Acta Dermatovener (Stockholm) 61: 535–542, 1981

TREATMENT OF DARIER'S DISEASE WITH AN ORAL AROMATIC RETINOID (Ro 10-9359): A CLINICAL AND LIGHT AND ELECTRON MICROSCOPIC STUDY

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Abstract. An aromatic retinoid (Ro 10-9359) cleared 6 of 8 patients with Darier's disease in 4–6 weeks. After discontinuing the treatment, remission was maintained for up to 5 months. In 2 severe cases continuous treatment proved necessary in order to maintain the improved state. Acantholysis, various types of dyskeratosis and acanthosis with downward proliferation of rete ridges were seen by light microscopy before treatment. Electron microscopy revealed a decreased number of desmosomes and other cellular attachments and conspicuous microvillous changes of cell surface in separated basal cells. Keratinization in epidermal cells was premature and characterized by abnormal aggregations of keratinization products. Under the influence of retinoid there seemed to appear a new generation of basal cells better able to form intercellular attachments, after which the progress of keratinization in the entire epidermis shifted towards normal, and acanthosis decreased.

Key words: Darier's disease; Retinoids; Acantholysis; Dyskeratosis

Darier's disease is a rare disorder of keratinization, believed to be transmitted as an autosomal dominant trait. Isolated cases within a family also occur, obviously resulting from an incomplete penetrance of the mutant gene, or from spontaneous mutation.

The pathogenetic events in Darier's disease leading to suprabasal acantholysis and various signs of dyskeratosis in epidermal cells, e.g. corps ronds and grains seen in light microscopy, are still obscure. Ultrastructural studies have revealed a breakdown of the tonofilament–desmosome complex, abnormal aggregation of tonofilaments (2, 12, 15) and early appearance of keratinosomes and keratohyalin granules (2, 15). Thus, acantholysis and dyskeratosis are suggested to be the morphological outcome of a hereditary error in the synthesis, organization and maturation of the tonofilament–desmosome complex (2). On the other hand, the importance of cytoplasmic vacuolization has been emphasized (8). Recent studies have furthermore disclosed some defects in cell-mediated immunity (13, 17).

Oral vitamin A and topical vitamin A acid (tretinoin) have been of some benefit in the treatment of Darier's disease. Recently an oral aromatic retinoid (Ro 10-9359), another analog of vitamin A, has been introduced to the therapy, with promising results (1, 10, 14). The present paper reports the treatment results obtained in 8 patients, with clinical, light microscopic and ultrastructural observations.

MATERIALS AND METHODS

Characteristics of the 8 patients treated are shown in Table I. The doses of aromatic retinoid used during treatment are given in Table II.

Biopsy specimens for light microscopy were taken from all patients before treatment. During treatment, samples from 6 patients were taken at 2 and 4 weeks during the initial clearing phase. Patients 3 and 6 who showed a slower response were examined thereafter at one-month intervals up to 5 months of treatment, and patient 3 even at 10 and 11 months of treatment. Samples for electron microscopy were taken from patients 4, 7 and 8 before and after 2 and 4 weeks of treatment, and from patient 6 before and after 2 and 8 weeks and 5 months of treatment. Patient 3 was examined after 5, 10 and 11 months of treatment.

Samples for electron microscopy were fixed in 2.5% glutaraldehyde (cacodylate-buffered) and post-fixed in 1 % osmium tetroxide. Specimens were embedded in epoxy resin, sectioned, and stained with 5% uranyl acetate and lead citrate.

