PSORIASIS
THERAPY AND PATHOGENESIS

P. C. M. VAN DE KERKHOF
PSORIASIS
THERAPY AND PATHOGENESIS

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OP WOENSDAG 8 JUNI 1983
DES NAMIDDAGS TE 16 00 UUR

DOOR

PETRUS CORNELIS MARIA VAN DE KERKHOF

GEBOREN TE AALST

krips repro meppel
The investigations described in this thesis have been carried out at the in-patient and out-patient departments of the department of Dermatology (Prof. Dr. J.W.H. Mali) at the University of Nijmegen. Laboratory studies have been carried out at the section of Biochemistry (Dr. P.D. Mier), the section of Flowcytometry (Dr. F.W. Bauer) and the section of skin barrier function analysis (Ing. G.J. de Jongh) of the department of Dermatology (Prof. Dr. J.W.H. Mali), University of Nijmegen, the Netherlands.
Aan mijn ouders,

Elly en de kinderen Esther en Frans
DANKWOORD

Dankbaar ben ik jegens mijn ouders. Door hun goede voorbeeld werd ik gestimuleerd bij mijn studies. Mijn vrouw Elly is in de jaren dat dit proefschrift werd voorbereid een ware steun geweest, waarbij onze kindertjes Esther en Frans op een geheel eigen wijze een vrolijke noot hebben meegespeeld. Mijn leermeesters in de klinische dermatologie, mijn collega's en de verpleegkundige staf (in het bijzonder Zr. A. Ph. Groëls en Zr. C.P.M. van Bakel) ben ik veel dank verschuldigd. Het onderzoek voor dit proefschrift is mogelijk geweest door de prettige samenwerking.

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Tenslotte wil ik allen danken die op nog andere wijze hun bijdrage hebben verleend bij de totstandkoming van dit proefschrift.
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INTRODUCTION

Psoriasis, as one of the most common of all skin diseases, is described in every text-book of dermatology; perhaps the most detailed account of the clinical picture is given by Baker and Wilkinson (1979). In brief, it is characterized by sharply defined erythematous plaques surmounted by fine silvery scales (fig. 1). The presentation may vary along a spectrum whose extremes are a chronic plaque type ('psoriasis vulgaris') and an unstable generalized form ('generalized pustular psoriasis'). Histologically, we see abnormalities in various regions of the skin (fig. 2); these include dilatation and tortuosity of the capillaries, moderate dermal infiltrate which focally penetrates the epidermis, hyperproliferation, and in places absence of granular layer with accompanying parakeratosis.

The prevalence of psoriasis is about 2% of the world's population; although there is some disagreement on this point, no gross differences between individual ethnic groups have been established. Overt lesions may appear at any age, but the disease most commonly begins in young adults. The course is unpredictable but usually chronic; complete spontaneous remissions are uncommon. It is generally agreed that psoriasis has a genetic basis, but detailed family studies have established that this cannot be explained in terms of a single allele. Many observations support the concept of a multifactorial determination. However, in contrast to the classical 'inborn errors of metabolism' psoriasis also manifests a strong dependency on non-genetic factors such as emotional stress. It has already been suggested in 1883 by Weyl, that the skin manifestations represent a peripheral projection of disruptions in the central nervous system.

From time to time reports of systemic abnormalities have appeared in the literature (Mier and Cotton, 1976; Krueger, 1981). These include changes in plasma levels of various metabolites, endocrine abnormalities, and more recently, abnormalities in various immunocompetent cell types. However, in general these are seen as statistical differences between large groups rather than diagnostic features of the disease.

Although it is, at the moment, impossible to achieve a permanent 'cure' for psoriasis, many therapeutic approaches will effect a more
Figure 1 A typical psoriatic lesion
Figure 2 The histology of the psoriatic lesion. (a) overview (H.E. staining) (b) penetration of leucocytes into the epidermis (H.E. staining) (c) tortuosity and dilatation of a capillary (alkaline phosphatase staining)
or less temporary remission. All those in common use are quite empirical; the most popular include the following:

(i) Local fluorinated corticosteroids are widely employed, especially in the chronic plaque type of limited extent. The metabolic effects of corticosteroids are so diverse that no agreement has been reached as to the specific 'target' in the lesion.

(ii) The combination of dithranol, tar bath and UVR ('Ingram' therapy) is especially valuable in the more widespread disease. It has been proposed that this therapy acts via inhibition of enzymes of intermediary metabolism, curtailing supplies of energy and metabolites required for cell division.

(iii) Methotrexate is of value in all forms of 'difficult' psoriasis. Its position as a cytostatic agent (inhibiting the enzyme dihydrofolate reductase) leads to the obvious concept that it blocks DNA synthesis in the basal cell layers of the epidermis, but other targets cannot be ruled out.

(iv) Photochemotherapy (PUVA) has more recently become popular. It is also claimed to be of value in all forms of psoriasis. It is clear that cross-linking of DNA occurs, which may block transition from $G_2$ to $M$ phase of mitosis in basal cells of the epidermis; again, however, additional targets cannot be excluded.

The pathogenesis of psoriasis remains completely unknown. Roughly, theories may be grouped according to which of the above abnormalities are regarded as 'primary'.

(i) The increased proliferation may be regarded as primary; thus the incomplete keratinization is the logical result of reduced transit time.

(ii) Conversely, many authors have suggested a primary fault in the keratinizing process; the hyperproliferation is consequently a normal response to impaired barrier function.

(iii) A further alternative is the dermal compartment; it is clear that both epidermal abnormalities may be secondary to alterations at the dermal level.

(iv) More recently, several authors have proposed that a systemic 'fault' could result in impaired stability of cutaneous homeostasis. Examples include endocrine factors (Mali, 1979) and
bone-marrow derived cells (Mier et al, 1980).

The aim of this thesis is to fill certain of the gaps in the above data. In particular we have investigated the resolution of the lesion during therapy (chapters II-IV); secondly, we have examined certain local and systemic factors which may influence cutaneous homeostasis (chapters V-VI) and finally, we have attempted to clarify the interrelationship of the various skin compartments in the pathogenesis of psoriasis (chapters VII-X).

REFERENCES


CHAPTER II

LOW DOSE PUVA MAINTENANCE IN PSORIASIS
FOLLOWING INGRAM THERAPY

P.C.M. van de Kerkhof and J.W.H. Mali

SUMMARY

Following the initial treatment of severe psoriasis with conventional Ingram therapy, it is shown that PUVA maintenance at a mean dose-rate of 28.6 J/cm²/month increases the average period of remission from 7 weeks to more than a year.

Surprisingly, the patients who withdrew from maintenance therapy whilst still in remission have so far continued (10 months) to show an extremely low relapse rate.

PUVA is now accepted as one of the most useful therapies for the treatment of psoriasis, being effective, convenient and relatively free from immediate side-effects. However, certain recent reports of neoplastic changes as possible longterm sequelae following very high doses of PUVA (Bridges and Strauss, 1980; Hofmann et al, 1979; Stern et al, 1979) suggest that it is advantageous to use the minimum cumulative dose compatible with therapeutic success.

In this Department, PUVA has been employed for the clearance of established lesions. An alternative approach is now being explored, namely the use of conventional treatment such as Ingram therapy to achieve initial clearance of lesions, followed by minimal-dose PUVA to maintain the remission. Although this investigation is still in progress, certain of our observations seem sufficiently striking to warrant preliminary publication.

MATERIALS AND METHODS

A total of 27 patients have so far participated in this study, which started January, 1978 (table 1, PUVA-maintenance group). These were selected as being particularly 'difficult' patients, all fulfilling the following criteria: (a) a long history of psoriasis (b) failure to obtain satisfactory control on an out-patient basis (c) more than 30% of the body surface involved at the time of examination.
Table 1: Composition of the PUVA-maintenance and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>M/F</th>
<th>Mean age</th>
<th>Mean duration of psoriasis</th>
<th>Classification of psoriasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUVA maintenance</td>
<td>27</td>
<td>16/11</td>
<td>34.0</td>
<td>16.4</td>
<td>24</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>12/18</td>
<td>37.5</td>
<td>10.7</td>
<td>27</td>
</tr>
</tbody>
</table>

All patients were admitted to the in-patient Department and were treated according to Ingram (1954) until free from lesions. This treatment consisted of tar-bath, exposure to mercury vapour lamps (UVA + UVB) and the application of dithranol paste, beginning at 0.05% and increasing to a maximum set by the tolerance of the individual patient. Fluorinated corticosteroids were in general avoided. This phase of treatment usually required about 5 weeks.

Within 3 days of completion of Ingram treatment, PUVA therapy was begun according to the dose-schedules for 8-MOP and UVA described by Wolff et al (1977) and using a Waldmann 6001 cabin (H. Waldmann and Co., Werk für Lichttechnik, Schwenningen). Initially each patient received one treatment per week. In those patients maintaining satisfactory remission for 4-5 months (no more than minimal involvement which appeared to be stable), this was reduced to one treatment on alternate weeks, and after a further 4-5 months to one treatment each 3 weeks. In the case of patients showing any indication of incipient relapse (i.e. the reappearance of significant lesions but less than the criteria defined below) the UVA dose was increased by 0.5 J/cm² for skin types I-II or by 1.0 J/cm² for skin types III-VI (Wolff et al, 1977), but the frequency of treatment was not changed.

Each patient was examined at intervals of 2-3 weeks. For the purposes of this study, a 'relapse' was defined as the reappearance of more than 2 palm-sized lesions or of multiple nummular lesions of an approximately similar total area; in these cases PUVA maintenance was discontinued. Of the patients remaining in remission, maintenance
therapy was eventually stopped at the discretion of the investigator or at the request of the patient. As far as possible (14 patients) follow-up of this latter group was continued after cessation of maintenance therapy. The average duration of PUVA maintenance (whole group) was 8.7 ± 5.8 months and the mean cumulative dose 249 ± 167 J/cm². This corresponded to an average dose-rate of 28.6 J/cm²/month.

The control group (table 1, controls) consisted of patients who satisfied the criteria described for the PUVA maintenance group, but had been admitted to the in-patient Department for Ingram therapy in the period immediately prior to the present investigation. The period between termination of therapy and 'relapse' (as defined above) for the control group was established by retrospective analysis.

RESULTS

The relapse-rate for the control group is shown in fig. 1. It is seen that clinical lesions had recurred in 50% of these patients within 7 weeks after completion of Ingram therapy. By contrast, in the PUVA maintenance group (fig. 2), only 3 relapses occurred in total; 75% of the patients were still free from significant lesions one year after discharge from the in-patient Department. It may be relevant that 2 of the 3 relapses were psoriasis pustulosa.

A remarkable and wholly unexpected finding has resulted from the follow-up of the 14 patients who stopped PUVA maintenance whilst still in remission and who remained available for regular control. Instead of reverting to the high relapse-rate characteristic of the control patients, the 'post-maintenance' group continues to follow the curve of fig. 2. So far 12/13 (92%) have remained free of lesions 4 months after withdrawal from maintenance therapy, and 7/9 (78%) who have completed 10 months without treatment are still in remission. These figures both differ from the control group at the levels P < 0.01.
Figure 1 Control group (n = 30). Percentage patients remaining in remission at various times after completion of Ingram therapy.

