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Immunohistochemical demonstration of keratins in oral mucosa lesions

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Abstract:

The immunoperoxidase reaction for total keratin labelling was carried out on 21 conventional histopathology specimens of human oral mucosa, using an antibody against keratins isolated from rat epidermis. Specimens containing orthokeratinized, parakeratinized and non-keratinized normal oral mucosa and homogeneous leukoplakia, leukoplakia verrucosa, verrucous carcinoma, carcinoma "in situ" and invasive carcinoma were studied. The intensity of the reaction was greater in orthokeratinized epithelia than in parakeratinized or non-keratinized mucous membrane, whereas the homogeneity of the reaction pattern did not depend on the stage of keratinization. Verrucous carcinoma and verrucous leukoplakia exhibited a similar pattern. Carcinoma "in situ" were only slightly positive. The method also allows the visualization of centers of incipient dyskeratosis in leukoplakia, the observation of early indifferentation in carcinomas and the detection of micro-invasive carcinomatous sites.

Resumen:

Se realizó la reacción de inmunoperoxidasa para marcación de queratina total en 21 biopsias de mucosa oral humana con procesamiento de rutina para diagnóstico histopatológico, usando anticuerpos contra queratina aislada de epidermis de rata. Se estudiaron casos que contenían mucosa normal ortoqueratinizada,paraqueratinizada o no queratinizada y cuadros de leucoplasia homogénea, leucoplasia verrugosa, carcinoma verrugoso, carcinoma "in situ" o carcinoma infiltrante.

Los epitelios ortoqueratinizados mostraron una intensidad de reacción mayor que las mucosas paraqueratinizadas y no queratinizadas, mientras que la homogeneidad de la reacción no depende del estado de la queratinización. Las leucoplasias verrugosas y los carcinomas verrugosos mostraron un patrón de reacción similar. Los carcinomas "in situ" fueron muy poco reactivos. El método permitió además, la visualización de disqueratosis incipiente en las leucoplasias, la observación de la indiferenciación inicial de los carcinomas y la detección de lugares de micro-infiltración carcinomatosa.

Introducción:

Immunohistochemical studies of intermediate filaments, have shown that they can be grouped into five different immunotypes. Each immunotype was found to be present in a specific cell stirpe: epithelial, connective, muscular, glial and neuronal (1-5). The intermediate filaments described in epithelial cells are, from the chemical point of view, identical to the well known protein called keratin (6).

Antibodies against keratin proteins have been used to study the biology of opithelial structures and the histogenesis and differentiation of neoplastic lesions (7-11). Thus Loening et al. (12) carried out a study of the distribution of keratins in epidermal and oral mucosa lesions, using antibodies against both total and three of the main keratin polypeptides. These authors were able to show great changes in keratin distribution which were in good correlation with the degree of dyskeratosis of the lesions. More recent work (13) has outlined the possible use of these techniques in the study of the altered keratinization in oral precancer and cancer.

Since antibodies against total kerating are now currently available it seemed of interest to evaluate the usefulness of the immunohistochemical detection of keratin in the analysis of routinely processed biopsies of the oral mucosa lesions. The aims of the present study were: a) to analyse the reaction patterns of normal oral mucosa with different types of keratinization, b) to enlarge on information a-

vailable for leukiplakic lesions and carcinomas and c) to characterize other lesions which have not previously been described as verrucous carcinoma and carcinoma "in situ".

Material and methods:

The complete procedure of isolation and characterization of keratin and production of antiserum was reported elsewhere (14). Keratins of the whole rat epidermis were purified using the technique described by Sun and Green (15) and characterized by means of polycrilamide electrophoresis and "in vitro" repolymerization to from electron microscopically controlled keratin intermediate filaments. Cross reactivity with commercially available anti-human keratin serum was controlled by means of an immunohistochemical technique applied to microtitter plates coated with the solubilized keratins. Antibodies against the rat keratins were raised in rabbits and the sera obtained was adsorved with spleen and assayed by immunodiffusion.