RESULTS

1. Clinical results

The treatment responses are presented schematically in Table III. Six of the 8 patients were cleared in 4–6 weeks. During treatment the lesions healed

Table I. *Characteristics of the patients*

	Patient							
	1	2	3[a]	4	5	6	7[b]	8
Age (years)	23	32	36	38	53	54	57	70
Sex	F	F	F	M	M	M	M	M
Heredity	–	+	–	+	–	+	–	–
Extent of the disease	2/4	1/4	3/4	2/4	1/4	4/4	1/4	1/4
Palmoplantar lesions	+	–	+	–	–	+	–	–
Nail changes	+	+	+	–	–	+	–	–
Duration (years)	14	17	30	15	1	30	2	35

[a] Patient had previously received X-ray treatment (cumul. dose 31 700 rad).
[b] Patient had previously received arsenic and PUVA treatment for psoriasis.

Table II. *Doses of Ro 10-9359 used for treatment and maintenance*

	Patient							
	1	2	3	4	5	6	7	8
Initial dose (mg/kg/day)	0.8	0.8	0.9	0.8	0.7	0.8	0.7	0.9
Reduced dose (up to 3 months)	0.4	–	0.6	0.5	–	0.6	0.3	0.3
Maintenance dose (after 3 months)	0.2–0.4	–	0.3–0.4	–	–	0.35–0.5	–	–

gradually, occasionally leaving some slight residual changes and brownish pigmentation on the sites of lesions.

There were 2 patients with extensive lesions and slower treatment responses. Patient 3 had extremely hypertrophied verrucous lesions, especially on the legs (Fig. 1*a*). After 8 weeks of treatment with aromatic retinoid, these lesions were markedly improved (Fig. 1*b*). On a maintenance dosage of 25 mg/day (0.37 mg/kg) the patient has remained fairly well, with a few lesions in the groins, up to the present, 2 years since initiation of the treatment. A temporary reduction of the dose resulted in the formation of new lesions on the trunk.

Patient 6 belongs to a family with several cases of Darier's disease, patient 2 being another representative. An extensive erythrodermic eruption had spread on the skin of patient 6 about 3 months before commencement of retinoid treatment. Aromatic retinoid had a distinct effect in this case too, but only after 4 months of treatment was a good clearing achieved.

All the patients complained of dryness of the lips, especially during the initial phase of the treatment. Transient alopecia occurred in patient 3 after 7 weeks of treatment. Serum transaminase and alkaline phosphatase levels have remained within normal limits in all patients. A slight fluctuation of

Table III. *Treatment responses*

	Patient							
	1	2	3	4	5	6	7	8
Clearing	+++	+++	++	+++	+++	++	+++	+++
Time needed for ++–+++ clearing (weeks)	4	4	10	4	4	16	5	6
Remission after discontinuation of treatment (months)	1.5	4	–	1.5	5	–	1	2

+++ = excellent clearing with, at most, slight residual changes. ++ = <25% residual lesions.

