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Regional Variations in Cytokeratin Expression in Palmo-plantar Epidermis

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The cytokeratin composition of palmo-plantar epidermis from sites with different degrees of mechanically induced thickening of the stratum corneum was analysed. The urea-soluble proteins of the stratum corneum were analysed by two-dimensional electrophoresis. Viable epidermal layers were analysed by immunofluorescence microscopy with polyclonal and monoclonal antibodies. A mouse monoclonal antibody specific for cytokeratin no. 9 was prepared for the study. Significant amounts of low molecular weight cytokeratins were found in suprabasal layers at sites with the most pronounced thickening of the stratum corneum. This was taken as evidence that palmo-plantar epidermis responds to mechanical stress with hyperproliferation. At sites where stratum corneum thickness is most increased this hyperproliferation appears to involve two different populations of cells—one capable of expressing high molecular weight, differentiation-related cytokeratins in the suprabasal epidermal layers, and one population that does not express these cytokeratins. At sites with intermediate epidermal hyperplasticity the high molecular weight cytokeratins were predominant in all suprabasal cells.

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Genetic skin disorders such as hereditary palmo-plantar keratoderma (Unna-Thost) (1) suggest that some of the mechanisms regulating stratum corneum thickness are unique for palmo-plantar epidermis. In normal palmo-plantar skin the thickness of the stratum corneum varies as a function of the degree of mechanical stress inflicted on different sites. The mechanisms of this important adaptive response are not known, although it may be assumed that variations in epidermal proliferation rate may be of importance (2).

In various conditions with epidermal hyperproliferation there are characteristic changes in the pattern of epidermal cytokeratin expression (3, 4). Thus, if the thickening of palmo-plantar stratum corneum at sites subjected to increased pressure and frictional forces is the result of a higher rate of epidermal proliferation, this may be reflected in the cytokeratin composition at these sites.

Cytokeratins always appear in pairs. A certain type I (acidic) cytokeratin of a given epithelium is always accompanied by a type II (basic-neutral) cytokeratin (for a general review, see ref. 5; for a review of cytokeratin terminology, see ref. 6). In the epidermis, basal cells express the cytokeratins No. 14 (K14, type I, 50 kDa) and No. 5 (K5, type II, 58 kDa) (7, 8). In addition to these two peptides, suprabasal cells produce K10 and K11 (type I, 56.5 and 56 kDa) and K1 (type II, 67 kDa) (7, 9, 10). The relative concentrations of K1 and K10/11 increase towards the granular layer. The K10/11 and K1 cytokeratins are considered to be markers of epidermal differentiation. In palmo-plantar epidermis an additional differentiation-related type I cytokeratin (K9, 64 kDa) has been identified (11, 12). In hyperproliferative skin disorders such as wound healing and psoriasis, the epidermal cytokeratin pattern is changed. There is a decreased expression of the differentiation-related cytokeratins, and cytokeratins identical with or closely related to those normally found only in basal cells become predominant also in supra-basal cells. Of these peptides, K6 (type II, 56 kDa) is closely related to K5, and K16 (type I, 48 kDa) closely related to K14. The K6 and K16 cytokeratins have been reported to be expressed exclusively in hyperproliferative tissues (6, 13).

In order to better understand the mechanisms that regulate palmo-plantar stratum corneum thickness, we have investigated regional variations in cytokeratin expression in normal palmo-plantar epidermis.

MATERIAL AND METHODS

Tissues

Samples of palmo-plantar stratum corneum for electrophoretic analyses were obtained from five 40–50-year-old male volunteers with normal skin. From each donor, samples were taken from the ulnar aspect of the palm, the medial and lateral aspects of the sole, and from the rear edge of the heel

