Structural Reactions of the Buccal Mucosa in Diabetic Parodontopathy

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Pronounced changes in the capillaries, hemodynamic disorders, epitheliocyte degeneration and atrophy develop in the buccal mucosa of patients with types I and II diabetes mellitus in the absence of inflammatory cellular infiltration or with facultative infiltration. The morphogenesis of pathological changes can be regarded as primary diabetic microangiopathy causing metabolic disorders with the development of degenerative and atrophic changes in all structural components of the buccal mucosa and development of diabetic parodontopathy, a primary degenerative process.

Key Words: *types I and II diabetes mellitus; buccal mucosa; biopsy; scraping off; ultrastructure*

Diabetes mellitus and peptic ulcer occupy a special place among the diseases which have a direct impact on the periodontal status [2,7]. Diabetes mellitus in the majority of cases is associated with chronic generalized periodontitis with frequent exacerbations and short remissions; in addition, the periodontium is often damaged under the effect of various unfavorable factors [1,8,9,11,12]. The main manifestations of diabetes are hyperglycemia and the resultant vascular disorders [10,15], leading to the development of severe ischemia of various organs [5,14].

We studied buccal mucosa epitheliocytes and endotheliocytes, specifically, vascular-epithelial interactions.

MATERIALS AND METHODS

Biopsy specimens (up to 1 mm^3) of the mucosa from the mandibular and maxillary alveolar processes (n=64) were collected during tooth extraction. Twenty-nine of these were collected from diabetics with types I and II disease, the rest formed the reference group of the same age and sex.

The material was fixed in 4% paraformaldehyde in Millonig phosphate buffer. Paraffin sections were stained by hematoxylin and eosin, by Schiff reagent, and after Van-Gieson.

Cytological material from the oral cavity (washout fluid) was also examined [13]. Oral washout fluid for electron microscopy of the cell elements was processed by the modified method [4] for bronchoalveolar lavage. After centrifugation the precipitate was embedded in gelatin, fixed in paraformaldehyde, and embedded in a mixture of epoxy resins. Semithin sections were stained with Azur II, ultrathin sections were contrasted with saturated ethanol solution of uranylacetate and lead citrate, and examined under a JEM 1010 electron microscope at accelerating voltage of 80 kV.

RESULTS

Diabetes was associated with significant changes in the periodontium in the majority of cases. Hypere-

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mia of the gingival mucosa and hemorrhages of different severity were observed. The interdental papillae were as a rule hypertrophic, bright red or cyanotic, which attests to chronic hemodynamic disorders. In case of stubborn lasting disease the interdental papillae shrank because of atrophy and the integrity of the dental ligamentous system was impaired: deep gingivodental pouches formed and granulation tissue growth was sometimes observed.

In some cases even the debut diabetes mellitus was associated with periodontitis (generalized bone tissue and mucosa atrophy without signs of inflammation). The gingivodental circular ligament was retained and there were no gingivodental pouches. This was followed by the development of pronounced atrophy of the maxillary alveolar processes with tooth mobility. Intact periodontium in diabetes mellitus was observed in just solitary cases, as a rule in young patients with short duration of the disease, in a compensated status and receiving adequate therapy.

Acanthosis with elongation of the epithelial processes, sharply plethoric subepithelial capillaries, edema and focal inflammatory cell infiltration of the lamina propria were observed in diabetics with disease history of 1-5 years (Fig. 1, *a*).

Patients with disease history of 5-15 years and/or with predominating atrophic changes in biopsy specimens presented with thinned mucosa of the alveolar processes, reduced and sclerosed subepithelial capillaries, pronounced stromal fibrosis (Fig. 1, *b*), atrophy of the multilamellar squamous epithelium with hornified foci and, importantly, without inflammatory cell reaction.