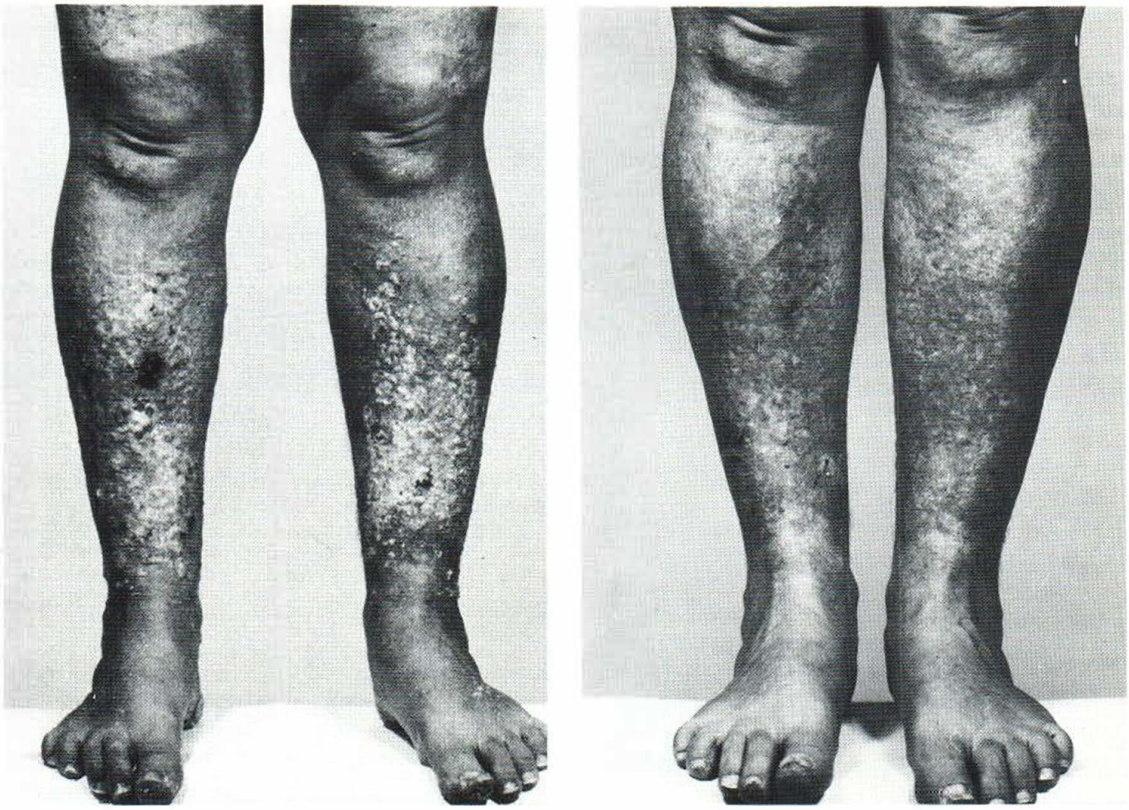

Fig 1. The legs of patient 3 (*a*) before treatment, (*b*) after 8 weeks of retinoid treatment.

serum creatinine, with a peak of 130 μmol/l, has occurred in patient 3.

2. Light microscopy

All biopsies taken before treatment revealed typical features of Darier's disease, e.g. acantholysis, hyper- and parakeratosis, dyskeratosis, acanthosis with downward proliferation of rete ridges and varying degrees of upper dermal inflammatory infiltrate. At 2 weeks of retinoid treatment the suprabasal lacuna formation had disappeared in patients 7 and 8. In these patients, however, some degree of hyperkeratosis, dyskeratosis, parakeratosis and downward proliferation of epidermal rete ridges were found.

At 4 weeks of treatment, acantholysis was no longer found in patients 4 and 5 either. Some epidermal downward proliferation was still discernible in patients 7 and 8, with occasional dyskeratosis and even one corps rond.

Two biopsy specimens were taken from each of patients 3 and 6 from variously affected areas at 4 months. Lacuna formation was found, but dyskeratosis was less evident than in earlier biopsies; corps ronds were only seen adjacent to an intra-epidermal sweat duct in patient 6. Varying degrees of hyper- and parakeratosis as well as epidermal downward proliferation were found in every biopsy specimen. These changes were not seen in samples taken from the same patient after 5 months of treatment. A specimen from a new lesion in patient 3 taken at 10 months of treatment revealed all the typical features of Darier's disease. A sample taken from the same area 4 weeks later showed only some acanthosis.

3. Electron microscopy

Before treatment. The acantholytic and dyskeratotic processes were more closely examined by electron microscopy. Suprabasal acantholytic clefts were found to be formed at sites where basal cells had completely lost their desmosomes and other