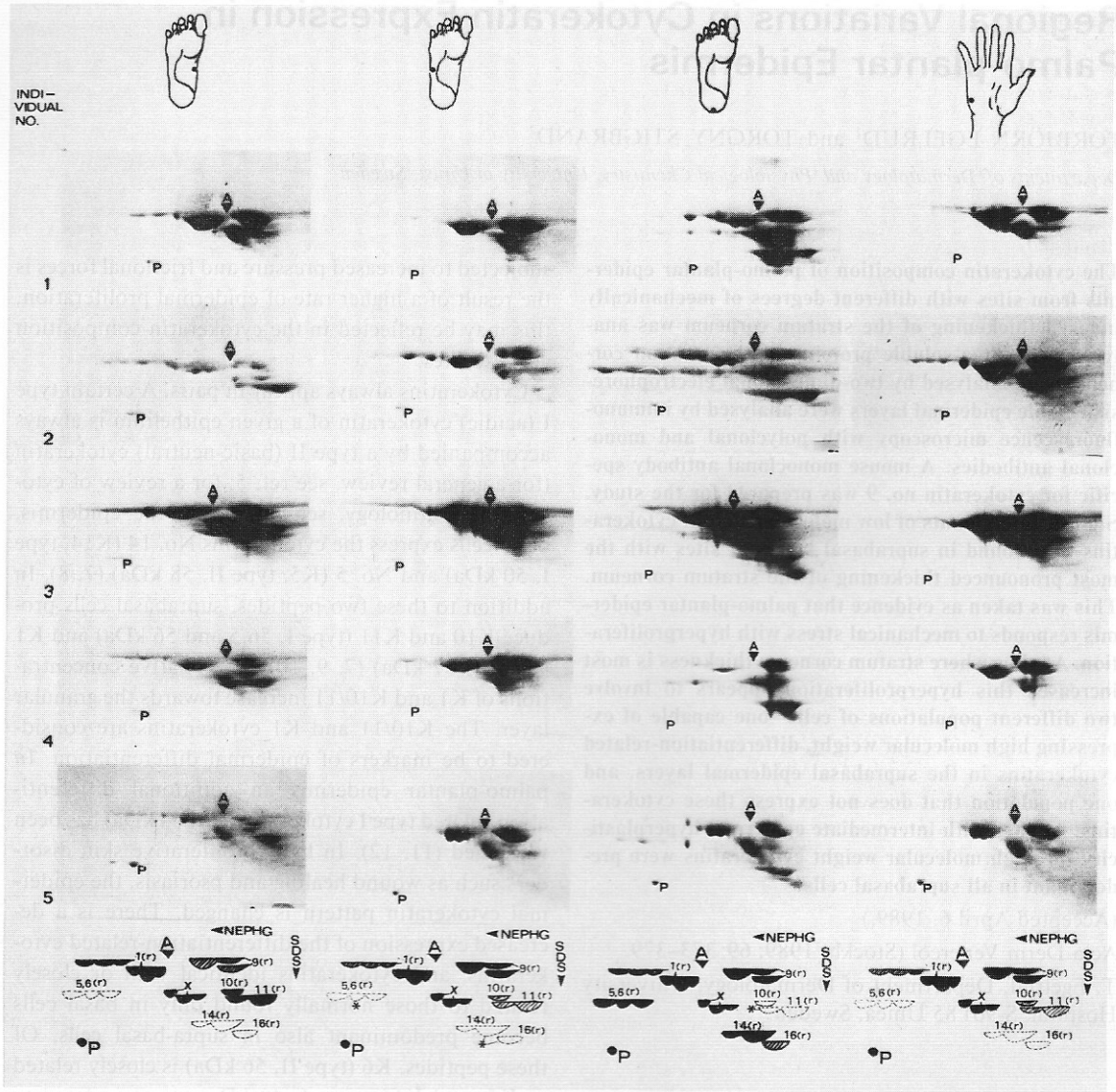


Fig. 1. Two-dimensional electrophoresis of urea-soluble proteins of stratum corneum from different palmo-plantar regions (schematically depicted at top of the figure). Each horizontal row of gels represents results for one individual. The suggested interpretation of the results for each region is shown in the scheme in the bottom of the figure. *A* and *P* are standard proteins; *A*, bovine serum albumin (Mw 66 kDa, isoelectric point of major variant 6.35); *P*, phospho-glycerokinase (Mw 43 kDa, isoelectric point of major variant 7.4). *NEPHG*, non-equilibrium, pH gradient; *SDS*, sodium dodecyl sulphate. *Filled spots*, major components; *partially filled spots*, minor components; *empty spots*, components not found at the specified site; *asterisks* indicate components found only in some individuals and in very small amounts. The numbering of cytokeratins was adopted from ref. 6. "*(r)*" after each cytokeratin number was added to emphasize that the cytokeratins of the stratum corneum may be modified forms of the cytokeratins synthesized in viable epidermal layers (7, 18). The identification of the K1- and K9-related cytokeratins was based on data from ref. 18.

