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MORPHOLOGY OF THE ORAL MUCOSA IN RATS EXPOSED TO HIGH CONCENTRATIONS OF PHOSPHORUS

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Attempts have recently been made to study the most general features of injury caused to cells, tissues, and organs by exposure to elementary phosphorus and its inorganic compounds [1, 2, 4]. The study of the morphology and function of the oral mucosa under these conditions is particularly interesting because of the known fact that phosphorus compounds are retained by the mouth fluids [3].

The aim of this investigation was to study the morphology of the oral mucosa in rats after long-term exposure to the atmosphere of a phosphorus factory.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 120-140 g. The animals were kept in the furnace room of a phosphorus factory. Rats in the experimental groups were poisoned for 4 h daily, 5 days a week. Control animals were kept in an animal house away from the factory. The animals were decapitated 1, 2, 3 and 4 months after the experiment began. The mucous membranes of the cheek, gum, and hard palate were dissected from underlying tissues, fixed in 10% neutral formalin solution, and embedded in paraffin wax. The animals' tongues were washed initially in the fixing solution, then fixed and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin and with picrofuchsin. Pieces of mucosa from the cheek, gum, and hard palate, and the tip of the tongue were fixed in 4% paraformaldehyde solution, postfixed in 2% OsO_4 solution, dehydrated with alcohol, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-1 ultrotome, stained, and examined in the GEIL 7A electron microscope.

EXPERIMENTAL RESULTS

The structural integrity of the epithelium of the oral mucosa was preserved 1 month after the experiment began. In some cases thickening of the epithelium of the buccal mucosa was observed along the line of contact of the teeth. A moderate degree of hyperkeratosis was observed on the dorsum of the tongue, and in some cases the proliferating stratum corneum sometimes reached as far as the apices of the filiform papillae. The surface of the tongue

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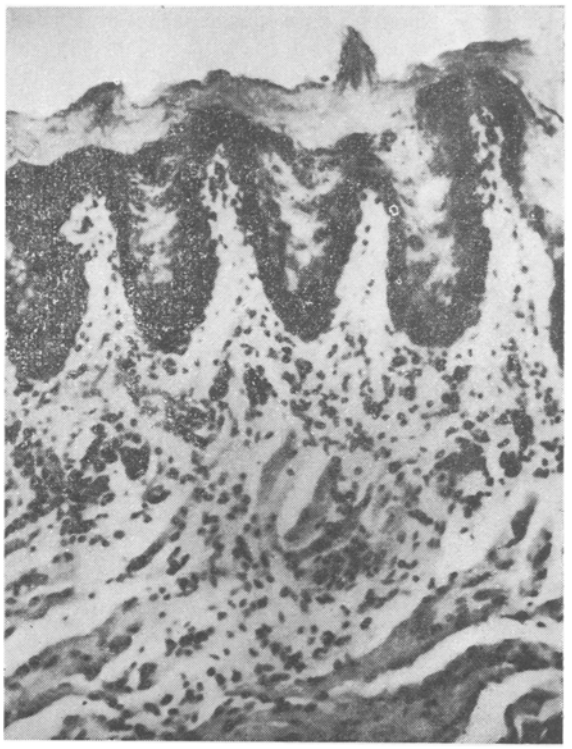

