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Keratin Polypeptides Distribution in Normal and Diseased Human Epidermis and Oral Mucosa

Immunohistochemical Study on Unaltered Epithelium and Inflammatory, Premalignant and Malignant Lesions

Thomas Löning[1], Marie-Jeanne Staquet[2], Jean Thivolet[2], and Gerhard Seifert[1]

[1] Institute of Pathology, University of Hamburg, Martinistraße 52
D-2000 Hamburg 20, Federal Republic of Germany
[2] ERA CNRS 788 et FRA INSERM 11, Laboratoire de Recherche Dermatologique et Immunologie, Clinique Dermatologique – Hôpital Edouard Herriot,
F-69374 Lyon Cedex 2 – France

Summary. Immune sera against total keratin and keratin polypeptide subunits were induced in guinea pigs, using the different bands of SDS polyacrylamide gel electrophoresis of fibrous proteins of stratum corneum, derived from normal human epidermis.

The distribution of the different polypeptides was studied in numerous human biopsies of normal epidermis, normal oral mucosa and epidermal and mucosal inflammatory, premalignant and malignant lesions using the indirect immunoperoxidase method.

Antisera against total keratin (TK) and against the keratin polypeptide of M.W. 55,000 dalton (55K) labelled all keratinocytes in normal and pathological conditions. These antisera may be useful for the histodifferentiation in diagnostic pathology.

Antisera against the keratin polypeptides of M.W. 67,000 (67K) and 62,000 dalton (62K) identified only keratin antigens in the spinous, granular and keratinized layer of normal epidermis and oral mucosa. No labelling of the basal layer was achieved with these immune sera. However, there were important differences in the distribution of these keratin antigens in altered epithelia which may be of value in the differential diagnosis of inflammatory, premalignant and malignant lesions of the skin and oral mucosa.

Key words: Keratin polypeptides – Epidermis – Oral mucosa – Epithelial differentiation – Malignant transformation.

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Offprint requests to: Dr. Thomas Löning, Institut für Pathologie, Universität Hamburg, Martinistraße 52, D-2000 Hamburg Federal Republic of Germany

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Introduction

The cytokeratins of epidermal cells consist of a number of polypeptide subunits (molecular weight 40,000 to 67,000 dalton; Matoltsy 1975; Baden et al. 1976; Brysk et al. 1977; Baynes et al. 1978; Thivolet 1980). Anti-keratin sera have been used for the study of epithelial differentiation in in vitro cultures of epithelia (Sun and Green 1978; Franke et al. 1978), in animal experiments (Schmid et al. 1979; Franke et al. 1979) and in human tissue of various epithelial origin (Oberle et al. 1979; Schlegel et al. 1980). Recently, specific antibodies against different polypeptides of the intermediate-sized filament system have been induced in guinea pigs and tested on normal human and rabbit epidermis (Viac et al. 1980). Some differences in filament distribution in the various compartments of the epidermis were thereby observed (Viac et al. 1980; Viac et al. 1980).

The demonstration of the cytokeratins in normal tissue and pathological conditions of the epidermis and oral mucosa is valuable, for the following reasons: 1. Its allows analysis and comparison of the keratinization processes in normal epidermis and oral mucosa. 2. It provides an additional morphological substrate for the identification and classification of epidermal and mucosal premalignant and malignant lesions.

In this study, the distribution of total keratin (TK) and three different cytokeratin polypeptides of molecular weight 67,000, 62,000 and 55,000 dalton (67K, 62K, 55K) was examined in 82 cases of normal epidermis and oral mucosa and in various inflammatory, premalignant and malignant lesions (lichen planus, leukoplakia, premalignant dyskeratosis, Bowen's disease, keratoacanthoma, basal cell carcinoma and squamous cell carcinoma) using the indirect immunoperoxidase technique (Bustamante et al. 1978; Thivolet et al. 1980).

Materials and Methods

1. Antigen Preparation. Keratin proteins were isolated according to the method of Baden and Lee (1978), modified by Viac et al. (1980). The fibrous proteins were analyzed by SDS polyacrylamide gel electrophoresis as described by Laemmli (1970). The distribution of the different keratin polypeptides is shown in Fig. 1.

2. Antibody Preparation. Keratin proteins purified from human stratum corneum (150 μg) were emulsified with Freund's complete adjuvant and injected intraperitoneally in adult female Hartley guinea pigs (400 g) using a method previously described (Viac et al. 1978). Keratin polypeptide antibodies were obtained by a recently published procedure (Staquet et al. 1979). The immune sera were absorbed with human erythrocytes and liver powder (Olson et al. 1972). There was no cross reactivity of immune sera with human serum albumin.

3. Material. The 82 specimens of the epidermis and oral mucosa were collected during the years 1979–1980 from the surgical department of the Dermatological Clinic (Pr. J. Thivolet) and from the Clinic of Maxillo-Facial Surgery (Pr. Dumas) of the Ed. Herriot Hospital (Lyon).

4. Immunoperoxidase Staining. The skin and mucosal biopsies were fixed in Bouin's solution for 24 h and embedded in paraffin. After cutting at 5 μ sections, keratin antigens were demonstrated by the indirect immunoperoxidase technique (Bustamante et al. 1978; Schmitt 1979; Thivolet et al. 1980) using the specific antisera described (diluted at 1/200) and a peroxidase-conjugated goat-anti-guinea pig-immunoglobulin serum (Nordic – diluted at 1/50). 3.3′ diaminobenzidine (Sigma) was used to reveal peroxidase activity (Graham and Karnovsky 1966). Control reactions with preimmune sera instead of the primary specific antisera were included.