DISCUSSION

Our findings for the control group are comparable to those of other workers who have studied patients with especially severe psoriasis. For example Briffa et al (1980a) reported that following Ingram therapy 74% of such patients relapsed to one-half of their pretreatment area after 5.4 months. It is seen from fig. 1 that 74% of our patients had begun to relapse after an average of 3.2 months; it is reasonable to assume that this relapse would reach the stage described by these authors some 2 months later.

The value of PUVA maintenance has already been established
following initial clearance with this regime (Wolff, 1977; Briffa et al, 1980b). The present investigation demonstrates that PUVA is equally for the maintenance of patients cleared with Ingram therapy, an approach which obviously necessitates far lower cumulative doses of UVA.

The observation that 'post-maintenance' patients show a very low relapse rate is surprising. Wolff (1977) remarked that certain patients 'remained free of lesions for months despite the fact that maintenance therapy was discontinued', but gave no figures to support this statement, and no further attention has apparently been paid to this aspect of PUVA treatment. Following initial clearance with PUVA
(but no subsequent maintenance) the relapse rate is identical to that after Ingram therapy only (Briffa et al, 1980a). It is, of course, possible that the relapse-curve following withdrawal from PUVA maintenance will prove to be sigmoid rather than exponential, but in any case a significant therapeutic advantage seems to be feasible.

It is difficult to postulate a satisfactory explanation at the cellular level without recourse to unlikely hypotheses such as the existence of a discrete sub-population of 'psoriatic' keratinocytes. Nevertheless, these findings strengthen the hope that psoriasis therapy may eventually be directed at modifying the long-term course of the disease rather than achieving a brief remission of symptoms.

ACKNOWLEDGEMENTS

I wish to acknowledge the skilful and painstaking work of zr. A.Ph. Groëls and her staff of nurses in carrying out the treatments described in this report.

REFERENCES


CHAPTER III

PUVA MAINTENANCE IN PSORIASIS

P.C.M. van de Kerkhof

We have recently reported a retrospective study of PUVA maintenance following Ingram therapy (van de Kerkhof and Mali, 1981). Two conclusions were reached; first, that the average period of remission was increased from 7 weeks (control group) to more than a year (PUVA group) and second, that patients who withdrew from maintenance therapy whilst still essentially symptom-free did not revert to the high relapse-rate of the control group but tended to remain in remission for long periods. However, whilst our article was still in press, the 'European PUVA Study' was published (Henseler et al, 1981). The authors confirmed the 'dramatic efficiency of PUVA in clearing psoriasis', but found no difference in remission rates between groups with or without subsequent maintenance treatment.

We therefore felt it desirable to re-evaluate and update our own study from 27 patients (May 1980) to 47 patients (January 1982). Using the dose-schedules and relapse criteria described previously, 13/20 patients were still in remission after 12 months continuous maintenance compared with 9/12 reported previously; these figures are not significantly different (chi-squared test). Following withdrawal from therapy, the actuarial remission curve is illustrated in fig. 1. Again this is in agreement with our earlier data.

The major difference between the European study and our own investigation is, of course, the treatment used for initial clearance of the lesions (PUVA and Ingram therapy respectively). Henseler and his colleagues comment on the adverse effect of pigmentation, the rationale of their approach being 'to clear the lesions before intense pigmentation raised the tolerance of skin to UV radiation'. It seems probable, therefore, that our success with PUVA maintenance is simply because we are able to begin this phase of treatment with relatively non-pigmented patients.
Figure 1 Actuarial remission curve after withdrawal of patients from PUVA maintenance (open circles). The control group of patients cleared with Ingram therapy who received no PUVA (filled circles) is taken from v.d. Kerkhof and Mali (1981).

REFERENCES


CHAPTER IV

METHOTREXATE MAINTENANCE FOLLOWING INGRAM THERAPY IN 'DIFFICULT' PSORIASIS

P.C.M. van de Kerkhof and J.W.H. Mali

SUMMARY

A retrospective analysis is presented summarizing 9 years' experience using a combined regime of Ingram therapy followed by methotrexate maintenance in the management of especially difficult psoriatic patients. The lesion-free period was extended from about 1 month (controls) to 1 year, and period before re-admission was extended from 5 months to more than 3 years.

In this Department we have, for more than twenty years used Ingram therapy for treating those psoriatic patients who cannot be managed satisfactorily on an out-patient basis. Generally this is very effective in achieving a total clearance of lesions, the average admission time being about 4 weeks.

However, as with any therapeutical approach, certain problems occur. First, certain patients consistently show a very rapid relapse following cessation of Ingram therapy; clearly repeated admissions every four months are hardly practical. Secondly, a few patients do not respond well to the Ingram regime; although clearance may eventually be achieved, this may require prolonged hospitalization. For this reason, from 1971 onwards, we have adopted the policy of maintaining these 'difficult' patients on a regime of continuous low-dose methotrexate, initiated just prior to completion of Ingram treatment. A retrospective analysis of our results up to the end of 1980 is presented below.

PATIENTS AND METHODS

Patients and Controls

A total of 1110 courses of Ingram therapy have been administered in our Department since 1971, involving 677 different patients. Of these, 108 courses have been followed by methotrexate maintenance involving 98 individual patients (table 1). Roughly two-thirds were selected for methotrexate maintenance because of a history of rapid
relapse (complete relapse within about 6 months), and about one-third of slow response to Ingram therapy; a few patients fulfilled both criteria.

Since patients given methotrexate maintenance represented a very 'preselected' group, it is difficult to provide an external control. We have therefore, as an alternative control, calculated relapse-curves for the total previous admissions (93) of patients within this group (46) where no maintenance therapy of any kind was given after discharge from our in-patient department.

Table 1: Composition of the methotrexate maintenance group

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>M/F</th>
<th>Mean age</th>
<th>Mean duration of psoriasis</th>
<th>Classification of psoriasis</th>
</tr>
</thead>
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<tr>
<td></td>
<td>98</td>
<td>51/47</td>
<td>44.7</td>
<td>V 86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P 8</td>
</tr>
</tbody>
</table>

Methods of treatment

Patients were treated with Ingram therapy (tar-bath, UVR and dithranol) as previously described (v.d. Kerkhof and Mali, 1981). During this period, all patients received a thorough physical and laboratory examination to identify any with contra-indications to methotrexate. These included dysfunction of bone-marrow, liver or kidney, alcoholism, psychiatric disturbance, pregnancy or desire for children during subsequent years, active infectious disease or active peptic ulcer.

In the patients with no contra-indications, methotrexate administration was begun during the second or third week of admission, using the dose-schedule described by Weinstein and Frost (1971), namely 5 mg at 12h intervals for a total of 3 doses, repeated at weekly intervals. Patients remained in the hospital and Ingram therapy was continued concurrently until all lesions were cleared. At this point (averaging 5 weeks after admission) the patient was discharged.
and Ingram treatment stopped. Methotrexate therapy was continued, with out-patient examination at 4-weekly intervals. Leukocyte and thrombocyte counts were obtained at each visit, and liver function tests and urinalysis each 3 months. After an average period of 10 months the dosage was gradually and progressively diminished in those patients showing no evidence of relapse, typically to 2.5 mg at 12h intervals. Appearance of any side-effects (see table 2) was also regarded as an indication for reduction of the dose; if this failed to reverse the adverse effect the patient was usually withdrawn from treatment. In a few patients a further reduction (minimum 1.25 mg) proved possible. The average period of treatment was 29 months, and the average cumulative dose was 1.18 g methotrexate.

Table 2: Side effects during methotrexate maintenance therapy  
(n = 108)

<table>
<thead>
<tr>
<th>Side-effects</th>
<th>Absolute number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver function disturbances</td>
<td>18</td>
</tr>
<tr>
<td>increased transaminases</td>
<td>13</td>
</tr>
<tr>
<td>increased alkaline phosphatase</td>
<td>5</td>
</tr>
<tr>
<td>increased BSP</td>
<td>9</td>
</tr>
<tr>
<td>both BSP and transaminase increased</td>
<td>4</td>
</tr>
<tr>
<td>Haematopoetic suppression</td>
<td>14</td>
</tr>
<tr>
<td>thrombocytopenia</td>
<td>4</td>
</tr>
<tr>
<td>leucopenia</td>
<td>12</td>
</tr>
<tr>
<td>combined</td>
<td>2</td>
</tr>
<tr>
<td>Subjective discomfort</td>
<td>39</td>
</tr>
<tr>
<td>nausea</td>
<td>27</td>
</tr>
<tr>
<td>headache</td>
<td>9</td>
</tr>
<tr>
<td>fatigue</td>
<td>14</td>
</tr>
</tbody>
</table>
Criteria of relapse

Two independent criteria were employed. The first was the 'beginning relapse', which was defined as the first entry in the case-history indicating the re-appearance of significant lesions. Although possibly not so precise as the criteria described in our previous investigation (v.d. Kerkhof and Mali, 1981), the area of involvement at this stage was comparable, i.e. about 2 hand-palm sized plaques or a corresponding surface area of smaller lesions. The second criterion was the 'complete relapse', which was defined as a state necessitating re-admission for in-patient therapy.

RESULTS

Actuarial remission curves following the completion of Ingram treatment, with (108 courses of treatment) and without (93 courses of treatment) subsequent methotrexate maintenance therapy, are shown in fig. 1 and 2; these employ the criteria for beginning and complete relapse respectively. It is seen that without maintenance, the 'therapeutical half-life' is about one month (beginning relapse) or 5 months for complete reappearance of lesions. Methotrexate lengthens these intervals to about 1 year or considerably more than 3 years respectively.

Further follow-up of patients in the maintenance group who eventually withdrew from therapy (21 because of adverse effects and 14 for other reasons) yielded the data illustrated in fig. 3. There is no significant difference between this curve and the control curve from fig. 1 (Chi-squared test).

The adverse effects during methotrexate maintenance are shown in table 2. All side effects were reversible although in 21 patients only after stopping methotrexate. The subjective side effects were extremely variable in their nature and severity. In general they were seen during the first few months of the treatment and either disappeared spontaneously or following reduction of the dosage.
Figure 1 Actuarial remission curves for Ingram therapy followed by methotrexate maintenance (open circles; 108 courses of therapy) and for Ingram therapy without any subsequent maintenance regime (closed circles; 93 courses). Criteria are for 'beginning' relapse.

Figure 2 As for fig. 1; criteria for 'complete' relapse.
DISCUSSION

It is clear from this study that the combined regime of initial clearance by Ingram therapy with subsequent methotrexate maintenance offers an extremely effective approach to the management of severe psoriasis over a long period of time. After discontinuation of methotrexate no 'rebound' phenomenon occurs.

This particular combination (Ingram and methotrexate) has not been described previously. Long-term methotrexate therapy per se has been reported by many authors, several of whom have advocated its benefits and many of whom have described its side-effects (Baker and Wilkinson, 1979). Used for the initial phases of treatment, a failure-rate of up to 25% has been quoted (Baker, 1977), a figure far higher than that encountered for Ingram therapy (McLennan and Hellier, 1961). It is more difficult to obtain quantitative data from the literature regarding the 'maintenance' phase, but we feel that the relapse curves shown in fig. 1 and 2 are compatible with the experience of other
centres. Problems with side-effects are also in line with earlier literature (Weinstein, 1971); the relatively small percentage of patients forced to stop treatment because of adverse effects (21 withdrawals over more than 3000 patient-months of maintenance) probably reflects the relatively low doses of methotrexate employed in this Department. It may be noted that our mean cumulative dose (1.18 g) is well below the threshold of 1.5 g suggested by Zachariae et al. (1980) as indication for liver biopsy.

