

CORNEOCYTE MORPHOLOGY AND FORMATION RATE IN LICHEN PLANUS AND EXPERIMENTAL PARAKERATOSIS IN SUBJECTS WITH AND WITHOUT PSORIASIS

Anders Johannesson and Hans Hammar

Department of Dermatology, Karolinska sjukhuset, Karolinska Institute, Stockholm, Sweden

Abstract. In a recent paper changes in corneocyte morphology and formation rate in psoriasis have been established. In the present study the specificity of these alternations were analysed in two situations. Early lichen planus papules were chosen as a model of hyperproliferation with hypergranulosis and orthohyperkeratosis. Hyperproliferation with parakeratosis was induced by nonanoic acid ($\text{CH}_3(\text{CH}_2)_7\text{COOH}$) as an experimental model in psoriatics and non-psoriatics. In lichen planus papules, the rate of corneocyte layer formation was 2.4 times that of the non-involved skin. In psoriasis, a similar rate was found in parts with hypergranulosis. A 13% decrease in corneocyte diameter took place in lichen planus lesions—similar to that found in psoriasis. The corneocytes in lichen planus were also thicker than normal cells, though their volumes were similar. Thickness decreased in more superficial locations of these corneocytes. Swelling was a finding during the histological procedure. Corneocytes in the lesion increased markedly in volume, due to swelling. The parakeratosis elicited by nonanoic acid showed cells with irregular membranes and nuclei with non-flattened and irregular borders. The rate of corneocyte layer formation was 2.6 times that of the normal skin. The epidermal reaction to the superficial damage by nonanoic acid did not differ between psoriatics and non-psoriatics. This type of parakeratosis was distinctly different from that present in psoriasis.

Key words: Rate of corneocyte formation; Corneocyte morphology and size; Lichen planus; Toxic contact dermatitis (nonanoic acid); Psoriasis

When the epidermis is stimulated, hyperproliferation and altered differentiation are responses which have been noted in various conditions, such as lichen planus and psoriasiform dermatoses (10-14). An interrelationship between these two changes has been found in psoriasis. Cox & Watson have reported on a positive correlation between mitotic index and the amount of parakeratosis (2). We have previously found a positive correlation between the amount of parakeratosis and the proliferative activity, estimated by the rate of corneocyte layer formation using the dansyl chloride method (6). Re-

cently, a new method for evaluation of the relative rate of corneocyte layer formation was introduced (8). A close relationship between corneocyte morphology and the formation rate was present in microscopic psoriatic lesions. It was also shown that the corneocytes formed at a pulsative rate (8).

In the present investigation, the specificity of the changes earlier found in the initial psoriatic lesion was evaluated. Other situations were sought where a disturbance is present in the epidermal differentiation and/or change in the proliferative activity. Lichen planus (11) was chosen, since the papules in this disease are well demarcated and the diseased skin can be clearly distinguished from the non-affected skin, at both the macro- and microscopical level. A toxic contact dermatitis elicited by nonanoic acid ($\text{CH}_3(\text{CH}_2)_7\text{COOH}$) was chosen as an experimental model of parakeratosis. This compound is used as a positive control for a toxic reaction in the standard patch tests at our clinic (17). Histological findings from these lesions have shown patchy hyperparakeratosis with some resemblance of the early psoriatic lesion, as shown in a preliminary investigation.

MATERIAL AND METHODS

Patients

Lichen planus. Five patients (30-53 years, one male) with active untreated lichen planus supplied 15 biopsies of minute papules on thighs and arms. The papules were identified under a magnifying glass and clearly distinguishable papules in interfollicular areas were chosen. The whole lesion was excised together with the surrounding uninvolved skin.

Experimental parakeratosis. Ten patients supplied 20 biopsies from the skin of the upper back from the border of a patch test with nonanoic acid. Seven were male and the age range was 30 to 69 years. Five of the patients were psoriatics hospitalized for treatment of an active disease with nummular and plaque lesions. In the psoriatics, the

tests were performed during treatment with UVB, anthralin and salt baths. The test area had not been affected and it was protected against all external influence during the period of study. Five of the subjects were hospitalized for treatment of contact dermatitis. They served as controls and tests were performed when the patients were almost cured. The test area had not been affected. The procedure was as described above.

