Ultrastructure of Epitheliocytes Oral Mucosa from Washings

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Electron microscopy of the main forms of epitheliocytes in washings from the oral cavity of patients with contact denture stomatitis and denture intolerance showed that most epitheliocytes were in a state of parakeratosis. These data are important for dynamic evaluation of the oral mucosa status during orthodontic treatment.

Key Words: *oral mucosa; epitheliocytes in washings; electron-microscopic analysis*

Relationship between the state of oral mucosa and viscera during exposure to different orthodontic constructions and materials have been described in detail [4-6,9-11,13].

Oral mucosa reacts to external factors [1-3,12] by hornification of the epithelium (hard palate, alveolar processes) or by leukocyte infiltration (cheeks, gingiva, soft palate). The intensity of these processes depends on changes in the internal or external exposures.

Analysis of cytological material obtained by washing helps to evaluate the status of the oral mucosa by the leukocyte/epitheliocyte ratio. The leukocytic formula and quantitative analysis of epithelial cells at different stages of development or degeneration are important.

We analyzed ultrastructure of the buccal mucosa epitheliocytes obtained by washing in patients in need of orthodontic treatment.

MATERIALS AND METHODS

Washing from the oral cavity were taken from patients with contact denture stomatitis and denture intolerance. Specimens for electron microscopy were prepared as described previously [7,8]. Centrifugate was fixed in 4% paraformaldehyde prepared on phosphate buffer (pH 7.2-7.4) at 4°C for 4 h. After fixation the pellet formed clumps or flakes, which makes impossible its embedding in epoxide resins. That is why the material in fixative was repeatedly centrifuged for 5 min at 600*g*, after which the fixative was carefully removed and the precipitate was washed in phosphate buffer pH 7.2-7.4. If the precipitate still disintegrated into flakes, the procedure was repeated, after which the tube walls were dried with filter paper to remove fluid. Several drops (depending on the volume of precipitate) of 10% gelatin in water heated to 40°C was mixed with the precipitate. The mixture was cooled to 4°C for 1 h and 1-mm fragments were cut on ice and fixed in 4% paraformaldehyde, after which the samples were treated routinely for electron microscopy.

Semithin (1 μ) sections were made in a Tesla-B microtome and stained with azur II. Ultrathin sections were made on an LKB III ultramicrotome, contrasted with uranyl acetate and lead citrate according to Reynolds, and examined under a JEM 100B electron microscope at accelerating voltage of 60 kV at magnifications of 5,000-25,000.

RESULTS

Electron microscopy of specimens from patients with denture intolerance or contact stomatitis showed several types of cells at various stages of hornification. Some cells, pricky layer epitheliocytes, had irregular stellate shape. Cytoplasmatic membrane formed large finger-like protrusions. The nuclei of oval cells with small invaginations lay centrally, and large chromatin granules were condensed along the nuclear membrane. The cytoplasm contained numerous tonofilament bundles, mitochondria with signs of degeneration, lipid drops,