It is of interest to compare the actuarial remission curves presented here with those found using PUVA-maintenance after Ingram therapy (v.d. Kerkhof and Mali, 1981). The pre-selection of 'fast-relapse' patients in the present study is reflected in a control value of only one month for 50% beginning relapse, compared with nearly two months in the previous investigation. The corresponding values for the groups on active maintenance are 1 year and 2 years (extrapolated) for methotrexate and PUVA respectively. The question of whether this latter difference is also a reflection of the pre-selection or whether it represents a real superiority of PUVA cannot, at the moment, be answered. The somewhat lower incidence of immediate side-effects in PUVA maintenance therapy must of course be balanced against the potential long-term hazard of carcinogenesis.

In conclusion, our present view is that Ingram therapy remains the treatment of choice for the initial clearance of severe psoriasis. In many patients some subsequent maintenance regime is desirable; the decision between PUVA and methotrexate cannot be made in terms of their relative efficacy but should rest on the relative clinical contra-indications regarding each individual patient.

ACKNOWLEDGMENTS

We wish to thank zr. A.P.H. Groels and her staff in carrying out the in-patient treatment as described in this report.


CHAPTER V

CALMODULIN LEVELS ARE GROSSLY ELEVATED
IN THE PSORIATIC LESION

P.C.M. van de Kerkhof and Piet E.J. van Erp

Levels of the intracellular calcium receptor, calmodulin, in the psoriatic lesion are more than thirty times higher than normal. By contrast, values in the clinically uninvolved psoriatic skin are unchanged.

Calcium is a major regulator of intracellular metabolism. It is now established that its effects are mediated via the activation of a specific receptor protein, calmodulin. The calcium-calmodulin complex influences enzymes such as adenyl cyclase, phosphodiesterase, protein kinases and phospholipase A₂ (Means and Dedman, 1980; Cheung, 1982; Wightman, 1982) and physiological processes such as endocytosis (Salisburry et al, 1981) and cell division (Chafouleas, 1982).

Although it is known that calmodulin is present in mouse (Murray and Rogers, 1978) and pig (Iizuka et al, 1982) epidermis, no values have been reported for human skin and possible changes in the dermatoses remain wholly unexplored. Since two prominent features of psoriasis, hyperproliferation and glycogen accumulation, would seem to be calmodulin-dependent, we have now measured calmodulin levels in psoriatic lesions, psoriatic 'uninvolved' skin and in the skin of healthy controls.

Patients were selected who had chronic, stable, plaque psoriasis and who had been untreated for at least 1 month. Controls were paid volunteers approximately matched for sex and age. Biopsies (3 mm diameter, about 0.2 mm thickness) were cut freehand using a razorblade in conjunction with a metal guard; in the case of the patients, one biopsy was taken from a lesion and one from a clinically normal site at least 20 cm from any plaque. All specimens were homogenized in 500 μl of a solution containing 50 mmol/1 tris, 3 mmol/1 MgCl₂ and 1 mmol/1 dithio-erythritol at pH 7.8, using an all-glass Potter-type homogenizor. The homogenate was centrifuged at 1000 g for 10 min and aliquots of the supernatant removed for DNA assay according to Kapuścinski and Skoczylas (1977). The remainder of the supernatant was heated at 65°C for 30 min to destroy endogenous proteases and
calmodulin determined using the calmodulin \(^{125}\text{I}-\text{RIA}\) kit supplied by NEN Chemicals GmbH (Doorn, NL). Levels were expressed as ng calmodulin per \(\mu\text{g}\) DNA.

The results of the individual measurements are shown in table 1. It is seen that the mean value for the psoriatic lesions is about 30 times higher than normal; the 'uninvolved' psoriatic skin, however, is not significantly different from the controls (Wilcoxon ranking test, \(P > 0.1\)).

Table 1: Calmodulin levels (ng per \(\mu\text{g}\) DNA) in normal and psoriatic skin

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Psoriatic lesion</th>
<th>Psoriatic uninvolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>100</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>335</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>109</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1221</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>548</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>116</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>230</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>28</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>691</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.0</td>
<td>331</td>
<td>12.2</td>
</tr>
<tr>
<td>(\pm) S.D.</td>
<td>(\pm) 6.2</td>
<td>(\pm) 358</td>
<td>(\pm) 5.9</td>
</tr>
</tbody>
</table>

The grossly increased calmodulin content of the lesions, although obviously not the primary genetic expression of psoriasis, may well be of importance in the development and maintenance of the overt lesion. The numerous points at which calmodulin interacts with cellular
metabolism makes it unwise to speculate in detail regarding pathogenetic pathways; however, it is tempting to extend the hypothesis of Voorhees and Duell (1971) by postulating either a direct influence on cyclic nucleotide levels or the modulation of protein kinase activity. Clearly more basic data are needed before this area of metabolic control can be clarified.

REFERENCES


CHAPTER VI

PLASMA ALDOSTERONE AND CORTISOL LEVELS IN
PSORIASIS AND ATOPIC DERMATITIS

P.C.M. van de Kerkhof


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The levels of plasma aldosterone were significantly raised in groups of patients with psoriasis and atopic dermatitis compared with a control group of patients with other skin diseases. Simultaneous assay of plasma cortisol indicated that these changes were not the effects of prior corticosteroid therapy. The reasons for the increased aldosterone levels are not clear, but our findings are compatible with a 'permissive' role for this hormone in psoriasis and atopic dermatitis.

Observation of psoriatic patients over long periods of time makes it clear that remissions and exacerbations tend to involve the skin as a whole rather than being purely local phenomena. Such a conclusion obviously implies the existence of some systemic factor which (regardless of its possible aetiologica significance) at least has a strong influence over the day-to-day state of the lesions. Several candidates for this hypothetical factor have been proposed; recent suggestions include the adrenocortical hormone aldosterone (Mali, 1979) and the blood monocyte (Mier, Gommans & Roelfzema, 1980). The former idea is of particular interest because of the relationship of aldosterone to the eccrine sweat gland (Sato & Dobson, 1970).

Preliminary work in this Department indicated two practical difficulties. First, the level of plasma aldosterone is rather labile, changing rapidly with the physical and emotional state of the individual (Albert & Hartmann, 1979; Nowaczynski, Sasaki & Genest, 1974). Secondly, the majority of patients have been exposed to topical corticosteroids; it is exceedingly difficult to rule out the possibility that percutaneous absorption might influence our measurements. The present investigation was therefore designed in such a way as to overcome these objections. In particular, we have avoided the use of an arbitrary 'control' group of non-hospitalized subjects and possible adrenocortical suppression has been monitored by the simultaneous measurement of plasma cortisol levels. Our findings are presented below.
MATERIALS AND METHODS

Selection of patients

In general this investigation included all patients hospitalized in this Department during the period August to December 1980 with the following exceptions:

(a) the presence of known systemic disease or intercurrent infection;
(b) the use during admission of any systemic drugs known to influence adreno-cortical function;
(c) the use of any systemic corticosteroid or the extensive use of topical fluorinated corticosteroid preparations, either during admission or in the 4 weeks immediately prior to admission.

A total of seventy-four patients fulfilled these requirements. The personal and clinical data of this group are summarized in Table 1.

Table 1: Summary of patients. 'Other' includes three patients each with contact dermatitis, polymorphic light eruption and urticaria; two each with dyshidrotic eczema, erythema multiforme, lymphoedema, nummular (non-atopic) eczema and sympathetic dystrophy; and a single example of six other dermatoses.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>M/F</th>
<th>Age ± s.d.</th>
<th>Treatment during admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis</td>
<td>33</td>
<td>16/17</td>
<td>39.6 ± 16.9</td>
<td>Ingram therapy or dithranol only</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>16</td>
<td>7/9</td>
<td>31.6 ± 15.0</td>
<td>Tar, with or without salt baths</td>
</tr>
<tr>
<td>Other</td>
<td>25</td>
<td>12/13</td>
<td>30.3 ± 12.6</td>
<td>Various</td>
</tr>
</tbody>
</table>

Sampling procedure and laboratory measurements

All patients followed the usual daily regime, namely rising at 07:00 and breakfasting at 07:30 followed by resting (usually in a sitting or
semi-recumbent position) prior to venepuncture. Blood (10 ml) was taken into heparin during the period 08:30 to 10:00, in general during the first week after admission. Plasma was separated by centrifugation and stored frozen.

Aldosterone and cortisol levels were determined by direct radioimmunoassay as described by de Man et al (1980). Aldosterone was measured in all seventy-four samples, and cortisol in forty-eight obtained during the latter part of the investigation. Transaminases, protein and alkaline phosphatase were determined in all specimens to exclude occult liver dysfunction, and urea and creatinine to establish normal kidney function.

Statistical analysis

Because of the markedly skew distributions of the values, a distribution-free procedure (Wilcoxon ranking test) has been used for the calculation of P values.

RESULTS

Preliminary statistical analysis of the aldosterone levels (all samples) showed no significant correlation with sex or age. However, the mean values for patients with psoriasis (13.5 ng/100 ml) and atopic dermatitis (14.3 ng/100 ml) were significantly higher than the mean of the residual combined group (10.7 ng/100 ml) at a level P < 0.025 and P < 0.05, respectively. The distributions of individual values within these three subgroups are shown in fig. 1a. Tightening of selection criteria by retrospective exclusion of patients using any oral medicaments (18), with the exception of hydroxyzine hydrochloride (Atarax) in patients with atopic dermatitis, and all patients with raised values for transaminases or alkaline phosphatase (4), yields the data illustrated in fig. 1b. Here the differences are still more clear-cut, the levels of significance reaching P < 0.005 for the psoriatic group and P < 0.025 for the atopics.
Figure 1 Individual plasma aldosterone levels (a) in all patients investigated and (b) after exclusion of certain patients for reasons listed in the text.

Corresponding values for plasma cortisol levels are shown in fig. 2a (all measurements) and 2b (selected group). Here the mean values for the psoriatic and atopic sub-groups are slightly lower than that of the residual combined group (0.05 < P < 0.1 in both cases).
DISCUSSION

Our aldosterone values for 'other dermatoses' seem to be in reasonable agreement with published data for healthy controls; for example Nowaczynski et al (1974) report 6.6 ± 3.5 ng/100 ml and Albert & Hartmann (1979) found a range of 5-19 ng/100 ml for 'resting' levels. However, it cannot be too strongly emphasised that in any investigation of an environmentally sensitive measurement (e.g. blood levels of vitamins, trace elements, hormones) comparison of an experimental group with an arbitrarily selected group of healthy controls may lead to erroneous conclusions. This does not, of course, preclude the use of a normal range for diagnostic purposes, since here one is looking for a gross abnormality in a single value. In statistical studies, however, even small differences may be
theoretically 'significant'. Such an observation is of little value unless all potential differences in environmental factors (diet, sleep/waking patterns, exercise and so on) can be ruled out, which is rarely possible.

The alternative approach - as employed here - is to study a single environmentally homogeneous group, and to base conclusions on internal correlations only. Thus we may safely conclude from fig. 1 that there is a real difference between the aldosterone levels of patients hospitalized for psoriasis or atopic dermatitis and those hospitalized for other skin disorders. Since it is extremely unlikely that a range of conditions as diverse as those listed in table 1 would result in consistently low aldosterone levels, we can further assume that the psoriatic and atopic groups are in fact raised. Similarly, the data in fig. 2 suggest a small but possibly significant decrease in plasma cortisol levels in the same groups. This latter observation is compatible with the prolonged and often extensive use of fluorinated corticosteroids by psoriatics and atopics, and indicates (despite the precautions noted in the selection of patients) a mild degree of adrenocortical depression.