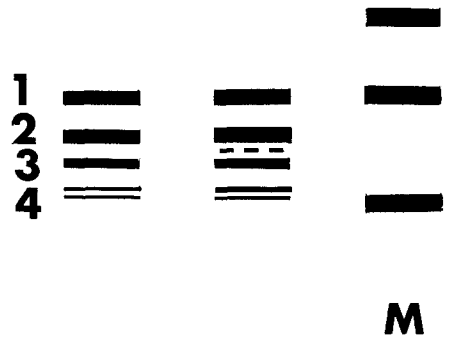

Fig. 1. SDS polyacrylamide gel electrophoresis patterns of fibrous proteins from normal stratum corneum of two different individuals (*1* = 67K, *2* = 62K, *3* = 55K, *4* = 48K, *M* = standard proteins used as markers)

Table 1. Distribution of keratin polypeptides in normal epidermis and oral mucosa

	67K	62K	55K	TK
Normal epidermis: 2 (number of cases)				
Keratinized layer	+++	+++	++	++
Granular layer	+++	+++	++	++
Prickle cell layer	+++	+++	++	++
Basal layer	–	–	+	+
Estimation of dyskeratotic cells	–	–	–	–
Normal ortho-keratinized oral mucosa				
Tongue: 1 (number of cases) Hard palate: 1 (number of cases) Gingiva: 2 (number of cases)				
Keratinized layer	+++	+++	++	++
Granular layer	+++	+++	++	++
Prickle cell layer	+++	+++	++	++
Basal layer	–	–	+	+
Estimation of dyskeratotic cells	–	–	–	–
Normal non-keratinized oral mucosa				
Buccal mucosa: 2 (number of cases) Floor of mouth: 2 (number of cases)				
Surface layer	++	++	++	++
Intermediate layer	++	++	++	++
Prickle cell layer	++	++	++	++
Basal layer	–	–	+	+
Estimation of dyskeratotic cells	–	–	–	–

Results

Normal Epidermis (Table 1)

In the normal epidermis, it could be seen that there were different staining properties of the various cytokeratin polypeptide antisera. Antisera against 67K and 62K labelled all epidermal layers except the basal cell layer (Fig. 2A). Antisera against 55K and TK, however, stained all epidermal cell layers including

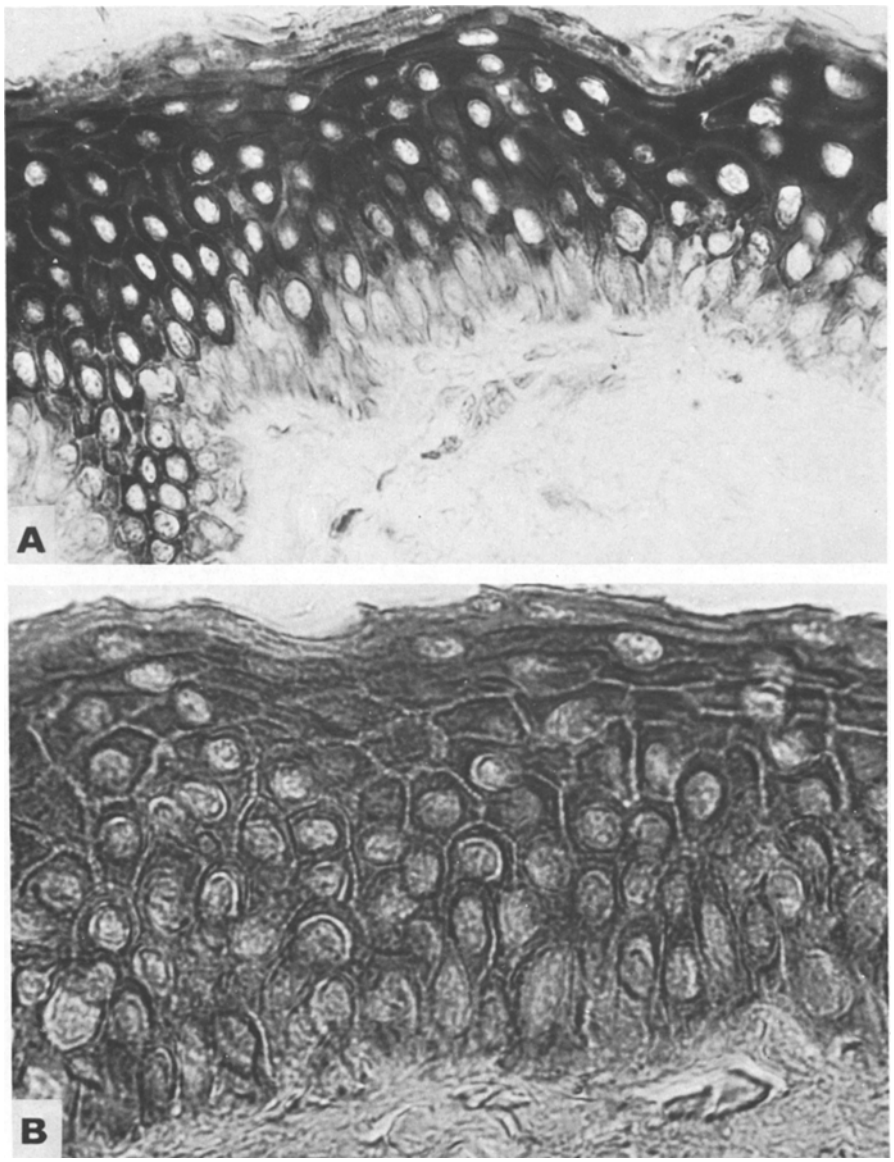

Fig. 2 A and b. Normal epidermis of the forearm. **A** The basal layer is not labelled by 67K-antiserum. **B** Staining of all epidermal layers by 55K-antibodies. Indirect immunoperoxidase method; ×645. No counterstaining

the basal cell layer. The upper Malpighian layers were labelled less intensively (Fig. 2B).