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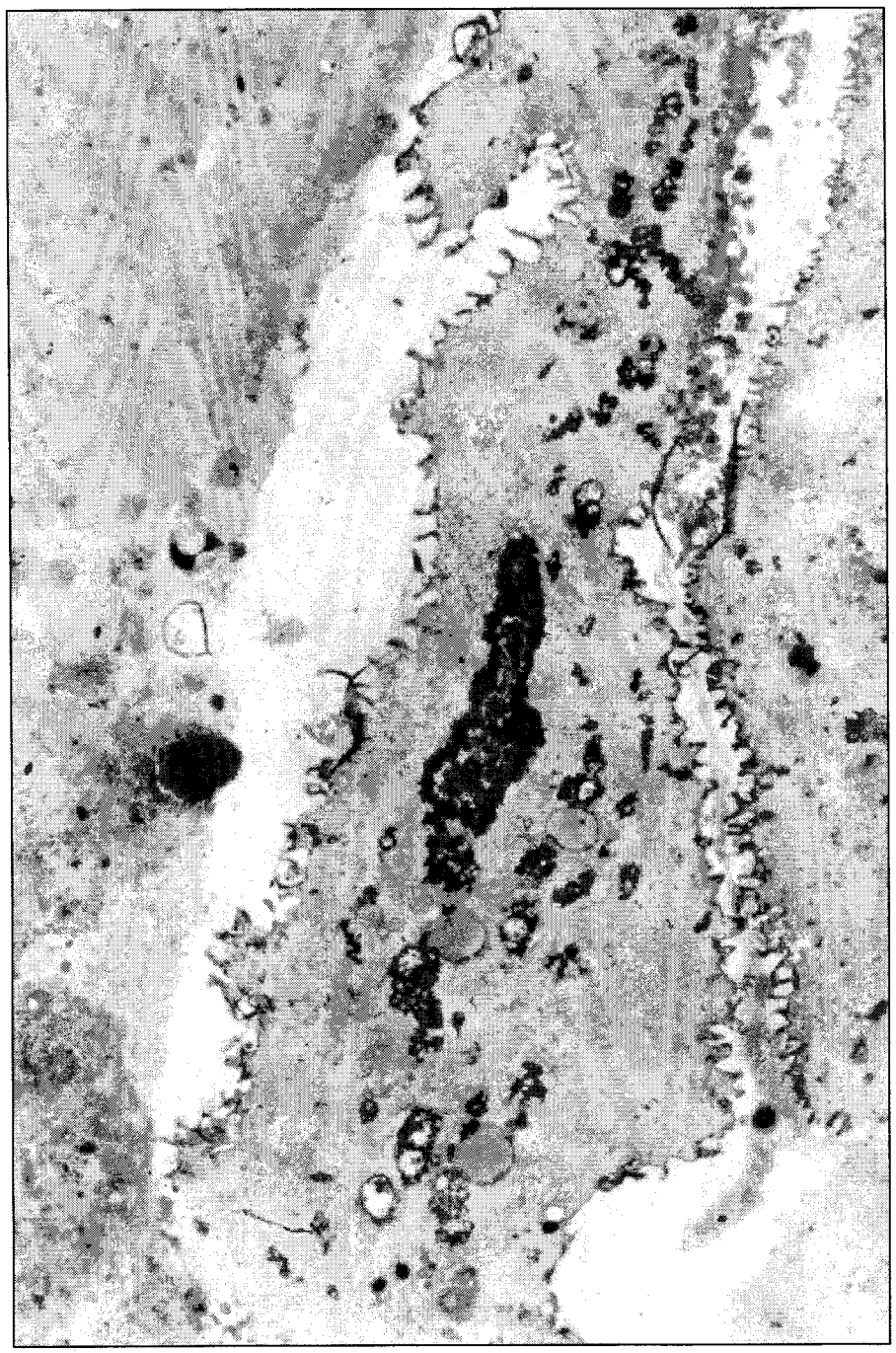

Fig. 1. Epitheliocyte in a state of parakeratosis from oral washing of a patient with contact acrylic stomatitis. Keratohyalin granules, destroyed mitochondria, and small lipid drops in the cytoplasm. ×2000.

glycogen and keratohyalin granules; elements of cytoplasmatic reticulum were seen.

The majority of cells were in a state of parakeratosis (Fig. 1). Cells were flattened, plasma membrane processes were reduced and shortened. The number of keratohyalin granules in these cells was increased and cytoplasmatic organelles were reduced. Elongated nucleus with condensed chromatin and clarified perinuclear zone of the cytoplasm are important characteristic features of cells in parakeratosis. Karyorrhexis was observed in some cells.

Cell population second in number are epitheliocytes in a state of keratinization (Fig. 2). These cells were irregularly shaped and occurred in washings as small plasts. Their cytoplasmatic membrane formed numerous processes of different shape and size. Neighboring cells in the epithelium formed zip profiles without coming in contact. Light keratinocyte cytoplasm contained fine granules and tonofilaments, small keratohyalin granules, solitary lipid drops, and degenerated mitochondria. The nuclei of such cells were almost lysed or fragmented. Individual or multiple microorga-