(schematically depicted in Fig. 1). The tissues were carefully loosened with a needle and removed with forceps. Biopsies of whole skin for immunohistochemical analyses were obtained from the same plantar sites as above, from one volunteer and from 5 autopsy cases. The latter, submitted to the Department of Forensic Medicine after sudden death, were 30–60-year-old males with normally appearing skin and had not been bedridden. Biopsies were taken within 24 h of death.

Two-dimensional electrophoresis

Samples of stratum corneum, 20 mg/ml, were extracted for 15 h at 37°C in sample buffer (14). This contained 9 M urea, 4%

Nonidet NP40, 5% β-mercaptoethanol, and ampholytes (Pharmalyte 3–10®, Pharmacia Fine Chemicals, Uppsala, Sweden) 2 ml/100 ml. Two-dimensional electrophoresis was performed in a Bio-Rad Minigel system (Bio-Rad, Richmond, Calif.) Non-equilibrium pH-gradient electrophoresis (NEPHGE) was run according to O'Farrel et al. (15) with a mixture of Pharmalyte ampholytes (4/5, pH 3–10; 1/5, pH 2.5–5). The second dimension (sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS–PAGE) was run according to Laemmli (16) in 10% gels. Gels were stained with Coomassie Brilliant Blue.

Immunochemical reagents

The antiserum and antibodies used were characterized by their ability to stain Western blots (17) of cytokeratins extracted from viable epidermis and separated by 2-dimensional electrophoresis as above.

1. Polyclonal rabbit antikeratin antiserum, R-antiker. This antiserum was obtained from a rabbit immunized with a keratin peptide fraction from human plantar stratum corneum. It reacted in Western blots with cytokeratins K1, K5/6, and K10/11, but not with K9 or K14/16. When used in immunohistochemistry the antiserum stained suprabasal cells intensely, but stained basal cells only weakly (see Figs. 2–5 A). Since it stained suprabasal cells intensely also in areas where K10/11 appeared to be absent (see Fig. 4 A, B), it was concluded that R-antiker was reactive with K1. The weak reaction with basal cells was taken as evidence that the reactivity of the antiserum against cytokeratin K5/6 did not contribute significantly to its staining properties.

2. Monoclonal mouse antibody K92 (MabK92) was obtained from Dakopatts (Copenhagen, Denmark). It stained suprabasal epidermal cells and reacted on Western blots with epidermal cytokeratins K10/11.

3. Monoclonal mouse antibody FE1 (MabFE1) The modified forms of K9 found in plantar stratum corneum (18) were purified by preparative SDS–PAGE followed by electroelution. BALB/c mice were immunized with 0.1 mg of purified peptide in complete Freund's adjuvant injected intra-peritoneally and given booster injections 6 weeks later on 3 consecutive days with the same amount of antigen. Hybridomas were produced as previously described (19) with cells of the X63-Ag8-P635 myeloma cell line (20). The identification of the K9-specific antibodies was made by an ELISA technique. In order to obtain antibodies recognizing determinants specific only for the K9 cytokeratins, clones with IgG non-reactive with extracts of total human epidermal cytokeratins from the trunk were selected. The monoclonal antibodies were purified and characterized as previously described (19). The antibody chosen for this study, MabFE1, reacted specifically with cytokeratin K9 on Western blots and stained suprabasal cells only in palmo-plantar epidermis. MabFE1 is of isotype IgG1/kappa.

Immunofluorescence microscopy

Ten µm freeze-cut sections of skin biopsies were fixed in acetone. Double staining of cytokeratins was carried out by initial incubations for 30 min with one of the monoclonal mouse antibodies (MabK92 1/10 or MabFE1 1/50 in phosphate-buffered saline) followed by incubations with the rabbit antiserum (R-antiker, diluted 1/50) for 30 min. After three washings in phosphate-buffered saline, bound antibodies were detected by incubation for 30 min with a mixture of goat anti-mouse IgG conjugated with fluorescein isothiocyantate (FITC-anti-mouse IgG; Becton-Dickinson, Mountain View, Calif.) and swine anti-rabbit IgG conjugated with tetramethyl-rhodamine isothiocyanate (TRITC anti-rabbit IgG; Dakopatts, Copenhagen, Denmark). The final dilutions of the second antibodies were 1/20.