Test performance. 0.04 ml 40% nonanoic acid ($\text{CH}_3(\text{CH}_2)_9\text{COOH}$) in 1-propanol solution was supplied to discs of the Al-test® (Imeco AB, Södertälje, Sweden). Two discs were applied on each patient for 48 hours. Occlusion was secured with Scanpore® tape. After 72 to 120 h the test area was well demarcated. It was brownish in colour and had a slightly wrinkled surface. Material was taken from the border region in one of the test areas at 72 h and in the other at 120 h.

Samples were collected without anaesthesia by a punch biopsy 1–2 mm thick and 2–3 mm in diameter which was excised, immediately frozen on solid carbon dioxide with the dermal side down, transported in the cold and kept in a deep-freezer, -90°C , until sectioned on a cryostat.

Sectioning. The cryostat sections were cut 6 μm thick, perpendicular to the surface. The biopsies were oriented in such a way that both the perilesional and lesional areas were displayed in the same section. The sections were attached to the slides as earlier described (8).

Staining. Corneocyte membrane were stained with 38 mM fluorescein-isothiocyanate (FITC) (Sigma, St. Louis) in water (1). Nuclei were stained with 3.4 mM 4-diamidinodiphenylamine dihydrochloride (DDP) (May & Baker, Dagenham, England) in a solution of 0.15 M NaCl in water (16). The FITC-DDP sections were processed as previously reported (8). Routine staining with haematoxylin & eosin (H & E) was also performed.

Readings. The sections were read and photographed in a Zeiss Universal microscope in unfiltered UV light and with phase contrast at 64, 100 and $\times 500$ with a Nikon camera, Kodak Panatomic film, ASA 32. Pictures were prepared for both UV and phase contrast micrographs and readings were made from blow-ups of negatives. The examination procedure is as described earlier (8).

Rate of corneocyte layer formation. Calculation was done as previously reported (8). The reference area was chosen at 0.24 ± 0.01 (SEM) mm outside the lichen planus lesion. In this part no dermal infiltrate or alterations of the epidermis were seen. In the diseased skin, observations were made in areas where the most distinct alteration was present. The analysis of the test skin was made in the border region of the patch test. The reference area was chosen at 0.15 ± 0.02 mm from the margin of notable changes in the horny layer.

Corneocyte diameter was estimated from the length of adjoining cells parallel to the surface on FITC-DDP stained sections from 6 lichen planus papules. In order to exclude any systemic error in the sampling procedure, groups of 15–20 adjoining cells in each of three-five opposite layers were counted. Clusters of cells were measured in three different areas in the six lesions. I: non-involved skin in up to 15 layers (100 cells); II: corneocyte layer number 6–10 from the bottom in the diseased skin (107

cells) or in 5 layers around the 15th layer (42 cells); and III: the 5 lowermost corneocyte layers in diseased skin (189 cells). The mean diameter of the corneocytes was estimated from the apparent length of the sectioned cells (L), assuming these to be circular in form. It was equal to

$$\frac{4}{\pi} \frac{1}{n} \sum L,$$

where n is the number of measured cells (8). The diameter data of the different patients and areas were submitted to an analysis of variance (15).

Corneocyte thickness was estimated on unstained sections and after FITC-DDP staining of the same cells whose diameters had been measured as outlined above. In the unstained sections, the thickness was measured on blow-ups of negatives taken in phase contrast. In the three areas I–III, five lines perpendicular to the surface were arbitrarily chosen. Along these lines corneocyte thickness was measured with a caliper. This was also done in area II in the 5 layers around the 15th layer, and in areas I and III as described above for diameter.