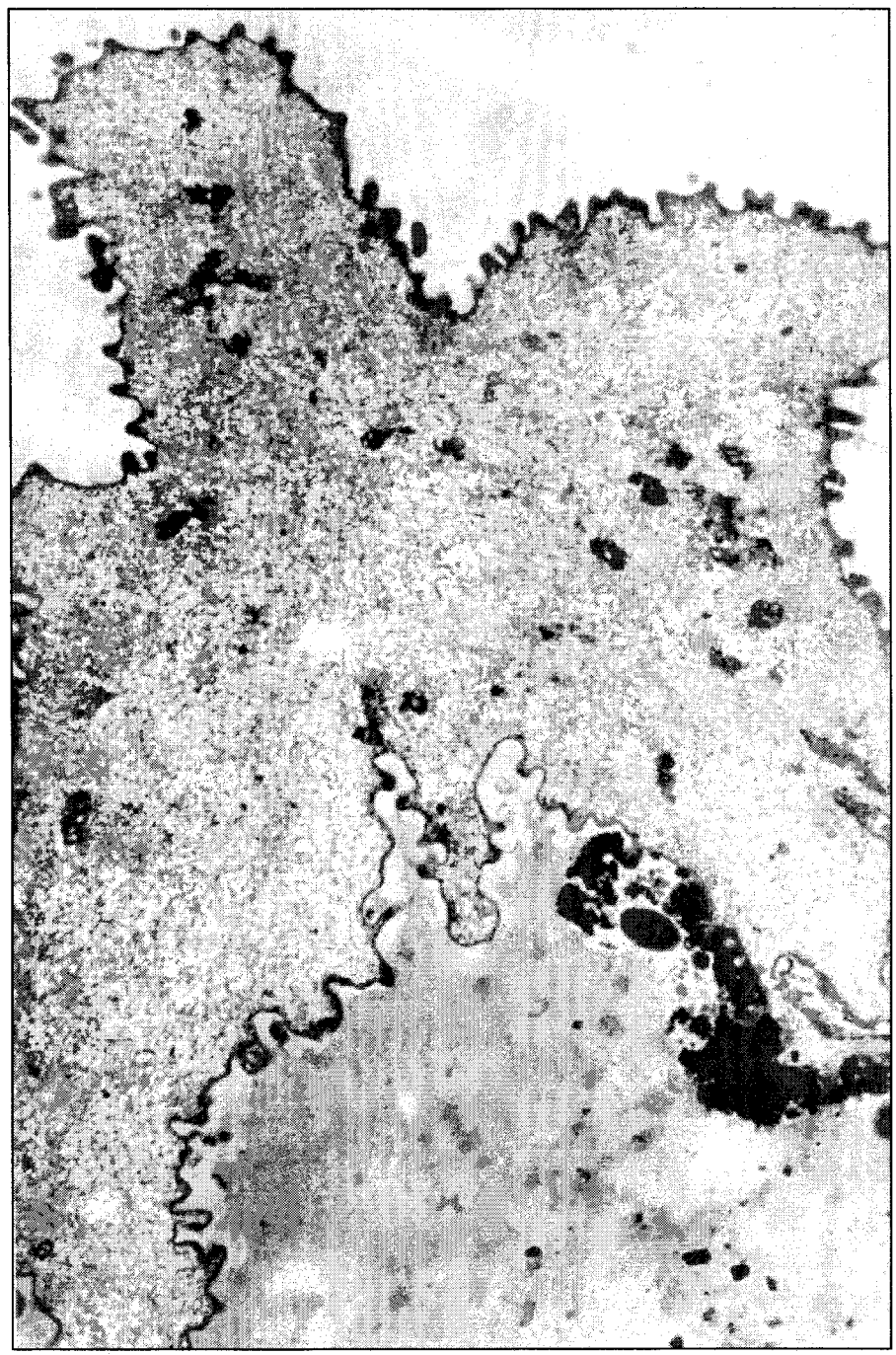

Fig. 2. Anulclear epitheliocyte of irregular shape from surface epithelial layer from a patient with denture intolerance. ×5000.

nisms (cocci, diplococci) were seen on the surface of solitary keratinized epitheliocytes, but no microflora was found in these cells.

Solitary cells in washings were presented by structures resembling horny scales. These irregularly shaped formations without organelles or nuclei rudiments contained mainly electron-dense substance. It is noteworthy that the ratio of epitheliocytes with different degree of keratinization was individual in each case.

Solitary oval granules with segmented nuclei were seen in some cases. Condensed chromatin was arranged along nuclear membrane and the cytoplasm contained numerous mitochondria, osmiophilic granules, and phagosomes.

Hence, the majority of exfoliative epitheliocytes in oral washings from patients with contact denture stomatitis and denture intolerance were in the state of parakeratosis, which indicated disordered hornification processes [1]. Few cells of the pricky layer were indicative of deeper local lesions of the epithelium. The results will help to develop morphofunctional criteria for evaluating the status of oral mucosa before and after orthodontic treatment and to correct it.

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