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CHANGES IN THE ULTRASTRUCTURAL ELEMENTS OF PERIODONTAL NEUROTROPHY UNDER CONDITIONS OF ACUTE SIMPLE COAGULATION DYSTROPHY IN THE EXPERIMENT

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ABSTRACT

Aim: To determine the role of damage to the ultrastructural elements of the periodontal nervous system in the pathogenesis of dystrophic periodontal disease.

Materials and Methods: The basis of the experimental part of the study was the preparation of ultrathin sections from blocks of gum tissue of white rats, which were prepared using the UMTP-3M device. The study and analysis of biopsy samples was carried out with the help of an electron microscope UEMV-100K.

Results: With the help of transmission electron microscopy, it was found that from the first minutes after the injection of hemolysate of isogenic erythrocytes into the rats, aggregates of erythrocytes, clumps of blood plasma, clusters of fibrin monomer masses, bundles of fibrin fibers, platelet and homogeneous were present in the connective tissue of the gums, and in particular in the lumens of hemocapillaries microthrombi, which confirms damage to the ultrastructures of the periodontium, which lead to the development of a pathological process, which is described when simple coagulation dystrophy is reproduced.

Conclusions: Coagulative damage to the ultrastructural elements of the periodontal nervous system is one of the important factors in the pathogenesis of dystrophic periodontal damage. Under these conditions, trophic disturbances occur, similar to those that occur when the integrity of the nerve is disturbed – neurotrophic mechanism of dystrophy.

KEY WORDS: Coagulation dystrophies, generalized decompensated thrombinogenesis, periodontium, nerves of the gingival mucosa membranes

INTRODUCTION

Many works in the modern medical literature are dedicated to the study of the mechanisms of periodontal tissue damage [1-9]. It is also known that the damage of parenchymal organs in many diseases has a coagulation-hypotrophic genesis [10-12]. As a result of the processing of scientific and informative material, protocols for providing therapeutic assistance to dental patients and taking into account our own experience, a classification of periodontal diseases is proposed, in which the varieties of coagulation periodontitis can be divided into the following types, of namely – coagulation periodontosis are distinguished: coagulation periodontosis (simple), inflammatory-coagulation periodontosis, immune-coagulation periodontosis, age-related coagulation periodontosis (physiological aging of the periodontium) [12-14]. One of the main mechanisms of the development of these periodontal diseases is the coagulation-hypotrophic mechanism. This coagulation-hypotrophic mechanism is responsible for the development of coagulation (degenerative) damage caused by the direct action of thrombin on periodontal structures and simultaneously includes three mechanisms of trophic reduction: enzymopathic, discirculatory and neurotrophic [10, 11, 15].

AIM

To determine the role of damage to the ultrastructural elements of the periodontal nervous system in the pathogenesis of dystrophic periodontal disease.