Normal Oral Mucosa (Table 1)

Observation of non-keratinized mucosa frequently displayed an unequivocally positive but foamy label of the epithelial cells (Fig. 3BD). In accord with the

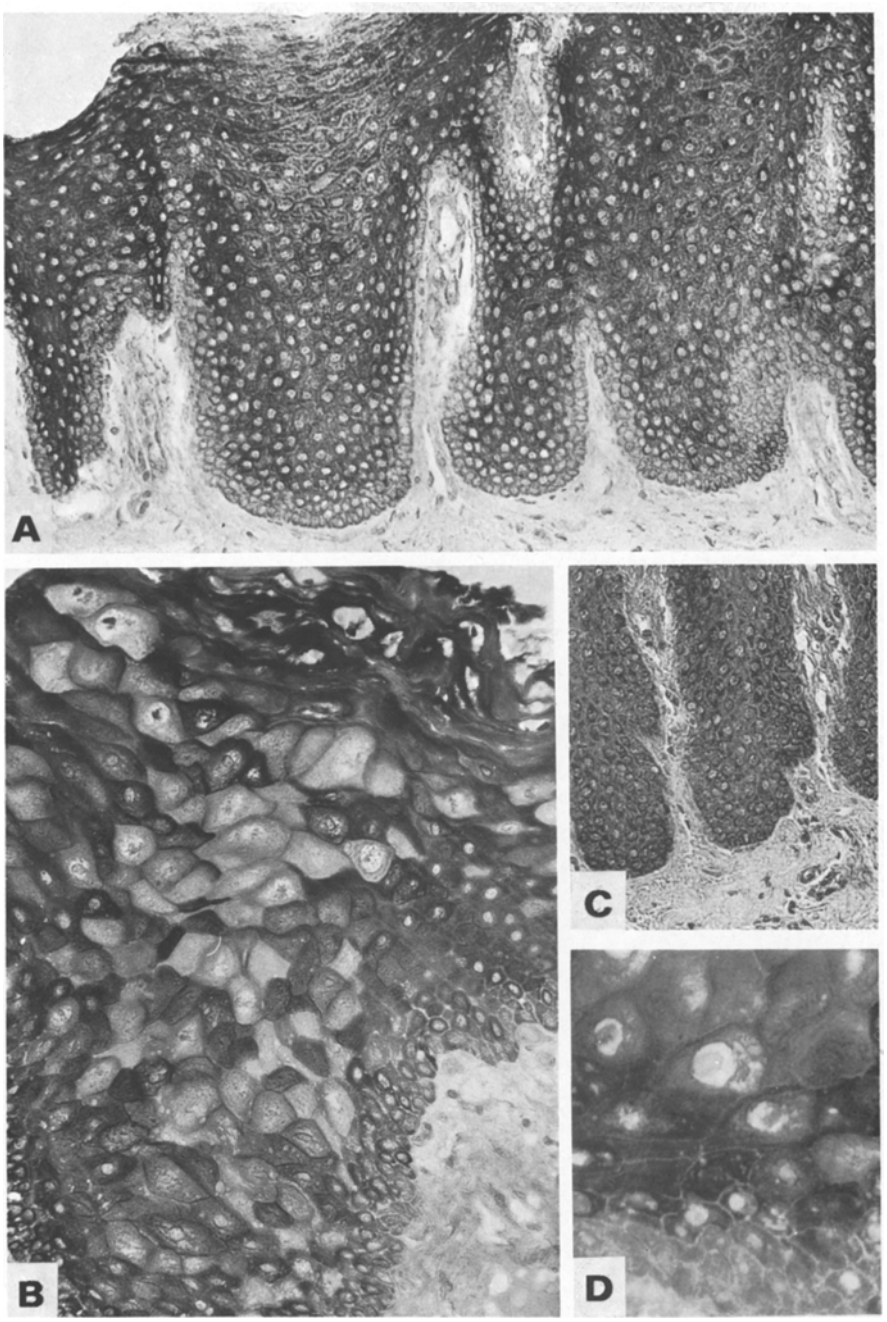

Fig. 3A–D. Normal oral mucosa. **A** and **C** Gingival ortho-keratinized epithelium. Negative basal layer in *A*(67K). Labelling of the basal layer in *C*(55K); ×102. **B** and **D** Buccal nonkeratinized mucosa. Foamy staining pattern of epithelial cells. 67K-negative basal cells (*D, at the bottom*). Indirect immunoperoxidase method; *B* × 160, *D* × 410. No counterstaining

Table 2. Distribution of keratin polypeptides in lichen planus and leukoplakia

	67K	62K	55K	TK
Lichen planus				
Skin: 3 (number of cases)				
Oral mucosa: 7 (number of cases)				
Keratinized layer	+++	+++	++	++
Granular layer	+++	+++	++	++
Prickle cell layer	+++	+++	++	++
Basal layer	++	++	+	+
Estimation of dyskeratotic cells	+	+	+	+
Leukoplakia without dysplasia: 11 (number of cases)				
Keratinized layer	+++	+++	++	++
Granular layer	+++	+++	++	++
Prickle cell layer	+++	+++	++	++
Basal layer	−	−	+	+
Estimation of dyskeratotic cells	−	−	−	−
Leukoplakia with dysplasia: 9 (number of cases)				
Keratinized layer	+++	+++	++	++
Granular layer	+++	+++	++	++
Prickle cell layer	+++	+++	++	++
Basal layer	−	−	+	+
Estimation of dyskeratotic cells	+−++	+−++	+	+

epidermis, the basal cell layer of the mucosa was not stained by antisera against 67K and 62K. The ortho-keratinized mucosa showed an identical aspect to the epidermis (Fig. 3AC).

Lichen Planus (Table 2)

In lichen planus, the staining pattern was often similar to that seen in normal keratinized epithelium. In lesions with a strong inflammatory reaction, however, the keratinocytes adjacent to the dermal cell infiltrate were intensively stained by 67K- and 62K-antisera. In addition, strongly 67K- and 62K-positive, dyskeratotic cells appeared in long-lasting lesions. Analogous observations could be made in lesions of the skin and oral mucosa (Fig. 4AB).