Although it is possible that individual values of aldosterone might have been influenced by the exact time of venepuncture, no systematic error could have resulted since the sampling time was unrelated to diagnosis. The increased aldosterone levels in psoriasis and atopic dermatitis cannot result from differences in sodium intake because all patients received a comparable diet. Although it is remotely possible that certain of the atopic patients lost sodium via skin exudation, such a concept is untenable for the psoriatic group.

Three possible explanations of our results may be considered. The first is a direct aetiological link. This can probably be discarded in view of the considerable overlap of actual ranges and because two distinct disorders are involved. Secondly, the presence of cutaneous lesions might directly influence the endocrine balance. Such a hypothesis would imply the existence of a feedback loop from the skin to the adrenal cortex, the signal possibly arising from the eccrine sweat glands. The third possibility is that of a 'permissive' role for aldosterone. Since the plasma level of this hormone may fluctuate very widely in any particular individual, and if we postulate that high
levels have an adverse effect on the disease process (again possibly via the eccrine sweat glands) it is clear that the hospitalized patients would represent a 'pre-selected' subgroup of the whole psoriatic and atopic population who, for possibly unrelated reasons (stress?), are secreting high levels of aldosterone. It will be of interest to investigate this last concept by long-term studies of individual patients.

ACKNOWLEDGMENTS

I wish to thank professor Th. J. Benraad and members of his staff for the aldosterone and cortisol measurements and for helpful discussions during the course of this work.

REFERENCES


CHAPTER VII

QUANTIFICATION OF ALKALINE PHOSPHATASE IN LESIONS AND UNINVOLVED SKIN OF PSORIATIC PATIENTS

P.C.M. van de Kerkhof, Helga van Rennes and P.D. Mier

Chapter VII was accepted for publication in Acta Dermato-Venereologica
SUMMARY

Alkaline phosphatase (ALP) has been quantified in psoriatic skin for the first time. Both the soluble and the particulate forms of this enzyme were grossly elevated in psoriatic lesions; by contrast, levels in the clinically uninvolved skin of the patient were normal. The changes in the lesion cannot be explained solely in terms of vasodilatation, since UVR-induced erythema was accompanied only by a modest increase in soluble ALP activity.

Many investigators have studied the histochemical localization of alkaline phosphatase (3.1.3.1, ALP) in normal human skin and in various pathological conditions (Fisher and Glick, 1947; Kopf, 1957; Pirilä and Eränkö, 1950). There is general agreement that a markedly increased staining of the capillary loop may be seen in the psoriatic lesion. Despite reports of structural changes in the capillaries of clinically uninvolved skin of patients with psoriasis (Madden, 1941), however, the intensity of ALP staining in the uninvolved skin seems to be normal (Wohlrab and Grüneberg, 1966).

We have recently developed a sensitive fluorimetric micro-assay for ALP and have described the properties of the cutaneous enzyme (Mier and van Rennes, 1982). Here we present the first quantitative data regarding ALP in psoriasis. For comparative purposes, ALP levels have also been determined during UVR-induced erythema of normal human skin.

MATERIALS AND METHODS

Subjects

Psoriatic patients were selected who had stable, chronic lesions which had not been treated for at least one week; biopsies were taken either from the central region of a well-established plaque or from the clinically healthy skin at least 20 cm distance from a lesion. Control specimens were obtained from the upper back of paid volunteers with no
personal or family history of psoriasis. In certain experiments a site (2 cm diameter on the back of control subjects was irradiated with 3 times the minimal erythemal dose using whole-spectrum emission from a xenon arc (XBO 150, Osram, Germany) 16h prior to biopsy.

**Biopsy**

All specimens were cut freehand using a razorblade in combination with a metal 'guard' (hole 4 mm diameter). No anaesthetic agent was employed. Biopsies averaged about 2 mg fresh weight; direct histological examination indicated that the central area included all epidermal layers plus some underlying dermis.

**ALP assay**

Biopsies were homogenized in 500 μl of bovine serum albumin solution (1 mg/ml) using an all-glass Potter-type homogenizer fitted with an ice-jacket. The homogenate was centrifuged to yield a clear supernatant ('soluble ALP'); the residue was washed once and resuspended in 500 μl bovine serum albumin solution ('particulate ALP').

ALP was assayed as described previously (Mier and van Rennes, 1982). Briefly, duplicate 20 μl samples were incubated with 20 μl of a solution of 0.5 mM 4-methylumbelliferyl phosphate at pH 9.8 containing 5 mM NaF to avoid possible interference from epidermal acid phosphatase. After 1 h at 37°C the reaction was stopped by the addition of 1 ml carbonate buffer (pH 10.5) and the 4-methylumbelliferone released was determined by fluorescence.

**RESULTS**

Levels of soluble and particulate ALP activity are shown in fig. 1 and 2 respectively. It is seen that both forms of the enzyme are grossly increased in the psoriatic lesion (P < 0.001 in both cases, Wilcoxon ranking test). By contrast, all specimens from the clinically uninvolved skin of the psoriatic patients fell within the normal range.

In the case of the irradiated control specimens, there was a relatively modest but statistically significant (P < 0.05) increase in
Figure 1 Levels of soluble ALP in the individual specimens

the soluble ALP activity; this was not accompanied by any change in the level of the particulate enzyme.

DISCUSSION

These data confirm the histochemical reports regarding ALP staining in psoriasis. Further, the sharp distinction between ALP levels in the psoriatic lesions and those in the irradiated control biopsies indicate that the psoriatic abnormality cannot be interpreted simply as a metabolic consequence of vasodilatation. Indeed, it is likely that there is in fact no change in the cellular levels of ALP during
UVR-induced erythema, since the slight increase in the soluble enzyme is compatible with the larger volume of plasma 'trapped' in the dilated vessels. Histochemical evidence suggests that the inflammatory infiltrate might contribute to the elevated ALP levels in the psoriatic lesion (Braun-Falco and Burg, 1970). However, since the infiltrate is well established at 16 h after UVR (personal communication, Prof. G. Volden, Trömso) and since the particulate levels of ALP in these specimens were normal, we may exclude this possibility as a major contribution in psoriasis.

Whether or not our findings are specific for psoriasis must, of course, await a more extensive investigation. The quantitative technique described here would in any case seem to offer a more
sophisticated approach to the study of dermatoses in which abnormalities of the dermal capillaries are a prominent feature.

REFERENCES


CHAPTER VIII

METABOLIC CHANGES AT THE MARGIN OF THE GROWING
PSORIATIC LESION

P.C.M. van de Kerkhof, Helga van Rennes, Rufi de Grood,
F.W. Bauer and P.D. Mier

Chapter VIII was accepted for publication in
The British Journal of Dermatology

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SUMMARY

Keratotome slices have been cut across the margins of rapidly-growing psoriatic plaques. Each slice was divided into 8 sections and 4 parameters measured on each section. These were percentage cells in S phase and the level of glucose-6-phosphate dehydrogenase (both related to epidermal proliferation), acid phosphatase (associated with keratinization) and alkaline phosphatase (a marker for dermal capillaries).

We show that disturbances in the epidermis extend only 2-4 mm into the 'uninvolved' skin, whereas the capillary is metabolically abnormal for a distance of about 2 cm ahead of the advancing edge of the plaque. This implies that changes in the capillary occur prior to those in the epidermis during the growth of the psoriatic lesion.

The psoriatic lesion is characterized by three signs: thickening, scaling and erythema. At the cellular level, these correspond to abnormalities in three more or less distinct regions of the skin: increased proliferation of the lower epidermis, incomplete keratinization (often with loss of the granular layer and parakeratosis) in the upper epidermis, and tortuous, distended capillaries in the dermal papillae. In each case the morphological changes are accompanied by characteristic alterations in the metabolic patterns of the tissues. However, the relationship between these phenomena remains speculative. Each of the three has, at one time or another, been nominated as the 'primary' expression of psoriasis (Mier and Cotton, 1976).

A popular experimental approach to this problem is the study of the margin of a growing psoriatic plaque, but different authors are by no means in agreement as to the sequence in which the various changes occur. Surprisingly, most of this work has been carried out employing histological or histochemical observation as the sole criterion of 'abnormality'. The use of more objective (preferably quantitative) parameters coupled with statistical evaluation of data might provide a more clear-cut answer.

In this investigation we have studied the events on the margins of
growing lesions by means of the following parameters:
(i) Cell cycle kinetics (percentage of cells in S phase) and glucose-6-phosphate dehydrogenase (G6PDH) activity. G6PDH occurs chiefly in the proliferative zone of the epidermis and is grossly elevated in the psoriatic lesion (Halprin and Ohkawara, 1966).
(ii) Acid phosphatase (ACP). This is found mainly in the upper epidermis (Mier et al, 1976) and is probably associated with the keratinization process. It is increased in the psoriatic plaque (Mier and v.d. Hurk, 1976).
(iii) Alkaline phosphatase (ALP). This is a well-established marker for the capillaries (Fisher and Glick, 1947; Kopf, 1957; Piralä and Eränkö, 1950). Biochemical assay has shown it to be almost exclusively dermal (Mier and v. Rennes, 1982b) and to be increased about 4-fold in the psoriatic lesion (v.d. Kerkhof et al, 1982).

MATERIALS AND METHODS

Materials

Glucose-6-phosphate and NADP were obtained from the Sigma Chemical Co. (St. Louis, USA) and 4’.6 diamidino-2-phenylindole. 2 HCL (DAPI) from Serva Feinbiochemica GmbH (Heidelberg, Germany). The sources of reagents employed for ACP and ALP assay have been described previously (Mier and v.d. Hurk, 1975; Mier and v. Rennes, 1982b).

Subjects and methods of biopsy

Patients with unstable progressive plaque psoriasis were employed for this study. No therapy (local or systemic) had been used for at least two weeks prior to biopsy. Control subjects were paid volunteers with no history of skin disease.

A growing lesion (5-10 cm diameter) on the back or upper arm of 3 patients was selected for investigation, and the edge marked with a fine-tipped felt pen. Care was taken to ensure that the skin outside this boundary appeared absolutely normal by macroscopical inspection. After cooling the skin surface with an ethyl chloride spray, a slice
of 8 x 16 mm was cut across the margin of the plaque (fig. 1) using a Castroviejo keratotome set for a depth of 0.2 mm in conjunction with a metal 'guard'.

The biopsy was spread into filter-paper moistened with phosphate-buffered saline (PBS) and dissected freehand into 8 consecutive sections, of which 2 were from the lesion and 6 of the clinically normal skin. Each section was then subdivided into 2 portions (fig. 1). The smaller portions were placed in ice-cold PBS for cell-cycle analysis and the larger were weighed and stored at -70°C prior to homogenization for biochemical analysis.

Figure 1 Scheme showing position and method of subdivision of keratotome slice

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Microbiopsies were cut freehand from the clinically uninvolved skin of 6 additional psoriatic patients at exactly 2 and 3 cm from the edge of a lesion, and from the back or upper arm of 11 control subjects, using a razorblade in conjunction with a metal guard with a hole 4 mm in diameter. No anaesthetic agent was employed. These samples were weighed and stored at -70°C for enzymatic analysis.