Corneocyte volume and effect of shrinkage. The thickness of the corneocyte was measured in two sections taken direct from the cryostat and compared with the measurements in the same sections after they had been attached to the slides by the procedure earlier described (7). No difference was found between these measurements. Thereafter, the attached unstained sections were used to measure thickness. The diameter of the cells was not affected by the attachment or staining procedures. The volume of the cells was estimated as indicated in Fig. 1. Volumes were also estimated from the stained sections. Analyses of covariance were performed to evaluate volume changes in layers and between areas (15).

RESULTS

Lichen planus

Histological analysis of the stained sections revealed the expected signs of lichen planus—band-like dermal infiltrate, liquefaction degeneration of the basal layer, hypergranulosis and hyperkeratosis. The diameters of the measured lesions ranged between 0.58 and 1.15 mm, mean 0.90 mm. The corneocytes in the perilesional skin and at the margin of the lesion were thin and oriented in parallel sheets. The hyperkeratosis was seen just superficial to the part where the basal layer of the epidermis was altered (Fig. 2a).

Corneocyte diameter. The mean corneocyte diameters of the diseased skin (areas II and III) were 29.3 ± 0.2 (SEM) μm and 29.9 ± 0.2 (SEM) μm , respectively. This was significantly smaller (13%) than the mean diameter of the uninvolved skin, which was 34.0 ± 0.2 (SEM) μm ($p < 0.02$). No difference was found between areas II and III or between patients. In this analysis of variance, the

F-tests were performed against interaction, as this was found to be significant. The reason for covariation between areas of skin studied and subjects was not further analysed. It was assumed that the interaction was due to variation in the age of the lesions.

Corneocyte thickness. The mean corneocyte thickness in the five lowermost layers measured on unstained sections was 1.00 ± 0.02 (SEM) μm in the perilesional skin and 1.36 ± 0.08 (SEM) μm in lesional skin. The thickness decreased exponentially towards the surface (Fig. 3). In perilesional skin a plateau value was found 3–5 corneocyte layers up from the bottom, whereas in the lesion itself this appeared higher up (Fig. 3). The mean thickness of corneocytes in the mid-portion of the horny layer was 0.62 ± 0.02 (SEM) μm in perilesional skin and 0.75 ± 0.06 (SEM) μm in lesional skin. The corneocytes in the FITC-DDP stained sections were thicker than were non-stained corneocytes. The swelling resulting from the staining procedure was prominent in lesional skin and particularly in the lowermost part, where corneocyte morphology changed considerably. The distribution of corneocyte thickness in relation to corneocyte diameter is given in Fig. 3*a, b*.

Corneocyte volume. In both unstained and stained sections the volumes decreased as the position of corneocytes became more superficial (Fig. 3*d*). In the basal corneal layers, the decrease in volume was exponential and similar to that in involved and perilesional corneocytes, since the slopes of regressions were not different in analyses of covariance. Adjusted means were not different in the unstained material, which indicated that the volume was the same in spite of differences in diameter and thickness in the two locations. In the stained material volumes varied, $p < 0.001$ ($F = 103$, degrees of freedom 1/87).

Rate of corneocyte layer formation in the lesion was 2.4 ± 0.2 (SEM) times that of non-involved skin, range 1.0–4.6. No difference was seen in the relative formation rate measured for the very thick cells located in the basal part of the horny layer, 2.4 ± 0.2 (SEM) compared with cells slightly thicker than normal located in the mid-zone of the horny layer, 2.6 ± 0.4 (SEM).

Nonanoic acid treated skin

Morphology. The Malpighian layers were slightly enlarged (26% increase in sectioned area) com-

