Acta Dermatovener (Stockholm) 61: 319–328, 1981

FINE STRUCTURE OF THE INTERCELLULAR SPACE OF PSORIATIC EPIDERMIS DURING RETINOID (RO 10–9359) AND RETINOID-PUVA TREATMENT

Lasse Kanerva, Jorma Lauharanta, Kirsti-Maria Niemi and Allan Lassus

Departments of Dermatology, Anatomy and Electron Microscopy, University of Helsinki, Finland

Abstract. The enlarged intercellular space in the lower malpighian layers of untreated psoriasis contains a fine granular and a coarse granular substance. Both types of this substance increase in amount during 2–6 weeks of retinoid (RO 10-9359) therapy. Retinoid stimulates the formation of these substances, which previously have been termed "mucus". After glutaraldehyde-osmium tetroxide fixation their mucoid nature cannot be ascertained, more specific cytochemical techniques being needed for this. A darker, more homogeneous substance in the upper spinous intercellular spaces increases in amount during retinoid therapy. Large amounts of keratinosome-derived lamellae are observed at the boundary of the stratum intermedium and the stratum corneum. Even after 14 weeks of treatment with retinoid or retinoid plus PUVA, at clinical remission, the histological and electron microscopical pictures still show some psoriatic changes, i.e. enlarged intercellular spaces with fine or coarse granular substance.

Key words: Psoriasis; Epidermis; Retinoids; PUVA; Electron microscopy

An aromatic retinoid (RO 10-9359) is a new substance which, when orally given, is effective in psoriasis and other diseases with disturbed keratinization. Its mode of action is not known, but several ultrastructural changes have been reported to be produced by topical or oral retinoid in various conditions. These include tight junction formation, gap junction growth, inhibition of desmosome and tonofilament formation, increases in the Golgi elements, keratinosomes, polyribosomes and endoplasmic reticulum, development of surface microvilli, formation of mucin granules, and development of intercellular canaliculi (7, 10, 16, 17, 18, 20, 21). Fine structural observations on the effect of oral retinoid on psoriasis have also been made. Orfanos & Runne (14) administered retinoid to 3 patients for 3 weeks. One of their main findings was the formation of intercellular mucous substance in the epidermis.

In order to further elucidate the effect of retionid therapy in psoriasis we have followed the fine structural changes in 8 patients for up to 14 weeks. We have also used the clinically highly effective therapy of retinoid followed up PUVA (RePUVA) and have studied biopsy samples fine-structurally. In the present paper we report a detailed study of the effect of retinoid and RePUVA therapy on the epidermal intercellular space in psoriasis.

MATERIAL AND METHODS

Eight patients with severe psoriasis were studied. The characteristics of the patients are summarized in Table 1. Four patients were treated with retinoid Ro 10–9359) for 14 weeks. The initial oral dose was 50–60 mg daily (0.5–1.0 mg/kg). This dose was gradually reduced to 0.3–0.5 mg/kg according to the clinical response. Four patients were treated for 4 weeks with the same dose of retinoid and thereafter with psoralen–UVA (PUVA) during the following 10 weeks. PUVA treatment was intially given three times a week and later once or twice a week depending on the clinical response. The dose of 8-methoxy-psoralen was approximately 0.6 mg/kg weight, followed 2 h later by irradiation in a high intensity cabin (PUVA-22, Airam Ltd. Helsinki, Finland).

Biopsies were taken from a psoriatic lesion before treatment and a nearby biopsy from the same area was taken after every 2 weeks of treatment.

The light microscopy specimens were stained with haematoxylin-eosin, PAS, Alcian blue at pH 3, toludine blue at pH 3, and Hale's colloidal iron stain.

For electron microscopy, 1 mm^3 or smaller blocks were fixed for 2 hours or longer in cacodylate (0.1 M) buffered 2.5% glutaraldehyde at 4°C and postfixed with 1% osmium tetroxide, stained *en bloc* in uranyl acetate for 2 hours at room temperature, dehydrated in a graded series of alcohol and embedded in Epon 812. Ultrathin sections were cut with an LKB ultramicrotome, stained with lead citrate and examined with a Jeol 100S electron microscope operated at 80 kV.

RESULTS

1. *Clinical observations*

The clinical response to both retinoid and RePUVA treatment was good; remission was achieved in all

Table I. *Characteristics of the patients*

Patients 1–4 on retinoid treatment. Patients 5–8 on RePUVA treatment

Patient	Age	Sex	Type of psoriasis	Previous treatments							Initial retinoid dose (mg/kg)
					Corticoids		PUVA		Retinoid		
				Dithranol	Local	Oral	Duration	Interval after last treatment	Duration	Interval after last treatment	
1	73	♀	vulgaris	×	×				6 mo	5 mo	0.8
2	71	♀	erythrodermia	×	×	×					0.9
3	52	♀	vulgaris	×	×		9 mo	1.5 yr	4 mo	1.5 mo	1.0
4[a]	72	♂	pustulosa	×	×	×	3 mo	3 yr	5 mo	1 mo	0.8
5	43	♂	vulgaris	×	×		2 mo	5 mo			0.8
6	27	♂	vulgaris	×	×		4 mo	2 yr			0.6
7	44	♂	arthropathica	×	×		1 yr	2 wk			0.5
8	62	♂	vulgaris	×	×		3 mo	2 yr			0.9

[a] Patient had also received methotrexate and hydroxyurea treatment 2 years earlier.

patients. The mean treatment period needed for remission with retionid was 11 weeks (range 10–14 weeks) and with RePUVA 8 weeks (range 6–10 weeks).