Leukoplakia (Table 2)

Leukoplakia without dysplasia showed many similarities to the normal orthokeratinized oral mucosa. The lower Malpighian cell layers, however, revealed a basaloid appearance and were not labelled by 67K- and 62K-antisera (Fig. 5A). In the dysplastic lesions, an irregular keratin pattern was present and dyskeratotic, 67K- and 62K-positive cells occurred (Fig. 5B).

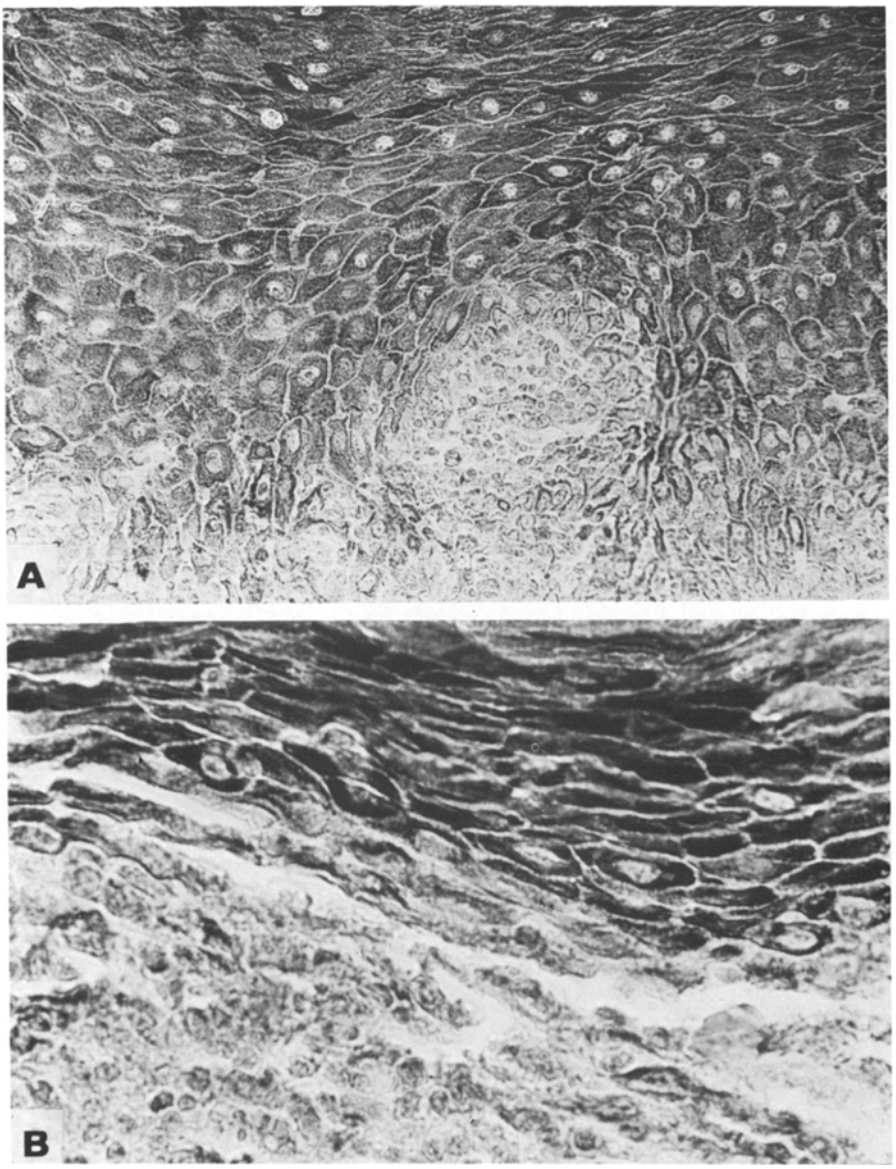

Fig. 4 A and B. Lichen planus of the buccal mucosa labelled by 67K-antiserum. **A** Staining of all epithelial layers. × 160. **B** Heavily labelled epithelial cells adjacent to the intense dermal inflammatory infiltrate. Indirect immunoperoxidase method; × 256. No counterstaining

Dyskeratosis of the Skin (Table 3)

The premalignant dyskeratoses revealed a mosaic of faintly stained and strongly 67K- and 62K-positive, atypical cells (Fig. 6A). The 55K- and TK-antisera showed weak staining properties in all the keratinocytes of these lesions.