MATERIALS AND METHODS

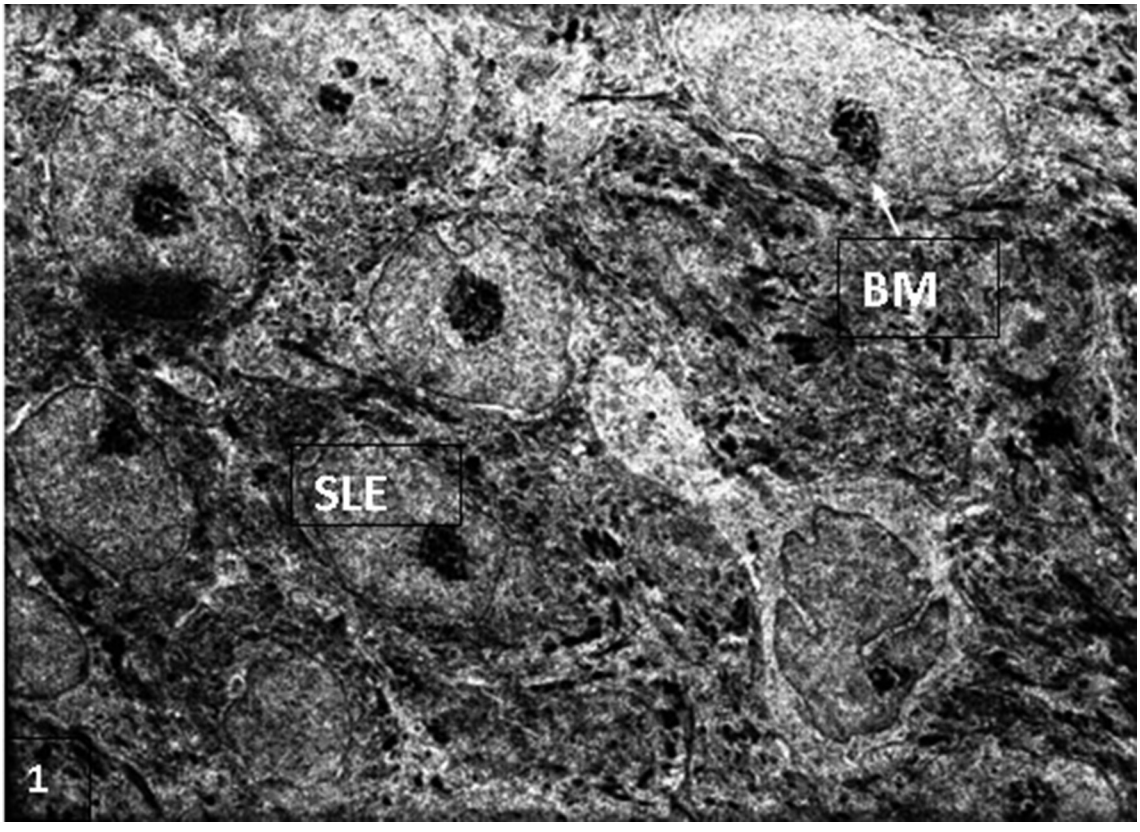
In an experiment on white rats, a model of acute simple coagulation dystrophy (generalized decompensated thrombinogenesis (GDT)) was reproduced by intravenous administration of hemolysate of isogenic erythrocytes in a dose of 25 ml/kg to white rats (55 rats) [16]. During

the research, the animals were killed by decapitation, at intervals of 15 and 30 minutes, and 2, 5, and 24 hours after the infusion of isogenic erythrocyte hemolysate, biopsies (tissue material of mucous membrane of the gums) were taken for the purpose of preparing electron microscopic samples for further studies. For the purpose of comparison, the gingival mucosa of the control group of 5 intact white rats was used. Bioptates of the gingival mucosa were fixed in a 2% solution of osmium tetraoxide in a 0.1 M phosphate buffer solution (pH 7.36) for 2 hours at (an ice-melting) temperature of 0 °C. Fixed blocks of gingival tissue were washed in chilled distilled water, dehydrated in solutions of increasing concentrations of ethyl alcohol and acetone, and soaked in a mixture of epon and araldite resins [17]. Ultrathin sections from blocks of gingival tissue were prepared using the UMTP-3M device. After that, the prepared sections were sequentially contrasted in solutions of uranyl acetate [18] and lead citrate [19]. Studying and photographing the material was carried out using an electron microscope UEMV-100K (Ukraine).

