituitary-gonadal axis, observed both in patients with CVG and in the control group, could perhaps explain the higher frequency of CVG among psychiatric patients with mental retardation or schizophrenia. Admittedly, these data must be considered with caution since our study was carried out in patients who had a complicated brain pathology that could influence the hypothalamo-pituitary regulation and/or who were on chronic treatment with psychotropic drugs, which are known to affect the endocrine system.

ACKNOWLEDGEMENTS
We thank Miss K. Downey for secretarial assistance and the nursing staff of the Messina Psychiatric Hospital for their assistance with this study.

REFERENCES
Initiation of the Effects of Acrivastine and Cetirizine on Histamine-induced Wheals and Itch in Human Skin

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The initiation of the antihistamine effect of a single dose of acrivastine (8 mg) or cetirizine (10 mg) on wheals and itch induced by histamine dihydrochloride (10 mg/ml) in the prick test was studied in a randomized cross-over design employing 20 healthy medical students. The prick test was performed before ingestion of the drug and after 15, 20, 25, 30, 60 and 90 min and 2, 3 and 4 h. Local symptoms (itching) were recorded on a visual analogue scale. The inhibitory effect of acrivastine on the histamine wheal was first noticed 20 min (p < 0.01) after ingestion of the drug and that of cetirizine after 60 min (p < 0.001). The maximum effect of cetirizine, at 4 h, was greater than that of acrivastine, at 3 h (p < 0.001). The suppression of itching was first noticed 25 min after ingestion with both drugs.

Key words: Prick testing; Histamine H1 antagonists.

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The time of onset of the action of the two new, non-sedative antihistamine drugs, acrivastine (1) and cetirizine (2), in suppressing the histamine-induced wheal and itch reaction in the human prick test was evaluated after a single oral dose. The two drugs have not been compared earlier in this respect.

MATERIAL AND METHODS

The volunteer test subjects were 20 healthy medical students (13 females and 7 males, mean age 27 years). Each subject received first either acrivastine 8 mg (Semprex®, Wellcome Foundation Ltd, London, UK) or cetirizine 10 mg (Zyrtec®, UCB S.A., Brussels, Belgium) according to a randomization code, a second test being performed with the other drug after a wash-out period of at least 72 h. The medication was given by a trained nurse between 8.00 and 10.00 a.m., following a fasting period from midnight onwards. The tablets (ordinary commercial preparations) were prepacked in non-transparent plastic containers which were coded A or B, one dose in each container. The test subject or the nurse could not see the tablet and the test subject was asked to swallow it quickly with a glass of water.

Histamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo., USA; 10 mg/ml in physiological NaCl) was used in the skin prick test performed on the volar forearm, the right and left forearm being used alternately. A histamine prick test was performed before administration of the antihistamine drug and after 15, 20, 25, 30, 60 and 90 min and 2, 3 and 4 h, by the same experienced person throughout, using the DHE prick lancet (Dome/Hollister-Stier, UK) which punctures the skin through a droplet of histamine solution, in a standardized manner. The size of the wheal was measured 10 min later and expressed as the mean of the maximum diameter and the maximum diameter perpendicular to it. Each test site was used only once. At the same time the subject was questioned about the severity of itching or other sensations at the test site, and the observations were marked on a 100 mm visual analogue scale.

The results were analysed statistically using the t-test for paired observations.

RESULTS

The beginning of the decrease in the size of the histamine wheal was noticed 20 min after the acrivastine dose (p < 0.01) (Fig. 1A), and the maximum effect was reached after 3 h. The subjective feelings of itching or tingling had decreased significantly 25 min after ingestion of the drug (p < 0.05) (Fig. 1B).

Cetirizine caused a significant decrease in the histamine wheal after 60 min (p < 0.001) (Fig. 1A), and its maximum antihistamine effect was reached at 4 h (the last observation time). The subjective feelings at the test site had decreased by 25 min after taking the drug (p = 0.05) (Fig. 1B).

The maximum antihistamine effect of cetirizine (at 4 h) was greater than the effect of acrivastine (at 3 h) (p < 0.001) (Fig. 1A).

Fig. 1. Effect of a single peroral dose of acrivastine (8 mg) or cetirizine (10 mg) on wheals (A) and local itching (B) induced by histamine dihydrochloride (10 mg/ml) in the prick test on the volar forearms of 20 test subjects. Mean and SEM, *p < 0.05, **p < 0.01, ***p < 0.001.
DISCUSSION

In a previous test with intradermally injected histamine, the antihistamine effect of acrivastine was observed 30 min after intake, which was also the first point of time at which the effect was evaluated (3). The peak effect was then reported to occur 2 h after intake. The present trial produced an antihistamine effect 10 min earlier (at 20 min), and peak inhibition occurred after 3 h, although there was no statistical difference between the wheal sizes at 2 and 3 h (Fig. 1A).

The initiation of the inhibitory effect of 10 mg cetirizine on histamine-induced wheals has been reported to appear as early as at 20 min after peroral intake (4), or, in another test, at 40 min or 60 min depending on whether the wheal size was compared with a placebo or baseline standard (5). Supporting the results of the latter evaluation, we first noticed an antihistamine effect after 60 min. It is not known why the histamine itch was relieved after only 25 min following cetirizine intake, whereas the decrease in the histamine wheal was not noticed until at 60 min.

Both acrivastine and cetirizine are quick-acting drugs after peroral intake and are suitable for “on-demand” medication.

REFERENCES

Papillomavirus DNA Typing Analysis in Condyloma Acuminatum Patients and Their Consorts

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The present study demonstrated human papillomavirus (HPV) infection by means of an in situ HPV DNA hybridization screening test (BIOHIT) in 8 male patients with genital condyloma acuminatum, their asymptomatic female consorts and 6 female patients with vulval condyloma acuminatum. The investigations revealed that all but one of the female consorts were infected by HPV but did not show any clinical sign of papillomavirus infection. HPV DNA typing analyses with the most common HPVs present in the genital tract (HPV 6, 11, 16, 18, 31 and 33) revealed the same HPV types in the consorts as found in their partners. (HPV 6 and/or 11 were detected in 4 pairs, HPV 18 and 31 in one pair each, and HPV 18/31 and 16/31 double infections in one pair each.) All cytological specimens of female patients with condyloma acuminatum were HPV-infected, the most frequent type being HPV 31. In the one patient with vulval condyloma acuminatum and carcinoma epidermoïdes cornecens, the “high-risk” HPV 18 was identified in both condyloma and carcinoma tissues. Key words: Human papillomavirus; In situ DNA hybridization; Sexually transmitted disease.

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Papillomaviruses are small, species-specific DNA viruses. Over 60 types of human papillomaviruses (HPV) have now been identified. The anogenital area is consistently infected by HPV types 6, 11, 16, 18, 31 and 33, together with less common types. It has become clear that types 6 and 11 (“low-risk”) are most often found in condyloma acuminatum. In contrast, high-grade dysplastic lesions and invasive carcinomas are much more frequently associated with the “high-risk” HPV 16 and 18 and the “medium-risk” HPV 31 and 33 (1-3).

The venereal transmission of HPV infection in men and women is well documented (4-6). Campion et al. have reported (7) that 19 (76%) of 25 women who for at least a year had been the sole sexual partners of men with preexisting condyloma acuminatum had similar lesions of the lower genital tract. Women with vulval condyloma acuminatum have been shown to display a 30% incidence of cervical intraepithelial neoplasia (CIN) (8).

In the present study, we have demonstrated the occurrence of cervical HPV infection by means of an in situ DNA hybridization screening test in asymptomatic female consorts of male patients with condyloma acuminatum and in female patients with vulval condyloma acuminatum. In the HPV-positive cases, further characterization was performed with specific HPV DNA probes corresponding to the most common HPVs present in the genital tract: HPV 6, 11, 16, 18, 31, 33.

PATIENTS AND METHODS

Male patients
Eight male patients (mean age 33.4 years) with condyloma acuminatum were investigated. The diagnosis was based on the characteristic clinical picture; in some cases histology was performed to support the clinical diagnosis. The location of the condylomas was penile in 6 cases and perianal in 2 cases. Homosexuality was not revealed in any case, and subjects were all HIV-negative. Condylomas were removed by excision.

Female patients
The 8 female consorts (mean age 30 years) were free of condyloma and they had only the one sexual partner. Six of them suffered from a slight vaginal discharge. The cytological specimens for HPVinvestigation were collected from the vagina and the cervix by means of the BIOHIT sample collection kit.

Five female patients (mean age 21.6 years) with vulval condyloma acuminatum were also investigated. Condyloma tissues and cytological specimens were investigated parallelly for the presence of HPV. In every positive case, HPV DNA typing was also performed.

One female (Z.E.), 40 years, suffered from recurrent vulval and perianal condyloma acuminatum. In 1989, a histological examination had revealed carcinoma epidermoïdes cornecens. Two years later, carcinoma epidermoïdes cervix uteri and carcinoma epidermoïdes cornecens vulvae were demonstrated. The condyloma and carcinoma epidermoïdes cornecens tissues were investigated retrospectively for HPV DNA.

HPV in situ DNA hybridization
BIOHIT HPV in situ screening and typing tests (BIOHIT OY, Helsinki) were used (9). The BIOHIT in situ screening test detects the most common genital HPV types. This is achieved with a mixture of biotinylated DNA probes of the most common HPV types. The BIOHIT in situ typing test is intended for the detection of HPV DNA 6, 11, 16, 18, 31 and 33 sequences in either paraffin-embedded or frozen human tissue biopsies and cytological smears. The biotinylated hybrids were detected with the streptavidin alkaline phosphatase method.

The paraffin-embedded sections of condyloma tissue were investigated by means of the BIOHIT in situ typing test. Gynecological smear were tested first with the screening test. If the result proved positive for HPV, then the HPV typing test was performed with those HPV types which were detected in the condyloma sections of the male consort or the female subject.

Tissue processing, pretreatments, hybridization and detection were carried out according to the manufacturer’s instructions.

RESULTS

All of the male condyloma biopsy specimens (8/8) were positive for HPV DNA. Four patients proved positive for HPV 6 and/or 11. The HPV 6 and 11 appeared as punctate granules located in both the nucleus and the cytoplasm of the infected cells (Fig. 1). One patient was positive for HPV 18 and one for type 31. HPV 31 gave a different staining pattern and was located in the nucleus as tiny dots scattered in the cytoplasm.
Double infections of HPV were registered in 2 patients (HPV 18/31 and 16/31). All but one of the female consorts were identified as being infected with HPV, as detected by the HPV DNA screening test. DNA typing analyses (e.g., Fig. 3) revealed similar HPV types in the female partners as found in the male patients with condyloma acuminatum (Table I). HPV 6 and/or 11 were detected in 4 pairs, HPV 18 and 31 in one pair each, and HPV 18/31 and 16/31 double infections in one pair each.

In the 5 female patients with vulval condyloma acuminatum, the typing analysis of their condyloma HPV DNA demonstrated the occurrence of HPV 6 alone in one patient, HPV 31 in another patient and multiple infections with HPV DNA 6/31/33, 18/31/33 and 6/33 in the remaining 3 patients (Table II). All cytological specimens proved positive for HPV DNA according to the screening test, and typing revealed similar typing data (Table II). At least one special HPV DNA type has been found that was common in the condyloma tissue and cytological smears of one of the female patients with condyloma acuminatum. In one patient with condyloma acuminatum and later carcinoma epidermoids cornescens, a mixed papilomavirus infection was demonstrated in both the condyloma and carcinoma tissue. The predominant HPV type was 18, and HPV 31 and 33 were detected with lower intensity.

**DISCUSSION**

Hybridization analysis of the virus DNA is one of the best procedures currently available for the diagnosis of HPV infection (10). In the in situ DNA hybridization technique, a cytological specimen or sections cut from frozen or formalin-fixed and paraffin-embedded biopsy material are probed directly without any DNA extraction. The detection of biotinylated hybrids with the streptavidin alkaline phosphatase method has proved to be a highly preferable modification (11, 12). In situ DNA hybridization, although less sensitive than the PCR method, has the advantage of not requiring special expertise or equipment. In addition, the lesion associated with the HPV infection can be assessed.

The BIOHIT HPV in situ screening test is simple, reliable and reproducible and allows the detection of latent infection. Its sensitivity and specificity have been found to be 95% and 88%, relative to another commercial HPV DNA kit (Enzo HPV probes). The BIOHIT HPV in situ typing test allows the identification of a specific type of HPV DNA and permits the detection of the association of different HPV DNAs with a sensitivity of 90% and a specificity of 87% as referred to the Enzo probes (13). It could be of clinical value in the diagnosis or prognosis of HPV infection, since there is a strong correlation between the presence of HPV and CIN (14).

In accordance with the literature data, “low-risk” HPV 6 and 11 were frequently detected in our male patients with condyloma acuminatum. However, “medium-risk” (HPV 31) and “high-risk” (HPV 18) types were also demonstrated in 4 cases. It was noteworthy that all but one of the female consorts were infected by HPV without any clinical sign of papillomavi-
Table I. HPV screening and typing data on condyloma tissue of male patients with genital condyloma acuminatum and on cytological smears of their female consorts

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Condyloma HPV type</th>
<th>Consortium</th>
<th>Age (years)</th>
<th>Cytological smears</th>
<th>HPV type</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. S.</td>
<td>42</td>
<td>18</td>
<td>R. S.</td>
<td>36</td>
<td>Positive</td>
<td>18</td>
</tr>
<tr>
<td>V. J.</td>
<td>47</td>
<td>6,11</td>
<td>V. E.</td>
<td>44</td>
<td>Positive</td>
<td>6</td>
</tr>
<tr>
<td>P. A.</td>
<td>25</td>
<td>6</td>
<td>S. T.</td>
<td>19</td>
<td>Positive</td>
<td>6</td>
</tr>
<tr>
<td>Sz. F.</td>
<td>30</td>
<td>6,11</td>
<td>S. F.</td>
<td>30</td>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>M. S.</td>
<td>34</td>
<td>31</td>
<td>T. A.</td>
<td>22</td>
<td>Positive</td>
<td>31</td>
</tr>
<tr>
<td>Sz. T.</td>
<td>23</td>
<td>18,31</td>
<td>B. K.</td>
<td>21</td>
<td>Positive</td>
<td>18</td>
</tr>
<tr>
<td>I. A.</td>
<td>23</td>
<td>16,31</td>
<td>S. T.</td>
<td>23</td>
<td>Positive</td>
<td>16,31</td>
</tr>
<tr>
<td>N. S.</td>
<td>48</td>
<td>6</td>
<td>N. S.</td>
<td>44</td>
<td>Positive</td>
<td>6</td>
</tr>
</tbody>
</table>

Table II. HPV screening and typing data on condyloma tissue and cytological smears of female patients with vulval condyloma acuminatum

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Condyloma HPV type</th>
<th>Cytological smears</th>
<th>HPV screening</th>
<th>HPV type</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. M.</td>
<td>23</td>
<td>6,31,33</td>
<td>Positive</td>
<td>31,33</td>
<td></td>
</tr>
<tr>
<td>A. A.</td>
<td>20</td>
<td>18,31,33</td>
<td>Positive</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>P. F.</td>
<td>20</td>
<td>6</td>
<td>Positive</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>B. M.</td>
<td>18</td>
<td>31</td>
<td>Positive</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>P. L.</td>
<td>17</td>
<td>6,33</td>
<td>Positive</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


Photopheresis in Systemic Sclerosis: Clinical and Serological Studies Using Markers of Collagen Metabolism

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Eight patients with progressive systemic sclerosis were treated with photopheresis or extracorporeal photochemotherapy given on 2 consecutive days every 4 weeks for 5 to 8 months. Previous treatment with immunosuppressive agents or D-penicillamine was discontinued for at least 4 weeks prior to photopheresis. Although IL-2 receptor density in peripheral blood T-lymphocytes decreased significantly in the initial phase of the photopheresis therapy, no substantial clinical improvement occurred. Although the intention had been to treat all patients for at least 8 months with photopheresis alone, it was necessary due to severe exacerbations to give additional immunotherapy to 4 patients, and 2 of these together with another patient wanted to discontinue photopheresis after 5 and 6 months, as they did not expect an effect. Three of the 4 patients with progression had RNP-antibodies, suggesting that they had their scleroderma as part of a mixed connective tissue disease. The clinical exacerbations were accompanied in all patients by a highly significant increase in serum aminoterminal propeptide of type III procollagen (PIIINP), which has been reported to correlate with involvement of skin and internal organs in systemic sclerosis. Similar but less significant increases were found in serum carboxyterminal propeptide of type I procollagen (PICP); there were no significant changes in serum cross-linked fragment of type III collagen. Plasma levels of 8-methoxypsoralen were all above 80 ng/l, showing that the lack of responses to photopheresis could not be due to poor absorption of the drug. Our data indicate that patients with the severe progressive form of systemic sclerosis at least in a number of cases may not be sufficiently controlled by photopheresis alone but should be treated with immunosuppressive agents. This may especially be the case if these patients have their scleroderma within the frame of a mixed connective tissue disease.