II. *Light microscopy*

In light microscopy we found enlarged intercellular spaces but no increase in mucous substances in the epidermis. The intercellular material did not stain with PAS, Alcian blue, Hale's colloidal iron or metachromatically with toluidine blue.

III. *Electron microscopy*

The present report concerns the main types of intercellular substances seen before or during retinoid therapy. They were as follows:

(*a*) A fine and a coarse granular substance seen before retinoid treatment and corresponding to what has been termed "mucous substance" (14).

(*b*) A darker, more homogeneous intercellular space substance that was seldom seen before treatment; this too has been termed a mucous substance (19).

(*c*) The keratinosome-derived lamellae in the intercellular space of the upper spinous layers and the stratum intermedium.

(*a*) *Fine and coarse granular intercellular material.* In the lower malpighian layers, two types of intercellular material were found: 1) A fine granular substance, with granules 80–150 Å in diameter embedded in an amorphous ground substance. This substance was also seen below the basal lamina (Fig. 1). 2) A substance containing coarser granules 120–150Å in diameter and less background amorphous substance (Fig. 2). The granules resembled ribosomes or beta particles of glycogen. Mixtures of these substances resulted in a sliding scale of materials of variable granularity.

During the first 2–4 weeks of therapy, these two types of material increased in the intercellular space (Fig. 3). Their fine structural appearance remained identical, although a change in their nature might have occurred (see Discussion). Vacuoles in the keratinocytes contained the same types of substances, and connections between the vacuoles and the intercellular space were sometimes observed, as before treatment. At 6 weeks and later there was a tendency to decreased width of the intercellular spaces, although enlarged spaces were still found (Fig. 4). Even after 14 weeks of treatment, some intercellular spaces were larger than in normal skin. After RePUVA treatment there was strong melanocyte stimulation, but the intercellular space substances corresponded to those seen after pure retinoid therapy.

(*b*) *Homogeneous dark material.* In the upper malpighian layers a darker, more homogeneous substance in the narrow intercellular spaces was rarely seen in untreated psoriasis. After 2–6 weeks of treatment this substance was encountered more frequently (Fig. 5). Keratinosomes were seen to protrude into the substance. Later during treatment the amount of this substance decreased.