RESULTS

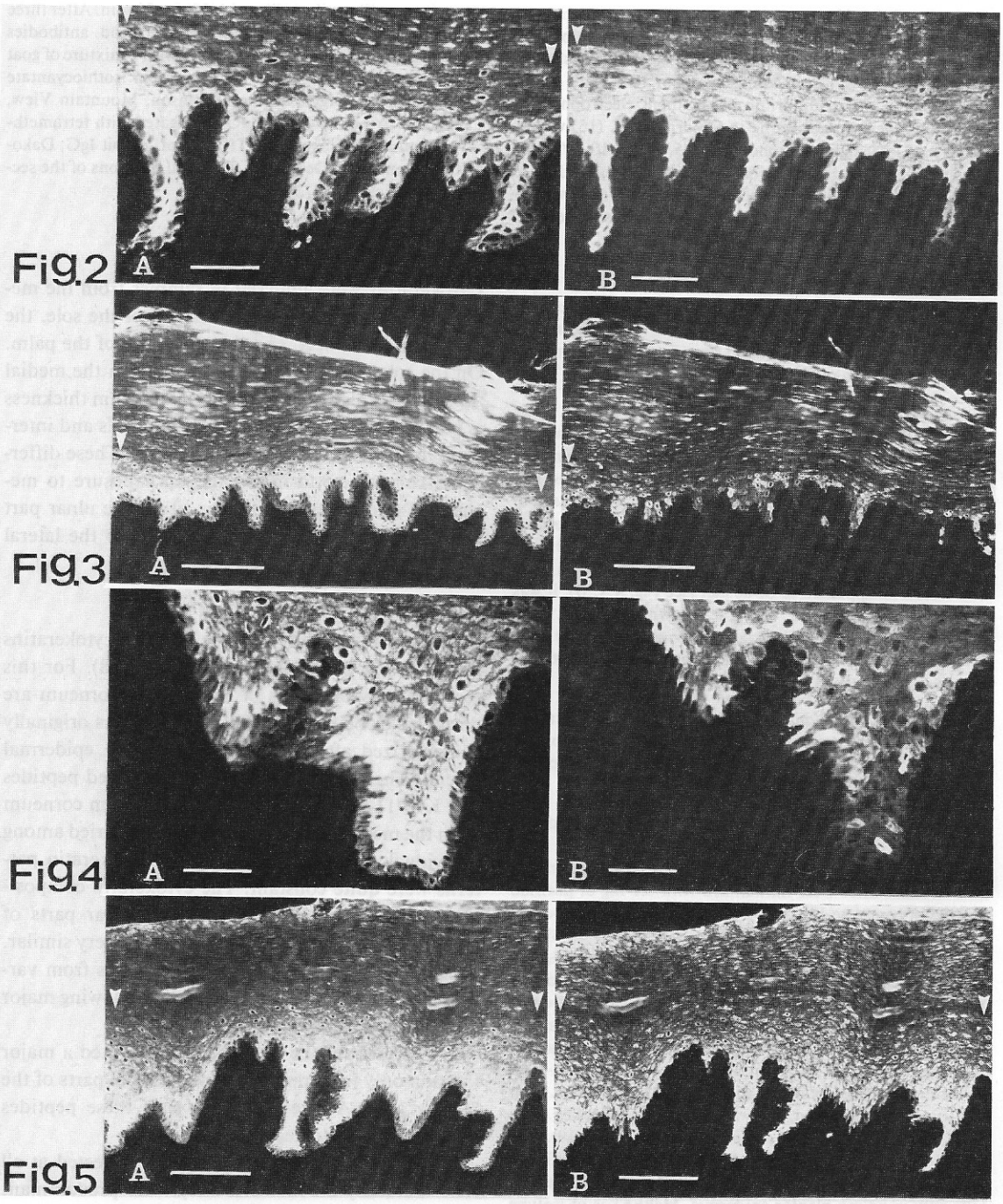
Cytokeratins were analysed in samples from the medial part of the sole, the lateral part of the sole, the rear edge of the heel, and the ulnar part of the palm. On the soles the epidermis is thinnest on the medial aspect of the plantar arch. Stratum corneum thickness is maximal at the rear margins of the heels and intermediate at the lateral parts of the soles. These differences correspond to differences in exposure to mechanical stress between plantar sites. The ulnar part of the palm corresponds anatomically to the lateral part of the sole.