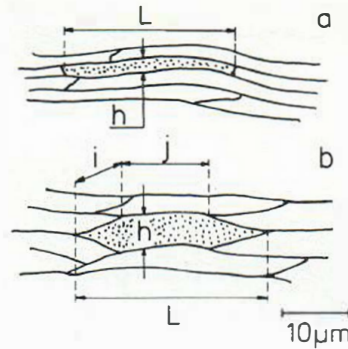


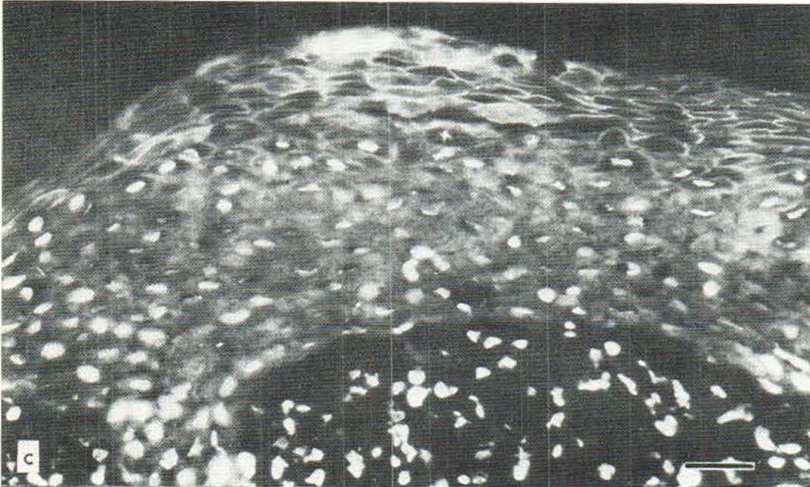
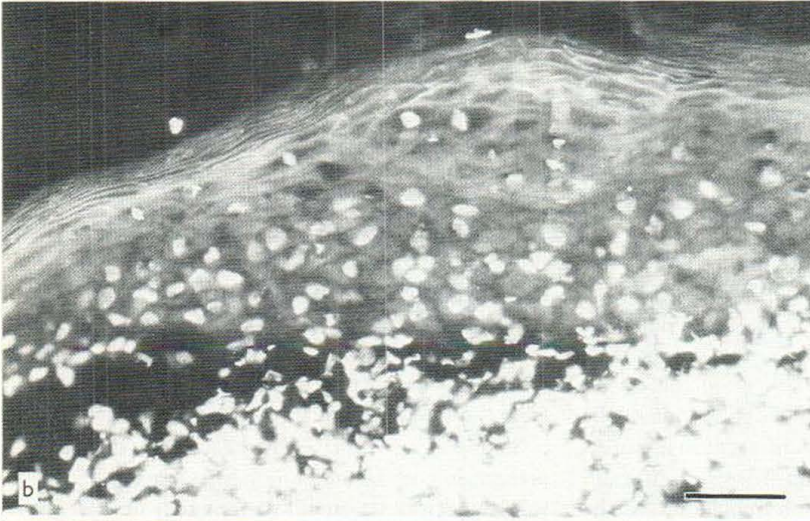
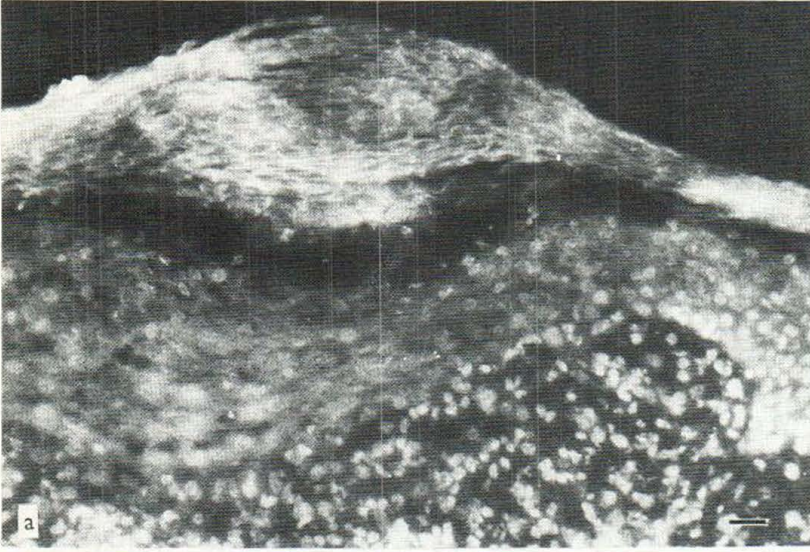
Fig. 1. Calculation of corneocyte volume in lichen planus. (a) Perilesional horny layer. The volume equals $\pi h (\frac{1}{2}L)^2$. (b) Basal part of the horny layer of the lesion. The volume is calculated on the indicated polygon rotated around its axis obtained by calculus and is equal to $\pi h (\frac{1}{2}L)^2 - \frac{1}{2}yL + \frac{1}{2}y^2$, where $y = (V(4x^2(h^2 + L^2) + h^2) - 4x^2L) : 4(1 - 4x^2)$ and $x = i : j$. The cell diameter was obtained from expected length (L), height (h) and the ratio ($i : j$) was taken as the mean 0.44. It was measured in 10 corneocytes sectioned centrally. The cell chosen had the diameters above the expected cell diameter of 29 μm .