(*c*) *Keratinosomes.* In untreated psoriasis,

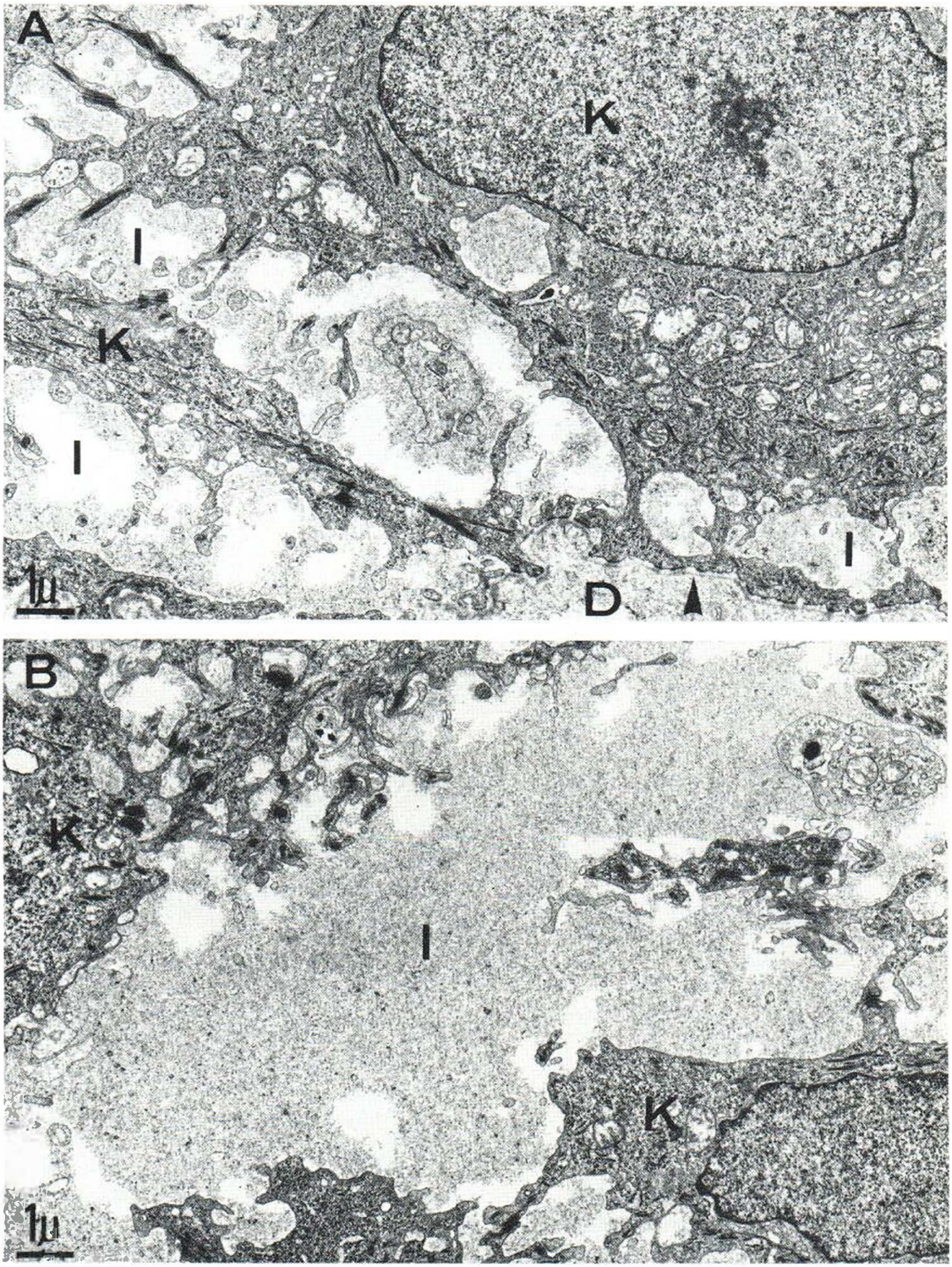


Fig. 1. Untreated psoriasis. Wide (Fig. 1 A) and very wide (Fig. 1 B) intercellular spaces (*I*) are seen between the keratinocytes (*K*) in the lower malpighian layers. The intercellular space contains a fine-grained substance that is also present below the basal lamina (arrowhead). *D*, dermis. ×9300.