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Photopheresis (PP) or extracorporeal photochemotherapy was introduced by Edelson et al. (1) for treatment of the leukemic phase of cutaneous T-cell lymphoma. In addition to killing tumor cells, animal studies (2) suggested that PP leads to suppression of pathogenic T-cell clones and could be suitable for treatment of autoimmune diseases. This was tried by Rook et al. (3), who in 1989 reported the successful use in a small number of patients including 2 with recent-onset systemic sclerosis (SS). Recently the same group together with others reported their results (4) of a 6-month multicenter prospective study comparing PP with D-penicillamine in SS, in which individuals receiving PP had the highest response rate. We did not want to question the results of the multicenter study (4), but found that we were obliged to publish some words of caution (5), as it was our experience that patients with the severe progressive form of the disease may not all be sufficiently controlled by PP alone.

In this article, we will give an extensive report of the treatment of 8 patients with severe SS with PP for 5 to 8 months, in which it was necessary to give additional therapy to 4 patients due to severe exacerbations of the disease. The clinical data are supplemented with results of our studies using markers of collagen synthesis and degradation.

PATIENTS AND METHODS

Eight patients, 7 females aged from 32 to 56 years and one 54-year-old male, all fulfilling the criteria of the American Rheumatism Association for SS (6), were chosen for treatment with PP. Three of the 8 patients had RNP-antibodies, suggesting that they had their scleroderma as part of a mixed connective tissue disease. All patients were in a state of progression and were given PP according to the procedures described by Rook et al. (4). The only difference was that we in order to optimize the therapeutic effect gave 8-methoxypsoralen (8-MOP) at fasting conditions (7). All patients were already being treated with other medications, but these treatments were discontinued for at least 4 weeks prior to PP. The patients continued with unchanged dosages of antacids, H2-blocking agents, calcium channel blockers, and omeprazol when necessary. The clinical data of the patients are shown in Table 1. One patient with RNP-antibodies had the clinical variant of SS, sclerodermatomyositis (8).

Two hours after the ingestion of 0.6 mg of 8-MOP/kg body weight, the patients underwent the discontinuous leukophoresis procedure at the Department of Clinical Immunology with exposure of removed leukocytes to ultraviolet radiation using a Therakos PP system as previously described by Edelson et al. (1). PP was given as two consecutive daily treatments every 4 weeks. We planned to treat all patients for at least 8 months without giving other medication besides the above-mentioned drugs, but due to severe exacerbations, it was found necessary to give additional immunosuppressive therapy to 4 patients, and 2 of these together with another patient wanted to discontinue PP after 5 and 6 months due to the lack of effect.

A general assessment of disease activity was performed at base line and monthly thereafter. The clinical investigations made prior to and following PP were besides evaluations of cutaneous involvement, oral aperture measurements, hand-closure, oesophagus motility, chest x-ray and lung function studies. Routine laboratory investigations were hemoglobin, leucocyte and differential counts, thrombocytes, erythrocyte sedimentation rate, serum creatinine, glomerular filtration rate, liver transaminase, alkaline phosphatase and anticardiolipin antibodies. Our patient with sclerodermatomyositis also had determinations of serum creatine kinase.

Prior to PP and 1 h after injection of 8-MOP, venous blood was drawn and serum was stored at −20°C for determinations of concentrations of 8-MOP by a HPLC method described by Bech-Thomsen et al. (7). Prior to the first of the two monthly consecutive PP treatments, sera were also stored for analyses of procollagen propeptides, cross-linked fragment of type I collagen and hyaluronan, and lymphocyte activity.
Table I. Clinical features of patients studied and latest treatment prior to photopheresis

<table>
<thead>
<tr>
<th>Pat. no.</th>
<th>Sex/Age</th>
<th>Duration of disease</th>
<th>Scleroderma type*</th>
<th>Antinuclear antibodies**</th>
<th>Latest treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/48</td>
<td>3</td>
<td>III</td>
<td>RNP</td>
<td>Prednisone + cyclophosphamide</td>
</tr>
<tr>
<td>2</td>
<td>F/32</td>
<td>3</td>
<td>SM</td>
<td>RNP</td>
<td>Prednisone + penicillamine</td>
</tr>
<tr>
<td>3</td>
<td>M/54</td>
<td>1</td>
<td>III</td>
<td>RNP</td>
<td>Prednisone + azathioprine</td>
</tr>
<tr>
<td>4</td>
<td>F/47</td>
<td>3</td>
<td>II</td>
<td>AC</td>
<td>Penicillamine</td>
</tr>
<tr>
<td>5</td>
<td>F/56</td>
<td>5&lt;</td>
<td>II</td>
<td>AC</td>
<td>Prednisone + cyclophosphamide</td>
</tr>
<tr>
<td>6</td>
<td>F/39</td>
<td>2</td>
<td>III</td>
<td>SS-A + Adrenaline (A-Ad)</td>
<td>Cyclosporin A</td>
</tr>
<tr>
<td>7</td>
<td>F/50</td>
<td>5&lt;</td>
<td>III</td>
<td>RNP</td>
<td>Penicillamine</td>
</tr>
<tr>
<td>8</td>
<td>F/46</td>
<td>5</td>
<td>II</td>
<td>SS-A + Adrenaline (A-Ad)</td>
<td>Penicillamine</td>
</tr>
</tbody>
</table>

* Type II represents limited cutaneous systemic sclerosis (ISSc) with lesions above wrists. Type III is diffuse systemic sclerosis (dSSc) with lesions involving trunk. SM is scleroderma morphenosis.

**) AC = anticyclic antibody-positive. Scl 70 = Scl 70 antibody-positive. A-Ad = antinuclear antibody-positive. SS-A = anti-SS-A antibody-positive. RNP = anti-RNP antibody-positive; these patients could have their scleroderma within the frame of a mixed connective tissue disease.

subsets were investigated by flow cytometry. Flow cytometry was also used for studies of IL-2 receptor presentation by T-lymphocytes (9).

The N-terminal propeptide of type III procollagen (PIIINP) was measured by a radioimmunoassay from Orion Diagnostica, Oulun saalo, Finland. This is an equilibrium assay based on polyclonal antibodies against a human aminopeptide of type III procollagen, a 44,000 Dalton trimeric protein (10). We found a normal reference range based on samples from 40 healthy persons of 1.8–4.1 μg PIIINP/l. The serum carboxyterminal propeptide of type I procollagen (PICP) was analysed using a newly developed equilibrium radioimmunoassay, also from Orion Diagnostica. This assay employs polyclonal antibodies against the carboxyterminal propeptide of the human procollagen molecule, a trimeric globular protein with a molecular weight of 100,000 (11). The reference range for 40 healthy persons was 40–172 μg PICP/l.

The degradation product of type I collagen, the pyridinoline cross-linked carboxyterminal telopeptide (ICTP), was measured by an equilibrium radioimmunoassay from Orion Diagnostica (12). Intra- and interassay coefficients of variation were less than 5% for the radioimmunoassays. Serum hyaluronan was determined by a radiometric assay for sodium hyaluronate using high affinity binding proteins from bovine cartilage (HA test, Pharmacia Diagnostics, Uppsala, Sweden), as described by Brandt et al. (13). A reference range for 30 healthy persons was 90–60 μg/l. Mean age of control persons was 49 ± 6.7 years (range 31–58 years). Intra- and interassay coefficient variations were less than 8%.

The leukocytes of EDTA-stabilized blood from 7 patients (no. 1 to no. 7) were stained using combinations of FITC- and phycoerythrin (PE-) conjugated monoclonal antibodies (mAbs): Leu-4A (FITC, Becton-Dickinson) + anti-CD25 (PE, DakoPatts). Leu-7 (FITC, Becton-Dickinson) + Leu-11A (PE, Becton-Dickinson), Leu-9A + B (FITC, Becton-Dickinson) + Leu-2C (PE, Becton-Dickinson), Leu-M3 (FITC, Becton-Dickinson) + Leu-16 (PE, Becton-Dickinson). The mAbs were used at concentrations suggested by the suppliers, and staining was allowed for 15 min at room temperature followed by lysis of red cells and fixation in lysing solution (BectonDickinson). After washing in phosphate buffered saline, pH 7.2, the cells were analyzed in a FACScan flow cytometer (Becton-Dickinson). Lymphocyte gating was performed using forward and side scatter parameters to exclude Leu-M3 (CD14) stained monocytes. Fluorescence signals were logarithmically amplified, and compensation for spectral overlap was adjusted using calibrating beads (Becton-Dickinson). FACScan research software was used to calculate the frequencies of lymphocyte subsets. The relative fluorescence of anti-CD25 stained CD4 + cells (IL-2 receptor density) was estimated by histogram analysis of FL2 after gating for CD3 + lymphocytes.

RESULTS

The clinical course of the patients is shown in Table II, to-
Fig. 1. Changes in serum aminoterminal propeptide of type III procollagen (PIIINP) prior to and following treatment in 4 patients experiencing exacerbations after photopheresis. Arrows indicate time of monthly courses of two consecutive photopheresis treatments. Time of previous and concomitant therapy can also be seen from the charts. The horizontal line represents the upper limit of normal controls.

together with the results of analysis of PIIINP. The case stories of patients nos. 1–4, who had severe exacerbations, follow below. Three of these 4 patients had RNP-antibodies. The individual sequential measurements of PIIINP ran parallel with the clinical course and can be seen for patients nos. 1–4 in Fig. 1. In general PICP would parallel with PIIINP, as illustrated by the results from patient no. 5, shown together with the data on serum hyaluronan (Fig. 2). But in contrast to PIIINP the results for PICP did not in all cases reach pathological values. ICTP varied without significant changes. Serum hyaluronan values were in general within normal range but increased in 2 patients after PP. IL-2 receptor density in peripheral blood T-lymphocytes decreased significantly for 3–4 months when all patients received PP alone but was gradually restored later (Fig. 3). CD4-lymphocytes also had a trend to decrease in most patients but increased in patients nos. 1 and 5. The other lymphocyte subsets showed no consistent pattern of change.

Investigations of 8-MOP showed that all patients had plasma levels over a recommended level for PUVA-treatment (14) of 30 ng/l. The lowest level was 87 ng/l, the highest 829 ng/l. The average level (n = 171) was 497 ± 151 ng/l.

The detailed case stories appear in our preliminary report (5). In spite of the fact that we had planned to treat all patients for at least 8 months with PP alone, it was mandatory due to severe exacerbations to give additional immunotherapy in the form of prednisone/cyclophosphamide or cyclosporin A to 4 patients (patients nos. 1–4). Two of these together with another patient wanted to discontinue PP after 5 and 6 months as they did not expect an effect. In spite of immediate improvement after re-administering 40 mg prednisone and 100 mg cyclophosphamide daily to patient no. 1, her lung function deteriorated further and she died 8 months after start of PP.

Patient no. 2 had the subtype of SS of sclerodermatomyositis. She had been well controlled on 25 mg prednisone and 100 mg cyclophosphamide daily. Her skin had softened and all signs of muscular involvement had disappeared together with normalization of serum creatinekinase. During PP all symptoms reappeared and serum creatinekinase rose from 59 μg/l to 1988 μg/l. After discontinuation of PP and reintroduction of prednisone/cyclophosphamide she again improved dramatically. Fig. 4 shows the moderate sclerodactyli in a 54-year-old male with a disease duration of approximately 1 year. Fig. 5 shows his hands by the end of PP treatment. Besides stiffening of the fingers, the fibrosis of the skin came to include arms, legs and trunk. After the severe progression of his disease, PP was first supplemented by cyclosporin A 3 mg/kg/day and later with 40 mg prednisone and 100 mg cyclophosphamide daily before progression was arrested.

Patients nos. 5 to 8 were found unchanged in clinical and laboratory status, and patient no. 5 wanted to continue with PP after the end of the trial. She is still on treatment after one
and a half year without significant changes. Patient no. 7, although unchanged in assessment in skin involvement and in her various subjective symptoms, died immediately after her 8th course of PP from cardiac arrest. Patient no. 8, although unchanged, wanted to discontinue PP after 6 months as she, as already stated, did not expect an effect.

![Image of patients hands before photopheresis](image_url)

*Fig. 4. Hands of patient no. 3 before photopheresis. At this stage the skin could be folded at the dorsum of her hands.*
to imagine that this would still be the case several months after discontinuation of the drug.

Our data on IL-2 receptors should indicate that PP did have an immunomodulatory effect on our patients. IL-2 receptor levels have been reported to decline during successful cyclosporin A treatment of dermatomyositis (21). In these cases the receptor levels correlated with clinical disease activity and serum creatine kinase concentrations. It should, however, be noted that we cannot rule out the possibility of redistribution of autoreactive T-cells to affected skin areas upon cessation of previous medical immunosuppression (22). Our data on 8-MOP concentrations in plasma rule out the possibility that a poor absorption could be the reason for a poor clinical effect.

Although PP may compete favourably with immunosuppressive drugs and penicillamine concerning side-effects, and if accepted, according to the multicenter study (4) with penicillamine as regards to efficacy on skin symptoms, we agree with Fries et al. (15) that in that case the huge differences in costs will favour the use of penicillamine. We found that PP was not sufficient in our severely progressive patients. Treatment with cyclophosphamide and corticosteroids seems to be the method of choice if there are lung symptoms (23), and our patient no. 1 had a severe exacerbation of pulmonary dysfunction following change from this therapy to PP. Besides having an effect on pulmonary vital capacity, prednisone and cyclophosphamide have also been shown to decrease skin scores (23). In our hands they almost normalized scleroderma and gave an exacerbation during PP treatment.

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Evaluation of Phototoxic Properties of Some Food Additives: Sulfites Exhibit Prominent Phototoxicity

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Additives are used widely to enhance the quality of food products. To identify possible phototoxic properties, 13 food additives (benzoic acid, sodium benzoate, 4-hydroxybenzoic acid, 4-hydroxybenzoic acid methyl ester, 4-hydroxybenzoic acid ethyl ester, 4-hydroxybenzoic acid propyl ester, p-hydroxybenzoic acid n-butyl ester, benzyl alcohol, sorbic acid, potassium sorbate, propionic acid, sodium disulfite and sodium sulfite) were evaluated in vitro by means of a photohemolysis test using suspensions of human erythrocytes. Irradiation was performed with various light sources differing with regard to their spectral irradiance. Sodium sulfite and sodium disulfite induced photohemolysis up to almost 100%, the effect depending on the concentration of the compounds and UV dose administered. Radiation rich in UVB was most effective; a sunlight-simulating lamp induced photohemolysis to a lesser degree. All other substances tested did not cause significant photohemolysis. As sulfites are frequently encountered, they may contribute to UVB sensitivity. The clinical significance of these findings has to be established by further work. Key words: Photohemolysis; UV. (Accepted May 3, 1993.)