pared with perilesional skin. The granular layer was present at 72 h and clearly enlarged at 120 h.

At 72 h the horny layer in the treated area was hyperplastic, with parakeratosis surrounded by orthokeratotic cells. One or two layers of non-nucleated corneocytes were interposed between the parakeratotic area and the granular layer. At 120 h the interposed layers were increased in number. The horny layer had a tendency to detach easily from the remainder of the epidermis.

Small perivascular infiltrates were occasionally present in both treated and perilesional skin. There was no accumulation of inflammatory cells near the epidermis. In the FITC-DDP stained sections of the lesion, the horny layer showed irregular membranes and the parakeratotic nuclei were non-flattened or irregular in shape (Fig. 4*a, b*).

The rate of corneocyte layer formation in the parakeratotic part of the horny layer was 2.6 ± 0.1 (SEM) times that of the reference area. No increase in formation rate preceded the formation of nucleated cells. The orthokeratotic cells interposed between the parakeratotic area and the reappearing granular layer could not be completely delineated. No precise measure of their formation rate could therefore be obtained. It was our impression that it might be slightly increased. No difference could be detected between psoriatics (2.7 ± 0.2) and non-psoriatics (2.5 ± 0.1).



DISCUSSION

Lichen planus

One of the main features of lichen planus is the destruction of the basal layer. Despite this fact, Marks et al. (11) found a decreased transit time of keratinocytes through stratum Malpighii, together with a greatly increased [^3H]thymidine labelling index in the lesion and at its margin, compared with normal controls. Their interpretation was that there is a repopulation of keratinocytes from the margins and from sweat ducts into the lesion.

Ebner et al. (3) also found a greatly increased labelling index in the lesion and in the dermal portion of the eccrine sweat ducts. The labelling index at the margins was also increased, but not to the same extent as in the other sites mentioned. Their data were interpreted as showing that a repopulation from the margins was less probable, in comparison with that from the sweat ducts.

The increased rate of corneocyte layer formation of 2.4 times in the lichen planus lesion compared with that of the perilesional skin found in this study is of the same magnitude as the threefold increase in desquamation rate earlier found by Hammar (5). These data, together with the shortened transit time found by Marks et al. (11), favour an increased epidermal cell turnover in the lichen planus papules as the net result of the two simultaneous processes: destruction and hyperproliferation. The change in the thickness of the horny layer occurred at the margin of the lesion (Fig. 2*a*). This means that the rate of corneocyte formation was not increased outside the lesion.

An increased labelling index at the margin of the lesion has been found as indicated above. The daughters of such germinative cells should lead to

hyperkeratosis on top of these if they moved perpendicular to the surface. The result of the present study, which showed an increased corneocyte formation just inside the lesions but not at its margins, favour the theory of a repopulation of cells to the lesion from its margin. It would be valid if the increased labelling index at the margin found by others were consistent with an enhanced formation of cells in the germinative compartment.

The combination of hyperproliferation and hypergranulosis is also reported in lichen simplex and lamellar ichthyosis (4, 13, 14). The hypothesis may be proposed from this study that hypergranulosis, together with orthohyperkeratosis, is a histological sign of moderately increased proliferation. Biologically this could mean that the process which maintains orthokeratosis, i.e. the time for the formation of the keratohyalin granula and the subsequent terminal differentiation, cannot be speeded up over a certain limit while still maintaining orthokeratosis. By extension of the granular compartment from about one layer up to about 3-4 layers in lichen planus, the transit rate can be increased 3-4 times, though the time spent in the granular compartment will remain about the same.