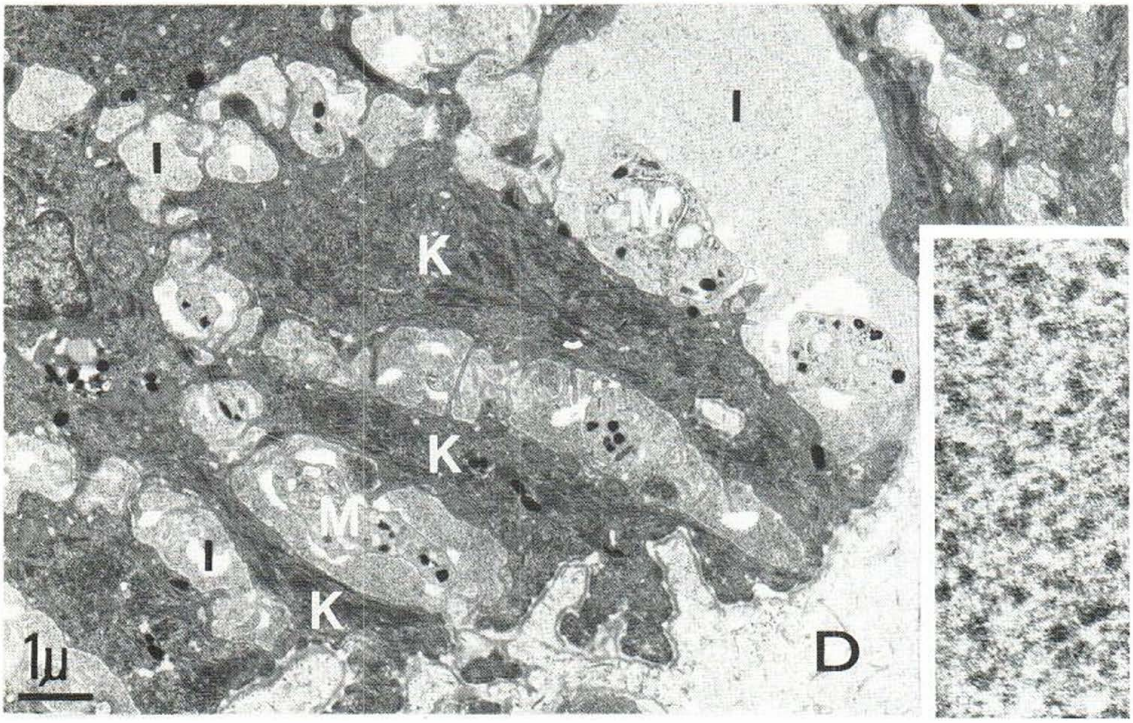


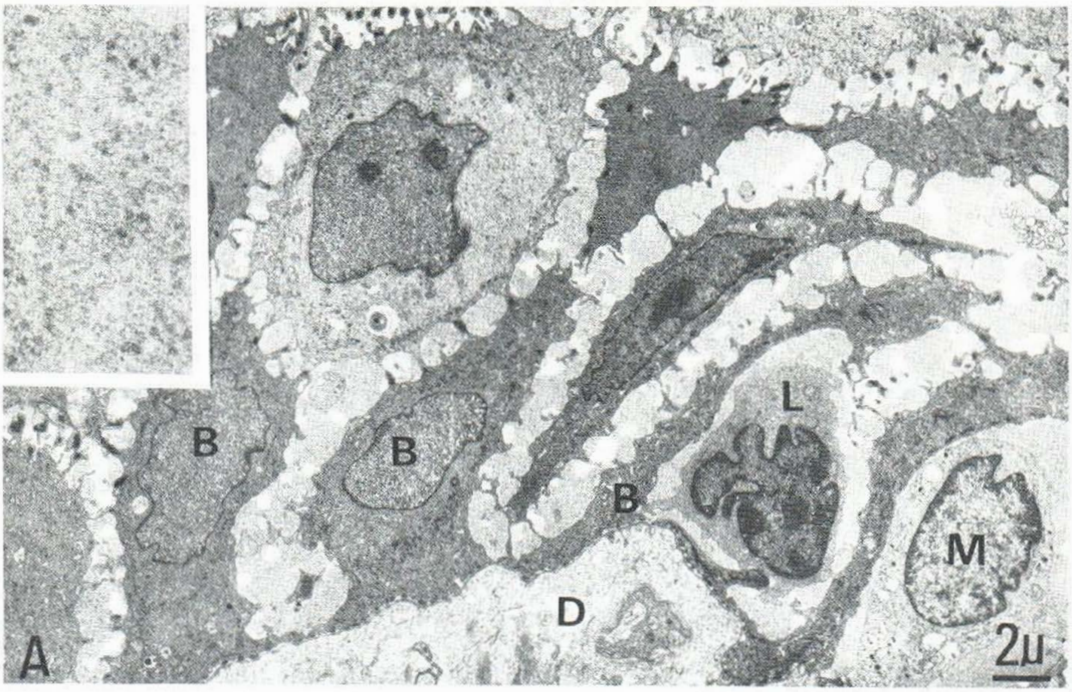
Fig. 2. Untreated psoriasis. Dermo-epidermal junction. Wide intercellular spaces (*I*) are seen betweeen the lower epidermal cells (*K*). Processes of melanocytes are abundantly seen (*M*). The intercellular space material is coarsely granular, as is seen in the larger magnification (inset, ×120000). *D*, dermis. ×9500.

keratinosomes were seen to protrude into the intercellular space in the upper stratum intermedium layers. During the initial retinoid treatment (2–6 weeks) the number of keratinosome-derived lamellae was clearly increased between the upper stratum intermedium layers, the transitional cell layers and the lower horn cell layers (Fig. 6). Sometimes these lamellae formed an almost continuous band intermingled with a few desmosomes. In later stages (8–14 weeks) the lamellae decreased in number.

DISCUSSION

The present study confirmed the previous electron microscopical findings of large intercellular spaces between the keratinocytes in psoriasis (3, 13). It was also known that in untreated psoriasis the intercellular space contains a fine-grained substance (3, 13). It increases during retinoid therapy (14) and was best seen in our study during the first 6 weeks. Thereafter the amount of intercellular substance decreased, giving the epidermis a more normal appearance. However, even in clinically cured skin there were wide intercellular spaces containing granular substance.

The present study shows that one must be careful when analysing the fine structure of retinoid-induced intercellular substance. It is quite possible that the substances that we observed are different, although they have the same fine structural appearance. Before retinoid treatment we found a fine granular substance in the intercellular space and below the basal lamina. This we consider a proteinous substance derived from the dermal vessels. After 2 weeks of retinoid treatment this type of substance was usually not seen below the basal lamina, indicating reduced leakage of vessel fluids. On the other hand, we found an increased amount of fine-grained substance in the intercellular space. This fine granular substance is probably identical with the "mucous substance" of Orfanos & Runne (14). Fine structurally it cannot be distinguished from the proteinaceous vessel fluid, but the large amount