Electron microscopy of cell populations in the mucosa biopsy specimens of the alveolar processes showed degenerative changes in the epitheliocytes and endotheliocytes and certain synchronization of their ultrastructural changes in the course of disease development. Degenerative changes of epitheliocytes involved the nuclear and cytoplasmic compartments: the number of nucleoli in the basal and pricky cells decreased; karyopyknosis, reduction of protein-synthesizing organelles, disorganization of mitochondrial cristae, decreased number of tonofilaments, and destruction of the cell-cell contacts were sometimes observed. Endotheliocytes of the subepithelial microcirculatory bed were characterized by sharp thinning of the cytoplasmic processes, condensation of the cytoplasmic matrix, decreased number of membrane organelles, and decreased pinocytic activity.

Polymorphism of structural changes in the mandibular and maxillary alveolar processes mucosa was noted in the reference group; the degree of multilamellar squamous epithelium keratinization was different.

Electron microscopy of the oral washout fluid showed two variants of ultrastructural changes in cell populations associated with the pathological changes in the mucosa in diabetes: predominating inflammatory or atrophic changes.

Epitheliocyte polymorphism, observed in patients with the former variant, reflected different degree of keratinization of these cells (hornification). The majority of epitheliocytes had a nucleus (Fig. 1, *c*), their shape was irregular (stellate) with numerous cytolemma invaginations; sometimes the nuclei had signs of different stages of degeneration. The cytoplasm contained numerous short tonofilaments, few mitochondria with different degree of cristae disorganization, small lipid droplets, solitary elements of the Golgi complex, and glycogen grains and keratohyalin granules. In addition, macrophages, lymphocytes, and granulocytes were present in the washout fluid. Granulocytes were oval, had lobular nuclei with marginal heterochromatin lumps and numerous lysosomes, mitochondria, and solitary autophagosomes.

Some epithelial cells were in a state of parakeratosis, which was associated with flattening of the cells, shrinkage and shortening of the cytoplasmic membrane processes, increase in the number of keratohyalin granules, decrease in the number of membrane cytoplasmic organelles. An important feature of epitheliocytes in a state of parakeratosis was elongated nucleus with compact heterochromatin and devastation of the perinuclear cytoplasm.

Epitheliocytes in a state of keratinization predominated in the washout fluid in atrophic variant of structural changes in the buccal mucosa. Hornified epitheliocytes, integrating by means of numerous desmosomes, often looked like small plasts. Keratinocyte nuclei were lyzed or presented by separate heterochromatin lumps, the cytoplasm contained numerous short tonofilaments; small keratohyalin grains, solitary lipid droplets, destroyed membrane organelles were seen in some cells. Solitary or numerous microorganisms, mainly cocci and diplococci, were located near the cytolemma of some hornified epitheliocytes (Fig. 1, *d*). No microflora was seen in the epithelium.

Solitary cells in the washout fluid were presented by structures resembling horny scales without cytoplasmic organelles and nucleus, with electron dense matrix; these were the rudiments of degenerative epitheliocytes. On the whole, electron microscopy of the cell populations in the oral washout fluid from patients with diabetic parodonto-