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Food additives, e.g. smoke, alcohol, vinegar, oil or spices, have been used for centuries to enhance the quality of food products. In the last decades, significant technological advances in food processing have been accompanied by a formidable increase of such additives. Today, more than 2,500 compounds are intentionally added to foods to produce a desired effect (1). Thus, humans are exposed to food additives on a large scale.

Only a few studies have addressed possible phototoxic effects of food additives. Some dyes (rose bengal, erythrosine, phloxine) have been found to inactivate yeast cells due to a photodynamic action (2). It has also been reported that photoactivated tartrazine causes hemolysis (3). A phototoxic compound may induce skin reactions, and phototoxicity can be regarded as a prerequisite of photoallergenicity. Even more important, it has been suggested that chronic exposure to phototoxic compounds may be related to photocarcinogenesis. These considerations prompted us to assess in vitro the phototoxic potential of various food additives, which are mainly used to inhibit microbiological growth and to prevent enzymatic and nonenzymatic discoloration.

MATERIAL AND METHODS

UV sources

Irradiations were performed with the following lamps: 1) UVASUN 5000 (Metzhaus, Munich, Germany), emitting in the range of 320-460 nm (maximum approx. 375 nm); UVB irradiance at a distance of 50 cm was 42 mW/cm²; 2) TL 20 W/12 light bulbs (Philips, Hamburg, Germany) with a main emission between 275-365 nm (maximum approx. 315 nm); irradiance was 1.0 mW/cm² for UVB and 0.4 mW/cm² for UVA at a distance of 40 cm; 3) SOL 3 sunlight-simulating lamp equipped with H2 filter (Hönle, Martinsried, Germany) with a main emission between 290-800 nm (broad maximum between approx. 400-700 nm); irradiance was 0.95 mW/cm² for UVB and 10.5 mW/cm² for UVA at a distance of 40 cm.

Dosimetry

UVA or UVB intensity (or dose) was measured with an integrating instrument (Centra-UV, Osram, Munich, Germany).

Test substances

Tests were performed with sodium benzoate, potassium sorbate, benzoic acid, sodium disulfite, sodium sulfite, sorbic acid (Merck, Darmstadt, Germany), benzyl alcohol, p-hydroxybenzoic acid n-butyl ester (Sigma, St. Louis, USA), 4-hydroxybenzoic acid, 4-hydroxybenzoic acid methyl ester and 4-hydroxybenzoic acid ethyl ester, 4-hydroxybenzoic acid propyl ester and propionic acid (Roth, Karlsruhe, Germany). The purity of sodium disulfite and sodium sulfite was 98% as declared by the manufacturer. Test substances were dissolved in distilled water or methanol and further diluted in TCM buffer (3 g Tris, 0.3 g KCl, 7 g NaCl, 0.147 g CaCl₂, 2 H₂O; 0.2 g MgCl₂, 2 H₂O, dissolved in 1000 ml distilled water; pH 7.4). 280 mosm/kg). Absorption spectra were recorded with an UV-VIS spectrophotometer UV 2100 (Shimazu, Tokyo, Japan).

Methods

The photohemolysis test was performed as previously described (4). Briefly, suspensions of human erythrocytes from at least three donors or correspondingly prepared erythrocyte-free samples were incubated with the test substances at concentrations of 10⁻³, 10⁻⁴ or 10⁻⁵ mol/L. Both substance-free erythrocyte samples (blanks) as well as samples containing the test substances were exposed to 0, 25, 50 or 100 J/cm² UVA from the UVASUN 5000 apparatus, to 0, 100 (37.5), 200 (75), 400 (150), 800 (300) or 1600 (600) mJ/cm² UVB (UVB) from the TL/2 light bulbs or to 0, 9 (0.45), 25 (2.25), 50 (4.52) J/cm² UVA (UVB) from the SOL 3 lamp. 100% hemolysis was obtained by exposure of the erythrocytes to distilled water. Supernatants were recovered by centrifugation. After incubation with Drabkin's solution (Sigma, St. Louis, USA), hemolysis was determined by reading of absorbance at 550 nm with a MR 70 Microplate reader (Dynatech AG, Denkendorf, Germany). Hemolysis was calculated for each radiation dose on the basis of the absorbance data according to the formula:

\[
\% \text{ hemolysis} = 100 \times \left( \frac{\text{(test sample) (blank)} - (erythrocyte-free sample)}}{\text{100 \% hemolysis} - \text{(blank)}} \right)
\]

Results are given as mean. In order to exclude equivocal results, only photohemolysis >5% was regarded as being meaningful positive finding.

The pH of the test samples was measured with a pH-meter (Orion, model SA 520, Colora, Munich).
RESULTS

At exposure to the TL/12 lamps, sodium sulfite and sodium disulfite in the $10^{-3}$ mol/l concentration caused a UV dose-dependent hemolysis up to 64.1% or 99.7%, respectively (Fig. 1). A phototoxic effect also at $10^{-4}$ mol/l was found for sodium disulfite with two out of three erythrocyte suspensions from different donors. Irradiation with the SOL 3 lamp in the presence of sodium disulfite also induced photohemolysis (Fig. 2), whereas exposure to the UVASUN 5000 apparatus had no such effect. No hemolysis occurred when erythrocytes prepared as indicated above were incubated with sodium sulfite or sodium disulfite ($10^{-3}$ mol/l) preirradiated with the maximum of the radiation doses used. Changes of the pH values of solutions with sodium sulfite or sodium disulfite before and after exposure were $<$0.2. None of the other 11 compounds tested exhibited a significant phototoxic action in this assay.

The absorbance spectrum of sodium disulfite is shown in Fig. 3. The absorbance peaks at 209 nm. Sodium sulfite showed a similar absorbance spectrum (data not shown).

DISCUSSION

Sodium sulfite as well as sodium disulfite exerted prominent phototoxic effects in this in vitro assay, sodium disulfite being the more phototoxic agent. This is probably a concentration effect, as the disulfite ion almost completely separates into its two constituting sulfate units in aqueous solutions (5) according to the following equation:

$$\text{S}_2\text{O}_3^{2-} + \text{H}_2\text{O} \rightarrow 2\text{HSO}_3^{-}.$$  

The other agents tested were not phototoxic in the photohemolysis test used.

The action spectrum of sulfite phototoxicity seems to lie predominantly in the UVB range. Although the highest UVB dose administered with SOL 3 was 4.5 J/cm², irradiation with this source yielded lower photohemolysis than 1.6 J/cm² UVB from the TL/12 light bulbs. This may be a photoinhibition due to the other wavelengths emitted by the SOL 3 lamp, possibly attributable to a photodegradation of sulfite or sulfite-induced photoproducts. No photohemolysis was observed after irradiation...
tion with the UVASUN apparatus, which is rich in UVA and free of UVB. This is notable, as most phototoxic substances have their action spectrum in the UVA range (2).

The UV-induced autoxidation of SO$_3^{2-}$ to SO$_3^{-}$ includes the intermediate SO$_3^{3-}$, SO$_2^{2-}$ and SO$_2^{-}$ radicals generated during a radical chain reaction (5, 6). The sulfite radical (SO$_3^{-}$) was shown to react readily with free polyunsaturated fatty acids (PUFA) (7, 8) but seems to be rather non-reactive to PUFA micelles (8) and therefore, probably, also to cell membranes. So, SO$_3^{-}$ or SO$_2^{-}$ could be responsible for membrane damage. To clarify the exact nature of the sulfite-dependent phototoxic reaction, photoproducst induced by UVB irradiation will have to be identified as well as their reaction with membrane components.

A variety of sulfiting agents have been employed for centuries in food processing. These agents are currently used to sanitize food containers and fermentation equipment to reduce microbial spoilage of food and to prevent enzyme-catalyzed oxidative discoloration and non-enzymatic browning during preparation, storage and distribution of foods (1). As antioxidants, they are added to e.g. fruits, vegetables, potatoes, shellfish, beer and wine. Disulfites are also used as preservatives in drugs. Estimations are that 2–3 mg of sulfites is consumed each day by the average citizen in the United States, and an additional consumption of 5–10 mg of sulfite per day occurs in wine and beer drinkers (9).

To our knowledge, up to now phototoxic effects of sulfites have not yet been reported. Phototoxicity has been studied in one model system, photohemolysis, which of course limits the conclusions that can be drawn regarding clinical significance. However, sulfites are ubiquitous contactants. So, encountered not only as preservatives, but also as airborne pollutants, their phototoxic potential may be biologically relevant. The question arises whether sulfite phototoxicity increases UVB sensitivity and thus even augments the risk of photocarcinogenesis. Such effects have been proposed to be possible sequel of the ingestion of photosensitizing food (10). Further in vitro and in vivo studies are needed to clarify these issues.

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REFERENCES

Induction of Type IV Hypersensitivity to Contact Allergens in Guinea Pigs by In vitro Haptenized Allogenic Peritoneal Exudate Cells

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The induction of type IV hypersensitivity to contact allergens in guinea pigs has been studied by using allogenic peritoneal exudate cells (>90 % macrophages), which had been incubated primarily in vitro with dimethylchlorobenzene, formaldehyde, potassium dichromate, nickel II sulphate or para-aminobenzoic acid. In these guinea pig sensitization experiments Freund's complete adjuvant was used. In all haptenes investigated the sensitization rates of the presented method were parallel to the known contact allergenicity in humans and, apart from the potassium dichromate results, comparable with those of the guinea pig maximization test. Because of its alternative sensitization procedure, in which only few or no allergen molecules escape the effective presentation pathway, the authors conclude that this method could be developed into a predictive test assay for the evaluation of the contact allergenicity of water-soluble substances. Key words: Animal assay; Contact allergy; Contact dermatisis; Macrophage; Predictive test.

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A lot of substances which come into contact with human skin must be regarded as possible sensitizers. To minimize or avoid the resulting hazard of contact allergy it is advisable to use predictive test methods evaluating the allergenicity of substances or compounds before their application. The tests most widely used for this purpose are guinea pig sensitization assays of a similar kind: an induction phase, followed by a resting period of about 2 weeks and subsequently a challenge test to prove whether sensitization has occurred or not. For induction, the hitherto most propagated test methods use "direct" application of the substance in question by intradermal injection, epicutaneous application or both (1, 2). However, in these application routes non-definable amounts of allergen may bypass the Langerhans' cells (LC). If the immune system is faced by allergens without involvement of the LC, specific unresponsiveness is often induced (3–5). Thus, it seems to be important to have induction procedures in which all allergen molecules are presented by sufficient accessory immune cells. Because of the technically complicated preparation of LC, we decided to choose the functionally and ontogenetically similar macrophages (paraffin oil-induced peritoneal macrophages). This way of inducing contact allergy has already been studied in some allergens in inbred guinea pigs by von Blomberg et al. (6). In the present study, we investigated the question whether the use of outbred guinea pigs and the application of allogenic haptenized peritoneal exudate cells (PEC), respectively, also cause a type IV hypersensitivity or not.

The following aspects have been studied: 1) the agreement of the sensitization results of our experiments with the known allergenicity of the tested substances in humans and with "reference assays" in animals; 2) the influence of some methodological factors on the sensitization rates (duration of the resting period between induction and challenge, different numbers of haptenized PEC); and 3) the reproducibility of the experiments. After promising preliminary experiments with potassium dichromate (7) we used five different haptenes in our study: one weak sensitizer – para-aminobenzoic acid (PABA) (Ferak Berlin, Germany) – three substances of medium sensitizing potential nickel II sulphate (Laborchemie Apolda, Germany), potassium dichromate (Laborchemie Apolda, Germany), formaldehyde (Laborchemie Apolda, Germany) – and one very strong sensitizer – dimethylchlorobenzene (DNCB) (Berlin-Chemic Berlin, Germany).

MATERIAL AND METHODS

Animals

Outbred guinea pigs (Dunkin-Hartley, Marx, Falkenberg) of either sex, weighing 300–400 g, single-housed in plastic cages, were used.

Peritoneal exudate cell production

Under aseptic conditions 10 ml of subliquid paraffin oil were injected into the peritoneal cavity of every animal in PEC breeding (32 animals in total). After 7–10 days PEC were harvested in cooled (2–4°C) Eagle medium (Institut für Immunpräparate und Nährmedien Berlin, Germany) and were filtered twice by centrifugation (380 g, 10 min). Then the cells were counted and probes for cell differentiation were taken. Two staining techniques were applied to identify the PEC: the Pappenheim method and a method for staining of the non-specific alphanaphthyl acetate esterase (8). Only samples with at least 90 % macrophages were used.

Haptenization procedure

PEC (in a concentration of 10⁶ cells/ml) were incubated at 37°C for 30 min in Eagle medium in the presence of a maximum non-toxic concentration of allergens (unless otherwise stated – see Table 1). The maximum non-toxic concentrations were determined in preliminary experiments. In order to dissolve DNCB we added 0,1 % acetone (Laborchemie Apolda, Germany). During haptenization procedure the cells were kept in suspension by careful motion. The cells were then washed twice again. Subsequently we estimated the cell viability by the trypan blue method (9). Only cell suspensions with at least 90 % vital cells were applied.

Immunization

Guinea pigs received totally 1,5 x 10⁹ or 1,5 x 10⁹ haptenized cells by six subcutaneous injections (4 into their extremities, 2 behind their ears). To increase the sensitivity of the method, in addition, all animals...
Table I. Sensitization rates (sensitivity/total number of animals) of experimental sensitization of guinea pigs by subcutaneous injection of allogenic peritoneal exudate cells (PEC) in vitro haptenated with p-aminobenzoic acid (PABA), nickel II sulphate, potassium dichromate, formaldehyde or dimethoxybenzene (DNCB).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Number of animals</th>
<th>Number of PEC per animal</th>
<th>Hapten concentration in PEC incubation</th>
<th>Challenge test concentration</th>
<th>Sensitization rates (animals with positive test results/total number of animals tested) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 week</td>
</tr>
<tr>
<td>PABA</td>
<td>20</td>
<td>$1.5 \times 10^7$</td>
<td>$1.4 \times 10^{-4}$% (10 μM)</td>
<td>2.0% aqu. $^a$</td>
<td>0/20</td>
</tr>
<tr>
<td>Nickel II sulphate</td>
<td>20</td>
<td>$1.5 \times 10^7$</td>
<td>0.3% (10 mM)$^a$</td>
<td>0.5% aqu. $^a$</td>
<td>3/10</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>20</td>
<td>$1.5 \times 10^7$</td>
<td>0.6% (3.5 mM)$^b$</td>
<td>0.5% aqu. $^a$</td>
<td>5/10</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>10</td>
<td>$1.5 \times 10^7$</td>
<td>1.5% (50 mM)$^c$</td>
<td>1.0% aqu.</td>
<td>0/10</td>
</tr>
<tr>
<td>DNCB</td>
<td>10</td>
<td>$1.5 \times 10^7$</td>
<td>0.001% (50 μM)$^c$</td>
<td>0.05% aqu. $^c$</td>
<td>10/10</td>
</tr>
</tbody>
</table>

$^a$ plus 70% ethanol, $^b$(NISO$_2 \times $ H$_2$O), $^c$animal died during experiment, $^d$ plus 0.1% acetone, $^e$ plus 2% acetone.

were given a single subcutaneous injection of 0.1 ml undiluted Freund's complete adjuvant (Institut für Impfstoffe Dessau, Germany) into their neck.