**Cell-cycle analysis**

This was performed as described by Bauer et al (1980). Briefly, cells were isolated by trypsinization in the presence of dithioerythritol, stained with propidium iodide and DNA histograms prepared using an ICP 11 impulse cytophotometer (Phywé, Germany). Percentage cells in S phase were calculated by an on-line computer system (Hewlett Packard 9810 A). All samples were processed on the day of biopsy.

**Preparation of extracts**

The second portion of each section of the psoriatic slices and the intact microbiopsies were homogenized in 1 ml of 1 mg/ml aqueous bovine serum albumin solution (BSA), using an all-glass Potter type homogenizer fitted with an ice-jacket. The homogenate was centrifuged (1000 g, 10 min) to yield a clear supernatant ('extract') and the residue was discarded. Samples were processed within 1 week of biopsy and the subsequent assays performed immediately after homogenization.

**Biochemical measurements**

The DNA concentrations of the extracts were determined as the fluorescent complex with DAPI according to Kapuściński and Skoczylas (1977) with modifications described by Mier and van Rennes (1982a).

G6PDH was assayed by fluorescence measurement of the NADPH released during the reaction of glucose-6-phosphate with NADP. Duplicate 20 μl aliquots of the extract were incubated (30 min, 37°C) with 100 μl of a reagent containing 50 mmol/l tris (pH 7.6), 10 mmol/l MgCl₂, 6 mmol/l EDTA, 0.2 mmol/l glucose-6-phosphate and 0.1 mmol/l NADP. The reaction was stopped by the addition of 500 μl of ice-cold carbonate-bicarbonate buffer (0.2 mmol/l, pH 10.5) and the NADPH determined by fluorescence. Preliminary experiments were carried
out to verify linearity with incubation time and tissue concentration over the ranges employed. G6PDH activity was calculated as nmol NADPH released per min per μg DNA.

ACP was determined by hydrolysis of 4-methylumbelliferyl phosphate at pH 3.6 as described previously (Mier and v.d. Hurk, 1975) but using 20 μl aliquots instead of 100 μl as originally reported. Enzyme activity was calculated as nmol 4-MU released per min per μg DNA.

ALP was assayed by hydrolysis of 4-methylumbelliferyl phosphate at pH 9.8 in the presence of 5 mmol/l NaF as described by Mier and van Rennes (1982b). Activity was expressed as pmol 4-MU released per min per mg fresh weight of tissue.

RESULTS

Normal ranges

The shaded areas in fig. 2 a-d represent the mean ± standard deviation for each parameter in biopsies from healthy controls. Values for percentage S phase (2.7 ± 0.8, n = 60) are taken from Bauer et al (1980). Ranges for G6PDH and ACP (0.87 ± 0.31 and 1.56 ± 0.59 nmol/min/μg DNA respectively) were established during this investigation using 11 control specimens; for comparison with earlier literature these have also been calculated on a fresh weight basis, yielding mean values of 0.95 and 1.72 nmol/min/mg respectively. The normal range for ALP (51 ± 16 pmol/min/mg fresh weight, n = 24) includes 13 specimens reported previously (v.d. Kerkhof et al, 1982) plus 11 additional specimens.

Psoriatic biopsies

Each point in fig. 2 a-d represents the mean ± standard error of the mean of 3 independent experiments. It is seen that the values of all 4 parameters for section 1 and 2 (i.e. those falling within the lesion) are markedly elevated, the increases ranging from a little over 2-fold for ACP to about 4-fold for G-6-PDH and ALP.

The epidermal parameters (percentage S phase, G-6-PDH and ACP) follow a very similar course outside the lesion, all entering the
Figure 2 Mean values (+ SEM) of (a) percentage S phase, (b) G-6-PDH, (c) ACP and (d) ALP. Numbers on the horizontal axis correspond to the sections illustrated in figure 1 and the shaded areas indicate normal ranges.
normal range at about section 4, i.e. 2-4 mm from the edge of the clinically involved skin. The behaviour of the capillary marker, ALP, is strikingly different; it is seen from fig. 2d that the ALP level is still distinctly elevated more than 1 cm away from the lesion. Microbiopsies taken 2 cm from the lesion indicate ALP levels to be in the upper part of the normal range (61 ± 11 pmol/min/mg, n = 6). At a distance of 3 cm, however, the activity is no longer elevated (44 ± 5 pmol/min/mg, n = 6).

DISCUSSION

Both the normal ranges established here and also the values for the plaque (section 1 and 2) are in reasonable agreement with earlier literature. Although no measurements have been made on the 'distant' uninvolved skin during the present investigation, previous workers have established that all 4 parameters in the uninvolved skin are essentially normal (Bauer et al, 1981; Halprin and Ohkawara, 1966; Mier and v.d. Hurk, 1976; v.d. Kerkhof et al, 1982).

Our data for the sections outside but immediately adjacent to the lesion make it clear that metabolic disturbances associated with the dermal capillaries extend to a much greater distance than those associated with the epidermis. Since we have selected patients whose lesions were in a rapidly-growing phase, we may reasonably conclude that the dermal changes in fact occur prior to those in the epidermis. This is in agreement with the views of authors such as Civatte (1924) and Pinkus and Mehregan (1966) but is in conflict with other workers including Christophers and Braun Falco (1970).

It is obviously tempting to assume that temporal primacy implies causality, and to conclude that the 'cause' of psoriasis must be sought in the dermis. However, although our findings are certainly compatible with such a hypothesis (for example Cotton and Mier, 1964), other explanations are by no means excluded. Further approaches which may throw light on this question include the provocation of lesions in previously uninvolved psoriatic skin and the time-course of biochemical events during therapeutic clearance of established plaques. These studies are currently in progress in our laboratories.
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Dermatology, 94, 535.
CHAPTER IX

RESPONSE OF THE CLINICALLY UNINVOLVED SKIN OF PSORIATIC PATIENTS TO STANDARDIZED INJURY

P.C.M. van de Kerkhof, Helga van Rennes, Rufi de Grood, G.J. de Jongh, F.W. Bauer and P.D. Mier

Chapter IX was accepted for publication in The British Journal of Dermatology
SUMMARY

Test sites on healthy controls and on the clinically uninvolved skin of psoriatic patients were stripped with tape, and 8 parameters quantified at intervals during the subsequent healing process.

In the control groups, the stratum corneum regenerated at a constant rate and the underlying skin showed elevations of metabolic activity peaking around days 2-4. In the psoriatic groups, we observed that (i) the response of the keratinizing zone is identical to that of the controls (ii) the proliferative response is initially normal but remains elevated rather longer than usual (iii) the dermal capillaries (indicated by alkaline phosphatase activity) show a gross hyper-reactivity which is already apparent after 1 day and which persists for more than a week.

These findings support our previous conclusion that metabolic alteration of the dermal capillary precedes epidermal hyperplasia in the pathogenesis of the psoriatic lesion.

We have recently studied the sequence of changes in the marginal zone of growing psoriatic plaques, and showed that a dramatic increase in the level of capillary alkaline phosphatase (ALP) precedes any apparent disturbance of epidermal metabolism (v.d. Kerkhof et al, 1983). It may be assumed that this elevation of ALP is in response to mediators diffusing from the fully-developed lesion, but it is by no means clear whether it is a mandatory step in the series of events leading to epidermal hyper-proliferation. In an attempt to throw more light on this question, we have now examined an alternative model, namely the behaviour of 'uninvolved' psoriatic skin following sellotape stripping.

In addition to the 4 parameters measured in our previous experiments (acid phosphatase, glucose-6-phosphatase dehydrogenase, alkaline phosphatase and percentage cells in S phase), this approach also permits quantification of the barrier function of the stratum corneum in terms of water loss, carbon dioxide loss and electrical properties (de Jongh, 1981). Our findings are reported in this communication.
MATERIALS AND METHODS

Subjects and experimental design

Patients were selected with chronic, stable plaque psoriasis. None had received either systemic or whole-body (UVB, PUVA) therapy for at least six months prior to investigation. Some had been using local applications of corticosteroid preparations, but these were asked to discontinue treatment from one week before investigation until the end of the experiments. For ethical reasons, patients in whom the disease was in an active phase were avoided, in order to minimize the possibility of an overt Koebner response to the stripping procedures. Control subjects were healthy paid volunteers with no history of psoriasis.

The investigation comprised 3 independent sets of experiments: enzyme studies, cell cycle kinetics and barrier function measurements. The patient and control groups were sex- and (approximately) age-matched in each case, the relevant data being summarized in table 1. Most subjects in fact participated in more than one set of experiments, the groups formulated in table 1 being drawn from a total of 11 individual patients and 14 controls. Patients and controls were in all cases investigated in pairs to exclude possible bias resulting from changing environmental conditions.

Stripping procedure

Areas selected for investigation (upper back or volar aspects of forearms) were at least 15 cm from any lesion. Flexible plastic templates were prepared with circular holes of appropriate diameter spaced at least 5 cm apart; these were fastened to the skin with surgical tape prior to stripping to ensure that damage to the stratum corneum did not extend beyond the designated test sites.

Exposed test areas were stripped with consecutive applications of 'Tesafilm' pressure-sensitive tape (Beiersdorf B.V. Aalsmeer, NL). Removal of the stratum corneum was considered to be complete when the whole area appeared to be glistening; this usually required 20-35 applications of the tape, no difference being observed between
Table 1: Composition of experimental groups; data for controls in parentheses

<table>
<thead>
<tr>
<th>Investigation</th>
<th>No.</th>
<th>M/F</th>
<th>Age</th>
<th>Years of psoriasis</th>
<th>Percentage body-surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme measurements</td>
<td>6</td>
<td>5-1</td>
<td>38.2 ± 10.5</td>
<td>20.2 ± 9.2</td>
<td>10 ± 5</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(5-1)</td>
<td>(35.7 ± 13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cycle kinetics</td>
<td>8</td>
<td>6-2</td>
<td>40.4 ± 13.5</td>
<td>19.5 ± 10.3</td>
<td>8 ± 5</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(6-2)</td>
<td>(37.3 ± 15.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barrier functions</td>
<td>8</td>
<td>4-4</td>
<td>32.6 ± 19.7</td>
<td>14.7 ± 13.2</td>
<td>6 ± 6</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(4-4)</td>
<td>(37.5 ± 15.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
patients and controls. Microscopic examination of the tape surface confirmed that few or no cells were removed after this point.

**Enzyme studies**

Six sites on the upper back were stripped. A biopsy was cut from the centre of one site at each time interval, the first being immediately after stripping ('day 0') and the others after periods of 1, 2, 4, 6, and 9 days. All biopsies were taken using a razor blade in conjunction with a metal guard. No anaesthetic agent was employed. The specimens (average weight about 2 mg, thickness about 0.3 mm) were weighed and stored at -70°C prior to analysis.

The processing of the biopsies and all biochemical measurements were exactly as described previously (v.d. Kerkhof et al, 1983). In brief, the specimens were homogenized in 1 ml aqueous bovine serum albumin solution and centrifuged. Duplicate 20 μl aliquots of the supernatant were used for fluorimetric determinations of the levels of acid phosphatase (ACP), glucose-6-phosphate dehydrogenase (G-6-PDH) and of ALP. DNA concentrations were measured in duplicate 50 μl aliquots for use as a reference variable for the epidermal enzymes (ACP and G-6-PDH).