Electrophoretic analyses

These results are shown in Fig. 1. (The cytokeratins are modified during keratinization (7, 18). For this reason the cytokeratins of the stratum corneum are discussed as being 'related to' cytokeratins originally characterized after isolation from viable epidermal layers.) The relative amounts of K9-related peptides and K10/11-related peptides in the stratum corneum from the medial part of the plantar arch varied among individuals. For the other sites the cytokeratin patterns were quite constant. The cytokeratin compositions of stratum corneum from the ulnar parts of palms and the lateral parts of soles were very similar. When the cytokeratin patterns of samples from various plantar sites were compared, the following major differences were found:

1. Peptides related to K10/11 constituted a major fraction only in samples from the medial parts of the plantar arch. At the two other sites these peptides were minor or undetectable.

2. Peptides related to K9 could be detected at all sites. Laterally on the soles they were predominant among the acidic (type I) cytokeratins. On the medial part of the plantar arch the acidic fraction was made up by these peptides together with peptides related to K10/11. At the rear margins of the heels, low molecular weight peptides apparently related to K14/16 constituted a major fraction of the acidic cytokeratins together with K9-related peptides.



Figs. 2–7. Immunofluorescence microscopy of epidermis from different plantar regions. Double staining with polyclonal rabbit antiserum to cytokeratins (R-antiker) and monoclonal antibodies MabK92 and MabFE1. In Figs. 2, 3, 5, and 7, *arrowheads* indicate the transition between viable epidermal layers and the stratum corneum. *Fig. 2.* Medial sole. *A,* R-antiker; *B,* MabK92; *bars,* 50 μm. *Fig. 3.* Medial sole. *A,* R-antiker; *B,* MabFE1; *bars,* 150 μm. *Fig. 4.* Lateral sole. *A,* R-antiker; *B,* MabK92; *bars,* 50 μm. *Fig. 5.* Lateral sole. A part of the stratum corneum was removed to facilitate sectioning. *A,* R-antiker; *B,* MabK92; *bars,* 150 μm. *Fig. 6.* Rear edge of the heel. Only the deepest parts of the epidermis are shown. *A,* R-antiker; *B,* MabK92; *bars,* 150 μm. *Fig. 7.* Rear edge of the heel. The stratum corneum is only partially shown. *A,* R-antiker; *B,* MabFE1. *Arrows* in *B* indicate rete ridges not stained by MabFE1. *Bars*=300 μm. The results shown in Figs. 2–7 are summarized in Table I.