Skin testing
After a resting period of 1–8 weeks, serial skin tests were performed. We used oclusive epicutaneous application of 100 μl of an aqueous solution (except of PABA: 70% ethanol, Laborchemische Apotheke, Germany) of the allergen in the maximal non-irritative concentration (Table I). The test was performed on the shaved left or right flank. We used adhesive patch test tape (Leucotec R, Beiersdorf Hamburg, Germany) additionally fixed by a circular adhesive bandage. After 24 h the dressing was removed and the skin reaction was evaluated. A second evaluation was performed after 48 h. The reactions were read "blind" and evaluated in the following manner: 0 = no reaction, (+) = red or pink reaction without edema or papulae, = erythema plus edema or erythema plus papulae, ++ = vesicule or crustac plus erythema, +++ = erosion of the epidermis. Only crescendo or lasting reactions of both readings were evaluated as positive test results, a reaction reaching merely the (+)-level was evaluated as negative in every case.

Control animals
A group of 10–20 animals for every experimental series were treated with unhaptenized PEC and PCA and tested in the same way as the "allergen animals". The control animals for the DNCB experiments were given cells incubated with 0.1% acetone.

RESULTS

Differentiation of PEC
The PEC samples used in sensitization experiments stained by the Pappenheim technique had the following composition:

- macrophages: 91–98% (93.59 ± 2.31 %)
- neutrophilic granulocytes: 0–6% (2.59 ± 1.85 %)
- eosinophilic granulocytes: 0–3% (0.88 ± 0.87 %)
- lymphocytes: 0–4% (1.91 ± 1.00 %)
- cells, not differentiable: 0–3% (1.03 ± 1.12 %)

By staining the non-specific alpha-naphyl acetate esterase as a marker for dendritic cells, 94.37 ± 2.42% of the total PEC were identified as macrophages.

Sensitization experiments
The results of the sensitization experiments are shown in Table I. As expected, the weak sensitizer PABA exerted the lowest sensitization rate (one animal out of 20 after 8 weeks). The ubiquitous hapten nickel II sulphate induced altogether 30% (9/30) and 66% of 20/29 positive results after 2 and 8 weeks, respectively, while the percentage of sensitization was 42% (3/73) for potassium dichromate after 2 weeks. Four test series with 20, 20, 18 and 16 guinea pigs gave similar results, indicating a good reproducibility of the experiments. In the highest hapten concentration used, the common hapten formaldehyde induced no positive test result although this concentration seemed to be non-toxic. The other formaldehyde series performed with a ten times lower hapten concentration showed allergic eczematous test reactions in 53% (17/32) and 72% (23/32) of the animals after 2 and 8 weeks, respectively. DNCB acted as a positive control. All animals reacted positively in the patch test after 2 weeks.

In the control animals of all series we did not find any positive epicutaneous test results.

The time course of the test results
The sensitization rates increased significantly (chi-squared test, p < 0.05) until the second week in all series of the nickel II sulphate, potassium dichromate and formaldehyde experiments using 1.5 X 10^7 haptenized cells (except of the disregarded first series of the formaldehyde experiments). In nickel II sulphate but not in the other hapten we found an additional significant increase (chi-squared test, p < 0.05) until the 8th week (Table I).
The influence of the number of injected cells

In nickel II sulphate, potassium dichromate and formaldehyde all series using the higher number of cells for immunization (1.5 x 10^6 per animal) did not show significantly higher or lower sensitization rates than the corresponding series using 1.5 x 10^4 cells per animal. In contrast to the series carried out with 1.5 x 10^4 PEC per animal, in the series using the higher number of cells for immunization there was no influence of the duration of the resting period between immunization and challenge.

DISCUSSION

The most important antigen presenting cell (APC) in contact dermatitis is the LC (10, 11). LC can take up and process antigen, migrate most likely under the influence of TNF alpha to the draining lymph nodes, mature under stimulation by GM-CSF (12), are capable of expressing certain cytokines (13) and to present antigen sufficiently in complex with MHC molecules to initiate T-cell response. What we have done in our experiments is to replace the APC function of LC by macrophages in the primary phase of a type IV immune reaction. The other cells of the PEC used do not play an important role as APC. Some of them may have an APC function in special cases (14) but when few, as in our experiments, they are not expected to have a significant influence.

The principal usefulness of macrophages as APC also in type IV reactions to contact allergens is widely accepted (6, 7, 10, 11, 14, 15). Despite of some differences, macrophages and LC have a functional analogy in their ability of antigen handling, presentation and T-cell stimulation, and they most likely share the same bone marrow stem cell (16, 17). However, in this study allogenic macrophages were used. The question is whether MHC molecules must be identical between APC and T-lymphocytes as a prerequisite for a successful cooperation. The identity of the MHC molecules seems to be necessary only for the secondary immune reaction, but not for the primary one (14, 18-20). This study does not give a conclusive answer to this question. The results can be interpreted as a possibility to induce contact allergy with allogenic macrophages in outbred guinea pigs. On the other hand, it is possible that allogenic macrophages exert only a carrier function for hapten, i.e. after bringing the hapten into the organisms of the animals PEC are recognized as foreign cells and are killed Consequently. The hapten may then be processed by accessory immune cells of the host. But the completely negative test result of the first formaldehyde series (PEC incubated in the higher formaldehyde concentration) speaks against this carrier hypothesis. A more likely explanation for these negative test results has been provided by investigations showing that a low concentration of antigen treatment (0.0012 to 0.005%) inhibits the expression of HLA class II antigens in human monocytes (21).

Another fact speaking against the carrier hypothesis is that such low doses of allergen carried by the haptened PEC were capable of inducing the sensitization rates achieved. Even the amount of allergen carried hypothetically by the PEC before cell washing (simply the volume of the injected cells multiplied by the hapten concentration during haptenization procedure) was about 10 (nickel II sulphate) to 1000 (formaldehyde) times lower than for instance the lowest published intradermal induction doses in the guinea pig maximization test (22-24). Because of this fact and the difficulty in exact quantification of the low amounts of allergen finally bound by the PEC (after cell washing twice), we have not included control experiments with groups of animals treated with comparable doses of a non-cell-bound hapten.

An important question to discuss is whether it is possible to obtain reliable and realistic sensitization rates in bypassing the epidermis. Now it is known that especially keratinocytes beside their barrier function take an active part in immunological processes by expression of a variety of cytokines, adhesion molecules and MHC (25, 26). In fact, this disadvantage is connected with the advantage of the method presented, namely that only few or no allergen molecules bypass the APC. From our results we can conclude that this induction method leads to realistic sensitization rates, too. It could be shown that according to the known sensitizing potentials of the hapten in man the sensitizing rates found in this assay were in parallel. This means that DNBC as a compulsory sensitizer (100% after 2 weeks) was followed by formaldehyde, potassium dichromate and nickel II sulphate as medium sensitizers, while PABA as a weak sensitizer reacted only in one animal after 8 weeks. To evaluate the significance of the method presented we considered it necessary to compare it with other predictive guinea pig tests. Numerous tests with different procedures and varying sensitization results have been published. We prefer the comparison with the guinea pig maximization test (GPM) introduced by Magnusson & Kligman in 1969 (27) and the Tierexperimenteller Nachweis (TINA-test) developed in our department by Ziegler & Süss (28) because the first-mentioned method is internationally accepted, in the second method we are experienced and both methods use an occlusive patch testing as we did.

Comparison with other results

1. Para-aminobenzenoic acid. Goodwin et al. (23) studied PABA with three different methods: the modified Draize test, the GPM and the single injection adjuvant technique. The sensitization rates were 0% (0/10, 0/10, 0/20) and corresponded with those of our study. In the TINA-test 4 of 23 animals (18%) reacted positively to PABA. This result does not differ significantly from ours (chi-squared test p > 5%).

2. Nickel II sulphate. Achieving sensitization rates of maximally 40%, Roholm et al. (22) have demonstrated that in nickel sulphate the results of the GPM correlate especially with the intradermal induction concentration. This may be an explanation of the different results of different authors mentioned in this paper. Magnusson & Kligman reached 55% (11/20) (29); other authors had lower results: 35% (7/20) (30) or 25% (7/31) (31). Our results (30% and 66% after 2 and 8 weeks, respectively) are in agreement with the GPM results already mentioned. On the other hand our results after 2 weeks but not after 8 weeks agreed with those of the TINA-test (8/33; 24%).

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3. Potassium dichromate. Our results (31/73, 42.2% at 2 weeks) do not differ significantly (chi-squared test, \( p < 0.05 \)) from those of the TINA-test (28/48, 58%). However, in the GPMIT the sensitization rates were significantly higher: 75% (18/24) (29) and 100% (10/10) (23) (chi-squared test, \( p < 0.05 \)).

4. Formaldehyde. Andersen et al. (24) studied the dependency of the sensitization rates of formaldehyde on the induction and the eliciting concentration in the GPMIT. They found 17 positive reactions in 19 animals (89%). In the same test concentration (1%), but 10 times higher induction concentrations (intradermal 1%, epicutaneous 5%) they observed 50% positive reactions (10/20). Our results observed again with 1% test concentration are between these results (17/32, 53% after 2 weeks and 23/32; 72% after 8 weeks). Magnusson & Kligman (29) reported on 16/20 positive animals (80%). 100% positive animals were found in the GPMIT (10/10) by Goodwin et al. (23).

5. Dinitrochlorobenzene. DNCB proved to be a very strong allergen in guinea pig tests. In the GPMIT the following results were reported: 15/20 (32), 10/10 (23) and again 10/10 (33). The 15/20 results may be explained by the relatively low challenge concentration (0.01%). We also found a sensitization rate of 100% (10/10).

The test results depend on various factors influencing the sensitization procedure and the challenge process. One of them is the resting period between immunization and challenge. The test series with nickel II sulphate, potassium dichromate and formaldehyde show that sensitization rates after one week are low and not representative of the expected allergenic potential of the tested substances. On the other hand it should be noted that the sensitization rates of nickel II sulphate increased until the 8th week. A possible explanation could be that the serial patch tests could act like booster applications. In order to standardize the method a resting period of 2 weeks seems to be recommendable.

Another factor expected to influence the sensitization results might be the number of injected PEC. The present results suggest that an increase in the number of PEC does not necessarily increase the sensitization rate, however, \( 1.5 \times 10^7 \) seems to be a recommendable number of PEC for immunization.

The three test series with potassium dichromate with 18 to 20 animals each, using \( 1.5 \times 10^7 \) haptens sensitized PEC showed a good agreement of the sensitization rates, which confirms the good reproducibility of our method. The differences of the results after one week underline the recommendation not to test earlier than 2 weeks after induction.

In conclusion the sensitization rates yielded with this method corresponded in all tested haptens with the known allergenic potential in humans as well as to the test results observed with the GPMIT (except of potassium dichromate) and the available results of the TINA-test (PABA, nickel II sulphate, potassium dichromate). This in vitro/vivo method is suited for the detection of the contact sensitizing potency of various water-soluble substances.

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Keratinocyte Proliferation in Epidermal Keratinocyte Disorders Evaluated through PCNA/Cyclin Immunolabelling and AgNOR Counting

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The assessment of cell proliferation is important to our understanding of hyperproliferative disorders. In this work we evaluated the proliferation characteristics of epidermal keratinocytes in diseases with abnormal keratinization by two different methods (immunostaining for the proliferating cell nuclear antigen - PCNA and histochemical staining for nucleolar organizer region - associated argyrophilic proteins - AgNORs). Twenty-seven specimens from diseases with an abnormal keratinization were studied and compared with specimens of normal human skin. As compared with the latter, the numbers of PCNA-positive epidermal keratinocytes were increased in psoriasis, congenital non-bullous ichthyosiform erythroderma, epidermolytic hyperkeratosis and chronic dermatitis and decreased in ichthyosis vulgaris, X-linked ichthyosis and pityriasis rubra pilaris. In most cases a parallel modification of AgNORs was found. We conclude that although PCNA immunolabelling and AgNOR staining do not provide strictly correlated values, both appear as useful markers for the assessment of keratinocyte proliferation in epidermal disorders. Key word: Epidermal diseases.

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Cell proliferation is one of the most fundamental biological processes, the evaluation of which has many implications both for the pathophysiological understanding and the treatment of hyperproliferative, neoplastic or hyperplastic diseases. Several methods have been proposed for evaluating cell kinetics on histological material and some of them have been applied to the study of epidermal disorders. Two techniques recently introduced are histochemical staining for nucleolar organizer region-associated argyrophilic proteins (AgNORs) and immunohistochemical labelling for the proliferating cell nuclear antigen/cyclin (PCNA). These have been applied mostly to the study of several types of cutaneous tumours. On the other hand, epidermal disorders exhibiting an abnormal keratinization usually also comprise altered proliferation characteristics. In the present work we assessed for the first time the usefulness of PCNA immunolabelling and AgNOR histochemical staining in the study of epidermal diseases with an altered keratinization.

MATERIAL AND METHODS

Tissue samples

These included twenty-seven biopsies from several diseases characterized by an abnormal keratinization (Table 1) and 6 specimens of normal human skin obtained through plastic surgery. The specimens had been collected in our dermatopathology laboratory, fixed in formalin and embedded in paraffin. The diagnosis had been established by examination of hematoxylin-eosin-stained sections using well-established criteria (1), taking also into account clinical data.

PCNA immunolabelling and counting

This was performed on 5 μm-thick deparaffinized tissue sections using a monoclonal antibody to PCNA/cyclin (clone PC10, Dako, Copenhagen, Denmark) (2) and a labelled streptavidin-biotin-peroxidase method (kit LSAB, Dako, Copenhagen, Denmark) with aminosulphylcarbazole as chromogen. Counting of positive nuclei was performed under direct microscopic examination. Results were expressed as number of positive nuclei per mm² surface of epidermal section: the latter was evaluated with the aid of a semi-automatic image analyser (videoplan, Kontron, Munich). The mean number of PCNA-positive cell nuclei (± SD) was then calculated for each disease group studied.

AgNOR histochemical staining and counting

This was performed according to the method of Ploton et al. (3). Briefly, after deparaffinization the sections were washed in deionized

Table 1. Results of PCNA and AgNOR counting

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n°</th>
<th>PCNA ± SD</th>
<th>AgNOR ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis</td>
<td>6</td>
<td>1513 ± 185*</td>
<td>2.30 ± 25**</td>
</tr>
<tr>
<td>Epidermolytic hyperkeratosis</td>
<td>3</td>
<td>977 ± 968*</td>
<td>2.35 ± 40*</td>
</tr>
<tr>
<td>Congenital non-bullous ichthyos</td>
<td>3</td>
<td>974 ± 537*</td>
<td>2.16 ± 0.12*</td>
</tr>
<tr>
<td>Chronic dermatitis</td>
<td>6</td>
<td>715 ± 256*</td>
<td>2.77 ± 0.53**</td>
</tr>
<tr>
<td>Normal skin</td>
<td>6</td>
<td>555 ± 531</td>
<td>1.93 ± 0.14</td>
</tr>
<tr>
<td>Congenital palmoplantar keratoderma</td>
<td>2</td>
<td>552 ± 458*</td>
<td>2.36 ± 19*</td>
</tr>
<tr>
<td>Pityriasis rubra pilaris</td>
<td>3</td>
<td>444 ± 164*</td>
<td>1.89 ± 32*</td>
</tr>
<tr>
<td>X-linked ichthyosis</td>
<td>1</td>
<td>322*</td>
<td>2.65*</td>
</tr>
<tr>
<td>Ichthyosis vulgaris</td>
<td>3</td>
<td>113 ± 143*</td>
<td>1.66 ± 0.07**</td>
</tr>
</tbody>
</table>

a: mean (± SD) number of labelled cells per mm² of epidermal section surface; b: mean (± SD) number per nucleus.

Statistical significance (compared with normal skin by the Mann-Whitney U-test): * non-significant, ** p < 0.05, *** p < 0.01.

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Fig. 1. PCNA immunostaining of normal human epidermis: scattered basal and parabasal cells show nuclear staining (× 250).

water and incubated for 30–40 min in the dark with the staining solution; this was freshly prepared by mixing 1 volume of 2% gelatin solution in 1% formic acid with 2 volumes of a 50% aqueous solution of silver nitrate. For each specimen at least 150 basal cells were examined and the number of AgNORs counted by the same observer (EH) in order to avoid inter-observer variation. All dots of basal cell nuclei that could be separated after appropriate focusing were counted individually. The mean number of AgNORs (± SD) was then calculated for each disease group studied.