Peroxidase-antiperoxidase (PAP) technique was performed as described by Sternberg (16) on 21 biopsies formaline fixed and routinelly processed for histopathology. The following lesions were studied: homogeneous leukoplakia (9 cases), verrucous carcinoma (5 cases), carcinoma "in situ" (2 cases) and invasive carcinoma (5 cases). Normal non-keratinized, para-keratinized and orthokeratinized mucosa (3 cases of each condition) were also available from the biopsy blocks. Paraffin sections were treated with 1.5 per cent hydrogen peroxide to eliminate endogenous peroxidase and sucessively incubated with the anti-keratin serum (1:100), goat anti-rabbit immunoglobulin serum, from DAKO-Chemetron Lat. Bs. As., (1:250) and anti goat rabbit PAP complex from Cappel Lab. Inc. P.A. (1:250). Sites of peroxidase binding were revealed with diaminobenzidine reaction. One section of each block was pre-treated with a solution of trypsin 0.05 per cent and Ca_2 0.4 per cent at room temperature during 10 minutes, before serum incubation. Adjacent control sections of each case were simultaneously processed by replacing the first antiserum with normal rabbit serum and with the antikeratin serum adsorbed with keratin.

Results

Ortho-keratinized mucosa

A very weak or no stain was observed in the basal cells. Lower spinous layers showed a moderate and homogeneous reaction which increased in intensity towards the surface. The upper malpighian layer exhibited a strong reaction. The pattern of this reaction was homogeneous in some areas and foamy in others (Fig. 1). No relation could be observed between these different patterns and the degree of keratinization as evaluated by the thickness of the stratum corneum. The stratum corneum generally failed to stain positively for the reaction except in those cases pre-treated with trypsin in which a strong stainning was observed.

Para-keratinized mucosa

The reaction of basal cells was almost negative. Prickle cells stained with different degrees of intensity thus producing a definite foamy appearance, although the general stainning increased towards the upper layers. When parakeratinized and orthokeratinized epithelia were present in the same section the latter showed a stronger reaction in the malpighian strata. Parakeratinized layers were negative or had areas of patchy stainnig in sections not pretreated with trypsin.

Non-keratinized mucosa

Non-keratinized areas were always stained fainter than the parakeratinized or orthokeratinized areas when they coexisted in the same section. The pattern of the reaction was similar to that of the para-keratinized epithelia with a definite foamy appearance.

Homogeneous leukoplakia

Homogeneous leukoplakia were classified on the basis of Pindbog's criteria (17) in the H&E stained sections. All cases showed hyperkeratosis or hyperparakeratosis and no signs of epithelial dysplasia. Five cases also showed a marked acanthosis, 2 cases exhibited epithelial atrophy and 2 cases were verrucous hyperkeratosis.

These cases differed in their pattern of keratin distribution. In two cases (one with acanthosis and the other with epithelial atrophy) a very strong reaction was found in some isolated basal cells (Fig. 2)

Malpighian layers were homogeneously stained in three cases (the atrophyc leukoplakias and one of the acanthotic cases) whereas the other six specimens showed a distinct foamy reaction.

The stratum corneum showed areas of weak reaction which alternated with others of strong and patchy stainning (Fig. 3). All trypsin pretreated sections showed a deeply stained horny layer.

Central areas of deeply stained cells were observed in the widened rete pegs of the verrucous leukoplakias, thus revealing sites of greater differentiation without a complete definition of a stratum corneum.

Verrucous carcinoma

Verrucous carcinoma showed an intense, irregular and foamy reaction in all epithelial strata with the exception of basal strata wich were weakly positive. The epithelial infolds showed central areas of strong reaction surrounding cornified layers with a similar but more advanced stage of keratinization to that observed in verrucous leukoplakias (Fig. 4).

Carcinoma "in situ"

Carcinomas "in situ" were virtually negative (Fig. 5). In one case in which the carcinomatous transformation was originated in a verrucous caricinoma, a clear decrease of the reaction intensity was evident in the undifferentiated area.

Invasive carcinoma

A very irregular distribution of labelled cells was also present in the infiltrating epithelial cords, the amount of strongly possitive cells being much greater in the Grade I and II carcinomas than in the more undifferentiated cases.