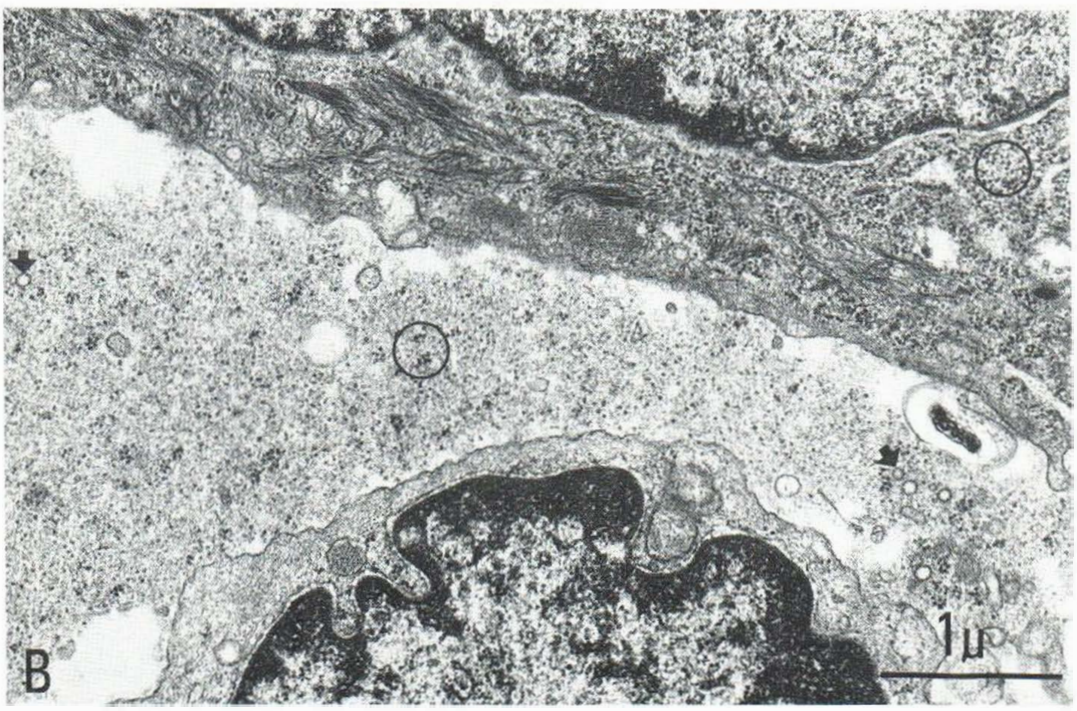


Fig. 3. Two weeks of retinoid treatment.
Fig 3A. Note the very large intercellular spaces between the lower epidermal cells. *A* lymphoid cell (*L*) and melanocyte (*M*) are also surrounded by a space filled with granular substance. *B*, basal cells. *D*, upper dermis. ×3 600). Inset: Greater magnification of intercellular substance. ×44 200).

Fig. 3B. Greater magnification of the intercellular space containing granular substance. The "granules" closely resemble the ribosomes or glycogen particles in the keratinocytes (circle). Note also the probable cell organelles (arrow) in the intercellular space. ×21 900).

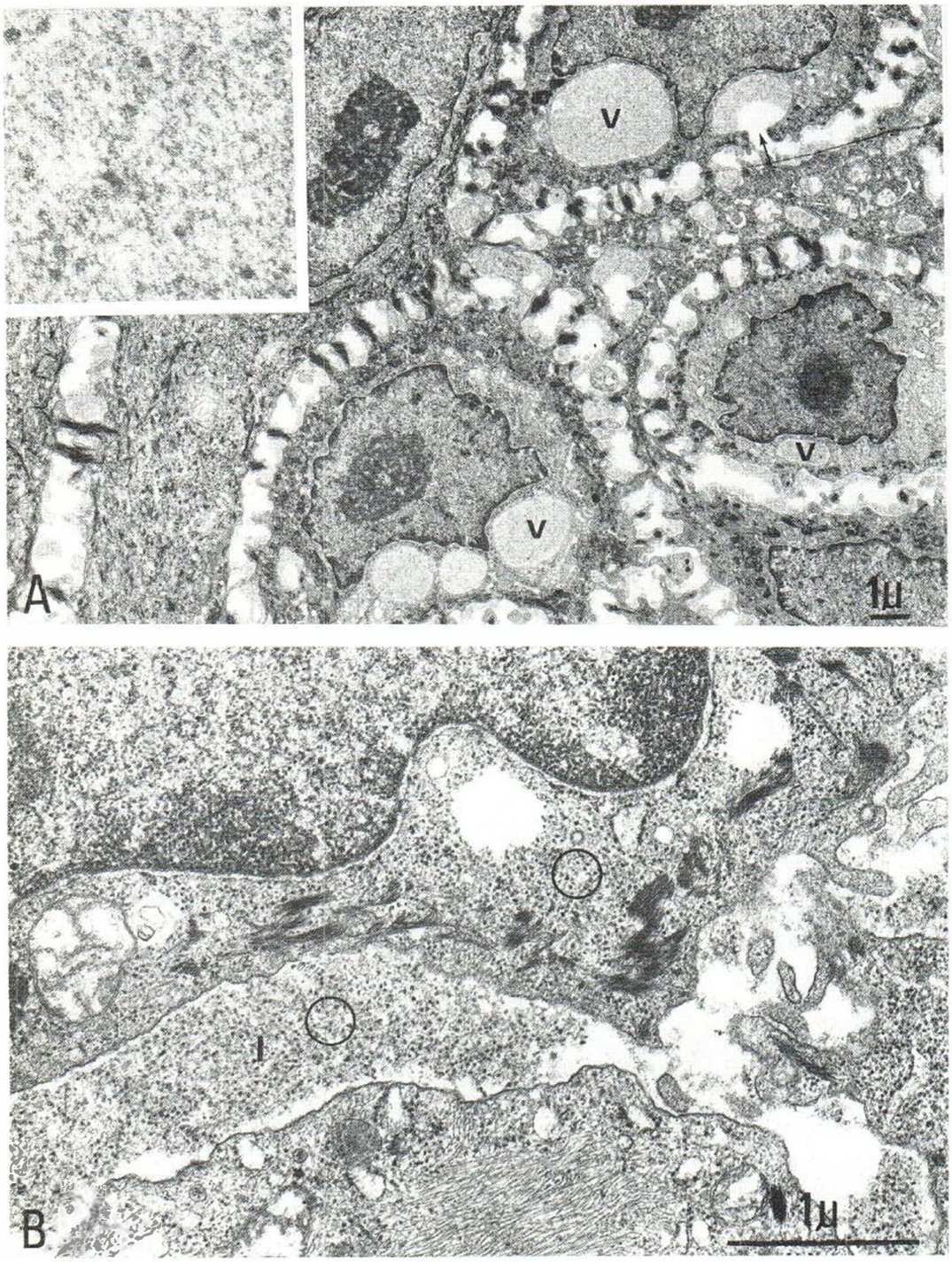


Fig. 4. Six weeks of retinoid treatment.
Fig. 4A. Even after 6 weeks of retinoid treatment, large intercellular spaces are observed in the lower epidermal layers. The keratinocytes contain large vacuoles (*V*), at least some of which have connection with the intercellular space (arrow). ×5 600. Inset: Larger magnification of intercellular substance. ×78 500.
Fig. 4B. The intercellular space (*I*) at higher magnification. The intra- and extracellular granular substances seem to be identical (circle). ×28 800.