Statistical comparison
This was performed by the non-parametric U-test (Mann-Whitney).

RESULTS

PCNA/cyclin immunolabelling

This revealed in normal skin a variable number of positive nuclei, usually within the basal epidermal layer and occasionally also of the para-basal layer (Fig. 1). The labelling intensity was somewhat variable from one nucleus to another not only among the various specimens but also within the same section; however, every effort was made to count positive nuclei in a homogeneous manner, considering the overall staining intensity of each section. In the diseases studied the appearance of the labelling was generally similar to that obtained on normal skin; however, in some diseases an obvious increase in the number of labelled nuclei was seen; these were present not only within the basal cell layer but also within the malpighian layer. This was particularly the case in psoriasis lesions (Fig. 2). The results of PCNA counting are shown in Table 1.

AgNOR histochemical staining in normal skin

This revealed a variable number of black dots within nuclei, of rather uniform size and shape. Basal cells as a rule contained more AgNORs than suprabasal ones. No major differences in the appearance of AgNORs between normal and diseased skin were detected (Figs. 3–4). The results of AgNOR counting are shown in Table 1.

DISCUSSION

The assessment of cell proliferation in histological material can
be achieved by a variety of methods, each one of which has advantages and disadvantages (4, 5). The simplest one is the direct microscopic counting of cells in mitosis, expressed as a percentage of mitotic cells ("mitotic index") or as number of mitoses per 10 high-power fields ("mitotic count"). This method can be performed in routinely-stained sections but has the disadvantage of considerable inter-observer variations. A refinement of the evaluation of cell proliferation was achieved by the study of $^3$H-thymidine incorporation. $^3$H-thymidine is a pyrimidine analogue incorporated in DNA-synthesizing cells, thus providing a good estimation of the fraction of cells in S phase of the cell cycle (but not of the length of S phase). The disadvantages of this technique, that has been applied to the study of epidermal disorders (6), include the necessity of preincubating the freshly-excised tissue with $^3$H-thymidine, as well as the potential hazards resulting from the manipulation of radioactive material. The latter problem has more recently been circumvented thanks to the generation of antibodies to pyrimidine analogues, such as $S'$-bromodeoxyuridine (BrdU) or $S'$-iododeoxyuridine (7). BrdU labelling yields results equivalent to those obtained with $^3$H-thymidine (8) and its handling is simple and rapid. One of the most widely used tools for studying cell proliferation is Ki-67; this monoclonal antibody recognizes a poorly-characterized antigen of the nuclear matrix expressed by all cycling cells (i.e., in late G1, S and G2/M phases but not in G0/early G1) (9). Some authors have used this reagent for the study of epidermal diseases (10, 11). However, a major disadvantage — that in our opinion severely hampers its use on the skin — is its (cross)reactivity with an as yet unknown cytoplasmic antigen of basal keratinocytes that occults the specific nuclear labelling (12, 13); besides, Ki-67 is only applicable to frozen tissue sections, thus limiting its utility in diagnostic pathology.

More recently, antibodies to other antigens reflecting cell
proliferation have been generated. PCNA/cyclins belong to a family of such cell-cycle regulatory proteins. PCNA was first detected in proliferating but not resting cells of human tissues by autoimmune antibodies from patients with systemic lupus erythematosus (14). Cyclin was discovered through two-dimensional gel electrophoretic studies of proliferating and quiescent cells (15). Later the identity of these two proteins (PCNA & cyclin) was shown (16). PCNA is a 36 kDa nuclear acidic non-histone protein identified as an auxiliary protein of DNA polymerase-d (17, 18). Although differences could exist between various cell lines, the expression of PCNA/cyclin seems to be highly increased during the late G1 and early S phases of the cell cycle (19-21); this also appears to be the case in cultured human keratinocytes where PCNA expression is correlated with DNA synthesis (22). Several anti-PCNA/cyclin monoclonal antibodies are currently available (PC10, TOB7, 19A2, 19F4) and some of them can be used on formalin-fixed tissue sections. The number of PCNA-positive cells seems to correlate with Ki-67 counts in nodal lymphoid neoplasms (non-Hodgkin leukaemias/lymphomas) (23, 24) and with H-thymidine counts in cultured epidermal keratinocytes (23).

Up to now, only limited data have been made available concerning the expression of PCNA in normal and diseased epidermis. It appears that PCNA is expressed in normal skin by a small percentage of basal keratinocytes (25); an increased number of PCNA-positive cells has been reported in cases of psoriasis (26), keratoacanthoma, Bowen's disease, melanoma, verrucous and squamous cell carcinoma (27-29).

On the other hand, nucleolar organizer regions (NORs) are loops of DNA occurring within nucleoli that encode for ribosomal RNA; these are located on human acrocentric chromosomes (n's 13, 14, 15, 21 & 22) and are associated with AgNORs that control their transcription. AgNORs comprise the nucleolar proteins C23 or nucleolin, B23 and a subunit of RNA-polymerase 1; they can be visualized and counted on tissue sections by a simple histochemical technique using a solution of silver nitrate. The number of NORs (and therefore of AgNORs) increases with an increased transcriptional or proliferative activity of the cell (30, 31). AgNOR counts have been reported to correlate with Ki-67 immunoreactivity in lymphomas (32) and breast cancer (33, 34). In dermatopathology, AgNOR counting has so far been applied to the study of benign and malignant neoplasms of melanocytic (reviewed in 35), fibrohistiocytic (36) and epithelial (37-39) origin.

Previous investigations have addressed the question of the proliferative profile of epidermal diseases, by studying mitotic counts, H-thymidine incorporation and Ki-67 immunostaining. The present study was prompted by the fact that within the group of epidermal diseases very few data have been published regarding PCNA expression and virtually no data at all regarding AgNOR counts. In our experience, PCNA immunolabelling proved to be a rapid procedure giving generally reproducible results. The slight variations in staining intensity observed among the different specimens are presumably due to the (inevitably) variable duration of fixation. The results we obtained with PCNA are on the whole in keeping with those obtained in previous works using mitotic counts, H-thymidine/BrdU incorporation or Ki-67 immunolabelling. Psoriasis, the most extensively studied disease, exhibits and increased mitotic count (40, 41), increased H-thymidine (6, 42) and BrdU uptake (43, 44) and Ki-67 immunolabelling (10, 11). In accordance with these results, psoriasis showed in our study the highest increase of PCNA expression and AgNOR numbers (p < 0.05 and 0.01, respectively). Chronic dermatitis ("lichen simplex") has been reported to show an increased H-thymidine uptake (45) and Ki-67 immunolabelling (10); in keeping with this, we found an increase both of PCNA and AgNOR counts that could be related to the epidermal hyperplasia seen histologically in this disease. Congenital nonbullous ichthyosiform erythroderma and epidermodysplastic hyperkeratosis, characterized by an increased H-thymidine uptake (6, 46) and increased mitotic counts (41), also showed increased PCNA and AgNOR counts. Conversely, ichthyosis vulgaris and X-linked ichthyosis, considered to comprise a normal or reduced epidermal turnover - evaluated through H-thymidine uptake (6), ki-67 immunolabelling (9), mitotic counts (41) or by their transit time (47) - showed reduced PCNA expression. Interestingly, we found that both PCNA and AgNOR values of PRP were significantly decreased as compared with psoriasis (p < 0.05). This finding suggests that the two diseases, despite clinical similarity, bear distinct cell kinetic characteristics; this is consistent with a different histological picture (1). Remarkably, an increased H-thymidine uptake has been reported in PRP (47); the cause for this discrepancy is presently unknown but the heterogeneity of PRP (48) provides a possible explanation.

On the other hand, we found AgNOR counting somewhat more problematic than PCNA counting. However, AgNORs were usually readily visualized, being uniformly sized, shaped and stained; this fact, along with the case in observing basal cells and distinguishing them from suprabasal or inflammatory cells rendered AgNOR counting easier in epidermal diseases as compared with tumoural proliferations, disorders where AgNOR counting has up to now been applied. When the values of PCNA and AgNOR counting were compared, it was found that in most diseases these varied in a similar way; thus, when compared with normal skin, AgNOR counts were increased in the case of psoriasis (p < 0.01), congenital nonbullous ichthyosiform erythroderma, chronic dermatitis (p < 0.01) and epidermodysplastic hyperkeratosis and decreased in the case of PRP and ichthyosis vulgaris (p < 0.05). The diseases in which AgNOR counts were increased despite reduced PCNA counts included X-linked ichthyosis and congenital palmpoplantar keratoderma. The unique case of X-linked ichthyosis studied does not allow conclusions to be drawn. With respect to congenital palmpoplantar keratoderma we were unable to find in the literature data concerning epidermal kinetics. It is relevant to remember here that the number of AgNORs is not correlated solely to the proliferative activity but seems also to reflect the transcriptional (metabolic) activity of the cell; therefore a discrepancy between AgNOR and PCNA counts as observed in the present study could be due to the fact that epidermal keratinocytes may show an increased metabolic activity unrelated to their proliferative capacity.
ACKNOWLEDGEMENT

We are indebted to Jeanne Soum and Josette Croisier for skilful technical assistance.

REFERENCES


Acta Derm Venereol (Stockh) 53
Scanning Electron Microscopy of Scales from Pityriasis Amiantacea

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The scanning electron microscope has been used to examine scales taken from the scalp of 3 patients with pityriasis amiantacea alone, 3 psoriatic patients with pityriasis amiantacea and one patient with both atopic dermatitis and pityriasis amiantacea. Samples from 2 patients were additionally studied by different fixation techniques and in the frozen hydrated state, but no cementing of the scales could be observed. There was no evidence of an infective agent.

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The aetiology of pityriasis amiantacea (PA) is obscure; the clinical features may be seen alone or in patients with psoriasis, seborrhoeic dermatitis and lichen planus (1). In this study, scales were examined by the scanning electron microscope using a variety of preparative techniques to see if any cementing substance could be visualized.

PATIENTS AND METHODS

Samples of scalp scale were collected from patients who presented as out-patients with the characteristic appearance of PA, see Table I. The samples were taken from the centre of patches by clipping the hair close to the scalp under the adherent scales. Initial fixation was in buffered 10% formalin, then after washing in distilled water the samples post-fixed in 1% (w/v) osmium tetroxide for several hours. After washing the samples were dehydrated through a graded series of ethanol solutions before critical point drying in carbon dioxide. The samples were mounted on aluminium stubs with colloidal silver adhesive, sputter-coated with 20 nm platinum and examined in a Jeol JSM-35CF scanning electron microscope operating at 10 kV.

In addition, samples from 2 patients (nos. 6 and 7) were processed by either omission of the formalin fixation in the process detailed above or cryofixation as described by Jones & McHardy (2).

RESULTS

The electron microscopy findings in the three groups of patients with PA showed marked similarities. All showed overlapping scales which were adherent to the hair encasing them like a sheath (Fig. 1). Parakeratosis was evident (Fig. 2). No cementing substance was apparent between the scales after standard chemical fixation (Fig. 3). Frozen hydrated specimens (Fig. 4) had a similar appearance to chemically fixed samples (Figs. 1-3).

DISCUSSION

The diagnosis of PA is clinical. Alibert is credited with the initial description (3). As part of a detailed study of 71 patients, Knight biopsied 18 patients and summarized the histological features as spongiosis, parakeratosis and variable acanthosis (4). The inflammatory infiltrate was lymphocytic.

A predisposition to PA in patients with psoriasis has been claimed (5) but not substantiated in another series (6) which had a greater proportion of patients with seborrhoea.

For many decades the patchy nature of the condition has prompted a search for an infective aetiology but although yeasts have been demonstrated (7) and cultured (8) no specific pathogen has been identified (4). In this study, the first to use scanning electron microscopy of this condition, no infective agent has been demonstrated. The material studied was hair and scale taken from within the plaques. Therefore, despite the fact that the scanning electron microscope only reveals features on the surface of the sample examined, because the samples came from within larger plaques our investigations were visualizing areas deep within the overall accumulation of scales. Once it was apparent that no cementing substance was visible between the scales processed by standard fixation, two alternatives were used to assess whether the preparation method had removed material. Scales were placed directly into the lipid fixative osmium tetroxide to assess if any lipid had been removed in the formaldehyde step. The cryofixation method retains water-soluble material and this was used to

Table I.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PA only</td>
<td>6</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>PA only</td>
<td>8</td>
<td>m</td>
</tr>
<tr>
<td>3</td>
<td>PA only</td>
<td>31</td>
<td>F</td>
</tr>
<tr>
<td>4</td>
<td>PA and psoriasis</td>
<td>14</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>PA and psoriasis</td>
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<td>F</td>
</tr>
<tr>
<td>6</td>
<td>PA and psoriasis</td>
<td>19</td>
<td>F</td>
</tr>
<tr>
<td>7</td>
<td>PA and atopic dermatitis</td>
<td>18 months</td>
<td>F</td>
</tr>
</tbody>
</table>

Fig. 1. Entangled hairs ensheathed by scales from patient no. 5. Specimen prepared by standard fixation method. Magnification ×11.
assess if any water-soluble material had been removed before the formaldehyde had time to take effect. There were no significant differences in the appearance of the scales whichever of our preparative methods was employed. In this study we could find no morphological evidence for an intercellular cementing substance causing accretion in these cells.

ACKNOWLEDGEMENTS
We are grateful to Dr W J McHardy, at the Macaulay Land Use Research Institute, Aberdeen, for his help in the cryofixation studies and Mrs Deborah Marshall for expert technical assistance.

REFERENCES
Acanthosis Nigricans and Bile Duct Malignancy

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A case of acanthosis nigricans associated with a metastatic adenocarcinoma is presented. To our knowledge this is the first one reported in the literature. The primary tumour is unknown, but it is presumably a cholangiocarcinoma. The skin changes preceded the detection of malignancy by 2 years, during which the skin lesions progressed though the patient was still in good health. If malignant acanthosis nigricans is suspected and the underlying malignancy cannot be found at the initial screening, repeated screenings are necessary because of the time factor. Key words: Paraneoplastic syndrome; Tripe palms; Internal malignancy.

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Acanthosis nigricans (AN) is characterized by hyperpigmented, velvety hyperkeratoses with papillomatosis mainly on axillae, neck and groin. AN develops in obese individuals or it may accompany endocrine diseases, often with insulin resistance and hyperinsulinemia, but sometimes AN can be a marker of internal cancer (1). If AN is associated with internal malignancy (MAN), there will often be a diffuse keratoderma of the palms – “tripe palms” – and soles. Esophageal involvement and oral manifestations may be seen in addition to the usual cutaneous findings (2–4). MAN usually appears in persons past 40. The malignant tumour is most frequently an adenocarcinoma of the gastrointestinal tract or a pulmonary carcinoma, and the skin changes may precede, accompany or follow the diagnosis of the underlying neoplasm. Often, however, it will follow an aggressive course (5, 6).

The following case report depicts a patient who developed marked AN in a very short time, the skin changes preceded the diagnosis of a metastatic adenocarcinoma by 2 years even if screening for internal malignancy was carried out.

CASE REPORT

In the spring of 1990, a 52-year-old obese woman developed skin changes round the neck, in the axillae, under the mammae, and on the abdomen (Fig. 1). The skin was thickened by a papillary overgrowth which was hyperpigmented, almost black, and the affected areas were covered with skin tags (Fig. 2).