Basal cells of epithelial islands were weakly but unequivocally stained. A case of early invasive stage (micro-invasive carcinoma) showed a distinct reaction in the epithelial cells protruding into the connective tissue in the area of microinfiltration (Fig. 6).

In other cases of more advanced infiltration, an area of clearly decreased stainning was observed between the irregularly possitive carcinoma and the strongly positive normal mucosa.

Discussion

The normal mucous membrane showed a reaction pattern similar to that described by Loening et al. (12), when they used total keratin antiserum. However, these authors described the foamy label of malpighian layers only in the cases of non-keratinized mucosa and a more homogeneous pattern in orthokeratinized mucosa and skin. In our series the foamy appearance was also found in some orthokeratinized cases and in all cases of parakeratinized mucosa. This particular pattern could be in relation not only with the stage of keratinization but also with the speed of differentiation and turnover. The presence of this foamy appearance in verrucous carcinomas with a prominent horny layer gives some support to this hypothesis. The intensity of the reaction seems to be more closely associated to the stage of keratinization since the parakeratinized epithelia were also less reactive than the orthokeratinized mucosa when both were available in the same specimen.

Homogeneous leukoplakia without histological signs of dyskeratosis also showed a less intense reaction of malpighian layers than the normal oral mucosa. On the other hand, orthokeratinized strata of normal mucosa were always negative in sections not pre-treted with trypsin, whereas hyperkeratotic layers of leukoplakias exhibited a strong patchy reaction, thus indicating a less compact, although greater in size, horny layer. This could be interpreted as a qualitative modification of the production mechanisms of the corneal substance.

The presence of strongly positive cells in the basal layer of some homogeneous leukoplakia could be a very early sign of dyskeratosis, not evident in the routine hematoxilin-eosin stained slides. The total absence of reaction towards the employed technique in the cases of carcinoma "in situ" and the more weakly stained intermediate zone in the border of the invasive carcinoma, indicates the sensitivity of this method to detect areas of initial epithelial undifferentiation. Once the infiltration occurs the amount of strongly possitive cells could be an additional aid in establishing the tumoral grade of differentiation. The present results indicate that under controlled conditions such as the comparison of sections with and without trypsin pre-treatment, and within the limitations of PAP reactions in routline histopatholoy specimens, the use of antibodies against total keratins is an easy and useful tool to the evaluation of oral epithelial lesions.

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Figure 1: Orthokeratinized mucosa showing a very weak staining of the basal cells, strong and foamy reaction in malpighian layers and an almost negative stratum corneum. Keratin-PAP staining without trypsin pre-treatment. No counterstaining (X 140).

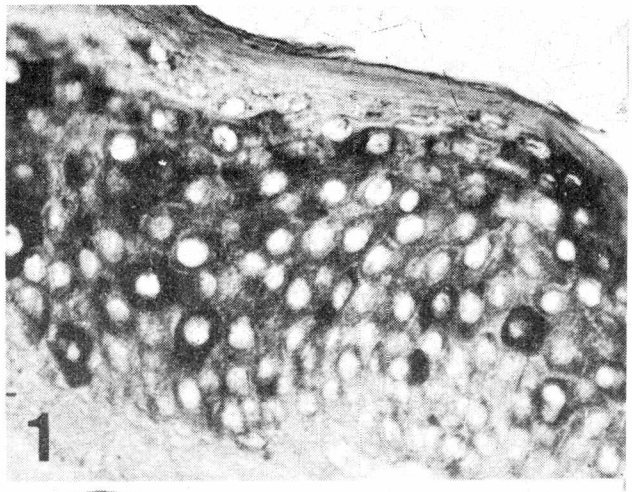
Figure 2: Homogeneous leukoplakia with a weak foamy reaction for keratins in malpighian layers and a very strong reaction in isolated basal or para-basal cells. Keratin-PAP staining with trypsin pre-treatment. No counterstain (X 140).

Figure 3: Homogeneous leukoplakia. An intense patchy reaction is present in the hyperkeratotic layers. Malpighian layers exhibit a weak and foamy stain. Keratin-PAP method without trypsin pre-treatment. No counterstain. (X 80).