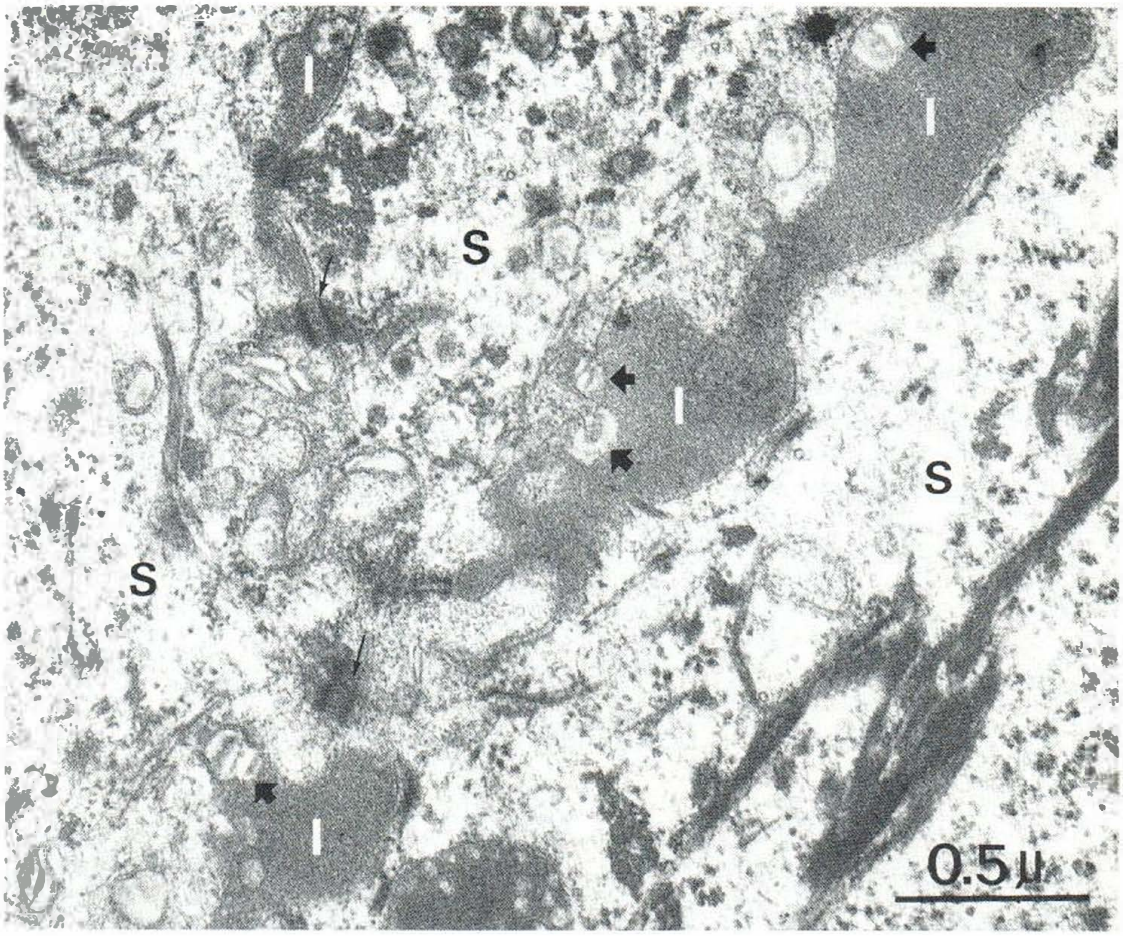


Fig. 5. Two weeks of retinoid treatment. The boundary between three spinous cells (S) in the upper stratum spinosum. The intercellular space is filled with a moderatately dark homogeneous substance (*I*). This substance also protrudes between the outer membrane thickenings of the desmosomes (fine arrow). Keratinosomes (heavy arrow) are seen to protrude into this dark substance. ×48 200.

present above the basal lamina indicates that it is formed in the keratinocytes. Its formation is probably stimulated by retinoid administration. The coarse-grained substance was probably of the same quality before and during retinoid treatment, but during retinoid treatment it was more often intermingled with small vesicles and sometimes also with other cytoplasmic organelles which might have leaked from the cytoplasm of the keratinocytes. Vitamin A has previously been shown to alter the permeability and stability of the lipoprotein cell membranes (2, 6, 8, 11). This could explain an extrusion of cytoplasmic constituents into the intercellular space. In the upper malpighian layers we found a darker substance that resembled the mucous substance of Wolff et al. (19). It was relatively rarely encountered, however, and was not seen in the lower epidermal cell layers in the present study. The amount was so small that it would not have been detected in light microscopy. The mucous stainings were light microscopically negative.