On the basis of the characteristic clinical picture and the histological findings, a diagnosis of AN was made. Apart from these severe skin lesions the patient was asymptomatic. A slight hypertension had been treated with hydrochlorothiazid from August 1988 and with atenolol from January 1989. At the same time there was a progressive loss of scalp and body hair, which lead to alopecia universalis from September 1989. In the autumn of 1990, another department carried out a thorough evaluation in order to reveal an underlying malignancy, but laboratory findings, x-ray of the chest, thyroid gland scintigraphy, i.v. urography and mammography were all normal. X-ray of colon showed diverticulosis and liver scan only showed cholecystolithiasis. Gastr-
copy and gynecological examination also were normal. The patient was referred to our department in the spring of 1992. In the meantime considerable progression of the patient's AN had occurred. Now, there were pronounced tripalms, and furthermore AN changes had developed periorally and in the cavum nasi (Fig. 3). The patient was still in good health, and except for the skin changes she was quite asymptomatic. Blood levels of insulin and glucose were normal.

A new screening for internal malignancy was commenced. Chest x-ray supplemented with CT scan now revealed multiple, round infiltrates in both lungs, consistent with metastatic disease. Abdominal scan supplemented with CT scan revealed a $12 \times 7$ cm large mass in the right liver lobe as well as glands around porta hepatitis. Liver biopsy guided by ultrasound was performed 3 times before it was successful in getting representative material; at this time light microscopy showed liver tissue with adenocarcinoma, which was most likely a cholangiocarcinoma. It was not possible to operate, and cancer chemotherapy was considered to be of no help and so treatment with etretinate (Neo-Tigason®) was started. The patient remained in good health for another 6 months, but thereafter there was loss of appetite, severe loss of weight and pruritus. The retinoid treatment did not have any effect upon the severe skin lesions.

In the spring of 1993 the tumour in the liver was still growing and alkaline phosphatase was increasing.

DISCUSSION

Previously, 3 cases have been reported of AN associated with adenocarcinoma of the gallbladder, but to our knowledge this is the first one involving cholangiocarcinoma (7).

MAN is a well-known paraneoplastic syndrome. The term is used to describe the indirect effects of cancer that are secondary to the production of biologically active substances in the neoplastic tissue. Because of the fact that many cutaneous paraneoplastic syndromes are proliferative skin disorders, growth factors have been investigated. Ellis et al. (8) hypothesize, from a case report on a patient with a Clark’s level II malignant melanoma and skin lesions, that proliferative paraneoplastic skin disorders – such as AN, the sign of Leser-Trelat and multiple acrochordons – may have been caused by a production of epidermal growth factor (EGF) or alpha-transforming growth factor (αTGF) by the melanoma. Similarly, androgenic steroids and various peptides have been proposed as causative factors of AN (9).

Tripalms occur in patients with or without AN. In approximately 90% of the reported cases tripalms occurred in patients with cancer, most commonly gastric or pulmonary carcinomas (10, 11). It is well-known that the activity of AN often follows the underlying malignancy: AN may regress after radical operation, and it may progress in cases of recurrence or metastases of the tumour (12, 13).

If MAN is suspected, a careful screening for internal malignancy should be carried out, and in case the tumour cannot be found at the initial screening, repeated screenings are necessary because of the time factor. Unfortunately, the malignant disease is often widely advanced when finally detected.

Fig. 3. The patient got triple palms.

REFERENCES

Neutrophil Dermatosis of the Face Associated with Aortitis Syndrome and Hashimoto’s Thyroiditis

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This is the first reported case of neutrophilic dermatosis of the face (Sweet’s syndrome) associated with aortitis syndrome (Takayasu arteritis) and Hashimoto’s thyroiditis. The patient was a 39-year-old Japanese female for whom corticosteroid therapy was effective.

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We report a case of a woman with aortitis syndrome and Hashimoto’s thyroiditis, who developed elevated erythematous plaques on the face. Histological findings were compatible with the diagnosis of neutrophilic dermatosis of the face, a variant form of Sweet’s syndrome.

Sweet’s syndrome is associated with a variety of systemic conditions; however, we are not aware of any previously published report in English on these associations.

CASE REPORT

A 39-year-old Japanese woman was admitted to our hospital. She had a 5-year history of intermittent fever of undetermined origin, cough and general fatigue. On admission, she complained that an eruption had appeared on her face. Skin examination revealed several discrete erythematous and edematous plaques, 1 cm in diameter, some of which were pustular in appearance, on the right cheek (Fig. 1). Skin biopsy showed an intact epidermis, edema of dermal papillae and a diffuse, moderately dense dermal infiltrate of predominantly polymorphonuclear leukocytes.

The major abnormal laboratory data were as follows: erythrocyte sedimentation rate, 124 mm/h; leucocyte count, 9300/mm³ (neutrophils 82%); serum protein, 8.3 g/dl; with gammaglobulin, 36.6% (IgG 3358 mg/dl, IgA 633 mg/dl, IgM 213 mg/dl); thyroid test 1280 (normal <80); microsome test 500 (normal <100); TSH 4.01 (0.5–4.0) U/ml; T₄ 133 (75–165) ng/dl; T₃ 14.6 (4–11.5) μg/dl; free T₄ 1.87 (0.8–2.1) ng/dl.

Hashimoto’s thyroiditis was diagnosed on the basis of the presence of goiter and the high thyroid test and microsome test titers. The thyroid scintigram with ⁹⁹Tc showed swelling of the thyroid gland, but there were no space occupied lesions.

Aortitis syndrome was suggested by the presence of murmurs in the carotid arteries. The angiographic findings of arterial ectasia of the thoracic artery and right carotid artery and arterial ectasia and stenosis of the left carotid artery were consistent with aortitis syndrome.

The patient was treated with prednisolone at an initial dose of 50 mg daily. Systemic corticosteroid therapy led to the resolution of both the systemic complaints and the cutaneous lesions. The dose of prednisolone was gradually decreased to 20 mg daily, and the patient was discharged in good condition.

DISCUSSION

Our case showed clinically edematous erythematous plaques on the face, and histologically a dense neutrophilic infiltrate in the dermis was observed. Therefore the lesions were diagnosed as a neutrophilic dermatosis of the face, a variant form of Sweet’s syndrome.

Although the etiology of Sweet’s syndrome remains unknown, it is most commonly suggested to be a hypersensitivity reaction to various agents. Sweet’s syndrome has been found to be associated with a variety of systemic conditions. Notable among these are respiratory tract infections and myeloproliferative disorders (especially acute myeloid leukemia). This syndrome is also associated with such auto-immune diseases as Sjögren’s syndrome (1), rheumatoid arthritis (2) and subacute cutaneous lupus erythematosus (3).

Hashimoto’s thyroiditis is an auto-immune disease. From the clinical stand-point, the most important findings for the diagnosis of this disease are the presence of antithyroglobulin and antimicrosomal antibodies. In this disease, the thyroid function may be reduced, normal or even increased depending on the residual thyroid cell function, and early in the disease, the patient is metabolically normal. Our patient showed thyroid enlargement in conjunction with a high thyroid and microsome test, the serum TSH mildly elevated, but the free T₄ index in the normal range. So we diagnosed that our patient was in the early or subclinical phase of Hashimoto’s thyroiditis. Recently, it has been established that both Bacedow’s disease and Hashimoto’s thyroiditis have as their underlying cause a deficiency in suppressor T-lymphocyte function (4). Although the association of Sweet’s syndrome and Bacedow’s

Fig 1. Discrete, erythematous and edematous plaque on the right cheek.
disease has been reported (5), to our knowledge, the association of Sweet’s syndrome and Hashimoto’s thyroiditis has never been reported.

Aortitis syndrome is a rare chronic inflammatory arteritis affecting mainly the aorta and its branches. Many skin manifestations have been reported to accompany this syndrome, such as erythema nodosum, erythema induratum and pyoderma gangrenosum (6, 7), and most cases of pyoderma gangrenosum associated with aortitis syndrome have been observed in Japan (6). The clinical and histological similarities of pyoderma gangrenosum and Sweet’s syndrome have led to the suggestion that these diseases might represent two extremes of one neutrophil-mediated hypersensitivity reaction (8). We conclude that neutrophilic dermatosis of the face (Sweet’s syndrome) should be considered among the skin manifestations which can be associated with aortitis syndrome.

REFERENCES
Toxic Pustuloderma Induced by Ofloxacin

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A patient with drug-induced toxic pustuloderma is presented. The patient, who was asthmatic and who was being treated with ofloxacin for bronchitis and pharyngitis, developed intense erythemas followed by subcorneal pustulation associated with fever and a neutrophil leukocytosis. The diagnosis was confirmed by oral readministration of ofloxacin, with the result that pustular eruptions were induced. This form of drug eruption had not previously been attributed to ofloxacin. Key words: Neutrophilic dermatosis; Sterile pustulosis; Subcorneal pustule; Spongeform pustule; Drug reaction.

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Generalized pustulosis as a form of drug eruption is a rare entity. Toxic pustuloderma (TP) (1), first reported as a variant of severe drug-induced toxic erythema, is characterized by a generalized erythema with sterile subcorneal pustulation, fever and a peripheral blood leukocytosis. The clinical symptoms of acneiform generalized exanthematous pustulosis (AGEP) (2), initially reported in the French literature (3), seem to be identical with those in TP. Drugs and acute viral infections not related to psoriasis are considered possible etiologic factors in TP/AGEP (2, 4). In recent years, the reported cases of TP/AGEP following drug ingestion have been on the increase (2, 5-19).

The new fluoroquinolone antibiotic ofloxacin has gained widespread use. Although much information has accumulated about the possible adverse effects of quinolones (20, 21), pustular dermatoses are uncommon (12). In particular, no established case of subcorneal pustular eruption by ofloxacin has been reported. We here report a typical case with TP/AGEP caused by ofloxacin, which was confirmed by a readministration test.

CASE REPORT

A 64-year-old Japanese female, with no personal or family history of psoriasis, had received theophylline and ketotifen for bronchial asthma with no adverse reactions. The patient was admitted because of asthma attacks and pharyngitis. She was treated with ofloxacin 300 mg daily for a week. Simultaneously, aminophylline, 125 mg/day; bromhexine HCl, 4 mg/day; and hydrocortisone, 100 mg/day, were intravenously administered for a week. On the 5th day after administration of ofloxacin, superficial pinhead-sized pustules within areas of a widespread erythema (Fig. 1) appeared over the entire body with high fever (38.8°C). Some pustules had a tendency to coalesce.

Laboratory examination showed marked leukocytosis (18500 WBC/μl) with 86% of neutrophils. Although a mild serum hypalbuminemia was present, serum calcium values were within normal limits. Additional routine examinations, including liver and kidney functions, were normal. *Staphylococcus epidermidis* was cultured from the contents of pustules. Serologic tests for streptococcal antibodies, hepati-

Fig. 1. Pustular eruption on the thigh.
Fig. 2. Photomicrograph showing subcorneal pustule and spongiform pustules of Kogoj. A predominantly lymphocytic perivascular infiltrate was observed in the papillary dermis. (H&E ×100.)

DISCUSSION

A generalized sterile pustular reaction can be observed in association with a variety of dermatoses, such as generalized pustular psoriasis (including acrodermatitis continua and impetigo herpetiformis), subcorneal pustular dermatosis, pustular bacterid, pustular necrotizing angiitis, Sweet’s syndrome, erythema multiforme, and halogen exposure (15). Pustular psoriasis and subcorneal pustular dermatosis have both been reported to be triggered by drugs (22, 23). In the present case, there was no personal or family history of psoriasis; nor were there any clinical features supportive of pustular psoriasis or subcorneal pustular dermatosis. Among these pustular dermatoses, we concluded that our patient’s eruption was TP/AEGP. The difficulty in diagnosing the patient’s condition was also rapidly solved once she was challenged by ofloxacin.

TP/AEGP has been described as a clinical entity and is characterized by the sudden onset of intense erythemas followed by sterile pustulation (2, 15). According to the clinical analysis in the 12 cases with previous drug reaction, the time between the beginning of drug administration and the occurrence of skin symptoms varied from a few hours to 10 days (2). Skin biopsy shows subcorneal and spongiform pustules containing predominantly neutrophils, and sometimes associates with a leukocytoclastic vascitis with perivascular deposits of C3 and immunoglobulins in upper dermis. The lesions often resolve after several weeks without any systemic treatment. The etiology of TP/AEGP remains unknown. Enteroviruses, such as echovirus 11, 30 and coxsackievirus A9, are known as the triggering agents of pustular dermatoses (2, 4). Food poisoning (15), hypersensitivity to mercury (2), and PUVA-therapy (24) are also believed to play a role in initiating the disease. Most frequently TP/AEGP is induced by drugs, in particular β-lactam antibiotics, macrolides, other antibiotics and other drugs (2). The new quinolone antibiotic norfloxacin has caused a subcorneal pustular eruption (12). Pustular reactions have not been documented to ofloxacin, although hypersensitivity leukocytoclastic vascitis has been reported (20, 21).

We conclude that our patient presents an adverse drug reaction to ofloxacin in the form of a generalized pustular eruption. The entity of this clinical picture is recognized as TP/AEGP, a severe form of toxic erythema. However, the evaluation of diagnostic criteria needs to be tested further, mainly in terms of specificity for differentiating TP/AEGP from pustular psoriasis (2). In addition, the precise mechanism inducing pustular eruption seems to be a matter of importance and needs further clarification.

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Interferon Alpha-2b Treatment of Symptomatic Chronic Vulvodynia Associated with Koilocytosis

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In an open study with identical criteria for diagnosis, 16 female patients with typical symptoms of chronic vulvodynia associated with acetowhitening of the vestibular epithelium and koilocytosis in biopsy specimens received recombinant interferon alpha-2b 5 MIU intrareosionally 3 times weekly for 3 weeks or subcutaneously 3 times weekly for 8 weeks. Three months after end of therapy, clinical symptoms had disappeared in 7 (70%) of the 10 patients treated intralesionally, compared with only one (16%) of the 6 patients treated subcutaneously. In addition, the last 3 (30%) patients in the first group had a partial response to therapy. The acetowhitrning persisted in all patients. The koilocytosis remained unchanged in 13 (81%) of the 16 patients. Our results indicate that recombinant interferon alpha-2b administered intralesionally seems to be efficacious in reducing clinical symptoms of vulvodynia with suspected human papillomavirus infection but does not eliminate the infection. Subcutaneous administration had neither symptomatic nor antiviral effect. A placebo-controlled study is needed, but with our present knowledge we recommend intralesionally administered interferon as a symptomatic treatment of vulvodynia. Key words: Vulvitis; Human papillomavirus.

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Vulvodynia is characterized by vulvar discomfort, especially the sense of burning. One subset of vulvodynia is vestibulitis, and infection with human papillomavirus (HPV) may be associated in some cases (1). Diagnosis is often stated late in the course of the disease and treatment is a major problem. Surgical procedures in the form of CO2-laser treatment (2–5) and vestibulectomy with vaginal advancement (2, 3, 5–9) have been performed with some success. Especially vestibulectomy has shown good results but is quite mutilating. Treatment with interferon may be a more acceptable therapy for the patient. This treatment modality has been tried with subcutaneously/ intramuscularly (sc/im) (10) as well as intralesionally (il) (11–13) administered doses. We have treated a group of females, all with symptomatic chronic vulvodynia, acetowhitrning of the vestibular epithelium and koilocytosis, with either sc or il administered recombinant interferon alpha-2b (rIF-α-2b) (Intron-A, Schering-Plough). Our data indicate that il administered rIF-α-2b, in contrast to sc administered rIF-α-2b, alleviates vulvodynia; it does not, however, affect the acetowhitrning or the koilocytosis.

PATIENTS AND METHODS

The study took place from June 1989 to October 1992 in two departments. Only two doctors were involved in the treatment and control of the patients. Patients included were non-pregnant women, older than 18 years, presenting with typical symptoms of vulvodynia for at least 6 months associated with acetowhitrning of the vestibular epithelium and koilocytosis in biopsy specimens taken before treatment. All patients had frequent experiences of vulvar burning, external dyspareunia and a tendency of fissuring at the posterior commissure. The symptoms could present spontaneously as well as following mechanical provocation, e.g. coitus, gynecological examination, use of tampons, bicycling or use of tight clothing.