Figure 4: Verrucous carcinoma. Strong reaction in cornified layers and surrounding keratinocytes in an epithelial infold. Keratin-PAP staining method without trypsin pre-treatment. No counterstain. (X 85).

Figure 5: Carcinoma "in situ". A: H& E stain showing nuclear hyperchromatism, increased mitotic activity in the basal layer. B: Immunoperoxidase stain in an adjacent section. A weak reaction of superficial layers and a negative-reaction in the other ephitelial cells (X25).

Figure 6: Micro-invasive carcinoma. A positive stain for keratins with a central core of stronger reaction is present in the malignant proliferation protruding into connective tissue. Keratin-PAP staining with no trypsin pre-treatment. No counterstain. (X 80).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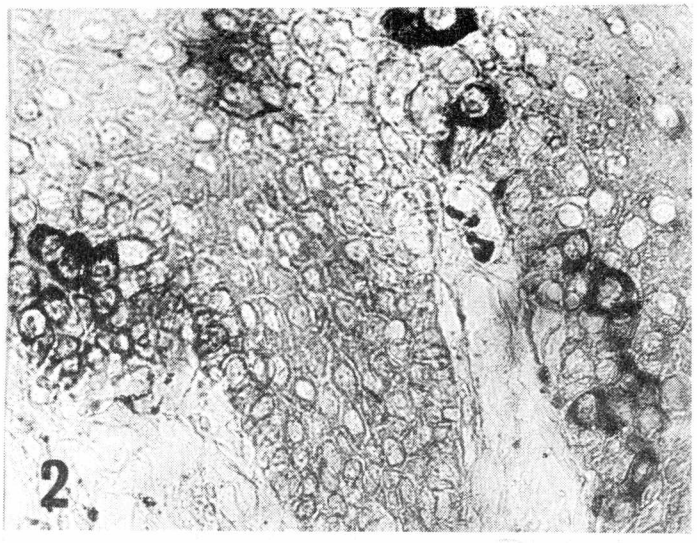
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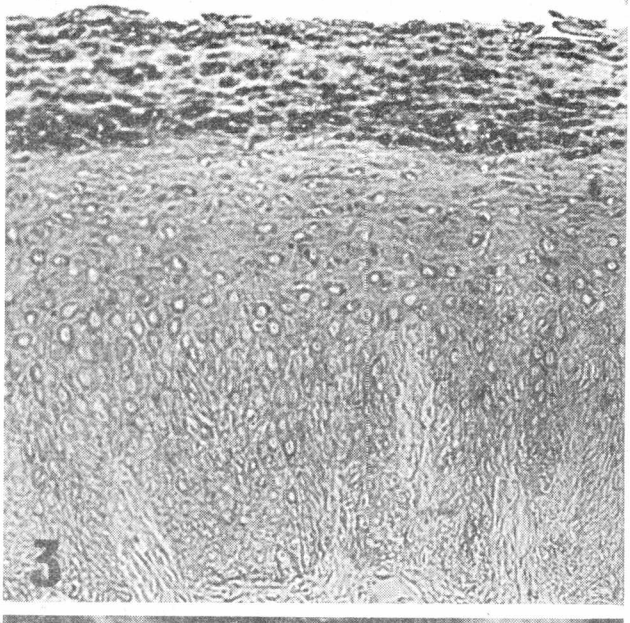
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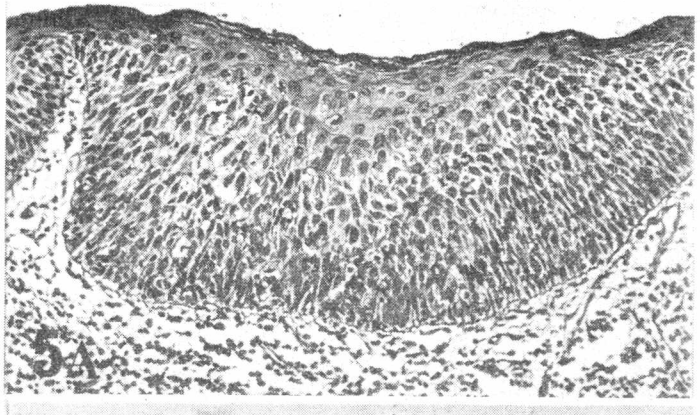
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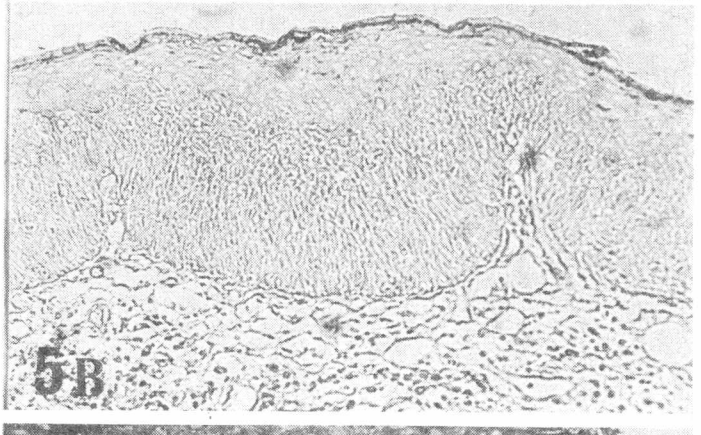
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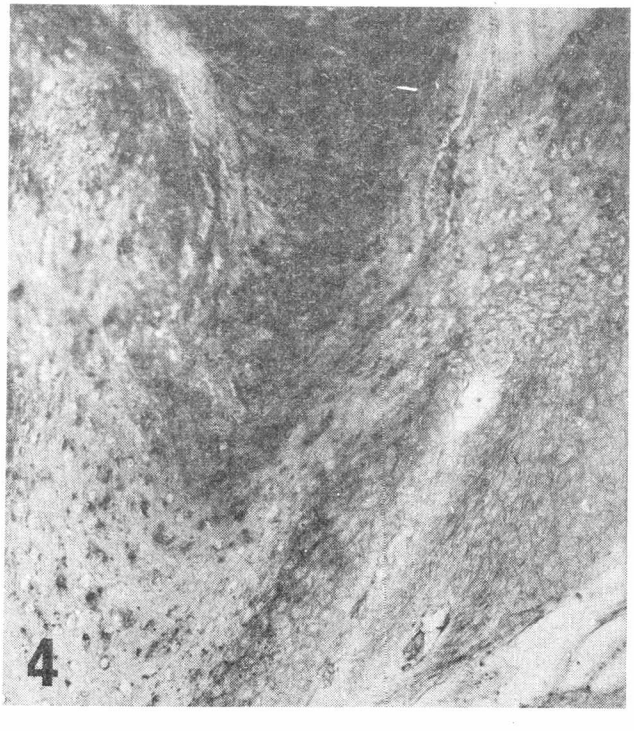
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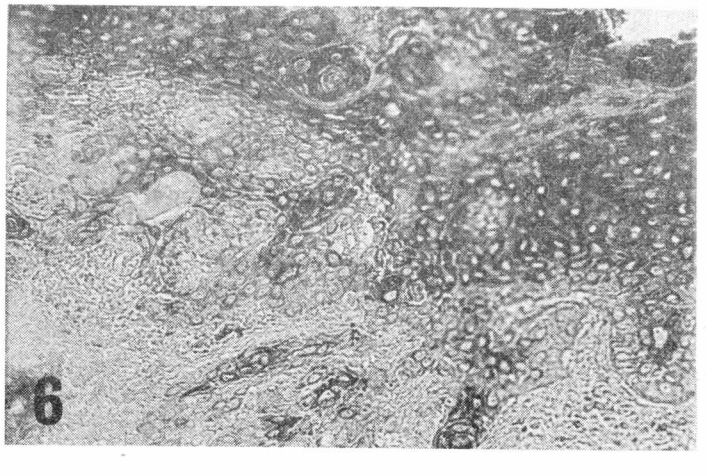
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