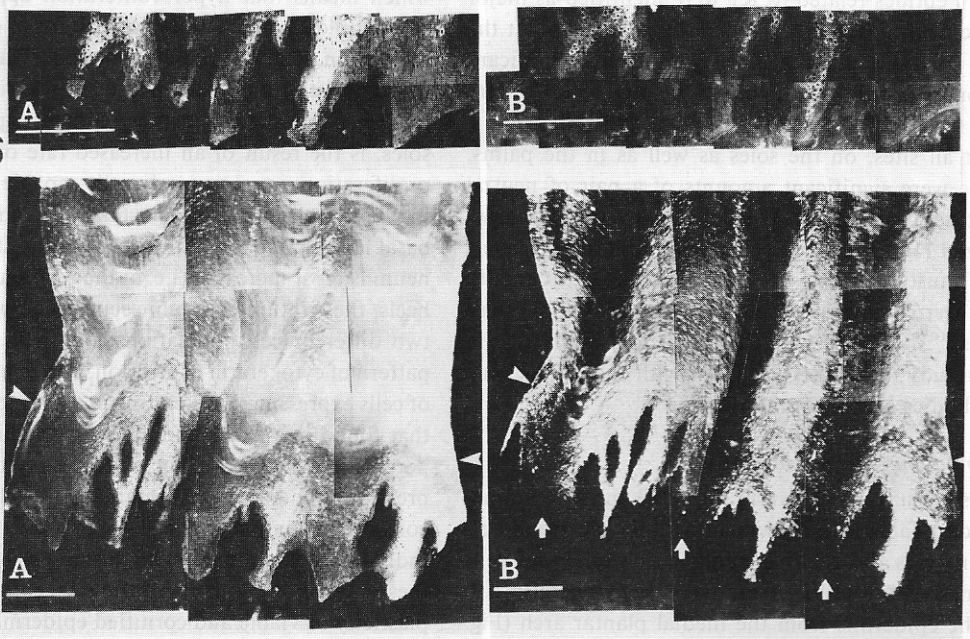


Fig. 6

Fig. 7

Table I. *Summary of the results presented in Figs. 2–7*

Site	Antibody[a]	Staining pattern	Fig.
Medial	R-antiker	Strong homogeneous staining of suprabasal cells, weak staining of basal cells	2*A*, 3*A*
Medial	MabK92	Homogeneous staining of suprabasal cells	2*B*
Medial	MabFE1	Irregular distribution of strongly stained and unstained cells in suprabasal layers	3*B*
Lateral	R-antiker	Strong homogeneous staining of suprabasal cells, weak staining of basal cells	4*A*, 5*A*
Lateral	MabK92	Only a small number of suprabasal cells significantly stained	4*B*
Lateral	MabFE1	Staining of all suprabasal cells	5*B*
Posterior	R-antiker	Strongly stained suprabasal areas alternating with areas with the same staining intensity as basal cells. The weakly stained areas the same as those not stained with MabK92 or MabFE1	6*A*, 7*A*
Posterior	MabK92	Areas with irregular, rather weak staining alternating with unstained areas	6*B*
Posterior	MabFE1	Strongly stained suprabasal areas regularly alternating with unstained areas	7*B*

[a] R-antiker stains cytokeratin No. 1 (and possibly Nos. 10/11). MabK92 stains cytokeratin Nos. 10/11. MabFE1 stains cytokeratin No. 9.

3. Peptides related to K1 were identified as major basic-neutral (type II) cytokeratins at all sites. At the rear margins of the heels, however, significant amounts of peptides apparently related to K5/6 were also found.

At all sites, on the soles as well as in the palms, there were significant amounts of a pair of neutral peptides ("*X*" in Fig. 1) that have not yet been characterized (18, 21). They occur only in cornified layers and must therefore be derived from a cytokeratin synthesized in viable epidermal layers. On Western blots, "*X*" reacted with the rabbit antiserum used in this study (R-antiker), but not with the monoclonal antibodies MabK92 and MabFE1.

Immunofluorescence microscopy

These results are shown in Figs. 2–7 and are summarized in Table I. In agreement with the electrophoretic analyses the relative number of suprabasal cells stained with MabFE1 (reactive with cytokeratin K9) in the epidermis from the medial plantar arch (Fig. 3 B) varied among individuals. All other staining patterns showed only minor inter-individual variations. The most striking results were obtained with biopsies from the rear margins of heels (Figs. 6 and 7). At this site there were regularly appearing suprabasal epidermal areas that apparently did not express any of the differentiation-related cytokeratins K1, K9, or K10/11. This vertical stratification was seen also in the stratum corneum (Fig. 7 B). Sections from the rear margins of heels stained with haematoxylin and eosin after fixation in formaldehyde showed no morphological evidence of keratinization disturbances, i.e. there was a uniform stratum granulosum and no parakeratosis (results not shown).

DISCUSSION

The finding of significant amounts of the low molecular weight cytokeratins K5/6 and K14/16 in the stratum corneum from the rear margins of the heels suggests that these were the only cytokeratins expressed in the vertical columns of cells at this site that were not stained by the antibodies reacting with the differentiation-related cytokeratins K1, K9, and K10/11. This indicates that these parts of the epidermis may be hyperproliferative, in analogy with what is known from studies on hyperproliferative skin disorders (3, 4).