Application for 3 min of acetic acid 3% on the mucosal epithelium of the minor labia showed acetowhitrning in all cases. The acetic acid test and biopsies for histopathologic examination were performed before treatment, immediately after, 1 month and 3 months after treatment.

Treatment response was characterized according to the patient's subjective judgement as complete response (CR) (total disappearance of vulvodynia), partial response (PR) (reduction of vulvodynia) or no response (NR) (vulvodynia unchanged) 1 and 3 months after conclusion of the treatment. The patients registered side-effects during and after treatment.

The patients were treated in two groups with interferon, differing in the method of administration and in total dose. Six patients were treated with rIF-α-2b 5 MIU sc 3 times weekly for 8 weeks. Interferon was diluted in 1.0 ml of isotonic saline and injected in the thigh. Ten patients were treated with rIF-α-2b 5 MIU il 3 times weekly for 3 weeks. The intralesional injections were performed as shown in Fig. 1.

Fig. 1. Localization of intralesional injection with interferon alpha-2b.
Table I. Effect of treatment with interferon alpha-2b (IF-alpha-2b) administered subcutaneously (sc) and intramuscularly (im) 3 months after therapy

<table>
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<tr>
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<th>Symptoms</th>
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</tr>
<tr>
<td>im</td>
<td></td>
<td>7/10</td>
</tr>
</tbody>
</table>

The 5 MIU of interferon were diluted in 0.6 ml of isotonic saline. Centrally in each minor labium 0.3 ml of the solution was injected and distributed upwards and downwards, 0.15 ml in each direction. Using a 1.0 ml syringe and a 27 gauge needle. In order to reduce local pain EMLA cream was applied 5–7 min prior to the injection. To disinfect the side-effects, all patients received 1 g of acetaminophen at the time of injection and, if needed, additional 1 g twice to 3 times on the day of treatment.

Blood tests were done before and weekly during the treatment. These were haematology (red blood cell count, white blood cell count with differential, platelet count) and clinical chemistry (alkaline phosphatase, alanine aminotransferase, total bilirubin). Before treatment all the patients had negative or normal tests for cervical cell atypia (Smear), pregnancy, venereal diseases (chlamydia, gonorrhoea, Trichomonas vaginalis and Gardnerella vaginalis, Candida albicans). All patients were HIV-antibody negative. Using Student’s t-test the two groups receiving different treatments were statistically comparable (p < 0.05) as to age and period of symptoms (Table II).

RESULTS

The response to treatment and the results of the histologic examinations are shown in Table I. Only one (16%) of the 6 patients treated with IF-alpha-2b sc had symptomatic relief, while the rest had no response. In 7 (70%) of the 10 patients treated with IF-alpha-2b im symptoms disappeared 1 to 3 months after stopping therapy. In addition, a reduction in severity of the symptoms was obtained in the remaining 3 patients treated im. The aceto-whitening persisted in all patients. The koilocytosis was unchanged in 13 (81%) of the 16 patients treated.

All patients experienced typical flue-like side-effects, especially after the initial injections. The symptoms declined in most of the patients during the treatment course. None of the patients stopped therapy due to side-effects or due to no effect of the treatment. None of the patients developed leukopenia, thrombocytopenia or signs of hepatotoxicity.

Table II. Patient’s age and the duration of symptoms at the time of treatment with interferon alpha-2b (IF-alpha-2b) subcutaneously and intramuscularly

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Mean (range) in years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
</tr>
<tr>
<td>Subcutaneous (sc)</td>
<td>28.5 (22–52)</td>
</tr>
<tr>
<td>Intramuscular (im)</td>
<td>30.2 (19–39)</td>
</tr>
</tbody>
</table>

DISCUSSION

Vulvodynia is “chronic vulvar discomfort, especially that characterized by the patient’s complaint of burning, stinging, irritation, or rawness” (14). The aetiology is multifactorial and treatment often has to be directed against different factors depending on the symptoms of the individual patient (15). At the moment vulvodynia is divided into six subsets. One of these is vulvar vestibulitis, which may be associated with HPV infection (16). Vulvodynia constitutes a therapeutic problem and several treatment modalities have been tried, e.g., locally with podophyllin, podophyllotoxin, 5-fluorouracil and surgical measures as CO2-laser and vestibulectomy with vaginal advancement. Vestibulectomy has proved to have a high cure rate (3,5–8) but is rather mutilating and must be regarded as the final attempt after failure of other methods. Interferon may have an effect on diseases in which the pathophysiology is assumed to involve viral and immunological factors, and a beneficial effect of IF-alpha interferon in HPV-associated vestibulitis has been reported (10). IF-alpha-2b has proven to have some effect in treating genital warts (17–19).

The sc method of administering the interferon is obviously much less uncomfortable to the patient. Based on this we decided to use this method in the first 6 patients diagnosed in the departments. If the results were unsatisfactory – and this was the fact – the next patients should be treated im. In both groups we used higher doses compared to most previous reports to prevent that too small doses would result in treatment failure. The sc treatment was given for 8 weeks while the im was given for 3 weeks based on the assumption that the effect of interferon may be increased when injected locally.

Our results indicate that im administration of IF-alpha-2b induces a symptomatic beneficial effect in some women with chronic vulvodynia, whereas sc administration of IF-alpha-2b has no effect. Our patients have been followed for 12 to 24 months after treatment, and we cannot exclude the possibility that symptoms may recur.

We established the diagnosis based on the presence of typical symptoms (vulvar burning, external dyspareunia and fissuring) combined with the patients’ history (frequent negative microbiological tests and non-effective treatments), aceto-whitening of the epithelium and histology (koilocytosis).

Previously HPV DNA has been found in 24 of 50 women with vulvodynia and subclinical HPV infection (koilocytosis in biopsy specimens) using a Southern-blot assay (20). Neither im nor sc administration of IF-alpha-2b had antiviral activity, which indicates that the aetiology of vulvodynia is multifactorial. A placebo-controlled study will be needed to substantiate our findings and to exclude the possibility that the mere injection of an indifferent solution or the intensive care of the patient may reduce the vulvar symptoms. In view of the data presented we recommend im IF-alpha-2b as a symptomatic treatment in women with vulvodynia.

REFERENCES


Supervised Four-week Heliotherapy Alleviates the Long-term Course of Psoriasis

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1Social Insurance Institution, Helsinki, 2Department of Dermatology, University of Turku and 3Department of Dermatology, Helsinki University Central Hospital, Helsinki, Finland

The long-term effects of psoriasis heliotherapy were studied in a randomized cross-over trial with a 2-year follow-up. We allocated 95 patients randomly to receive a 4-week heliotherapy course, either at the onset or in the middle of the follow-up period. After a highly significant immediate alleviation of psoriasis about 50% of the patients still had a reduction of psoriasis 6 months later and about 25% one year later. A favourable carry-over treatment effect was still observed during the second follow-up year. Taking advantage of the cross-over design, the effect of heliotherapy was calculated to be statistically significant during the first follow-up year, and the apparent long-term alleviation of psoriasis after the heliotherapy was reflected in a significant period effect. The alleviation of psoriasis was accompanied by a significant decrease in the use of antipsoriatic treatments. Key words: Climatherapy; Psoriasis therapy; Follow-up studies.

(Accepted May 6, 1993.)


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Sun-bathing in a suitable climate (heliotherapy) is a popular form of treatment for psoriasis in patients from North European countries. The immediate alleviating effect on psoriatic symptoms of 3-4 weeks of heliotherapy is quite evident and has been substantiated in several studies (1-5). However, since a relapse of the skin condition often occurs after a median period of only 80 days (5), the cost-benefit of heliotherapy trips might be questioned, unless longer-lasting advantages, such as decreased disease severity or reduced need for anti-psoriatic therapy, can be demonstrated. We have examined this question in a randomized trial with a cross-over design and a 2-year follow-up of 95 psoriasis patients.

METHODS

Patients and study design
Psoriasis patients of working age with a mild to severe condition were referred to the study by dermatologists from various parts of Finland. Inclusion criteria included a minimum disease duration of 2 years and previous experience of alleviation of skin symptoms during natural or artificial ultraviolet (UV) exposure. Exclusion criteria included sensitive skin type I (6) and diseases incompatible with long-distance travel or southern climatic conditions. A total of 106 eligible patients, 70 men and 36 women, were randomized into two groups, G1 and G2, of equal size. The randomization was carried out in blocks of 2 patients in order of the arrival of their applications. Eleven patients, 5 in group G1 and 6 in group G2, withdrew before entering helio-

therapy, due to family and work commitments, complete temporal remission of psoriasis, or other diseases unrelated to psoriasis. In addition, 3 patients withdrew 8-12 months after heliotherapy, but their data was included in the analysis.

The follow-up schemes for patient groups G1 and G2 are shown in Fig. 1. Both groups were followed for a period of 2 years, but the timing of the heliotherapy was different. The G1 patients received their 4-week heliotherapy course upon their inclusion in the study and were then followed up for an uninterrupted period of 24 months. In contrast, the G2 patients were initially followed for 12 months, after which they participated in the 4-week heliotherapy course, and were then followed up for another 12 months. During the follow-up, either before or after heliotherapy, all conventional anti-psoriatic therapies were allowed, but the patients were discouraged from arranging sun-vacation trips of their own.

Of the total of 95 patients entering heliotherapy, 48 patients belonged to group G1 (18 females and 30 males, mean age 40 years, range 22-61 years) and 47 to group G2 (14 females and 33 males, mean age 39 years, range 20-64 years). Skin type II (6) was present in 21%.

![Fig. 1. Follow-up schemes for randomized patient groups G1 and G2.](image-url)
type III in 59% and type IV in 20% of the patients. Psoriasis was of the plaque type in 73%, guttate in 24% and erythrodermic in 1%. The mean duration of psoriasis was 18 years, range 2–39 years. A total of 23% of the patients had joint complaints. The distribution of age, sex, skin phototype, type of psoriasis and duration of disease in the two groups was comparable, as were the percentages of patients receiving their heliotherapy either in November or in March.

**Heliotherapy**

Heliotherapy was undertaken in the Canary Islands, Spain, during winter months when UV radiation in Finland is negligible. Each patient received only one course of treatment. Altogether three 4-week heliotherapy periods were arranged, in November 1988, March 1989 and November 1989. The supervising staff consisted of one dermatologist, two nurses and a sports-and-leisure coordinator. In addition to supervised sunbathing, physical exercise and patient counselling sessions were arranged.

Depending on the skin type, sunbathing was started with either 0.5 or 1 h of noon-time exposure. This exposure time was increased daily to a level of 6 h by the end of the second week. Other anti-psoriatic treatments were avoided during heliotherapy, but antirheumatic or nonsteroidal anti-inflammatory drugs were not restricted at any time. Topical glucocorticosteroid or dithranol were allowed in cases of troublesome scalp psoriasis.

**Psoriasis assessment and treatment records**

During heliotherapy, the supervising dermatologist recorded each patient’s psoriasis severity index (PSI) and use of therapy. One dermatologist (E.S.) examined the patients at 2-month intervals in the pre-and post-heliotherapy periods. The PSI scoring system involved recording of scaling, infiltration and the area of psoriasis, with a maximum PSI value of 60. In the post-heliotherapy follow-up, two relapse definitions were used in parallel, viz. time until recurrence of either 50% or 100% of the initial PSI level at the onset of heliotherapy. The disease experience over a period of time was measured by calculating each patient’s mean PSI score over each full follow-up year; from these individual annual scores, mean values and standard deviations were calculated for the whole patient group and termed the group year score (GYS). For some of the comparisons, PSI data from only the last 6 months of each year were used to calculate a group half-year score (GHYS), in order to exclude the immediate (first 4 months) effects of a preceding heliotherapy period.

Furthermore, the time until institution of antipsoriatic therapy was recorded using two criteria: firstly, the start of any antipsoriatic treatment other than plain emollient, including use of mild tar preparations or the occasional application of a corticosteroid ointment; and secondly, the institution of a specific anti-psoriatic treatment, such as phototherapy, photochemotherapy, systemic retinoid, dithranol ointment, or a self-administered sun-bathing trip. To record the use of anti-psoriatic treatments, the patients filled in weekly questionnaires at home. The term weeks on treatment was introduced to estimate therapy usage and was defined as the number of weeks a patient had been using a particular anti-psoriatic treatment on at least one out of the 7 days of the calendar week.

**Statistics**

Group differences in background variables were tested by the chi-squared test. Changes in PSI within groups were analysed using the Wilcoxon one-sample rank sum test. Use of therapy comparisons within the groups were analysed using the sign test, and comparisons between the groups were made using the Mann-Whitney test.

The 2 × 2 cross-over design was employed in the analysis of the logarithm of the PSI scores with split-plot analysis of variance (7, 8). The treatment and period effects were separately calculated, as well as their interaction (therapy × period), to evaluate if the effect of heliotherapy was dependent on the period during which it had been given. The logarithmic transformation normalized the PSI score distribution. The logarithmic PSI scores of different groups were also compared by the Mann-Whitney test. All statistical tests were two-sided.
Table I. Mean weeks on active treatment in the pre-heliotherapy year and the 2 post-heliotherapy years in patient groups G1 and G2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G1 First post-heliotherapy year</th>
<th>G1 Second post-heliotherapy year</th>
<th>G1 Pre-heliotherapy year</th>
<th>G1 Post-heliotherapy year</th>
<th>G2 Pre-heliotherapy year</th>
<th>G2 Post-heliotherapy year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticosteroid (topical)</td>
<td>14</td>
<td>17</td>
<td>28</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tar</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dithranol</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV B</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUVA</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UVB + UVA (SUP)</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinoid (oral)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-arranged heliotherapy trip</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any treatment</td>
<td>25</td>
<td>27</td>
<td>38</td>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** p < 0.001, * p < 0.05; the sign test (intra-group comparisons) and the Mann-Whitney test (inter-group comparisons).

RESULTS

Remission and relapse rates

The median PSI values in the two patient groups are shown in Fig. 2. At the onset of heliotherapy the difference between the PSI scores of the two patient groups G1 and G2 was not statistically significant. During heliotherapy, patients in both treatment groups (G1 and G2) showed a significant (p < 0.001) improvement in their PSI scores, from median values of 5.0 and 4.4 to 0.4 and 0.3, respectively (Fig 2.): the median PSI value for all patients was 4.7 at the start and 0.3 at the end of heliotherapy.

Fig. 3 shows the percentage of patients in remission defined as either PSI < 100% or < 50% of the pre-heliotherapy PSI during the first post-heliotherapy year. A PSI of 50% of the pre-heliotherapy value was reached in 85% of the patients at 6 months post heliotherapy, in 97% at 12 months, in 98% at 18 months, and in 100% at 24 months. The corresponding figures for a full relapse were 54%, 70%, 73%, and 73%.

Disease experience during the follow-up

The GYS and the GHYS are given in Fig. 2. The effect of heliotherapy was investigated taking advantage of the cross-over design of the study, by performing an analysis of variance. The effect of heliotherapy was statistically significant (p < 0.05), and there also appeared to be a significant (p < 0.001) period effect, but no significant therapy * period interaction was recorded. When the immediate (0-1 months) effect of the heliotherapy period was ruled out using the (months 6-12) GHYS, the results were essentially similar (Fig. 2). Furthermore, a significant therapy * period interaction effect was observed (p < 0.05). The period effects within GYS and GHYS and the interaction within GHYS were caused by the much lower PSI scores of group G1 in the second post-heliotherapy year as compared with the PSI scores of group G2 in the pre-heliotherapy year, thus also indicating the existence of a carryover effect within the group G1.