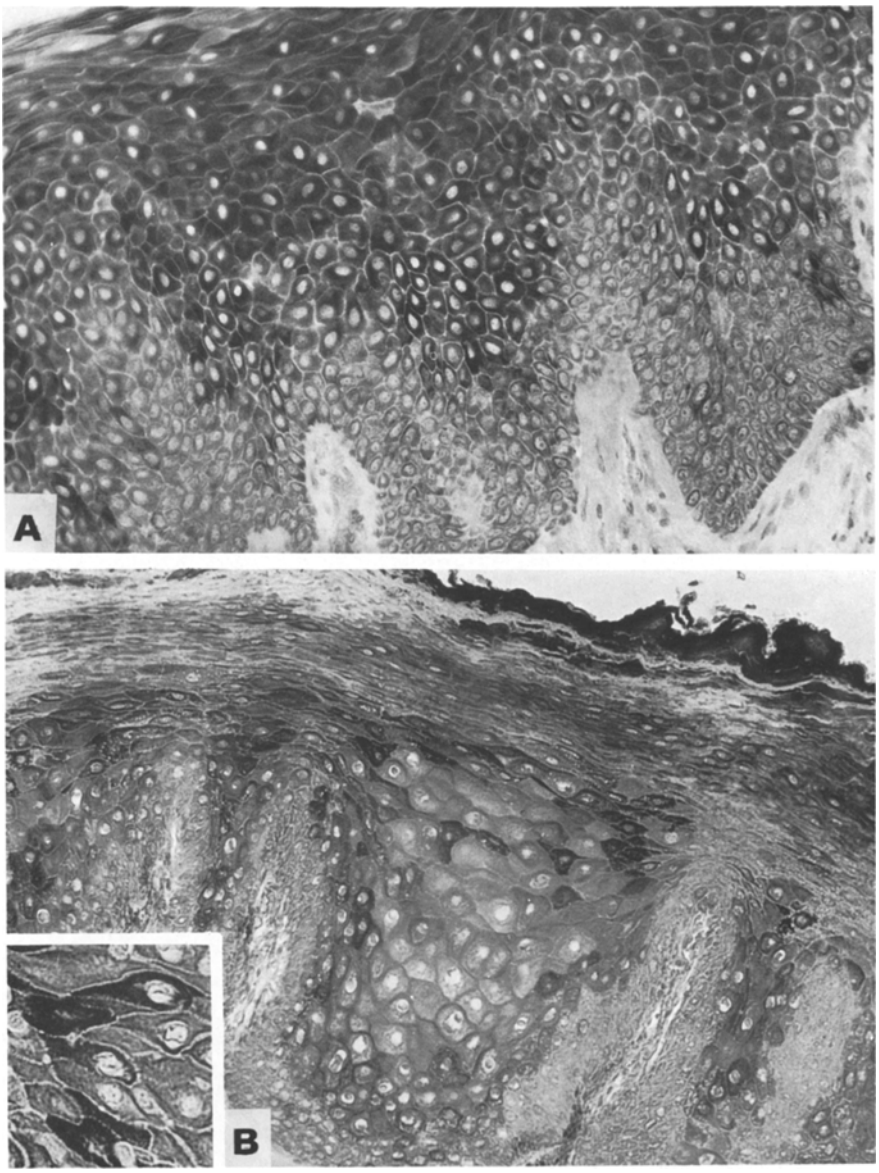

Fig. 5A and B. Oral leukoplakias stained by 67K-antiserum. **A** Leukoplakia without dysplasia (Gingiva). Hyperplastic non-labelled basal cell layer; ×160. **B** Leukoplakia with dysplasia (floor of the mouth). Negative basal cell layer. Irregular keratin pattern. Intensively stained dyskeratotic cells in the Malpighian layer (*Inset*). Indirect immunoperoxidase method; ×102, *Inset* ×256. No counterstaining

Bowen's Disease (Table 3)

In Bowen's disease, the occurrence of dyskeratotic, heavily stained (67K, 62K) epithelial cells was augmented in comparison with the previously mentioned dyskeratotic lesions (Fig. 6BC).

Table 3. Distribution of keratin polypeptides in premalignant epidermal dyskeratoses and Bowen's disease of the skin

		67K	62K	55K	TK
Premalignant dyskeratoses: 5 (number of cases)					
disturbed layering of epithelium	Keratinised layer Granular layer Prickle cell layer Basal layer	+	+	+	+
	Estimation of dyskeratotic cells	++	++	+	+
Bowen's disease: 4 (number of cases)					
disturbed layering of epithelium	Keratinized layer Granular layer Prickle cell layer Basal layer	+++	+++	++	++
	Estimation of dyskeratotic cells	+++	+++	++	++

Table 4. Distribution of keratin polypeptides in keratoakanthoma, basal and squamous cell carcinoma

	67K	62K	55K	TK
Keratoakanthoma: 4 (number of cases)				
Peripheral parts of tumour islands	−	−	+	+
Central parts of tumour islands	+++	+++	++	++
Estimation of dyskeratotic cells	+	+	±	±
Basal cell carcinoma: 4 (number of cases)				
Peripheral parts of tumour islands	±	±	+	+
Central parts of tumour islands	±	±	+	+
Estimation of dyskeratotic cells	±	±	±	±
Squamous cell carcinoma				
Skin: 14 (number of cases)				
Oral mucosa: 11 (number of cases)				
High differentiated (16 cases)				
Peripheral parts of tumour islands	−	−	++	++
Central parts of tumour islands	+++	+++	++	++
Estimation of dyskeratotic cells	+++	+++	++	++
Low differentiated (9 cases)				
Peripheral parts of tumour islands	±	±	+	+
Central parts of tumour islands	+	+	+	+
Estimation of dyskeratotic cells	++	++	+	+

Basal Cell Carcinoma (Table 4)

In basal cell carcinoma, the leading feature was the weak or absent labelling by antisera against 67K and 62K (Fig. 7A). The 55K- and TK-antisera showed weak staining of the tumour cells. Occasionally, circumscribed areas with complete keratinization were observed.