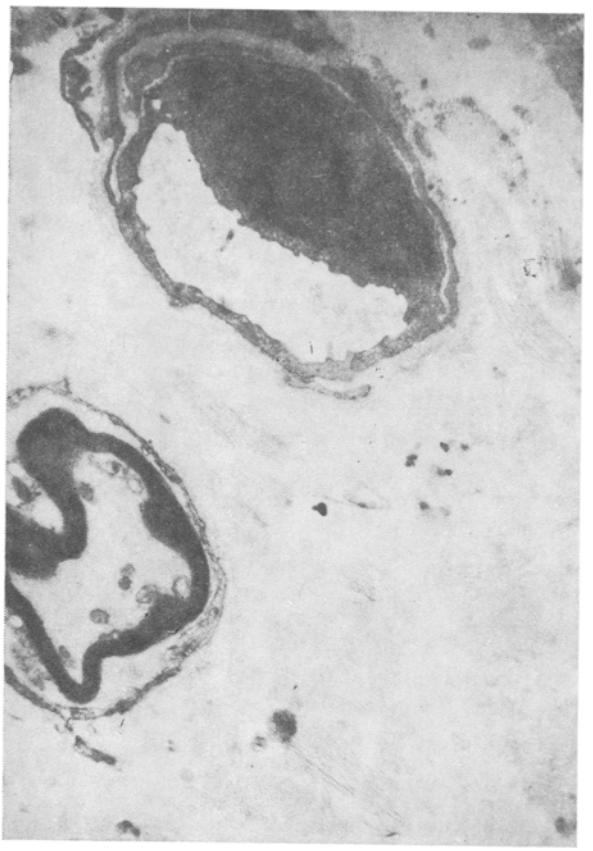

Fig. 1 Fig. 2

Fig. 1. Foci of concentration of lymphocytes and macrophages in connective-tissue layer of the tongue. Hematoxylin and eosin. 14 ×.

Fig. 2. Connective-tissue layer of lingual mucosa (exposure for 2 months to conditions of phosphorus manufacture). Capillary. Increased permeability of capillary wall, pericapillary edema of stroma. Loosening and disorientation of collagen fibers. 3800 ×.

appeared smoother in the experimental animals than in the controls. The stratum spinosum contained 7-12 rows of cells compared with 5-9 rows in the control. The number of rows of cells in the stratum spinosum of the epithelium and in the buccal mucosa was increased. At the ultrastructural level hyperkeratosis of the epithelium of the mucosa was manifested as enlargement of the tonofibrillary-keratohyaline complexes and hyperplasia of the endoplasmic reticulum in the cytoplasm of the epitheliocytes, and hypertrophy of the cell nuclei.

In the second month from the beginning of the experiment the morphological changes in the oral mucosa were more pronounced. The epithelium of the mucosa of the gum, cheek, hard palate, and tongue was thickened. The number of cell layers in the epithelium was greater both than the control and than at the previous time of investigation. As a result of massive hyperkeratosis, the proliferating stratum corneum completely covered the apices of the papillae of the tongue in seven fields of vision.

In the subepithelial connective tissue base of the gum and tongue hyperemia of the blood vessels, pavementing of the leukocytes, and their migration into the perivascular spaces were observed; sometimes small focal concentrations of lymphocytes and macrophages appeared (Fig. 1). Similar changes were found in the connective tissue of the submucosa of the cheek and the glandular zone of the hard palate, but in fewer cases. Edema of the perivascular ground substance and more intensive fuchsinophilia of the collagen fibers were observed in the submucosa of the cheek and tongue. There was an increase in the number of mast cells, which were located mainly alongside blood vessels.

Electron-microscopic investigation confirmed the presence of vascular disturbances in the form of increased permeability of the walls of the capillaries and postcapillary venules.

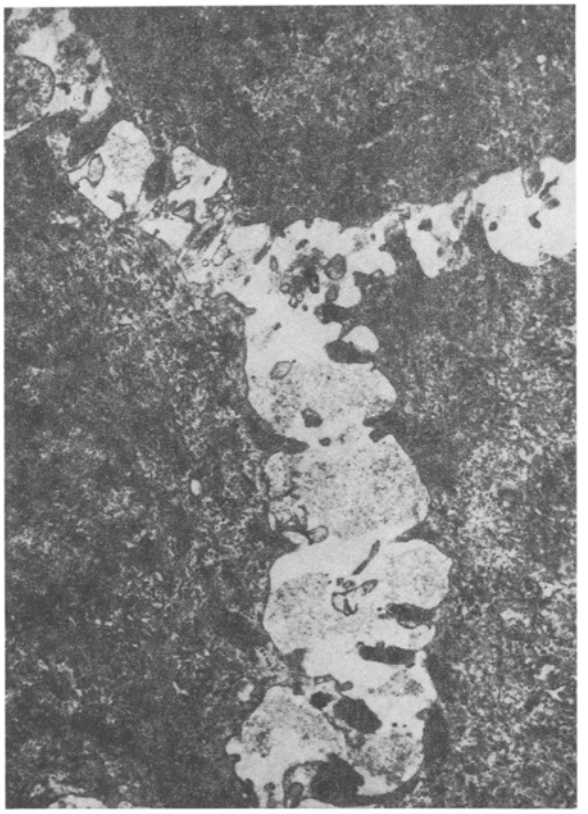

Fig. 3. Epithelium of lingual mucosa of albino rats (exposure for 4 months to conditions of phosphorus manufacture). Epitheliocytes of stratum spinosum. Widening of intercellular spaces and reduction in number of desmosomes as a result of intercellular edema. 4920 ×.

accompanied by outflow of plasma into the pericapillary space (Fig. 2). A picture of activation of endotheliocytes, increased microvesiculation, the formation of space between the endothelial cells, and loosening of the basement membrane were found. These changes were accompanied by perivascular edema of the stroma. The collagen fibers were disoriented and fragmented, and edema of the amorphous substance causing their adhesion was observed.