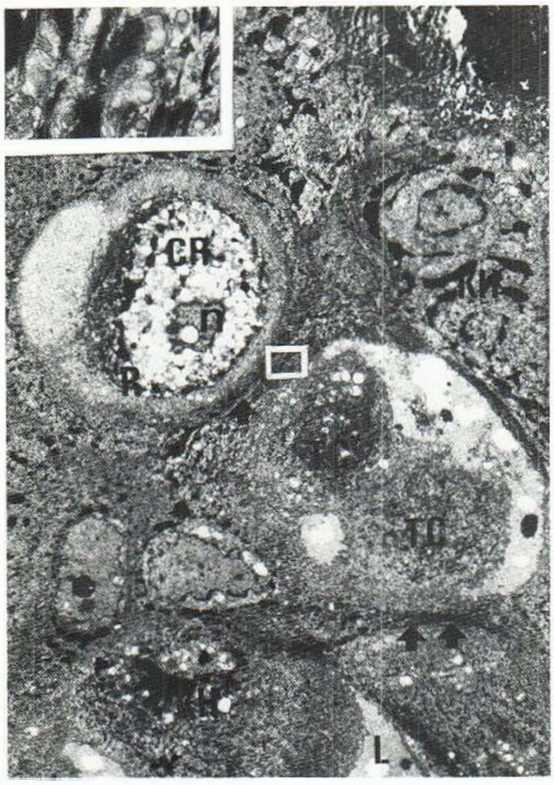

Fig. 2. Epidermis above a lacuna (*L*) before treatment. All keratinocytes show various types of dyskeratosis. There is a corps rond (*CR*) with vacuolized cytoplasm and pyknotic nucleus (*n*) inside a ring (*R*) of aggregated tonofilaments and keratohyalin granules. Note the different types of tonofilament clumping (*TC*). The keratinization lines (*arrows*) are premature and wrongly orientated. *KH*, keratohyalin granule. ×1900. *Inset:* Greater magnification of a keratinizing band shows that it consists of aggregated keratohyalin granules, tonofilament bundles and keratinosomes. ×15150.

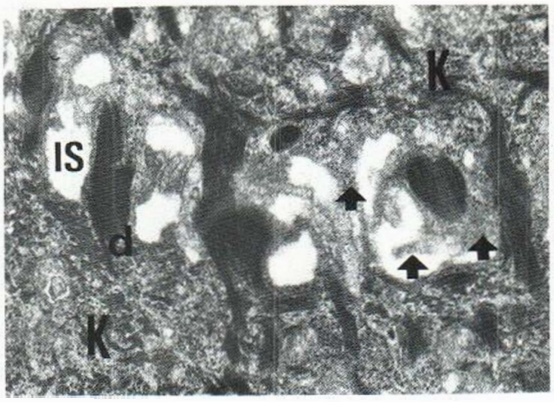

Fig. 3. After 2 weeks of retinoid treatment the intercellular space (*IS*) contains abundant fine granular substance (*arrows*) that makes the cellular outlines obscure. *K*, keratinocyte; *d*, desmosome. ×15150.

intercellular contacts. Basal cells had also separated from each other, but remained attached to the basal membrane with intact hemidesmosomes. Conspicuous microvillous changes in cell surfaces were seen between detached basal cells. Large gaps between keratinocytes with few and often stretched desmosomes and villous changes were found in places where there was no actual acantholysis. In a few separating cells there was some vacuolization originating mostly from mitochondria. Plasma membrane was obscure or disrupted at places. However, no significant leakage of intercellular components was discernible.

Throughout the epidermis, numerous signs of dyskeratosis were evident. In separating basal cells there were thick, electron-dense bundles of tonofilaments occasionally showing conspicuous clumping. The tonofilaments ran parallel to the cell surface, with no evident contact with it. Above or adjacent to the acantholytic areas there were keratinocytes with more considerably disturbed keratinization. Large keratohyalin granules and keratinosomes appeared markedly below the usual keratinizing layer, forming at places falsely orientated zones of keratinization (Fig. 2). Some keratinocytes showed condensation and aggregation of tonofilaments throughout the cytoplasm, and in some other cells tonofilaments assumed a peripheral or a perinuclear, circular distribution, being intermingled with keratohyalin granules and keratinosomes (Fig. 2). Keratinosome-derived lamellae were conspicuous beneath the horny layer. Many horny cells, especially in intermediate and the uppermost horny layers showed a fibrillar structure in a less electron-dense ground substance.