RESULTS

With the help of transmission electron microscopy, it was found that from the first minutes after the injection of hemolysate of isogenic erythrocytes into the rats, aggregates of erythrocytes, clumps of blood plasma, clusters of fibrin monomer masses, bundles of fibrin fibers, platelet and omogeneous were present in the connective tissue of the gums, and in particular in the lumens of hemo-capillaries microthrombic damaged organelles, precipitates, and coagulates were found in capillary endotheliocytes. The main substance and collagen fibers, which were adjacent to the capillaries, were disorganized. In the main substance, among the disorganized collagen fibers, there were mucoid and fibrinoid masses, and in the cells – precipitates, coagulates or continuous cytogel. Fibroblasts and macrophages had an increased electron density of the protoplast, significant numbers of autophagolysosomes were also found in them. Changes and degranulation of mast cells were observed, but this was accompanied by significantly less damage to non-cellular and cellular elements adjacent to them. At the same time, significant damage to the structures of epithelial cells was detected in the form of loosening (loss of contour) of the membranes of various organelles. An increase in the electron density of these cells and disruption of intercellular contacts were characteristic features. The most pronounced changes were found in the cells of the spinous, and especially the basal, layers of the epithelium, while they were less pronounced in the cells of the granular and horny layers. The state of the gum's mucosa ultrastructure of a white rat during the experimental simulation of simple coagulation dystrophy of the periodontium is shown in (Fig. 1).

The activity of mitosis at the cellular level decreased and was 10.25±0.57%, being at normal 16.67±0.41% (Fig. 2).



Disorganized cells of the spinous layer of the epithelium (SLE) and the basement membrane (BM) of the gums.

Fig. 1. The ultrastructure of disorganized cells of the spinous layer of the epithelium (SSC) and the basal membrane (BM) of the gingiva for 2 h of the experiment. Coll. x2000.

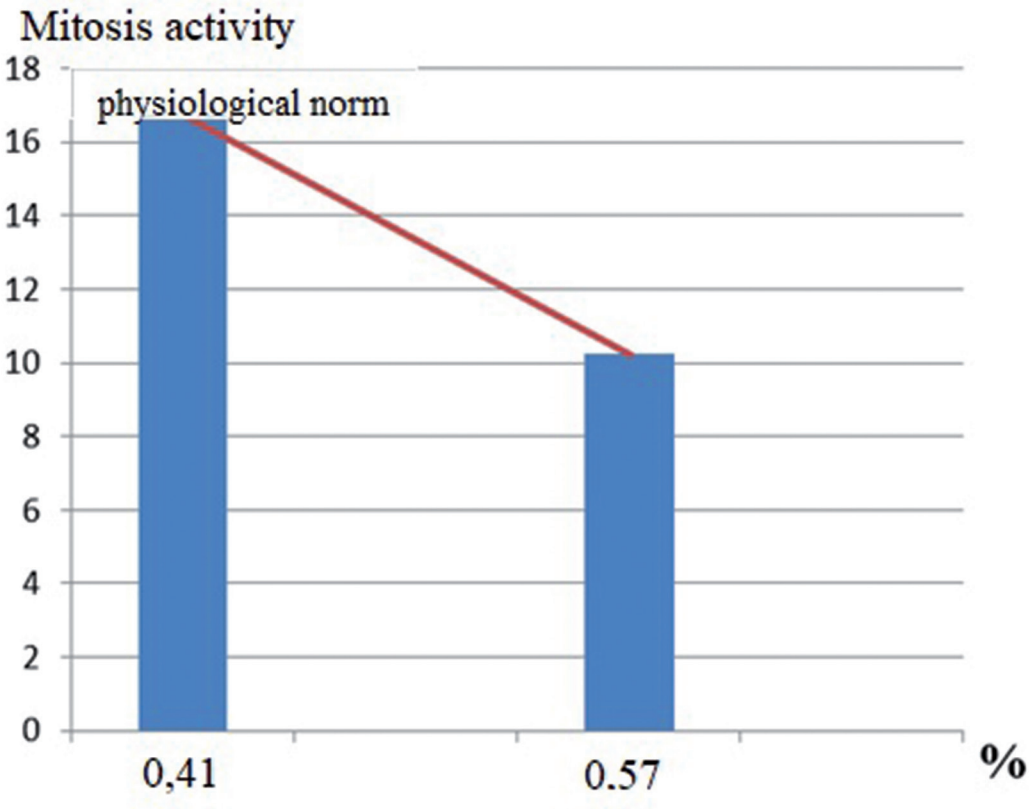


Fig. 2. Evaluation of mitotic activity compared to the physiological norm.