The corneocyte volumes in lichen planus seem to be the same in healthy as in involved skin. This indicates that the alteration present (Fig. 3) is associated with the terminal process of differentiation in cells passing the granular compartment. The decrease in corneocyte volume seen in both normal and in lichen planus lesions in the lowermost layers probably reflects a water exclusion process secondary to the ongoing maturation in this part. This seems to be of similar nature in normal and diseased areas, since the slopes of cell volume reduction are the same (Fig. 3*d*).

Nonanoic acid treated skin

The production of parakeratosis in the treated skin led to a minor increase in the rate of corneocyte layer formation. It is probable that the early appearance of granular cells as seen at 72 h indicates that the damage inflicted killed the upper layers of keratinocytes rather than disturbing the process of their terminal differentiation. This damage should correspond to the increased rate of formation as measured. The absence of an inflammatory cell reaction is noteworthy. The reaction to the treatment became limited in time and also restricted the perturbation of the cell population in the Malpighian

Fig. 2. Cryostat sections of a lichen planus papule stained with the fluorochromes FITC and DDP. The bar indicates 50 μm . (a). Border region of a medium-sized lesion. To the right, hyperplastic epidermis with a detectable basal layer. On top of this part a thin horny layer is present with parallel sheets of corneocytes. The hyperkeratotic mid-portion is located superficial to an area where the basal layer is hardly distinguishable. (b). A small lesion with normal looking epidermis to the left. At the bottom a dense dermal infiltrate is present, invading the epidermis, centrally and to the right. In this part the basal layer cannot be distinguished and the corneocytes overlying appear voluminous. (c). A medium-sized lesion with a hardly recognizable basal layer. The whole lesion is covered by voluminous corneocytes.

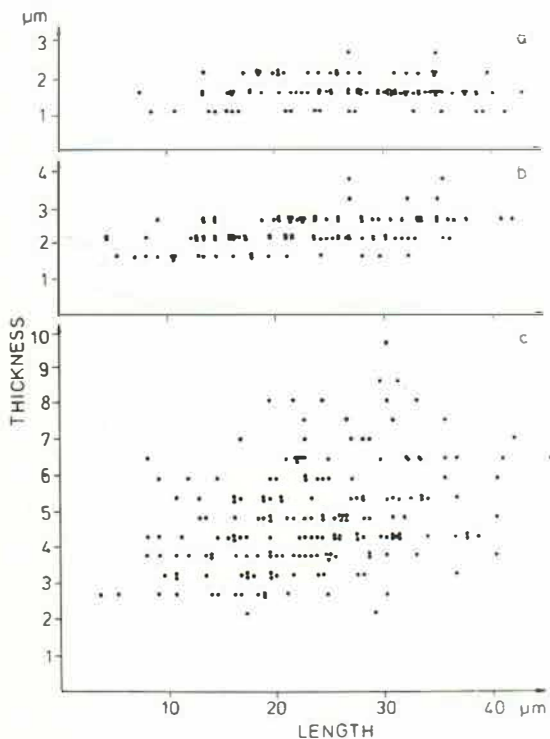


Fig. 3a. Distribution of corneocyte thickness in FITC-DDP stained sections (a) perilesional skin (area I), (b) lesional skin (superficial part, area II) and (c) lesional skin (basal part, area III). Periodicity is due to the resolution of the measurement $0.43 \mu\text{m}$.