At 2 weeks of retinoid treatment. Acantholytic lacunae were no longer found in patient 8 but the intercellular space still was wide, containing in places extracellular, fine granular substance in abundance (Fig. 3). Dyskeratotic cells were found in a few sections only. In stratum granulosum there seemed to be normal amounts of keratohyalin and keratinosomes.

In patients 4 and 5 lacuna formation was found, with amorphous gathering of fluid and freely floating acantholytic cells. In patient 7 there were only

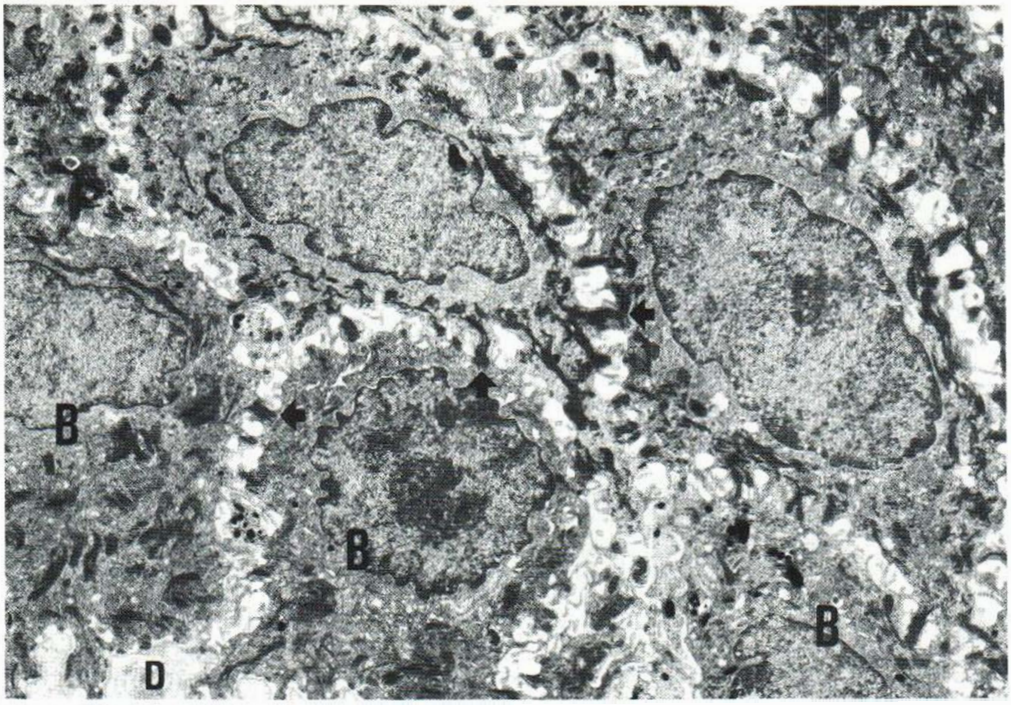

Fig. 4. Basal epidermis after 4 weeks of treatment. The cells are in closer contact than before treatment. The number of desmosomes (*arrows*) is increased. *B*, basal cell; *D*, dermis. ×4600.

minor suprabasal splits. At lower epidermal levels, dyskeratosis was manifested in these patients as a condensation of large masses of tonofilaments, and in the upper levels tonofilaments formed a peripheral aggregation band in the cytoplasm of some cells. Plasma membrane was occasionally damaged. Numerous microvilli protruding into the wide intercellular spaces seemed to have an obscure contour. Some mitochondria were disrupted, showing no distinct lamellar structure. Keratinosomes were abundant in the upper epidermal keratinocytes as also were keratinosome-derived lamellae beneath the horny layer. Stratum granulosum was poorly organized. Some keratinocytes showed precocious keratinization with marginal envelope, irregular accumulation of keratinosomes and scattered keratohyalin. In patient 4, keratinocytes with perinuclear cytolysis were seen.