Mitosis is an important component of the process of growth, development of cells and tissues, and thus is an important component of restorative (reparative) processes. In the case of dystrophic processes, which were artificially induced in the periodontal tissues of experimental animals, a decrease in the mitotic activity of cells was established, as a result of which a decrease in the growth point of tissues was observed in the periodontium at the cellular level.

With the help of transmission electron microscopy, it was found that from the first minutes after the injection of hemolysate of isogenic erythrocytes into the rats, aggregates of erythrocytes, clumps of blood plasma, clusters of fibrin monomer masses, bundles of fibrin fibers, platelet and homogeneous were present in the connective tissue of the gums, and, in particular, in the lumens of hemo-capillaries microthrombi, damaged organelles, precipitates, and coagulates were found in capillary endotheliocytes. The main substance and collagen fibers, which were adjacent to the capillaries, were disorganized. In the main substance, among the disorganized collagen fibers, there were mucoid and fibrinoid masses, and in the cells – precipitates, coagulates or continuous cytogel. Fibroblasts and macrophages had an increased electron density of the protoplast, significant numbers of auto-phagolysosomes were also found in them. Changes and degranulation of mast cells were observed, but this was accompanied by significantly less damage to non-cellular and cellular elements adjacent to them. At the same time, significant damage to the structures of epithelial cells was detected in the form of loosening (loss of contour) of the membranes of various organelles. An increase in the electron density of these cells and a violation of intercellular contacts were characteristic findings. The most pronounced changes were detected in the cells of the spinous, and, especially, the basal layers of the epithelium, while they were less pronounced in the cells of the granular and corneous layers (Fig. 3). It was often possible to observe vacuolar dystrophy of epithelial cells (Fig. 3).

At the 5th hour from the beginning of reproduction of periodontal coagulation dystrophy, the walls of a number of capillaries were destroyed, which led to continuous interpenetration of ultrastructural components of the main substance of connective tissue and blood plasma. In most cells of the connective tissue, the cytoplasm was saturated with residual bodies, vacuoles, precipitates, and coagulates. Mast cells were found in small numbers, and their electron-bright cytoplasm contained small amounts of secretory granules. Such secretory granules resembled partially or almost completely emptied containers. At this time, intercellular contacts were broken in the epithelial part of the mucous membrane, represented by large intercellular spaces, in which polymorphonuclear leukocytes and small disintegrating lymphocytes were often detected. In some places of the mucous membrane, peeling of epithelial cells was noted. At the 24th hour after the onset of acute simple coagulation dystrophy of the periodontium, the changes in the mucous membrane of the gums mostly resembled the changes that were detected at the 5th hour, but some of them were more pronounced, the lysis of microthrombi