**Cell cycle kinetics**

Stripping and biopsy schedules were exactly as for the enzyme studies, except that the specimens were floated onto cold physiological saline and processed immediately. Again, the analytical procedures were as described previously (v.d. Kerkhof et al, 1983). In brief, cells were isolated by trypsinization, stained with propidium iodide and the DNA distribution determined using an ICP 11 cytophotometer. Percentage cells in S phase were calculated using an on-line computer.

**Barrier function measurements**

Four barrier function parameters were determined simultaneously using techniques which have been described in detail by de Jongh (1981). These were:

(a) Transepidermal water loss (WL). This employed the ventilated chamber method, water being measured with a 'Meeco' electrolytic
moisture meter (Manufacturers Engineering & Equipment Corp., Warrington, USA). Values were averaged from 2 cups (diameter 1.4 cm) applied to the left arm.
(b) Transepidermal carbon dioxide loss (CDL). Again, the ventilated chamber method was used, in this case a single cup (diameter 2.8 cm) on the left arm. Carbon dioxide was determined using a Lira 202 S infra-red spectrophotometer (Mine Safety Appliances Comp., Pittsburgh, USA).
(c) Electrical resistance (R) and capacitive reactance (XC). Three cups (2.8 cm diameter) were applied to the right arm and filled with 15 mmol/l NaCl. These were connected to a bridge impedance meter (Radiometer type GB 11 C, Radiometer, Copenhagen). Impedance (magnitude and phase angle) was determined at a frequency of 25 Hz, and the values of R and XC computed.

Appropriate test sites were marked on the volar aspects of the forearms, and baseline parameters measured on the intact skin (suffix '0' below). Each site was then stripped in the usual way, and barrier functions measured at subsequent intervals of 1, 2, 4 and 8 days (suffix 't' below). It should be noted that in these experiments all sites were subjected to repeated measurements, in contrast to the enzyme and cell cycle kinetic studies where each individual site was biopsied once only. In the case of WL and CDL results were calculated as ratios of pre-stripping to post-stripping levels (i.e. \(\text{WL}_0/\text{WL}_t\) and \(\text{CDL}_0/\text{CDL}_t\)); for electrical parameters the reciprocal function was employed (\(R_t/R_0\) and \(\text{XC}_t/\text{XC}_0\)).

Statistical analysis
Evaluation of differences between the patient group and the control group for any individual time-point was carried out using the Wilcoxon ranking test. Additional statistical evaluation of barrier function data was provided by linear regression analysis of the plots (days 1-8) using a Hewlett Packard 9810 A computer.
RESULTS

Clinical assessment of response to stripping

No difference was seen between the psoriatic and the control group. In all cases an immediate erythema was observed following stripping. This was maximal after about 1 h and faded slowly during the following 8-9 days. After 1 day the stripped area was covered with a shiny translucent crust which flaked away during the latter half of the experimental period to leave a slightly scaly surface at day 9. By day 15 the skin seemed entirely normal except for a variable degree of hyperpigmentation; in some subjects this persisted for several months.

No overt Koebner reaction was seen in response to stripping. In 2 of the patients, however, lesions appeared specifically at the biopsy sites after about 3 weeks.

Enzyme studies

The behaviour of ACP was similar in the control and psoriatic groups (fig. 1a). In both cases a sharp but transient peak was found on day 4, the remaining values falling within the range for normal, unstripped skin. Differences between the groups were not statistically significant ($P > 0.05$ for all days).

The early response of G-6-PDH to stripping was also the same in the 2 groups (fig. 1b). The enzyme level had already exceeded the normal range by the second day, and a further increase was apparent on day 4. In the latter half of the experiment, however, the groups diverged sharply; the G-6-PDH activity of controls returned abruptly to normal, whereas that of the patients remained around maximal levels. Differences were statistically significant ($P < 0.05$) on days 6 and 9.

In the case of ALP the behaviour of the psoriatic group was strikingly different to that of the controls throughout the experiment (fig. 1c). It is seen that the response of the patients to stripping was of earlier onset, reached much higher peak levels and lasted longer than the control group. Significance levels were as follows: day 1, $P < 0.05$; day 2, not significant; day 4, $P < 0.05$; day 6, $P < 0.02$; day 9, $P < 0.05$. 
Figure 1 Enzyme levels following stripping. (a) ACP, (b) G-6-PDH and (c) ALP. Open circles represent the control group and filled circles the psoriatic group; vertical bars are S.E.M. Shaded areas indicate normal ranges (mean ± S.D.) and are taken from v.d. Kerkhof et al (1983)
Cell cycle kinetics

Both groups showed a sharp peak in percentage S phase on the second day after stripping (fig. 2). Remarkably, the psoriatic group differed from the controls in the appearance of a second, smaller peak on day 6 (P < 0.01).

![Figure 2 Percentage S phase following stripping.](image)

**Figure 2** Percentage S phase following stripping.
Symbols are as in fig. 1

Barrier functions

All 4 parameters indicated a trend towards restoration of the barrier function during the 8 days (i.e. a decrease in WL and CDL and an increase of R and XC). Appropriate mathematical transformations gave linear plots (fig. 3a-d; correlation coefficient better than 0.9 for all graphs), indicating that the newly-formed stratum corneum is growing in thickness at a uniform rate during the experimental period. No significant difference could be established between the data from the psoriatic and the control groups (P > 0.05 for all parameters).
Figure 3 Barrier functions following stripping. (a) H₂O loss,
(b) CO₂ loss, (c) electrical resistance and
(d) capacitive reactance. Symbols are as in fig. 1.
In all cases the value for normal stratum corneum is defined by the mathematical transformation as unity.
These findings permit certain generalizations. First, we can confirm that during 'normal' regeneration, transient metabolic disturbances occur in all cutaneous layers which are similar in direction and magnitude to those seen in the psoriatic lesion. Second, we have demonstrated that certain of these responses are quantitatively abnormal in the clinical uninvolved skin of the psoriatic patient. Finally, we show that this abnormal pattern of behaviour in the psoriatic is manifest in the dermal capillary long before any deviation is apparent in the epidermis. We shall discuss these data in more detail before commenting on their wider implications.

Our observations on the clinical behaviour of the stripped skin are in line with those of many workers from Pinkus (1951) onwards. In particular, we agree with Illig and Holtz (1966) that stripping does not elicit an overt Koebner response in patients with stable plaque psoriasis; this in contrast to the frequently positive reactions reported during active phases of the disease (Reinertson, 1958; Illig and Holtz, 1966).

Metabolic and physiological responses to stripping may be considered at 3 levels: keratinization, proliferation and dermal changes. The keratinization process has been monitored in this study by one enzyme (ACP) and by the barrier function measurements. Our conclusions are quite clear; we find no difference between the psoriatic and the control group. This is in agreement with the views of Marks et al (1979) who reported that the physical properties and ultrastructural appearance of stratum corneum from distant (as opposed to paralesional) uninvolved psoriatic skin was quite normal, and who concluded that 'no single feature can be identified... which would suggest that there is a primary abnormality in keratinization'. It is of interest that the regeneration of stratum corneum proceeded linearly with time. Extrapolation of the plots to unity (fig. 3) yields a regeneration period of about 2 weeks, a figure compatible with a transit time of about 4 weeks for the entire epidermis (Porter and Shuster, 1968).

At the level of epidermal proliferation (the enzyme G-6-PDH and percentage S Phase) our findings are again consistent; here we do find
a difference between patients and controls, but it is not manifest until the latter half of the experimental period. The peak in S phase on day 2 (both groups) is in agreement with earlier observations regarding the effects of stripping on normal skin (Williams and Hunter, 1957) but does not support the claim of an increased response in the psoriatic at this time (Wiley and Weinstein, 1979). The second peak (day 6, psoriatic group only) seems to indicate some degree of synchronization of the cell cycle, an observation in accordance with the views of Mali (1979).

ALP is found exclusively in the dermis (Mier and van Rennes, 1982) and has been used extensively as a histochemical marker for capillaries. The grossly exaggerated response of ALP to stripping in the psoriatic group (already apparent on the first day) must therefore be interpreted as an abnormal reaction of the psoriatic capillary; this may indicate an inherent defect of the vessel itself, although other explanations are by no means ruled out. It should be noted that the peak values in the psoriatics are remarkably similar to the levels found in the lesion itself (206 ± 94 pmol/min/mg; v.d. Kerkhof, van Rennes and Mier, 1982), control subjects reaching little over half of this value. The slight increases seen directly after stripping (day 0, both groups) are doubtless related to the immediate erythema, a similar effect being noted following ultraviolet radiation (v.d. Kerkhof et al, 1982).

We may now return to the question posed originally. Integrating the findings of this investigation and our previous study (v.d. Kerkhof et al, 1983) we may conclude firstly, that a disturbance of capillary function (marked by a large increase in ALP activity) seems to be a mandatory precursor of any epidermal alteration. Secondly, that this may proceed to a psoriatic lesion if (and only if) the patient is in an 'active' phase of the disease. This latter state, marked by a radial growth of existing lesions and a positive Koebner reaction, is presumably linked to systemic factors such as circulating hormone levels (v.d. Kerkhof, 1982). These conclusions suggest that psoriasis may represent an intrinsic defect in one of the interlocked group of homeostatic mechanisms collectively termed 'inflammation'. Such a concept is very close to the view of Stone (1968), who proposed that 'psoriasis is a defect in the ability of the upper dermis to
inactivate the mediators of inflammation'. Further work in this laboratory will provide data on the sequence of metabolic changes which accompany therapeutically induced remissions of the psoriatic lesion.

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CHAPTER X

METABOLIC CHANGES IN THE PSORIATIC LESION
DURING TOPICAL CORTICOSTEROID THERAPY

P.C.M. van de Kerkhof and Helga van Rennes
SUMMARY

Three marker enzymes were measured during treatment of psoriatic plaques with clobetasol propionate. It was established that the epidermal enzymes (acid phosphatase and glucose-6-phosphate dehydrogenase) returned to normal within 14 days; by contrast, the level of capillary alkaline phosphatase remained at the original level during the entire experimental period.

Our previous investigations into the pathogenesis of the psoriatic lesion (v.d. Kerkhof et al. 1983a and b) have established that abnormalities in the dermal capillary certainly precede, and may well be a mandatory precursor of epidermal dysplasia. In this communication, we present data regarding the levels of acid phosphatase (ACP; keratinizing zone), glucose-6-phosphate dehydrogenase (G-6-PDH; proliferation zone) and alkaline phosphatase (ALP; dermal capillary) during corticosteroid-induced regression of the established lesion.

MATERIALS AND METHODS

Materials

Clobetasol propionate, 0.05% in an ointment base (Dermovate®, Glaxo) was selected for this study. The sources of all reagents employed to biochemical measurements were as described previously (v.d. Kerkhof et al. 1983a).

Subjects and experimental design

Three patients with chronic stable plaque psoriasis participated in this study. None had ever received any systemic therapy, and none had used local therapy for at least one month prior to investigation. In each patient a handpalm-sized lesion was selected (two from the upper arm and one of the upper leg) and 6 prospective biopsy sites were 'mapped' onto paper prior to the experiment. All sites were at least
1 cm inside the margin of the lesion and 2 cm apart. Three healthy subjects (without history or signs of psoriasis) were employed as controls, an area of about 10 cm square being marked on the upper arm.