At 4 weeks of retinoid treatment. No acantholysis was found in patients 4, 7 and 8. The basal cells were in closer contact with each other and were provided with increased numbers of desmosomes (Fig. 4). However, in some areas microvillous formations were still encountered. Tonofilaments showed a rather normal distribution and orientation. In the middle epidermal cells, tonofilaments were mostly perpendicular to the cell surface, but some tonofilament bundles looked rather thick and clumpsy and their association with desmosomes was obscure. In patient 6 acantholysis and various signs of disturbed keratinization were noted.

After 4 weeks of retinoid treatment. In patient 6, besides the features of Darier's disease described above, giant cells were formed from keratinocytes with foamy cytoplasm. At 8 weeks of treatment there appeared abundant round keratohyalin granules. Superabundant colonies of keratino-some-derived lamellae were seen at the border of horny layer and between the lowest layers of stratum concernum (Fig. 5).

New lesions which at 10 months of treatment appeared in patient 3 despite a small maintenance dose revealed all the typical features seen before treatment (Fig. 6).

DISCUSSION

Our observations confirm that most cases of Darier's disease are cleared with aromatic retinoid

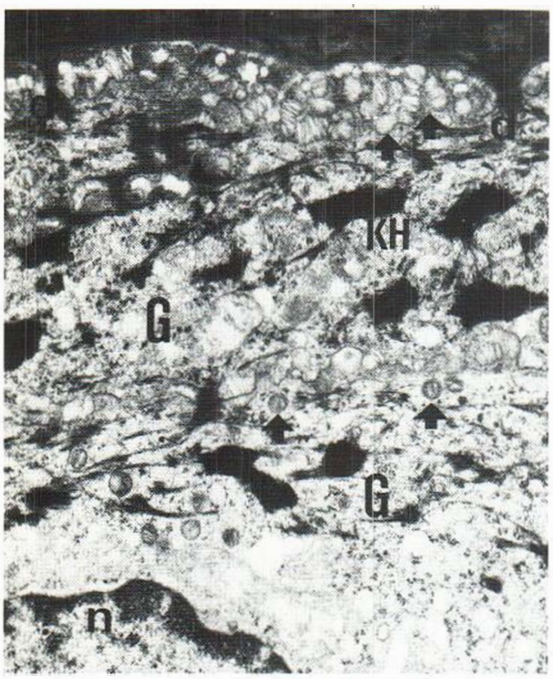

Fig. 5. The horny layer border in patient 6 after 4 months of treatment. Note the large number of keratinosomes (*arrows*) in the interspace between a horny cell and a granular cell (*G*) and even inside the granular cells. KH, keratohyalin granule; *d*, desmosome; *n*, nucleus. ×15600.

in 4–6 weeks, as has been reported previously (1, 14). The effect in hypertrophic and extensive cases is slower and complete clearing cannot always be achieved.

The initial dose of retinoid in previous studies has varied between 0.9 and 1.5 mg/kg/day (1, 10, 14). Judging by our findings, initial daily doses of 0.7–0.9 mg/kg seem to be adequate. Later during treatment the dose must be adjusted according to the clinical response. After clearing, the treatment can in many cases be interrupted and remission will nevertheless be maintained for even 5 months. This is obviously partly ascribable to prolonged elimination of the drug from the body. After initiation of a new treatment period, patients seem to respond more rapidly and at lower doses. Patients 1 and 3 have now been treated according to these principles for about 2 years.