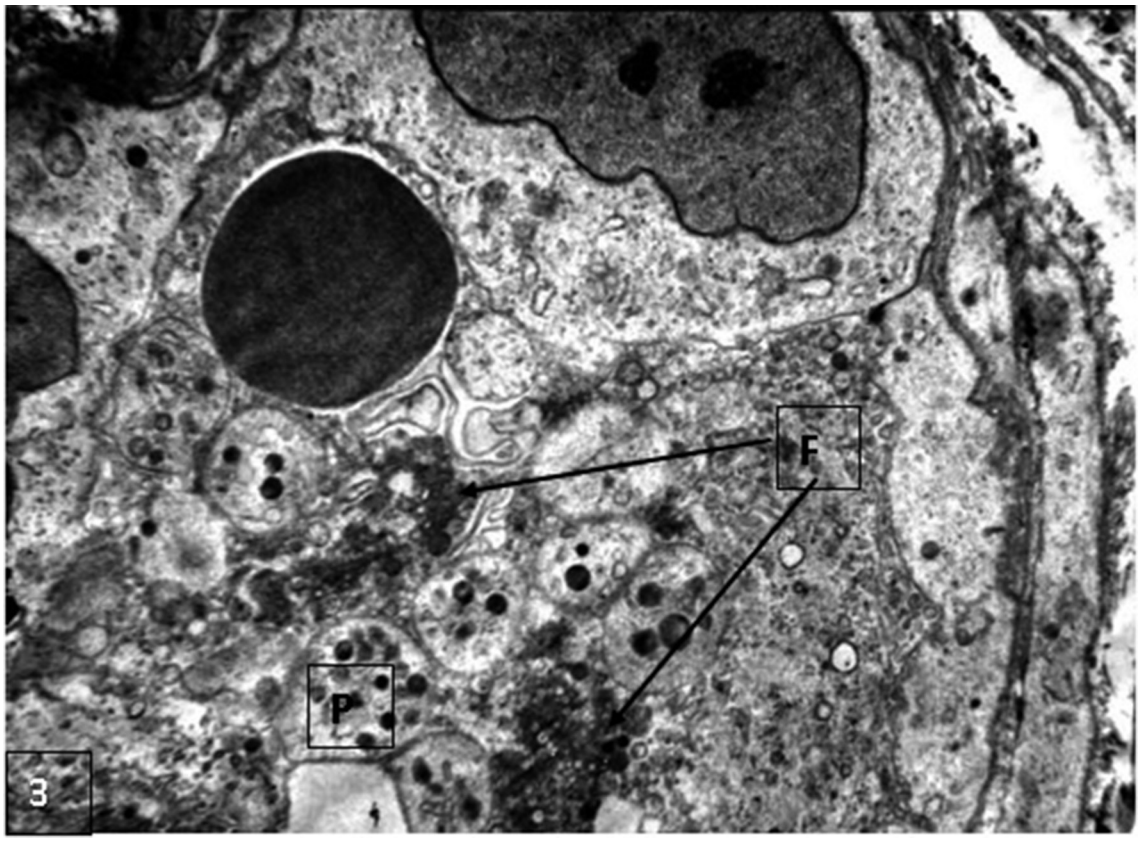
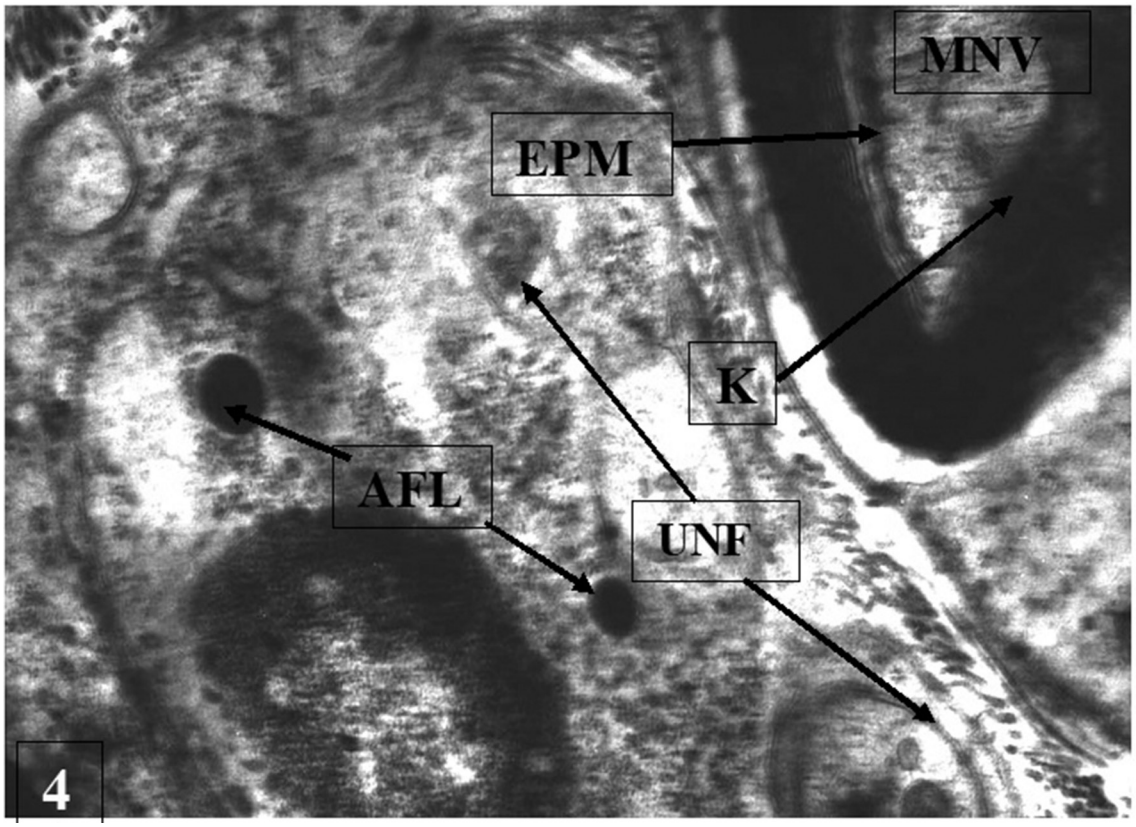