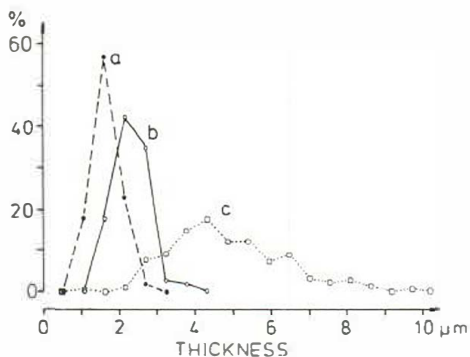


Fig. 3b. Relative distribution of corneocyte thickness in FITC-DDP stained sections in the three different areas (see Fig. 2a).

layers, irrespective of whether the test person was non-psoriatic or psoriatic.

Relations to psoriasis

The increased proliferation in lichen planus is in the same range as earlier found in the orthohyperkeratotic parts of the minute psoriatic lesion (9). A rate of corneocyte layer formation of 3.4 times that of the non-involved skin was found. In the orthohyperkeratotic parts of psoriasis we also find hypergranulosis, as in lichen planus. In the expanded granular compartment in lesions of psoriasis

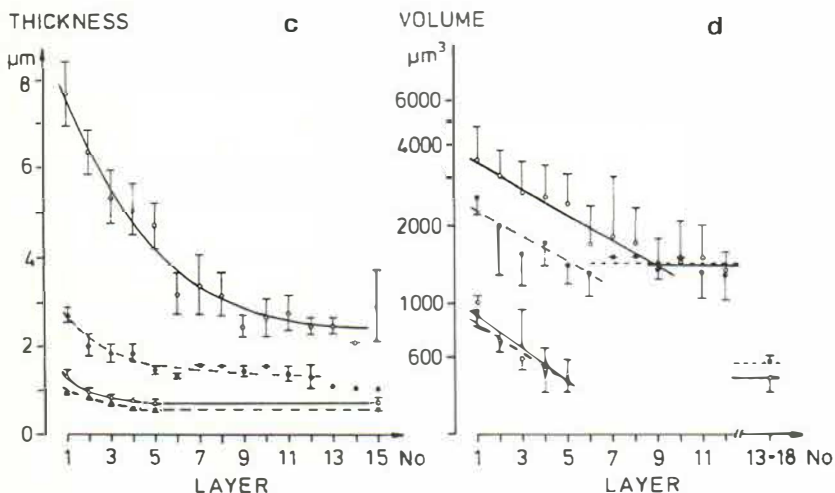


Fig. 3c-d. (c). Corneocyte thickness in relation to position of corneocytes in horny layer. Numbering of the corneocyte layers starts from the basal part. Means and standard errors are given. The upper solid and hatched lines represent FITC-DDP stained corneocytes from in-

involved and non-involved skin, respectively. The lower solid and hatched lines represent unstained lesional and normal corneocytes, respectively. (d). Corneocyte volume in relation to cell position in horny layer. Conventions as in Fig. 3c.