The selected lesions and the control areas were treated with clobetasol propionate ointment twice a day, applied thinly and evenly, without occlusion. Biopsies were taken free-handed, using a razor blade in conjunction with a metal guard (hole 4 mm diameter), immediately prior to the application of steroid and at subsequent intervals of 1, 3, 7, 11 and 18 days. Specimens were weighed and stored at -70°C prior to analysis.

Biochemical assays

All procedures were as described previously (v.d. Kerkhof et al. 1983). In brief, the specimens were homogenized in 1 ml aqueous bovine serum albumin solution and centrifuged. Duplicate 20 μl aliquots of the supernatant were used for fluorometric determinations of the levels of ACP, G-6-PDH and of ALP. DNA concentrations were measured in duplicate 50 μl aliquots for use as reference variable for the epidermal enzymes (ACP and G-6-PDH).

RESULTS

All 3 steroid treated lesions showed an excellent clinical response. A marked but transient reduction in the erythema was observed (day 1-3), followed by a return to its original level; this persisted until the end of the experimental period (day 18). Scaling and thickening diminished rapidly from day 1 onward, and had essentially subsided by day 7 or 11. No clinical reaction of any kind was seen on the treated areas of the control subjects.

The activities of the 3 marker enzymes are shown in fig. 1 a-c. It is seen that the epidermal parameters (ACP and G-6-PDH) of the lesion fall at a similar tempo, both reverting to the normal range after about 2 weeks of treatment. By contrast, the capillary marker (ALP) was uninfluenced by the steroid; remaining at the level of the original lesion during the whole investigation.
Figure 1 Enzyme levels during treatment with clobetasol propionate.
(a) ACP, (b) G-6-PDH, (c) ALP.
Open circles represent the control areas and filled circles the psoriatic lesions.
Vertical bars are SEM. Shaded areas indicate normal ranges (mean ± S.D.) and are taken from v.d. Kerkhof et al (1983a)
The activities of all three enzymes in the controls did not alter in response to steroid application.

DISCUSSION

Again, the use of quantitative biochemical parameters as 'marker' for tissue specific processes throw some new light onto an old problem. In particular it is seen that the corticosteroid employed rapidly 'normalizes' epidermal metabolism whilst leaving the capillary dysfunction unchanged. The lack of response in healthy skin makes it clear that the therapeutic effects are not mediated by a direct inhibition of these enzymes, despite reports that steroids are effective inhibitors of G-6-PDH in vitro (Raab and Siber, 1974; Cotton and v. Rossum, 1974).

Perhaps the most interesting aspect of our present finding is that it provides an insight into the rapid relapse of lesions following cessation of local corticosteroid therapy (Stevenson and Whittingham, 1963; Alexander, 1965; Champion, 1966). The situation after 18 days treatment with clobetasol propionate ointment is identical to that in the 'margin' zone of the untreated, growing lesion (v.d. Kerkhof et al, 1983α) and that following stripping (v.d. Kerkhof et al, 1983β), namely a gross elevation of capillary ALP underlying an apparently normal epidermis. In all three situations a lesion may follow rapidly.

REFERENCES


CHAPTER XI

GENERAL DISCUSSION
GENERAL DISCUSSION

As shown in the introduction, psoriasis is a multifactorially-determined disease in which diverse local and systemic abnormalities may become manifest. In our investigations we did not set out to find the 'cause' of this disease, but to study the inter-relation of selected parameters with respect to its pathogenesis and therapy. A synthesis of our findings is presented in this general discussion. This is in no sense a dogmatic claim to understand the whole situation, but rather an attempt to represent the available data in their simplest form.

1. THERAPY

Although much of the data provided by chapters II-IV has obvious clinical relevance, our concern here is with dynamic aspects; that is, the influence of the individual therapies on the metabolic events occurring during the transition from lesion to 'normal' skin and vice versa. Thus important data from this work are the relative clearing times and subsequent remission periods after discontinuation of treatment. Chapter X is an initial attempt to use the techniques developed in chapters VIII-IX to identify the 'target' of a therapeutic modality. In brief, we may summarize our conclusions as follows:

(i) Corticosteroids clear lesions within 10-14 days (chapter X), compared with an average period of 5 weeks for Ingram therapy (chapter II). Data from the general literature suggest that PUVA (used as initial therapy) is comparable to Ingram treatment (Henseler et al, 1981), whereas methotrexate is definitely rather slower in its effects (Baker, 1976).

(ii) Remission periods following PUVA can be extremely long (50% about 1 year) in comparison with either methotrexate or Ingram therapy (about 2 months, chapters II-IV). The 'rebound' effect following corticosteroids is, of course, well established.

(iii) The data of chapter X establish clearly that the primary target of corticosteroids is epidermal. The action of other
therapies is still entirely speculative. These data are summarized in table 1. It is already apparent that the mechanisms by which the various forms of therapy operate are not the same; indeed, the very different characteristics of corticosteroids and PUVA already suggest different target points in the lesion. However, more specific discussion of the actual target points of individual therapies must be deferred until a theoretical framework of the lesion and its pathogenesis is developed (below).

Table 1: Dynamics of some therapeutic methods

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Clearing</th>
<th>Remission</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+++</td>
<td>(-)</td>
<td>Epidermis</td>
</tr>
<tr>
<td>PUVA</td>
<td>(++)</td>
<td>+++</td>
<td>?</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>(+)</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Ingram</td>
<td>++</td>
<td>+</td>
<td>?</td>
</tr>
</tbody>
</table>

2. PATHOGENESIS

The following conclusions may be drawn from the data reported in chapters V-IX:

(i) In the established lesion, all morphological 'compartments' (capillary, proliferative zone, keratinizing zone) show grossly increased metabolic activity in terms of the markers selected. Values for clinically uninvolved skin were essentially normal.

(ii) In the margin zone of growing psoriatic plaques, we have shown that disturbances in the epidermis extend only a few mm into the uninvolved skin. By contrast, the capillary is metabolically abnormal for a distance of about 2 cm ahead of the advancing edge of the plaque.

(iii) The 'distant uninvolved' skin of the psoriatic patient was shown to react abnormally to removal of the stratum corneum. In comparison to controls, an early hyperreactivity of capillaries
was prominent, followed by a less marked deviation of the proliferative response.

(iv) Our data support the conclusions of many other workers that specific local changes accompany the appearance of psoriatic plaques (e.g. grossly increased calmodulin levels), but also indicate that systemic factors (e.g. aldosterone) may play a permissive rôle.

An attempt to integrate these data is presented in fig. 1. Here hypothetical cause-effect relationships are indicated by arrows; theoretical justification of these is considered in the following paragraphs.

Figure 1 A schematic presentation of data considered in this discussion

Epidermis $\rightarrow$ capillary

The data in chapters VIII-IX make it abundantly clear that a capillary alteration is fundamental to the development of the psoriatic plaque. This is compatible with the conclusions of several earlier authors using unrelated experimental approaches (Fleck, 1951; Eddy et al, 1964; Ryan, 1980). Two possibilities exist: either we may postulate an
intrinsic abnormality of the vessel, or we may suppose this to be secondary to the action of a local mediator. Since no convincing evidence exists to support the former idea, and in particular since our biochemical parameter (ALP) is essentially normal in 'distant uninvolved' skin (chapter VII), we may regard the capillary change in the lesion to be a 'normal' response to a pathological excess of a vasoactive mediator.

It is clear that this substance is secreted by the established plaque, since ALP elevation occurs in an annular zone in the clinically normal skin surrounding the lesion (chapter VIII). More precise localization of the source as the epidermis is provided by our findings of chapter IX, where it was shown that an identical response of capillary ALP was evoked by removal of stratum corneum. Again, our conclusion is compatible with previous observations, for example the report that dermal injury alone will not result in a Koebner response (Farber et al, 1965).

It should be noted that the early metabolic change discussed here (increased ALP activity) by no means correlates with the vasodilatation which is the classical histological marker for the capillary in the psoriatic lesion; indeed, we have shown that following UVR these two phenomena can be clearly distinguished (chapter VII). It is, of course, tempting to speculate on the chemical nature of the mediator(s) involved. Although no direct evidence exists, certain recent publications point to the possibility of an abnormality in the inflammatory eicosanoid pathway (i.e. arachidonic acid metabolites):

(i) The precursor enzyme, phospholipase A₂, is grossly abnormal in psoriatic skin (Forster et al, 1983).
(ii) Both arachidonic acid and prostaglandin levels are elevated in the psoriatic lesion (Plummer et al, 1978).
(iii) Prostaglandins (derived from arachidonic acid) are, in general, strongly vasoactive.

The data shown in chapters VIII-IX make it clear that hyperplasia and dyskeratinization in the epidermis can only occur subsequent to the
capillary changes noted above. Assuming a cause-effect relationship, we must ask whether this is a direct or indirect link. The difficulty with the former concept is the relatively long 'induction period' in the margin zone between the observation of increased ALP and the epidermal changes; the growth rate of a typical lesion suggests this to be several days or weeks, a period similar to that observed for the occurrence of a Koebner reaction. Such a time-lag is hardly compatible with the diffusion of a chemical mediator.

An escape from this impasse is provided by the well established fact that overt epidermal change occurs, and only occurs in the presence of a perivascular infiltrate. This is further strengthened by the observation of a quantitative relationship between the density of the dermal infiltrate and the mitotic rate of the overlying epidermis (Christophers et al., 1973). Thus we may reasonably conclude a two-step pathway as illustrated in fig. 1.

The individual steps may be considered briefly. With regard to the first, it should be noted that the most potent known chemotaxins belong to the group of mediators termed 'leukotrienes'; these, like the prostaglandins, are arachidonic acid derivatives, and have also recently been reported as increased in the psoriatic lesion (Brain et al., 1982; Grabbe et al., 1982). The nature of the second step is more obscure, but is presumably related to the physical penetration of neutrophils into the epidermis ('squirting' phenomenon; Pinkus and Mehregan, 1966). Remarkably, it has very recently been shown that the presence of peripheral blood leukocytes increases the proliferation of keratinocytes in tissue-culture (Ristow, 1982).

Regarding events within the epidermis itself, the evidence is more conflicting. Our studies of the response to stripping (chapter IX) suggest that hyperplasia precedes dyskeratinization, the latter being presumably the consequence of the reduced transit-time. However, no separation between these processes was noted in the margin zone (chapter VIII); at the present time it is perhaps better to leave this question open.

Systemic factors → local processes

Many purely local changes have been described in the psoriatic lesion;
indeed, much of the biochemical literature of the past two decades deals with this aspect of psoriasis (Mier and Cotton, 1976). Unfortunately very few of these changes seem specific to the psoriatic process, being simply related to the increased proliferation. Our observations regarding calmodulin (chapter V) are of particular interest for several reasons: firstly the change is of greater magnitude than commonly encountered (a thirty-fold elevation); second, the gross increase appears to be quite specific to psoriasis (van Erp, personal communication), and finally the key role of calmodulin in regulator enzymes such as phospholipase $A_2$ and adenyl cyclase offers room for intriguing speculation.

There is also strong evidence for the importance of systemic factors in the psoriatic process, some of which has already been mentioned in chapter I. In brief, observations to support the idea that the local homeostasis is regulated by systemic factors include the following:

(i) Growth or regression of all lesions on an individual patient is usually 'in phase'.
(ii) The Koebner response is an 'all or none' phenomenon (Pedace et al, 1969; Eyre and Krueger, 1982).
(iii) Most patients report a strong correlation between disease activity and stress, again suggesting a systemic, probably endocrine, regulation.