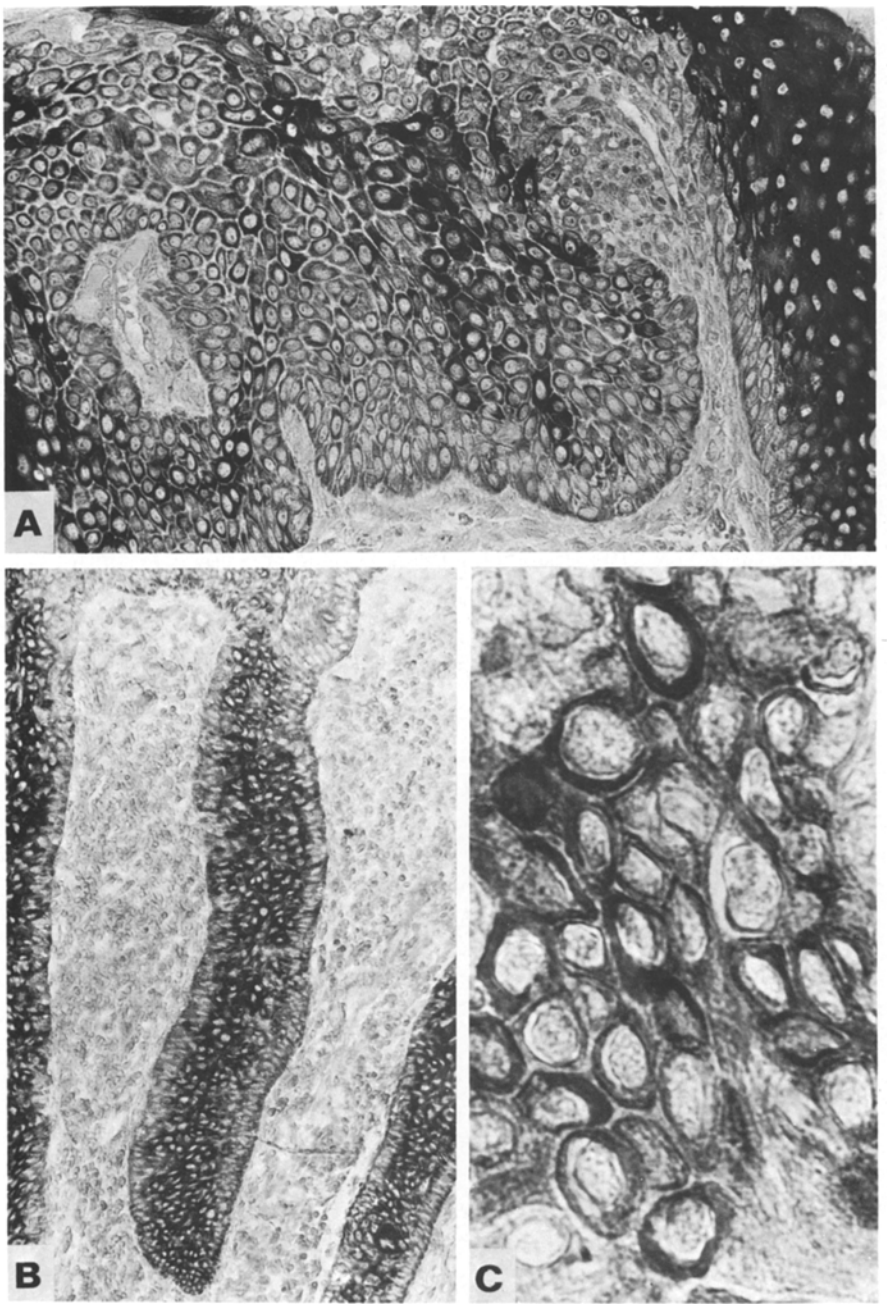

Fig. 6. **A** Premalignant dyskeratosis of the nose. Transition of normal epidermis (*on the right*) into a mosaic of heavily stained keratinocytes (67K) and negative epidermal cells; ×160. **B** and **C** Bowen's disease (back of the hand). Intense labelling of a great number of keratinocytes with marked cellular and nuclear polymorphism by 67K-immune serum. Indirect immunoperoxidase method; *B* ×102, *C* ×645. No counterstaining