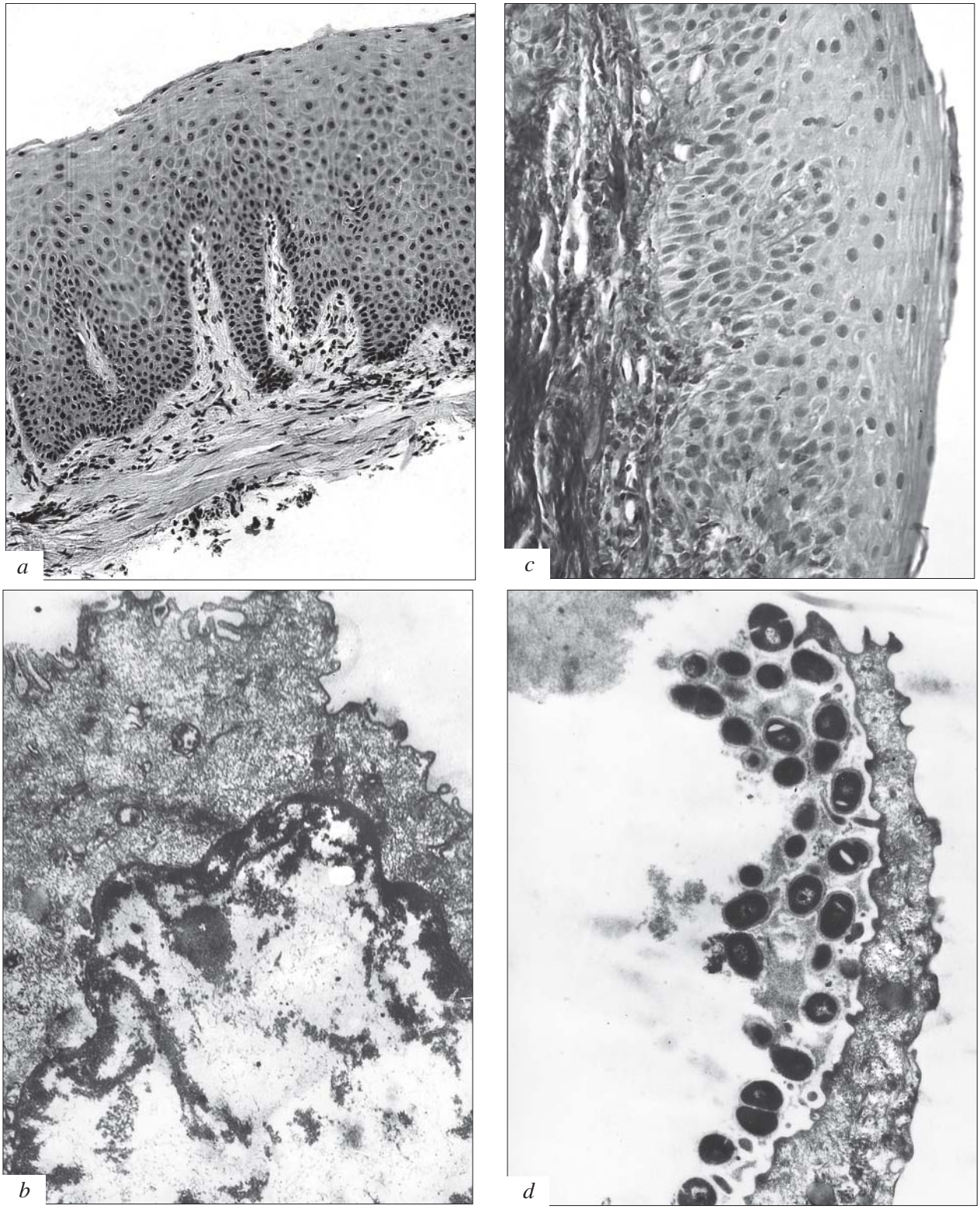

Fig. 1. Optical and ultrastructural characteristics of buccal mucosa cell populations in diabetic parodontopathy. *a*) acanthosis of the epithelial layer and minimum cell infiltration. Hematoxylin and eosin staining, ×350; *b*) fibrosis of lamina propria. Van-Gieson staining, ×650; *c*) degenerative epitheliocyte: chromatin margination, devastation of cytoplasmic matrix, ×6000; *d*) cocci and diplococci near the cytolemma of a hornified epitheliocyte, ×15,000. *a*, *b*: biopsy specimens of maxillary alveolar process mucosa; *c*, *d*: oral washout fluid, electronograms.

pathy showed predominating epitheliocytes with ultrastructural signs of keratosis and parakeratosis.

Hence, complex comparative analysis of structural changes in the alveolar process mucosa showed that diabetes mellitus was characterized by pronounced changes in the capillaries and hemodynamic disorders, significant degeneration and atrophy of epitheliocytes, without or with facultative inflammatory cell infiltration.

Based on the findings, the morphogenesis of pathological changes in the buccal mucosa in diabetes mellitus can be regarded as primary diabetic microangiopathy, causing metabolic disorders with the development of degenerative and atrophic changes in all structural components of the buccal mucosa and formation of diabetic parodontopathy, a primary degenerative process [3]. The inflammatory reaction is presumably secondary and reflects failure of defense reaction of the epithelial barrier and body in general [6].

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