For a balanced tissue growth, the rest of the epidermis at this site must also be produced at a higher rate, which means that hyperproliferation appears to be compatible also with a normal pattern of cytokeratin expression. It should therefore be conceivable that the thickened stratum corneum also at sites with intermediate hyperplasticity, such as the lateral parts of the soles, is the result of an increased rate of epidermal proliferation, involving only cells capable of expressing high molecular weight cytokeratins in the suprabasal layers. Where the thickness of the stratum corneum is most increased, i.e. at the rear margins of the heels, the stratum corneum seems to be produced by two different cell populations; one with a 'normal' pattern of cytokeratin expression, and one population of cells expressing cytokeratins in a manner similar to that found in various conditions with epidermal hyperproliferation (3, 4). The pattern of cytokeratin expression found at the rear margins of heels should be considered non-pathological, since it was found in all individuals examined. The histological picture at these sites showed no aberrations except for a hyperplasticity of viable and cornified epidermal layers.

This work has shown that a significant part of the keratin of the stratum corneum at sites exposed to greater mechanical stress than any other part of the body surface seems to consist of low molecular weight cytokeratins. The functional implications of this finding remain to be elucidated.

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REFERENCES

1. Costello MJ, Gibbs RC. The palms and soles in medicine. Springfield, Ill.: Charles C. Thomas, 1967; 104–118.
2. Wright NA. The cell proliferation kinetics of the epidermis. In: Goldsmith LA, ed. Biochemistry and physiology of the skin. Oxford: University Press, 1983; 1: 204–229.
3. Mansbridge JN, Knapp AM. Changes in keratinocyte maturation during wound healing. J Invest Dermatol 1987; 89: 253–263.
4. Weiss RA, Guillet GYA, Freedberg IM, Farmer ER, Small EA, Weiss MM, Sun T-T. The use of monoclonal antibody to keratin in human epidermal disease: Alterations in immunohistochemical staining pattern. J Invest Dermatol 1983; 81: 224–230.

5. Steinert PM, Parry DAD. Intermediate filaments: Conformity and diversity of expression and structure. Ann Rev Cell Biol 1985; 1: 41–65.
6. Moll R, Franke WW, Schiller DL. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982; 31: 11–24.
7. Fuchs E, Green H. Changes in keratin gene expression during terminal differentiation of the keratinocyte. Cell 1980; 19: 1033–1042.
8. Nelson WG, Sun T-T. The 50- and 58-kdalton keratin classes as molecular markers for stratified squamous epithelia: cell culture studies. J Cell Biol 1983; 97: 244–251.
9. Eichner R, Bonitz P, Sun T-T. Classification of epidermal keratins according to their immunoreactivity, isoelectric point, and mode of expression. J Cell Biol 1984; 98: 1388–1396.
10. Woodcock-Mitchell J, Eichner R, Nelson WG, Sun T-T. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. J Cell Biol 1982; 95: 580–588.
11. Knapp AC, Franke WW, Heid H, Hatzfeld M, Jorcano JL, Moll R. Cytokeratin no. 9, an epidermal type I keratin characteristic of a special program of keratinocyte differentiation displaying body site specificity. J Cell Biol 1986; 103: 657–667.
12. Moll I, Heid H, Franke WW, Moll R. Distribution of a special subset of keratinocytes characterized by the expression of cytokeratin 9 in adult and fetal human epidermis of various body sites. Differentiation 1987; 33: 254–265.
13. Weiss RA, Eichner R, Sun T-T. Monoclonal antibody analysis of keratin expression in epidermal diseases: a 48- and 56-kdalton keratin as molecular markers for hyperproliferative keratinocytes. J Cell Biol 1984; 98: 1397–1406.
14. O'Farrell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem 1975; 250; 4007–4021.
15. O'Farrell PZ, Goodman HM, O'Farrell PH. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 1977; 12: 1133–1142.
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–685.
17. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350–4354.
18. Egelrud T, Lundström A. Stepwise modifications of keratin polypeptides during keratinization in palmar-plantar epidermis. Acta Derm Venereol (Stockh) 1989; 69: 105–110.
19. Carlsson L, Folkersen J, Stigbrand T. Antigenic determinants of pregnancy-associated α2-glycoprotein and α2-macroglobulin defined by polyclonal and monoclonal antibodies. Molec Immun 1985; 22: 1073–1080.
20. Kearny JF, Radbruck A, Liesegang B, Rajewsky K. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J Immunol 1979; 123: 1548–1550.
21. Moll R, Moll I, Franke WW. Differences of expression of cytokeratin polypeptides in various epithelial skin tumors. Arch Dermatol Res 1984; 276: 349–363.