It has previously been shown that retinoids can cause a "mucous metaplasia", at least during cell differentiation (9, 10, 18). Also, a periodic acid silver methenamine positive mucous substance has been found in guinea pig skin after vitamin A acid treatment (19). Thus it is possible that retinoid directs the cellular differentiation processes towards a mucous direction also during the treatment of psoriasis (4, 19). Furthermore, retinoid has been

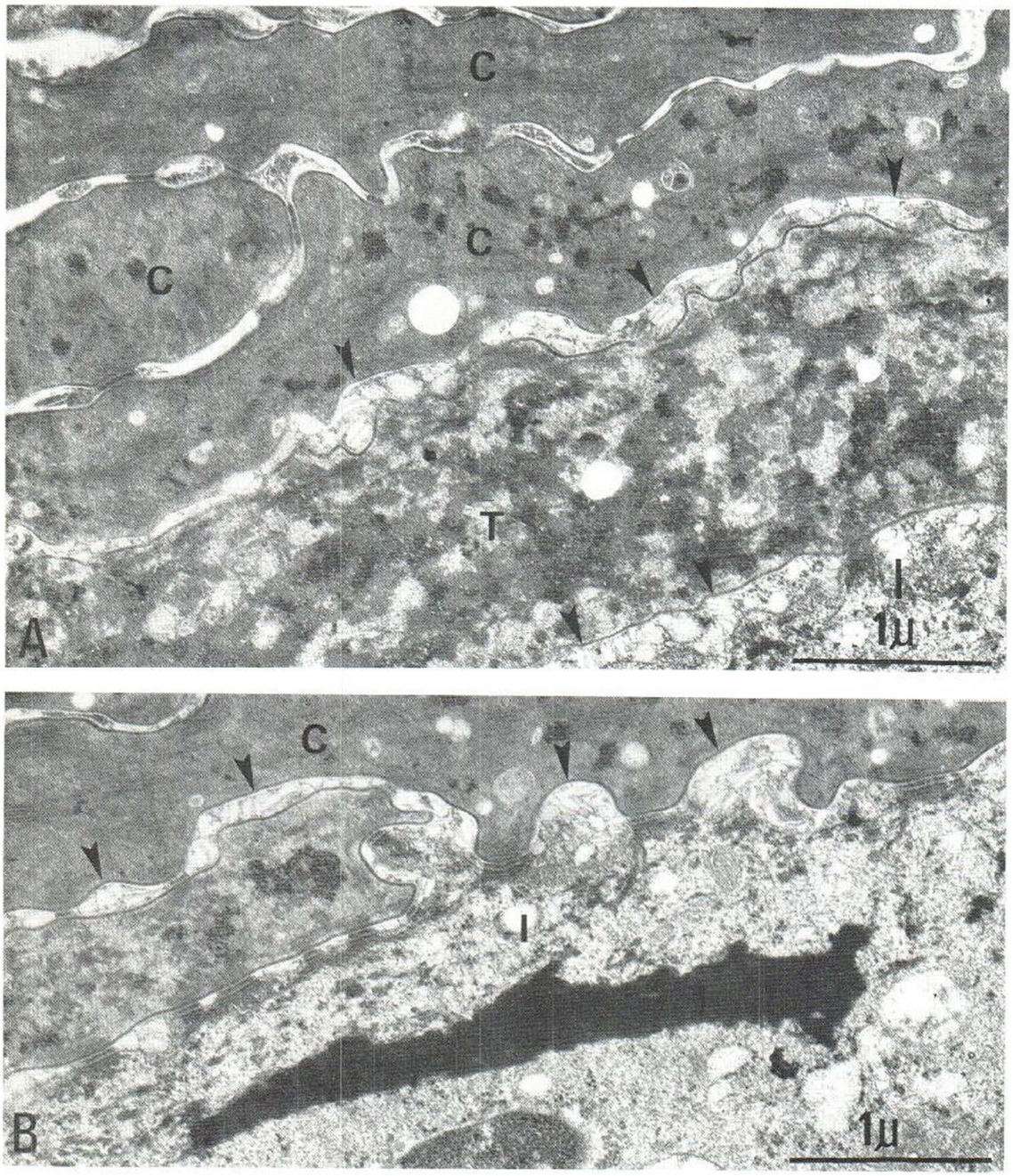


Fig. 6. Six weeks of retinoid treatment.

Fig. 6A. Boundary between the stratum intermedium (*I*), the transitional cell (*T*) and the stratum corneum cells (*C*). Note the very abundant keratinosome-derived lamellae (arrowhead) in the intercellular space. ×30 300.

Fig. 6B. Direct contact between the stratum intermedium (*I*) with keratohyalin and the stratum corneum (*C*). Wide intercellular space with abundant keratinosome-derived lamellae (arrowhead). ×30 300.

found to modulate the glycoprotein synthesis in cultured mouse epidermal cells (1). These observations indicate that the intercellular substance may be mucus, but this could not be ascertained with the present methods. The demonstration of mucin would require the use of fine structural cytochemical methods.

Whether a mucous metaplasia is beneficial during psoriasis treatment with retinoid is not known. Orfanos et al. (14, 15) speculated that retinoid has a mucogenic effect on the epidermis. According to them, mucin synthesis would cause an increase in the deficient glycoprotein-rich surface coats in psoriasis (12) and thus restore the suggested loss of "contact inhibition of growth", resulting in the normalization of keratinization in psoriasis. Whether the excessive amount of intercellular substance found by us represents a surface coat substance remains to be shown. Such a large amount of cementing substance would seem unnecessary for normal keratinocyte function.