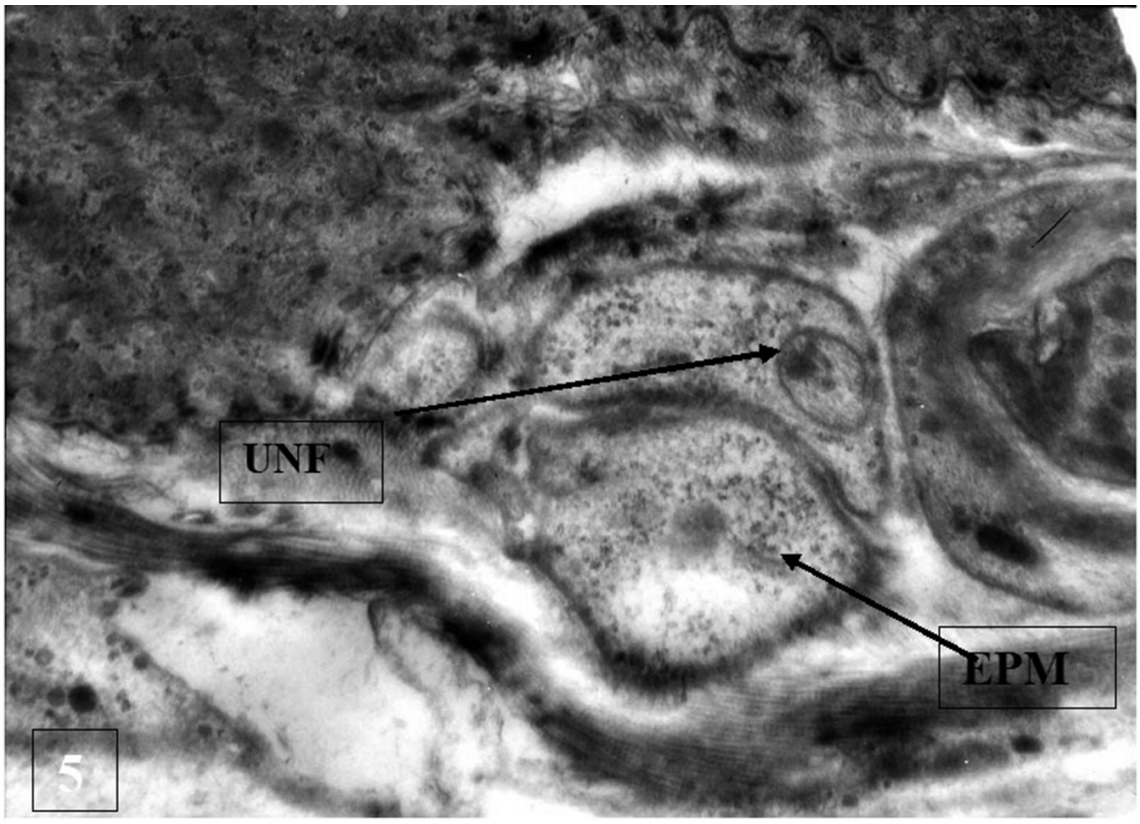