Concerning the mechanism of acantholysis in Darier's disease it has been suggested that the withdrawal of tonofilaments of the desmosomal attachment plaques precedes the breakdown of the desmosome (2) or, according to another explanation, the desmosomes are first pulled apart into two halves, after which the detachment of tonofilaments takes place (12). The assessment of any dynamic process on a purely morphological basis is rather difficult and is still complicated because the ascent of even normal keratinocytes from the basal layer upwards obviously causes considerable alterations in cellular attachments. However, the findings in our study suggest that at the margins of the acantholytic areas at least some tonofilament–desmosome complexes become stretched and are ultimately pulled apart into two halves. On the other hand, it seems probable that the basal cells in acantholytic areas have a deficient ability to even form desmosomes or other intercellular attachments. These pathological features may arise from a primary disorder in the metabolic processes of keratinization, which leads to abnormally aggregating tonofilaments that are unable to form tonofilament–desmosome complexes. As previously suggested, there may also be a disorder of the whole cell surface, i.e. plasma membrane and/or surface coat, and not merely of the desmosomes (12). The abnormal aggregations of tonofilaments and the pathological progress of keratinization might then be a result of a loss of control and co-ordination between epidermal cells. The plasma membrane disruption and microvillous changes seen in our study support the concept of some defect in cell surface properties. The possible role of accelerated epidermopoiesis in the formation of deficient desmosomes has also been emphasized (5).

Microvillar changes are also seen in other acantholytic conditions (9). Their co-existence with abnormal aggregation of tonofilaments is seen in both Darier's disease and Hailey-Hailey disease and thus this combination is not pathognomonic of the latter exclusively, although previously so suggested (9).

The deficient incorporation of amino acids in acantholytic cells points to a cessation of their protein synthesis (16). As an early sign of cellular death, some vacuolization in separated cells originating from mitochondria was seen in our study. The vacuolization, however, is not considered by us to be the primary fault triggered by the mutant gene, as was previously suggested (8), but rather a secondary phenomenon. Despite marked damage to the epidermis, the pathological keratinization processes in our patients seem to proceed at upper epidermal levels in areas adjacent to the acantholysis.

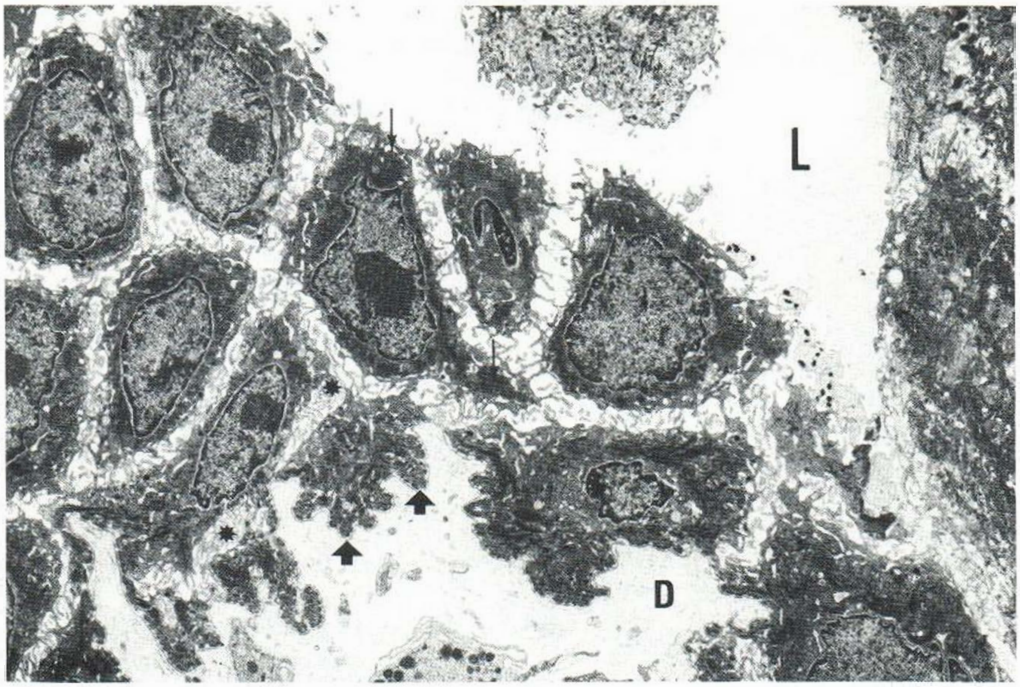

Fig. 6. A suprabasal lacuna (*L*) in a new lesion in patient 3 at 10 months of treatment (maintenance dose 10 mg/day). Microvillous changes (*asterisk*) of cell surfaces and tonofilament clumps (*thin arrows*) are conspicuous. There are no desmosomes but the attachment to basal lamina (*thick arrows*) is intact. *B*, basal cell; *D*, dermis. ×3400.