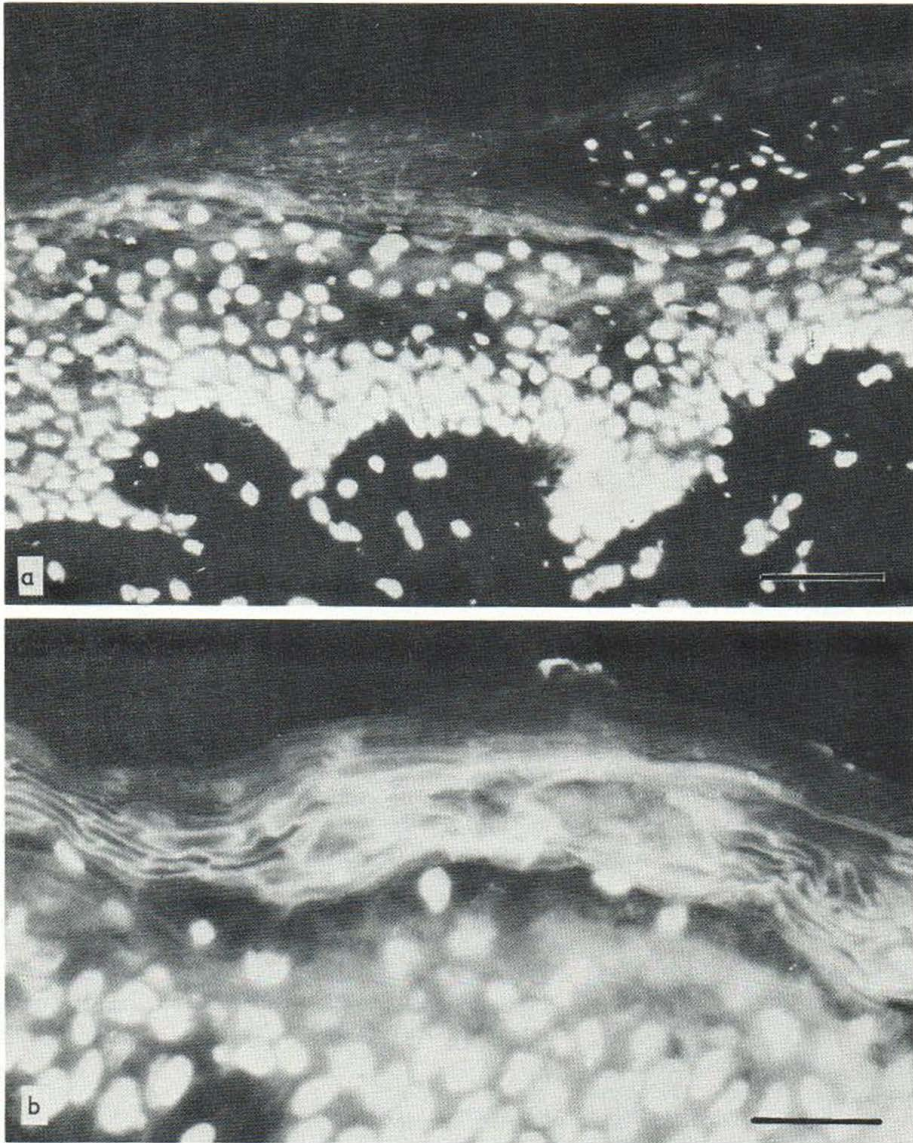


Fig. 4. Cryostat section of border region of a patch test treated with nonanoic acid, stained as in Fig. 2. (a). A thin normal looking horny layer (left), and hyperparakeratotic horny layer (right). The parakeratotic area show nuclei with varying forms. A few cells are present in the dermis.

Biopsy taken after 120 h. Bar $50\ \mu\text{m}$. (b). The corneocyte membranes show heterogeneous and bizarre forms in the mid-portion of the horny layer. Biopsy taken after 72 h. Bar $20\ \mu\text{m}$.

and lichen planus, the cells are less flat than in the normal case. The result is the formation of the thicker corneocytes with a decreased diameter, as we found in both diseases.

The swelling present in the lichen planus corneocytes, much larger than in normals, points to a biophysical alteration in the keratinization process

in these cells. The swelling pattern seen in lichen planus differs considerably from what has earlier been found in psoriasis (6, 8). In this condition, parakeratotic cells and the surrounding orthokeratotic corneocytes were thicker and had decreased diameters compared with the cells in uninvolved skin. The thicker cells did change in thick-

ness on their way up through the horny layer. This change was not so well marked as that shown in lichen planus (6). These findings indicate that the alterations of the keratinization process are different in the two diseases.

Apart from the discrepancies mentioned above, the pattern of the rate of corneocyte formation are different in lichen planus and psoriasis. In lichen planus the rate is raised but even in nature, while in the minute psoriatic lesion the rate was much increased and pulsative (8). The parakeratosis obtained by nonanoic acid was dissimilar to that of psoriasis. The reaction in the epidermis was also limited after nonanoic acid. In the psoriatic patient, this must mean that the damage leading to the formation of a minute lesion is located not in the superficial Malpighian layers but rather in its basal portion. The inflammatory response of mononuclear cells always present in minute psoriatic lesions (8) should also be considered important in this context.

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A. Johannesson, M.D.
Department of Dermatology
Karolinska Sjukhuset
S-104 01 Stockholm 60
Sweden