A prominent feature was the large amount of keratinosome-derived lamellar structures at the border of the horny layer. It has previously been shown that vitamin A acts as a lysosomal labilizer (5, 8). Thus, one target of the retinoids could be the keratinosomes, which belong to the lysosome family. The accumulation of keratinosomes at the boundary of the horny layer may account for the desquamative effect of retinoid by the lysosomal action of these granules, as speculated by Woo-Sam (20) and Tsambaos et al. (17).

ACKNOWLEDGEMENT

We wish to express our gratitude for the support of Hoffmann-La Roche and for the excellent technical assistance given by Mrs Liisa Erola.

REFERENCES

1. Adamo, S., DeLuca, L. M., Silverman-Jones, C. S. & Yuspa, S. T.: Mode of action of retinol. Involvement in glycosylation reactions of cultured mouse epidermal cells. J Biol Chem *254*: 3279, 1979.
2. Anderson, O. R., Roels, O. A., Dreher, K. D. & Schulman, J. H.: The stability and structure of mixed lipid monolayers and bilayers. II. The effect of retinol and α-tocopherol on the structure and stability of lipid bilayers. J Ultrastruct Res *19*: 600, 1967.
3. Bonneville, M. A., Weinstock, M. & Wilgram, G. F.: An electron microscope study of cell adhesion in psoriatic epidermis. J Ultrastruct Res *23*: 15, 1968.
4. Braun-Falco, O. & Christophers, E.: Psoriasiforme Epidermis-Reaktion der Meerschweinchenhaut durch örtliche Vitamin A-Säure-Applikation. Arch Klin Exp Dermatol *234*: 70, 1969.
5. Dingle, J. T., Glauert, A. M., Daniel, M. & Lucy, J. A.: Studies on the mode of action of excess of vitamin A. III. Release of a bound protease by the action of vitamin A. Biochem J *79*: 509, 1961.
6. Dingle J. T. & Lucy, J. A.: Studies on the mode of action of excess of vitamin A. 5. The effect of vitamin A on the stability of the erythrocyte membrane. Biochem J *84*: 611, 1962.
7. Elias, P. M. & Friend, D. S.: Vitamin-A-induced mucous metaplasia. An in vitro system for modulating tight and gap junction differentiation. J Cell Biol *68*: 173, 1976.
8. Fell, H. B., Dingle, J. T. & Webb, M.: Studies on the mode of action of excess of vitamin A. 4. The specificity of the effect of embryonic chick-limb cartilage in culture and on isolated rat-liver lysosomes. Biochem J *83*: 63, 1962.
9. Fell, H. B. & Mellanby, E.: Metaplasia produced in cultures of chick ectoderm by high vitamin A. J Physiol (Lond) *119*: 470, 1952.
10. Jackson, S. F. & Fell, H. B.: Epidermal fine structure in embryonic chicken skin during atypical differentiation induced by vitamin A in culture. Dev Biol *7*: 394, 1963.
11. Lucy, J. A., Dingle, J. T. & Fell, H. B.: Studies on the mode of action of excess vitamin A. 2. A possible role of intracellular proteases in the degradation of cartilage matrix. Biochem J *79*: 500, 1961.
12. Mercer, E. H. & Maibach, H. I.: Intercellular adhesion and surface coats of epidermal cells in psoriasis. J Invest Dermatol *51*: 215, 1968.
13. Nagy-Vezekényi, C. & Zs.-Nagy, I.: Studies on the ultrastructure of psoriasis and of the "normal" skin of psoriatics. Acta Dermatovener (Stockholm) *51*: 435.
14. Orfanos, C. E. & Runne, U.: Tissue changes in psoriatic plaques after oral administration of retinoid. Dermatologica *157* (Suppl. 1): 19, 1978.
15. Orfanos, C. E., Schaumburg-Lever, G., Mahrle, G. & Lever, W. F.: Alterations of cell surfaces as a pathogenetic factor in psoriasis. Possible loss of contact inhibition of growth. Arch Dermatol *107*: 38, 1973.
16. Peck, G. L., Elias, P. M. & Wetzel, B.: Effects of retinoic acid on embryonic chick skin. J Invest Dermatol *69*: 463, 1977
17. Tsambaos, D., Mahrle, G. & Orfanos, C. E.: Epidermal changes induced by oral excess of aromatic retinoid in guinea pigs. Arch Dermatol Res *267*: 141, 1980.
18. Wilkoff, L. J., Chopra, D. P. & Peckham, J. C.: Effect of retinoids on the differentiation of chick embryo metatarsal skin explants. J Invest Dermatol *72*: 11, 1979
19. Wolff, H. H., Christophers, E. & Braun-Falco, O.: Beeinflussung der epidermalen Ausdifferenzierung durch Vitamin A-Säure. Eine elektonenmikrosko-

pische Untersuchung. Arch Klin Exp Dermatol *237*: 774, 1970.
20. Woo-Sam, P. C.: The effect of vitamin A acid on experimentally induced comedones: an electron microscope study. Br J Dermatol *100*: 267, 1979.
21. Yuspa, S. H. & Harris, C. C.: Altered differentiation of mouse epidermal cells treated with retinyl acetate in vitro. Exp Cell Res *86*: 95, 1974.

Received December 3, 1980

Lasse Kanerva, M. D.
Department of Dermatology
University Central Hospital
Snellmaninkatu, 14
SF-00170 Helsinki 17
Finland