The primary target of retinoid in epidermal cells cannot be conclusively assessed on a purely morphological basis. However, it seems obvious that retinoid acts in particular on proliferating basal cells, inducing the development of a new generation of cells having the ability to form intercellular attachments and with kerainization processes shifted towards normal. Thus during improvement, the increased cohesiveness of the keratinocytes was the first change to be observed. However, even in clinically markedly healed skin, some signs of disorder in tonofilament behaviour were seen, with occasional microvillous changes in cell surfaces. Some downward proliferation of rete ridges was also occasionally seen after the disappearance of acantholysis and dyskeratosis.

Previous studies have provided some clues as to the possible mechanism of action of retinoids. It is known that they are capable of affecting epithelial differentiation considerably; in epidermal cultures even a mucous metaplasia with a reduction of keratinization products can be achieved (18). Biochemical studies have revealed that retinoids can modulate glycoprotein synthesis; in fibroblast cultures the adhesiveness of cells increased, parallel with a retinoid-induced incorporation of mannose into glycoproteins (4). Since glycoproteins are essential components of the cell surface (7) and are involved in cell adhesion and growth (4), this ability of retinoids might also in Darier's disease be responsible for some beneficial change in the basal cell surface or in its ability to form intercellular attachments.

Possibly related to the influence on glycoprotein synthesis, increased amounts of an intercellular substance have been found during retinoid treatment of psoriasis (11). This has been called mucus-like material, but its mucous nature has not been ascertained. Whether it is produced by keratinocytes under the influence of retinoid is still unknown, as also is its role in the improvement of psoriatic lesions. During treatment of Darier's disease an increase in intercellular material was only occasionally seen and it is therefore obvious that the increased cohesiveness of keratinocytes can hardly be attributed exclusively to changes in intercellular material, visualized by conventional electron microscopy. Fine structural histochemical

methods would be needed to evaluate changes in intercellular substance or in the cell surface coat, or their importance in the establishment of cellular cohesion in Darier's disease.

The disappearance of hyperkeratosis in two of our extensively affected cases was quite dramatic. As a conceivable mechanism for the rapid desquamation of psoriatic scale during treatment it has been suggested that retinoids enhance the activity of lysosomal enzymes in the intercellular space (11). Although the exact role of keratinosomes is still a matter of controversy, our study would seem to indicate that the above mechanism might also be involved in Darier's disease, since excessive amounts of keratinosome-derived lamellae were seen at the boundary of the horny layer in our patients during treatment. Furthermore, a recent study (6) has shown a decrease in intracellular lysosomal enzymes in Darier's disease during retinoid treatment. This could be explained by leakage of lysosomal enzymes into the intercellular space.

The improvement of psoriasis and Darier's disease during retinoid treatment possesses certain other common features at the ultrastructural level. According to the as yet unpublished findings of Kanerva et al. (forthcoming) and to the present paper the widened gaps between keratinocytes become narrower and the number of desmosomes increases in both diseases. However, whereas in psoriasis the number of tonofilaments increases during retinoid treatment, the abundant tonofilaments with a tendency to abnormal aggregation in Darier's disease show normalization. These changes are followed by a decrease in epidermopoiesis in both diseases. On the other hand, retinoids are known to stimulate the proliferation of normal epidermis (3). Thus, retinoids seem to have diverse and partly opposite effects on epidermal cells, the basic stage of proliferation and differentiation being obviously of importance in determining the direction of their influence.

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Received February 17, 1981

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