During the first year of the study, the patients randomized to an initial heliotherapy period (patient group G1) had a PSI GYS of 4.2 ± SD 3.3, which was significantly (p < 0.05) lower than the GYS of 6.2 ± SD 5.5 displayed by the patients randomized to an initial year of follow-up only (patient group G2). Group G2’s GYS of 4.5 ± SD 4.2 observed in the post-heliotherapy year was highly significantly (p < 0.001) lower than the same group’s pre-heliotherapy GYS. Due to the above-mentioned sustained influence of the previously instituted heliotherapy, the GYS of the G1 group of the second post-heliotherapy year did not differ significantly (p = 0.455) from the GYS of the first post-heliotherapy year within the same group.

Use of therapy

Cumulative percentages for the institution of treatment after heliotherapy are shown in Fig. 3. Some form of treatment was instituted at 6 months after heliotherapy by 89% of the patients, at 12 months by 97%, at 18 months by 99% and at 24 months by 100%. The corresponding figures for instituting a specific anti-psoriatic treatment were 55%, 79%, 86% and 91%. The use of different therapies in each follow-up year is detailed in Table I.

In both patient groups, the number of weeks on treatment in the (first) post-heliotherapy year (25 weeks in both cases) was very significantly (p < 0.001) lower than that (38 weeks) in the pre-heliotherapy year of the G2 patients. In contrast, no significant difference was discernible in use of therapy between the first and second post-heliotherapy year of patients in group G1, corroborating the aforementioned sustained influence of the previously instituted heliotherapy on psoriasis symptoms.
in the second follow-up year. The post-heliotherapy decrease in use of therapy was principally due to a significant reduction in the use of topical glucocorticoid ointments and systemic retinoids.

On the other hand, 21 patients in group G2 spent a total of 33 weeks on self-arranged sun-bathing trips in the first post-heliotherapy year, in contrast to only 5 patients spending a total of 8 weeks on sun-bathing trips in the preheliotherapy year. In patient group G1, 14 patients spent a total of 31 weeks of sun-bathing trips in the first post-heliotherapy year and 20 patients spent 47 weeks on such trips in the second post-heliotherapy year. However, when the median PSI values for the first post-heliotherapy year were calculated separately for the patients who had made sun-bathing trips and those who had not, no significant difference was found. Furthermore, in the patients who had participated in the self-arranged sun-bathing trips, the PSI scores, recorded at 2-month intervals, were similar before and after these trips ($p = 0.86$).

**DISCUSSION**

The immediate and short-term (up to 6 months) data of our study corroborate earlier reports by us (5) and others (1-4) to the effect that heliotherapy effectively clears psoriasis. However, our investigation goes beyond any previous study in respect of its randomized design, the total length of the post-heliotherapy follow-up (up to 2 years) and in the use of precise criteria for recording disease severity and therapy usage. The main characteristics of the randomized patient groups were comparable in all pertinent respects. The both randomization groups were, furthermore, comparable as to the percentage of patients receiving their heliotherapy either in November or in March, thus eliminating any influence of this variable in between-group comparisons. The study design, incorporating an initial full year of pre-heliotherapy follow-up in one of the patient groups, provided a unique opportunity to make a parallel comparison of non-heliotherapy-treated and heliotherapy-treated patients. Our design also allowed a comparison, within one of the patient subgroups, of disease and treatment use scores from a pre-heliotherapy and a post-heliotherapy year.

The somewhat unexpected finding of long-term (up to 2 years) alleviation of psoriasis severity was reflected both in the psoriasis severity scores and the therapy usage scorings (Fig. 2, Table I). This effect may be caused by a number of mechanisms which do not necessarily exclude one another. First of all, the patients received a high UV radiation dose during the heliotherapy period. In parallel studies (9), we have recorded a mean total load of 118 erythemal units (EU) of sunburning radiation during our heliotherapy courses—a large dose to be received in only 28 days. This intensive UV radiation may have long-term metabolic or immunological effects on the skin; we have e.g. reported earlier that a 4-week heliotherapy course induced a marked, sustained elevation of epidermal total urocanic acid (UCA) levels, possibly by induction of the UCA-forming enzyme, histidase (10). The significantly diminished use of glucocorticoid ointments in the post-heliotherapy period (Table I) may also have exerted a favourable influence on the patients’ condition. Long-term use of topical glucocorticoids is known to lead to cutaneous tolerance and a rebound effect with concomitant exacerbation of psoriasis (11), and a week of total withdrawal from the use of corticosteroid creams may, conversely, have had a favourable effect. Furthermore, the patient counselling provided during the heliotherapy courses may have led to a more consistent and stringent approach to home treatment, resulting in better disease control with a minimum of therapy expenditure. Interestingly, the post-heliotherapy increase in self-arranged sun-bathing trips did not influence the overall follow-up outcome. This is probably due to the short duration of the self-arranged trips (usually one week).

Psoriasis may also be influenced by psychosocial factors (12) and some of the curative effects of heliotherapy may be due, for instance, to psychological relaxation connected with freedom from usual responsibilities, and other factors such as psychological support from the heliotherapy staff and fellow patients. However, it is difficult to conceive that a psychological effect of this kind would last for up to 2 years after a heliotherapy trip. We have, in fact, been able to record favourable effects in the occurrence of psychosomatic symptoms only in the immediate post-heliotherapy period, with a reversal to initial levels within a period of 2-3 months (manuscript in preparation).

Irrespective of the reasons for the favourable outcome, our study demonstrates that, in addition to the substantial short-term (up to 6 months) curative effect of heliotherapy in psoriasis, a long-term (up to 2 years) alleviation of psoriasis severity can be achieved. This finding lends additional credibility to heliotherapy as a treatment modality in mild to moderately severe cases of psoriasis.

**ACKNOWLEDGEMENTS**

We thank dermatologists all over Finland for referring patients to the trial or participating in the clinical examinations during the heliotherapy periods, and staff members of the Social Insurance Institution for assisting in technical arrangements of the study. Our thanks are also due to the Departments of Dermatology at Helsinki, Tampere and Oulu Universities for providing the facilities for the clinical follow-up of patients. This study was supported by the Social Insurance Institution and the Psoriasis Association in Finland.

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LETTERS TO THE EDITOR

Is Vitiligo an Acquired Pigmentary Anomaly?

Sir,
I read with interest the article by H. Zachariae et al. (1) about autotransplantation in vitiligo. Although the aim of the authors was not to discuss the aetiology of vitiligo, I think that the following remark is relevant.

The statement that vitiligo “is an acquired pigmentary anomaly of the skin manifested by depigmented white patches” does not correspond to reality. Many authors (2-10) affirm that genetic factors are involved in the aetiology of this pigmentary anomaly. It seems that about 2/3 of the cases of vitiligo are sporadic (11), but this fact does not preclude the possible and probable hereditary character of many sporadic cases. It is known that there are cases of vitiligo in 2 or 3 successive generations; but there are also families with 2 or 3 affected siblings, whose parents were free of vitiligo.

The autosomal dominant character of inheritance of the former, as well as the autosomal recessive mode of inheritance of the latter, is evident. There are also cases where the inheritance seems to be polygenic. So vitiligo is very probably a heterogenic entity.

Finally, there are concordant cases of vitiligo in uniovular twins (7). All these facts speak in favour of the thesis that vitiligo is not an acquired pigmentary anomaly. It is an entity in whose aetiology genetic factors play an essential role. Unfortunately, there are few articles about the heredity of this common pigmentary disorder, whose diagnosis is very easy.

REFERENCES

Received March 25, 1993.

Professor Tibor Salamon, M.D., D.Sci., Department of Stomatology, Faculty of Stomatology, University Sarajevo, Zagreb, Buljanova 101, Croatia.

In response to the letter by Salamon
In our article about autotransplantation in vitiligo, we used the definition of vitiligo stated in the latest edition of "Andrews: Diseases of the Skin" (1), without any intention of discussing the etiology of the disease. The same textbook also refers to several mechanisms (which are not mutually exclusive) within the pathogenesis, and other textbooks also offer a discussion about genetic factors. These have been proposed as autosomal dominant suspected, autosomal dominant with variable expressivity, or polygenic. I do not think that our article should be a basis for a discussion of this matter but agree that there are rather few articles about the heredity of this common pigmentary disorder.

REFERENCE

Hugh Zachariae, Department of Dermatology, Merselsborg Hospital, Aarhus, Denmark.
Calcioptriol or Clobetasol Propionate Occluded with a Hydrocolloid Dressing for Treatment of Nummular Psoriasis

Sir,

Recently it has been reported that occlusive treatment of nummular psoriasis with hydrocolloid dressings together with either group III or IV corticosteroid ointments has been successful. It was considered an easy and rapid procedure and a supplement to other treatments (1, 2). However, relapses occurred faster applying this method.

During the last 5 years a new topical antipsoriatic vitamin D₃ analogue, calcipotriol (Dainonex®, Levens Kemiske Fabrik, Denmark) has appeared (3). It was, therefore, considered of interest to compare clobetasol propionate (Dermovate®, Glaxo, UK) with Dainonex® occluded with a thin hydrocolloid dressing (Contracet®, A/S Coloplast, Denmark).

Fifteen patients, 11 men and 4 women, average age 23 years (range 18–48 years) were included in the study. Patients with pruriginous psoriasis or a family history of atopy were excluded due to the well-known irritant side-effects of Dainonex® ointment.

The trial was performed single-blind, right/left on symmetric lesions of nummular psoriasis localized to different regions. Ointments were applied to the lesions and covered with hydrocolloid dressings. The treatment procedure lasted for 4 days and was repeated three times, resulting in a total treatment period of 12 days. Standardized photography was performed prior to the trial and after every single change of ointments and dressings. A clinical and photographic follow-up examination was also performed 2 weeks after the treatment period.

Two patients were excluded after the first two periods of treatment (8 days) because of intolerable local irritation from the Dainonex® ointment. Thirteen patients completed the trial and at the clinical and photographic assessment, erythema localized to the lesion area was found in 2 women and 1 man. Irritant reactions were only demonstrated in lesions treated with Dainonex® ointment. Lesions had disappeared on both sides and the erythematous reaction of Dainonex® ointment did not interfere with the treatment effect. Scaling disappeared in all patients, and the skin was found completely smooth. The patients’ subjective opinion of the treatment effect corresponded well with the objective assessment.

At the final follow-up examination 2 weeks post treatment, it was not possible in those patients who completed the study to differentiate between lesions treated with Dainonex® or Dermovate® ointment occluded with Conratect®. The erythematous reaction found in 3 patients at the end of the trial period had completely disappeared.

Previously, it has been reported that no significant difference exists between betamethasone valerate and calcipotriol ointment without occlusion (4). According to the results of the trial, the appearance of lesions seems to be the same in both groups. It was, therefore, concluded that the efficacy of a group IV corticosteroid ointment and calcipotriol ointment combined with hydrocolloid occlusion was equal. Application of calcipotriol occluded with hydrocolloid dressings has the benefit of avoiding steroid side-effects by repeated treatments and appears to be a possible treatment for some patients. Used as a supplement to daily treatment of psoriasis, it may restrict the amount of UV-light necessary for clearance.

ACKNOWLEDGEMENT

The study was kindly supported by A/S Coloplast, Kokkedal, Denmark.

REFERENCES


Received April 20, 1993.

P. Gamborg Nielsen, MD, Department of Dermatology, Varberg Hospital, S-432 81 Varberg, Sweden.
Psoriasis Exacerbation Induced by Interferon-α. Report of Two Cases

Sir,

Hepatitis C virus is an important cause of post-transfusion hepatitis and a common cause of acute sporadic hepatitis, which often leads to a chronic form. At present there is no therapy of established benefit for chronic hepatitis C. Recent studies have suggested that prolonged therapy with interferon-α may be useful in some patients with chronic hepatitis C (1).

We report the first 2 cases of psoriasis induced by interferon-α in patients treated for chronic hepatitis C.

Case 1

A 60-year-old man, affected with chronic hepatitis C (hepatitis activity index, HAI: 7), diagnosed in June, 1992, received 3 million units (MU) of rIFNα2b (recombinant interferon-α2b) subcutaneously three times a week. Psoriasis, confirmed by a skin biopsy, had been diagnosed 1 year before the chronic hepatitis C diagnosis but had not required treatment. He had no family history of psoriasis. During the first 6 weeks of treatment the psoriatic lesions flared up, with generalized plaques (psoriasis area and severity index, PASI, increased from 4 to 7). Interferon treatment was interrupted after 4 weeks followed by substantial improvement of psoriasis which, without therapy, returned approximately to its previous state (PASI: 4.5) in 2 weeks.

Case 2

A 42-year-old man developed a chronic hepatitis C (HAI: 4) in 1989. He was included in a clinical trial in which 3 MU of human lymphoblast interferon-α were injected subcutaneously three times a week. He had been affected with psoriasis, confirmed by skin biopsy, since 1980 and he had a positive family history of psoriasis (his father). Before treatment with interferon-α, psoriatic lesions were limited to elbows and scalp only (PASI: 0.8). Two weeks after the start of treatment with interferon he developed large plaques of psoriasis on elbows, knees, chest and scalp (PASI: 6.6). The patient continued treatment with interferon. The lesions were treated with topical drugs without further worsening.

DISCUSSION

Interferons were originally expected to be a rational choice of therapy for psoriasis as well as tumors and viral infections because of their antiproliferative property (2-4). Recombinant human interferon-α has been used in the treatment of malignant metastatic carcinoïd tumour, metastatic renal carcinoma, and more recently chronic hepatitis C and other diseases (1,5). Various cutaneous side-effects have been described after treatment with interferon-α, including itching, dryness and moderate hair-loss (5).

In 1986 Quesada & Guttermann reported the cases of 3 patients whose psoriasis was aggravated or induced during treatment with recombinant human interferon-α (3). Shirahara et al. described a rapid development of psoriasis on the warts of a hand after an intradermal injection of interferon-α. The presence of the Koebner phenomenon in the patient described was unlikely, because similar cutaneous lesions did not appear in the sites of dinitrochlorobenzene administration (2).

These reports suggest the possibility that interferons may participate in the pathophysiology of psoriasis. Interferon-γ and also interferon-α activity was demonstrated in suction blister fluid obtained from psoriatic skin but not in the blister fluid of unaffected skin. Abnormal serum levels of interferon have also been found in the sera of psoriatic patients (6). Moreover, the activity of psoriatic lesions has been associated with a reduction in cAMP/cGMP (cyclic adenosine monophosphate/cyclic guanosine monophosphate) ratio, while it is known that interferon-α alters the intracellular content of these nucleotides, inducing an increase in cGMP concentration (3,5).

In our opinion our cases and the previously quoted reports (2-5, 8, 9) suggest that interferon-α may play an aggravating role in psoriasis, while the mechanism through which this occurs is still unclear. Baker et al. (7) stressed that interferons involve those cells and mechanism that seem to be particularly altered in psoriatic lesions.

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Seborrheic Dermatitis and Daylight

Sir,
We have read with interest the short report "Seborrheic dermatitis and daylight" (Acta Derm Venereol (Stockh) 1991; 71: 538-539) (1).

The figure presented clearly shows the number of visits made by relationship between the number of gloomy hours per day per month and the seborrheic dermatitis outpatients. We do agree that the role of ultraviolet light or visible light on seborrheic dermatitis healing is not clear. The identification of a humoral factor acting via the retina has to be explored. The authors also mention that a relationship between melatonin secretion and sebum output has never been investigated. We have performed such a study (2) on 12 healthy male volunteers, during 30 consecutive hours. Sebum excretion was evaluated every hour using sebum-absorbent tape (Sebatape). Blood samples were collected every 2 h from 0.00 h to 24.00 h and then hourly until 07.00 h. Melatonin was assayed using a TECOVA Kit (ORUS Industries, France). We confirmed a circadian rhythm of sebum excretion. The elevation of sebum excretion was correlated with an increase in the number of sebaceous follicles. The acrophase of forehead sebum excretion occurred at 13.00 h. We failed to demonstrate any correlation between sebum excretion and plasma levels of melatonin. Therefore it seems very improbable that seborrheic dermatitis recurrences are influenced by melatonin secretion through sebum output.

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