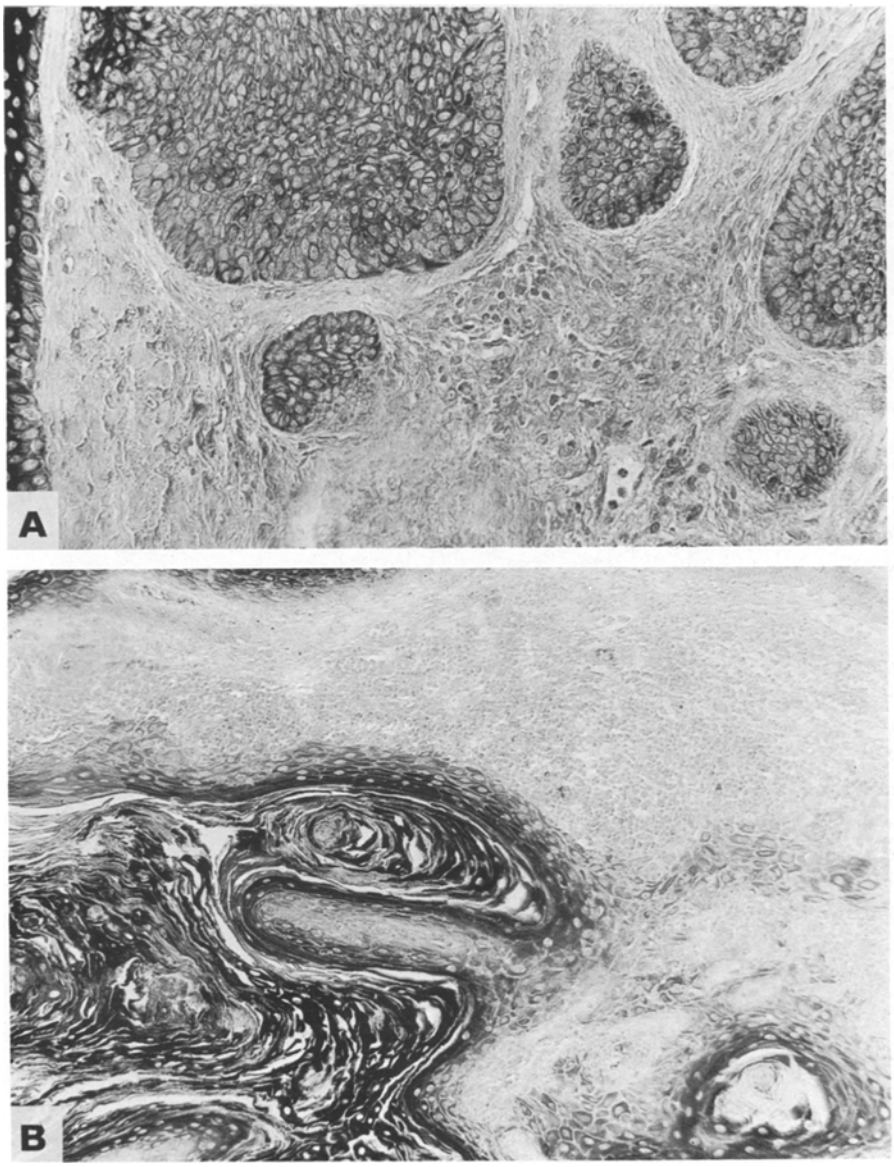

Fig. 7. A Basal cell carcinoma of the forehead. Faintly labelled tumour cells (67K). Circumscribed keratinized tumour region (*upper left corner*) adjacent to normal epidermis; ×160. **B** Keratoacanthoma (presternal skin) stained by 67K-immune serum. Transition of normal epidermis (*at the top*) into the strongly labelled tumour. Impression of a rather regular keratin pattern. Indirect immunoperoxidase method; ×102. No counterstaining

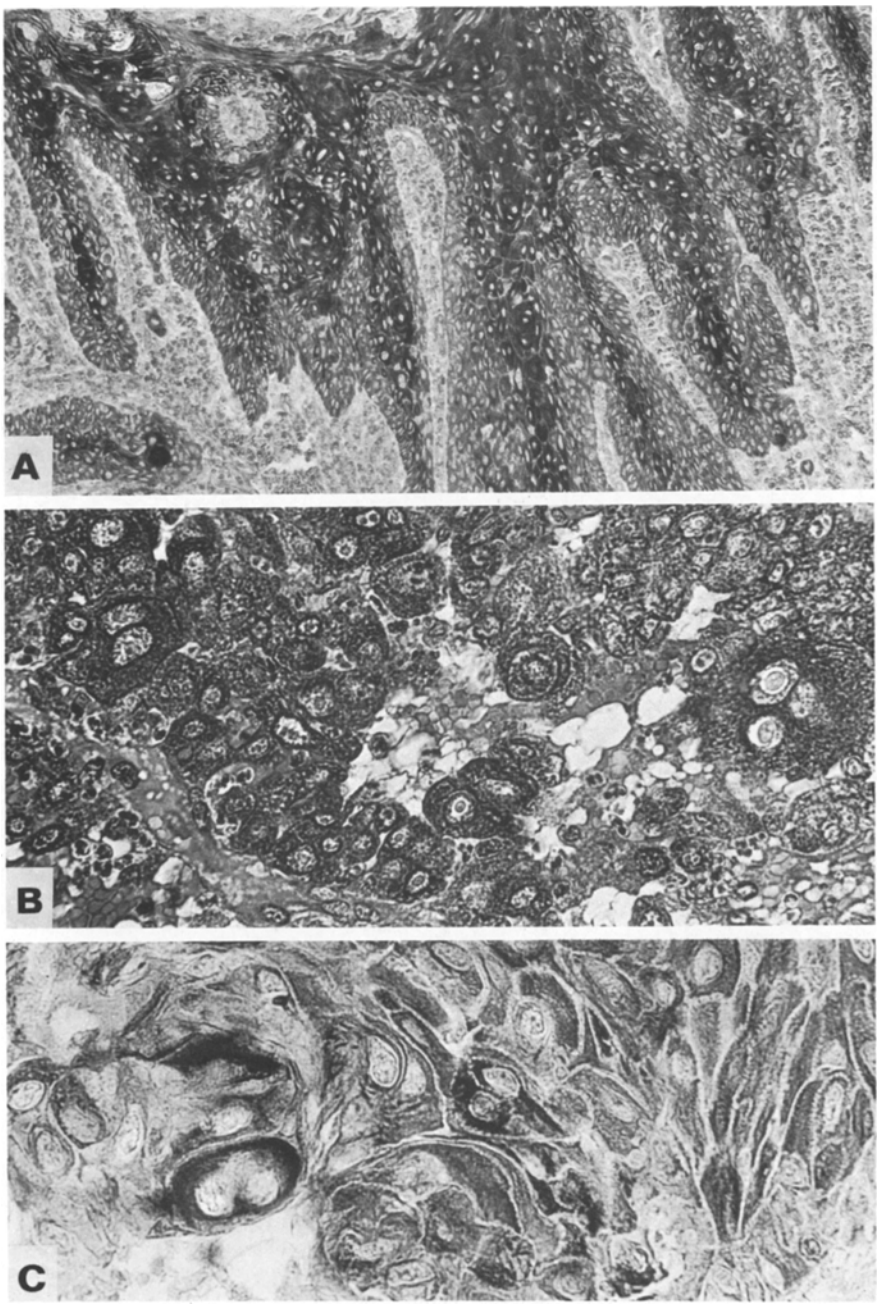

Fig. 8. A Well differentiated squamous cell carcinoma of the lower lip. Staining with 67K-antibodies. Irregular keratin pattern; ×64. **B** Poorly differentiated squamous cell carcinoma (Gingiva). Staining of all anaplastic tumour cells by 55K-antiserum. ×256. **C** Moderately differentiated squamous cell carcinoma (cheek). Labelling with 67K-immune serum. Heavily stained pleomorphic tumour cells adjacent to negative malignant cells. Indirect immunoperoxidase method; ×410. No counter-staining

Keratoacanthoma (Table 4)

The staining revealed a different distribution pattern with respect to the periphery and the centre of the tumour. The tumour islands showed a generally regular arrangement of non-labelled peripheral and heavily stained, 67K- and 62K-positive, central tumour cells (Fig. 7B). In contrast to the squamous cell carcinomata, only a moderate number of dyskeratotic cells were observed.