All the changes indicated above were found in the experimental animals but not in the controls.

In the third month after the experiment began changes in the oral mucosa continued to progress. Hyperkeratosis in the mucosa of the cheek and hard plate remained pronounced. In the lingual mucosa hyperkeratosis was found episodically and was moderate in degree. Compared with the results of poisoning for 1-2 months, the filiform papillae on the dorsum of the tongue were clearly visible and projected above the surface of the stratum corneum of the epithelium. The histological picture of the structure of the gingival epithelium was conflicting in character. Besides hyperkeratosis, there were also areas where the epithelial layer was thinner, usually on the free surface of the gum.

In the connective-tissue base of the gum, cheek, and tongue, hyperemia accompanied by increased porosity of the vessel wall and diapedesis of the blood cells into the surrounding tissues, perivascular edema also was found. The fibrillary structure here were loosely packed, and the swollen bundles of fuchsinophilic collagen fibers were disoriented and in some places fragmented.

Analysis of the electron-microscopic data at this stage of the investigation and their comparison with previous times showed that, besides the ultrastructural components of hypertrophy of the epithelial cells, there were also epitheliocytes with a reduced number of tono-

fibrils and keratin granules, profiles of the endoplasmic reticulum, and free polysomes in their cytoplasm. In some cases widening of the intercellular spaces and a reduction in the number of desmosomes in the stratum spinosum of the epithelium of the gum, teeth, and tongue were found. In the connective tissue, both in the mucosa and in the submucosa, microcirculatory disturbances and changes in the walls of the blood vessels similar to those described previously, but more pronounced and occupying greater areas of tissue, were observed. Foci of infiltrating cells were located mainly along the course of the vessels and consisted of lymphocytes, macrophages, and a few mast cells. Among the lymphocytes activated forms were frequently observed; the macrophages contained numerous primary and secondary lysosomes and phagolysosomes in their cytoplasm.

Toward the end of the experiment, in the 4th month, analysis of histological sections and of the electron-microscopic data confirmed the picture of morphological changes found in the epithelium and connective tissue at the previous time in most cases. At the same time, there were certain differences. Besides hyperkeratosis, areas of atrophy, accompanied by intensive desquamation of cells of the surface layer, appeared in the epithelium of the gum, cheek, and hard palate. Dystrophic changes, leading sometimes to necrobiosis and necrosis of the epitheliocytes of the stratum spinosum and stratum basale, also became more pronounced. Besides the patterns of disturbance of the microcirculation and connective tissue described above, small foci of perivascular sclerosis were formed in the connective-tissue layers of the gums, cheek, and sometimes, the hard palate.

Ultrastructural analysis showed widening of the intercellular spaces and a reduction in the number of desmosomes in epitheliocytes of the stratum spinosum of the gingival and buccal epithelium (Fig. 3). Dilation of the lumen of the vessels with accumulation of erythrocytes in them, edema of the stroma, and widening of the spaces between the endothelial cells were discovered in the connective tissue. Besides diapedesis of the erythrocytes and leukocytes, the dystrophic changes progressed in the endotheliocytes, with swelling of their cytoplasm, reduction of their organoids, and fatty degeneration.

The results indicate a definite time course of the morphological changes in the different tissue structures of the mucosa. In most experimental animals the surface epithelium and connective-tissue base of the mucosa in different parts of the oral cavity responded uniformly. At the beginning of the experiment an increase in the number of cells in the stratum spinosum and intensification of its keratinization, leading to the development of hyperkeratosis were observed in the epithelium of the mucosa. Toward the end of the experiment dystrophic and atrophic changes were observed in the epithelium, leading in some places to a decrease in thickness of the epithelial layer.

Disturbances of the microcirculation and dystrophic lesions in the walls of the blood vessels were observed in the connective tissue in the mucosa and submucosa, and by the end of the experiment they were pronounced.

These observations can be interpreted as the result of direct exposure of structures of the oral mucosa to elementary phosphorus and its inorganic compounds.

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