For these reasons, and following the suggestions of Mali (1979), we investigated aldosterone levels in a group of psoriatic patients (chapter VI). It was concluded that this hormone may well play a permissive role in the development of lesions. It is of interest that psoriatic manifestations form a 'spectrum', the extremes of which are chronic plaque psoriasis (an apparently local disorder) and psoriasis pustulosa, which can conform to all criteria of a systemic disease (chapter I). The position of the individual patient in this spectrum dictates optimum therapy.

Infiltrate -> capillary

One remaining point of criticism of the concept - as so far developed - is that the metabolic abnormality of the capillary remains after the
epidermis has normalized in response to corticosteroids. Perhaps the most likely explanation of this rather surprising observation is the influence of the established dermal infiltrate, which may persist for long periods after the lesion is clinically healed (Suurmond, 1965).

Although frankly speculative, this concept is wholly compatible with reports that blood-derived cells possess a high level of phospholipase A2 activity and are well-known secretors of inflammatory eicosanoids (Lewis and Austin, 1981).

3. CONCLUSIONS AND FUTURE PROSPECTS

Our conclusions regarding pathogenesis have been explicitly stated in the penultimate paragraph of chapter IX and do not need restating here. It may be noted that the additional data derived from the therapeutic section (chapters II-IV and X) are wholly compatible with this conclusion, and at certain points strengthen it considerably.

It is now clear that the 'interlocked group of homeostatic mechanisms' referred to in this earlier paragraph includes the elements depicted in fig. 1, and that these are indeed pathways prominent in the inflammatory situation in its broadest sense. It is also clear that, in our option, a deviation in arachidonic acid metabolism is central to the problem of psoriasis. The view of Stone (1968) might be updated to the statement that 'psoriasis is a defect in the ability of the upper dermis to inactivate inflammatory eicosanoids', with the proviso that the reverse situation (i.e. the overproduction of eicosanoids by the epidermis) is by no means ruled out.

We may finally attempt to interpret our therapeutic observations in the light of the pathogenetic concept presented in the previous section. The most complete picture so far available is with regard to local corticosteroids. Remarkably, the effects which can be predicted from fig. 1 and the data in chapter X are wholly borne out by clinical experience. Following cessation of 'successful' short-term treatment we are left with a situation where the epidermis is apparently normal but the capillary and infiltrate remain as in the original plaque; in a patient where systemic factors permit,
transition to a complete lesion must recur within weeks. Thus - used in this way - one might be justified as regarding local corticosteroid treatment as merely cosmetic.

The contrast with PUVA is striking. It is hard to escape the conclusion, even in the absence of laboratory data, that this therapy has a direct effect on capillary and/or infiltrate cells in addition to any possible action on the epidermis. The very recent observation that macrophages are extremely sensitive to PUVA (Verhagen et al, 1983) and the efficacy of PUVA in other diseases characterized by heavy infiltration by blood-derived cells (such as mycosis fungoides) both support such a concept.

Our knowledge of the mode of action of the remaining therapeutic approaches - methotrexate and Ingram treatment - is so scanty that further discussion must await the availability of concrete experimental data.

Future prospects are clearly linked to advances in basic research, for example elucidation of the physiological rôle of ALP. However, a number of experimental lines within dermatology are already demarcated by the various uncertainties remarked above. These include:

(i) Evaluation of 'marker enzymes' during additional therapies.
(ii) Development of a biochemical marker for infiltrate cells, for example peroxidase.
(iii) Direct verification that eventual normalization of capillary ALP parallels resolution of infiltrate.
(iv) More exact definition of the eicosanoid products of psoriatic epidermis.
(v) Further studies of circulating endocrine hormones, in particular along the time axis in individual patients.

It is clear, that just as clinical observations can provide clues regarding the pathogenesis of the psoriatic lesion, a better insight into the pathogenesis will form the foundation of future developments in therapeutic management.
REFERENCES


Chapter I reviews the established clinical, histological, epidemiological and genetic data regarding psoriasis. Common modes of therapy and current theories of pathogenesis are discussed. The aim of this thesis is defined in terms of the gaps in these data.

Chapters II-III provide data regarding the relapse times following Ingram therapy and the use of PUVA to extend these remission periods considerably.

Chapter IV is a similar investigation regarding methotrexate.

Chapter V is an example of a localized abnormality in cutaneous homeostasis in psoriasis. Calmodulin (a key activator of many enzymes) was shown to be 30-fold increased in the lesion, but normal in the uninvolved skin.

Chapter VI confirms that systemic factors may play a permissive role in psoriasis. Aldosterone levels in hospitalized patients were significantly higher than controls; on the other hand, cortisol levels were quite normal.

Chapter VII reports the first quantification of alkaline phosphatase (ALP) in psoriasis. This enzyme was shown to be grossly elevated in the lesion but normal in 'distant' uninvolved skin.

Chapter VIII follows the dynamics of the growing lesion. It was established that the capillary marker, ALP, becomes abnormal well before the appearance of overt epidermal change.

Chapter IX is an investigation of the response of clinically uninvolved skin to removal of the stratum corneum. Again, abnormalities in the behaviour of capillary ALP were observed prior to epidermal hyperreactivity.
Chapter X uses the enzyme 'markers' established in chapters VII-IX to evaluate the sequence of events which occurs during local corticosteroid therapy. It was shown that capillary ALP remains elevated long after apparent normalisation of the epidermis.

Chapter XI presents a theoretical model based on the data from the experiments above. We conclude that dermal change (capillary and infiltrate) plays a mandatory role in the development and maintenance of the lesion, and further speculate that abnormalities in the metabolism of inflammatory eicosanoids may be of fundamental significance in the etiology of this disease. Our observations regarding certain therapies are discussed in the light of the theoretical model.
SAMENVATTING

Hoofdstuk I gaat over de klinische, epidemiologische en genetische aspecten van psoriasis. Enige bekende therapieën en enkele hedendaagse theorieën over de pathogenese worden besproken. De doelstelling van dit proefschrift is een beter inzicht te krijgen op die punten waar onze kennis nog onvolledig is.

Hoofdstuk II-III betreffen een klinisch onderzoek naar de remissie perioden na Ingram therapie. Aangetoond werd dat PUVA onderhouds behandeling deze remissie perioden aanzienlijk kan verlengen.

Hoofdstuk IV behandelt een soortgelijk onderzoek over onderhouds behandeling met methotrexaat.

Hoofdstuk V is een voorbeeld van een plaatselijke ontregeling van het metabole evenwicht in psoriasis. Het Calmoduline gehalte (Calmoduline is een belangrijke activator van veel enzymen) bleek in de psoriasis plaque 30 x hoger te zijn dan zowel in de klinisch normale huid als in de huid van proefpersonen zonder psoriasis gemeten werd.

Hoofdstuk VI bevestigt dat Aldosteron van belang is in de pathogenese van psoriasis. Plasma Aldosteron spiegels bij klinische psoriasis patienten waren significant verhoogd t.o.v. een controle groep. Cortisol spiegels bleken echter normaal te zijn.

Hoofdstuk VII rapporteert de eerste quantitatieve bepaling van alkalisch Phosphatase (ALP) in psoriasis. Het enzym heeft een sterk verhoogde activiteit in de laesie, maar is niet afwijkend in de klinisch normale huid op een afstand van laesies.

Hoofdstuk VIII volgt de dynamiek van de groeiende laesie. Het markeerenzym van capillairen (ALP) wordt al afwijkend vóór het verschijnen van duidelijke epidermale veranderingen.
Hoofdstuk IX is een studie van de respons van klinisch normale huid op het verwijderen van het stratum corneum. Wederom werd een afwijkend gedrag van het ALP gevonden vóór een epidermale overreactie optrad.

Hoofdstuk X maakt gebruik van de 'markeerenzymen' uit hoofdstuk VII-IX. De volgorde van gebeurtenissen welke optreden tijdens uitwendige behandeling met corticosteroïden werd bestudeerd. Het markeerenzym van capillairen (ALP) bleek nog lang verhoogd te blijven na normalisering van de epidermis.

Hoofdstuk XI biedt een theoretisch model, dat gebaseerd is op de besproken experimenten. We concluderen dat dermale afwijkingen (capillairen en infiltraat) een conditio sine qua non zijn voor de ontwikkeling en het persisteren van de laesie. We speculeren over een fundamentele rol van arachidonzuur metabolieten in de aetiologie van psoriasis. Tenslotte worden onze therapie studies beschouwd vanuit dit nieuwe model.
CURRICULUM VITAE


STELLINGEN

I

Gedurende de ontwikkeling van de psoriasis laesie worden de verhoogde epidermale proliferatie en dyskeratinisatie voorafgegaan door een metabole verandering op capillair niveau.

(Dit proefschrift).

II

Na een ogenschijnlijk afdoende locale behandeling van de psoriasis laesie met gefluoronideerde corticosteroiden blijft de alkalisch phosphatase activiteit van de capillairen sterk verhoogd. Het is dan ook niet verwonderlijk dat na stoppen van deze behandeling in de regel snel een recidief volgt.

(Dit proefschrift).

III

De hypothese dat bij psoriasis de afwijkingen aan de huid het gevolg zouden zijn van een ontregeling op het niveau van het centrale zenuwstelsel (Weyl, 1883) is nog steeds actueel en dient met hedendaagse neurologische en biochemische methodieken verder getest te worden.

Het is vooralsnog onduidelijk welke neuronale circuits aangedaan zijn bij de torticollis spasmodicus. De sterk wisselende ernst van de symptomatologie door stimuli van verschillende aard (emotionele, vestibulaire, proprioceptieve, visuele en tactiele) suggereert dat een 'hogere orde systeem' is aangedaan.

J.J.M. van Hoof, ongepubliceerde mededelingen.

De 'Lymphocytic Infiltration of the Skin van Jessner en Kanof' is een verlegenheidsdiagnose.


Het gebruik van een thermoelement bij de cryotherapie geeft slechts een schijnzekerheid.

Alvorens men tot een chirurgische behandeling van inwendige haemorrhoiden besluit dient een conservatieve proctologische behandeling (zoals het aanbrengen van ligaturen en submuceuze injecties met scleroseringsvloeistoffen) tenminste eerst overwogen te zijn.
Bij de behandeling van de tromboflebitis doen hirudinum en heparinoiden houdende zalven weinig kwaad.

De 'bijsluiter' heeft een negatieve betekenis voor de volksgezondheid zolang alle ooit gerapporteerde bijwerkingen zonder enige opgave over de frequentie van optreden erin worden opgesomd.

Bij een onderzoek aan parameters welke sterk afhankelijk zijn van omgevingsfactoren verdient het de voorkeur een controlegroep te kiezen welke leeft in dezelfde omgeving als de experimentele groep.

Er bestaat geen correlatie tussen de kwaliteit van een wetenschappelijk onderzoek en de kwantiteit van de verantwoordelijke onderzoekers.

Het grote aantal wereldreizen, welke 'beroepshalve' door sommige onderzoekers worden ondernomen, maakt duidelijk dat zij zeker nog geen optimaal gebruik van de boekdrukkunst maken.

Stellingen behorende bij het het proefschrift 'Psoriasis: Therapy and Pathogenesis'.
Nijmegen, 8 juni 1983

Peter van de Kerkhof