Squamous Cell Carcinoma (Table 4)

The highly differentiated squamous cell carcinomata showed numerous dyskeratotic cells which were strongly labelled by anti-67K and -62K. The outer layers of the tumour islands were generally negative; but there was an irregular and spotted pattern of stained cells (Fig. 8AC). Anti- 55K and -TK stained all malignant epithelial cells.

Poorly differentiated squamous cell carcinomata, however, revealed a generally low or moderate labelling by all antisera. Nevertheless, the 55K- and TK-antisera stained unequivocally these anaplastic tumour cells (Fig. 8B). The features of squamous cell carcinomata of the skin and oral mucosa showed no differences.

Discussion

Since it has been possible to induce antibodies towards the different filament systems (Franke et al. 1979; Gabbiani 1979), a new view has been opened for the study of the normal and diseased human epidermis and oral mucosa.

It is the intermediate-sized type of filaments which play the central role in epithelial tissue (Sun and Green 1978); Schmid et al. 1979; Thivolet 1980). For this reason, they may provide a marker for cells of epithelial origin, and the distribution pattern of these filaments may be a helpful criterion for the exact classification of inflammatory, premalignant and malignant lesions.

In the normal epidermis, the basal layer and the upper cell layers appeared to be different with regard to the distribution of keratin polypeptide subunits. This finding is probably the expression of a modification of keratin polypeptides during keratinocyte division and differentiation (Viac et al. 1980; Thivolet et al. 1980).

In the normal oral mucosa, the ortho-keratinized regions showed an identical keratin pattern to the epidermis which correlates well with similarities on the ultrastructural level (Breathnach 1975; Squier et al. 1976). The foamy staining of the non-keratinized mucosa, however, may be due to the special arrangement of tonofibrils which are shorter, finer and fewer in number (Zelickson and Hartmann 1962). In addition, the distinct amounts of glycogen in this type of mucosa may also contribute to this feature (Squier et al. 1976).

Some differences in keratin polypeptides distribution can also be demonstrated in pathological lesions. In lichen planus, the regions without marked inflam-

mation revealed an identical appearance to normal keratinized epithelium (Oberle et al. 1979). However, heavily inflamed lesions showed an intense staining of all epithelial layers by 67K- and 62K-antisera in contrast to the normal absence of labelling of the basal cells by these immune sera (Viac et al. 1980). This may be due to the fact that the basal cell layer is severely altered in these lesions (s. for review: Lever 1975). In consequence, the keratinization process is extensively disturbed (Sümegi 1979). The remaining basally situated cells may even belong to the low stratum spinosum as described in bullous lichen planus (Ebner et al. 1973). In long-lasting lesions, dyskeratotic, strongly 67K- and 62K-positive cells appeared in the Malpighian layer, a fact, which has been interpreted as a criterion of premalignancy (Seifert and Burkhardt 1979).

The leukoplakias of the oral mucosa must be morphologically distinguished in lesions with and without dysplasia (Burkhardt and Seifert 1977; Who 1978). In leukoplakias without dysplasia, the well-known presence of more than one layer of cells with a basaloid character (Löning and Burkhardt 1978) is supported by the absence of staining with 67K- and 62K-antisera. In leukoplakias with dysplasia, the most impressive feature was the irregular keratin pattern and the occurrence of intensively 67K- and 62K-positive cells in the Malpighian layer. Antisera against 67K and 62K labelled dyskeratotic epithelial cells in dysplasias of the oral mucosa and in premalignant dyskeratoses of the skin. They represented the main cell type in carcinomata in situ like Bowen's disease. These strongly positive keratinocytes correspond to the cells with crowded swirled bundles of tonofilaments on the ultrastructural level (s. for review: Lever 1975; Burkhardt 1979). The morphologically established loss of polarity of these cells is apparently associated with a marked disturbance of the normal keratinization process.

The weak staining patterns of basal cell carcinomata are in good accordance with the generally accepted histogenesis of this type of carcinoma (Lever 1948; Pinkus and Mehregan 1969; Lever 1975).

Our preliminary analysis of keratoakanthomas revealed a generally more regular keratin pattern in comparison with squamous cell carcinomata. Features characteristic of normal keratinization were also observed by electron microscopy (Fisher et al. 1972). Our results have to be confirmed by a larger series of cases, but may be an additional aid in the differential diagnosis of these tumours.

Regarding squamous cell carcinomata, different degrees of differentiation can be distinguished by an estimation of the percentage of keratinized tumour areas (Broders 1920). The keratin antisera now provide a tool for an objective evaluation of keratin content and distribution. Our investigation of a large number of squamous cell carcinomata showed no differences between squamous cell carcinomata of the skin or oral mucosa. In highly differentiated carcinomata, keratin staining by 67K- and 62K-antisera displayed an irregular and spotted pattern of intensively positive adjacent to completely negative keratinocytes. This observation corresponds to electron microscopical findings demonstrating cancer cells with a decreased number of tonofilaments (Fisher et al. 1972) and malignant cells with clusters of swirled, densely packed tonofilaments (Chen and Harwick 1977; Burkhardt 1979). In poorly differentiated squamous

cell carcinomata the expression of keratin antigens was extremely reduced. This observation may be interpreted as either a lower or an atypical production of cytokeratin filaments.

Since 55K and TK seem to be common antigens to all epithelial cells, they turn out to be of value in differential diagnosis between tumours of epithelial and mesenchymal origin. This may be important in the identification of anaplastic tumours at the light microscopical level.

As 67K and 62K are only present in special compartments of the epithelium, their arrangement in premalignant and malignant lesions provides a morphological aid in diagnostic pathology.

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Note Added in Proof

Our results of the keratin polypeptides distribution in the epidermis were recently confirmed by Fuchs and Green (Fuchs E, Green H (1980) Changes in keratin gene expression during terminal differentiation of the keratinocyte. Cell 19:1033-1042).