Fig. 3. Ultrastructure of a vessel of connective tissue, the lumen of which is filled with clusters of bundles of fibrin fibers (F), platelets (P), erythrocytes for 24 hours of the experiment. Coll. X2000.



Myelin nerve fibers (MNV), electron-dense precipitate masses (EPM), coagulates (K). Unmyelinated nerve fibers (UNF) Autophagolysosomes (AFL)

Fig. 4. Ultrastructure of myelinated nerve fibers (MNV), which have electron-dense disorganized axoplasm and mesaxons in the form of electron-dense masses of precipitates (P) and coagulates (K). The axoplasm of unmyelinated nerve fibers (BMNV) is limited by a plasma membrane that does not have clear contours. Autophagolysosomes (AFL) in the cytoplasm of a neuroleumocyte, at 15 min of the experiment. Coll. x23000.



Electron-dense precipitate masses (EPM), Unmyelinated nerve fibers (UNF)

Fig. 5. Ultrastructure of unmyelinated nerve fibers (BMV), the membranes of which do not have clear contours. The presence of precipitates (P) in the cytoplasm of a neuroleumocyte at 24 hours of the experiment. Coll. x17000.

and the disintegration of individual endotheliocytes were observed in the capillary lumens. A marked increase in secretory granules was found in individual mast cells.

All the damage in the ultra-structures we found were identified by a number of researchers as accompanying the development of increased thrombin formation and attributed to the pathological process described in the reproduction of simple coagulation dystrophy [1, 2, 4, 6, 7]. Therefore, as is known, the main active process in this case is the development of generalized decompensated thrombino-genesis, and the result of thrombin-induced changes in the structure of proteins, namely: the conversion of fibrinogen to fibrin (in the blood and in PST), actin polymerization (the conversion of G-actin to F in cells -actin) and denaturation of other proteins (enzymes, receptors, regulators and structural proteins), so in fact they are primary coagulation damage to organs. At the same time, significant damage to the ultrastructure of the nerves of the mucous membrane of the gums was revealed. Thus, already 15 minutes after the onset of reproduction of acute simple coagulation dystrophy of the periodontium, the cytoplasm of neurolemocytes contained precipitates and coagulates. The myelin layer of myelinated nerve fibers did not have a clear structural organization and orderliness of mesaxons and was often represented by local clusters of homogeneous electron-dense masses (Fig. 4).

Cytoplasm of axons with disorganized myelin sheaths was mainly represented by lysed fibers and filled with precipitates and coagulates of disintegrating organelles. Most of the unmyelinated nerve fibers were found to be in a state of disorganization and disintegration. Their axoplasm is electron light, and the plasma membrane is loose.

The cytoplasm of neurolemocytes, which included unmyelinated nerve fibers, was disorganized and saturated with electron-dense homogeneous masses (Fig. 5).

DISCUSSION

Coagulation damage to organs, according to the data in the modern literature, simultaneously includes three mechanisms of trophic reduction – enzymopathic, circulatory and neurotrophic, which during the next 24 hours causes a significant increase in damage to cell structures up to their disintegration (secondary dystrophic damage) [1, 2, 10, 13, 14, 16]. That is, in the test animals, under the conditions of the experiment, all gum damage characteristic of dystrophic periodontal damage and, in particular, periodontal disease, occurred. The obtained results, considering existence of the thrombin-plasmin system in all environments of the body [10, 11, 15] confirm and expand the previously substantiated coagulation-trophic theory of the pathogenesis of periodontitis [1]. Cell damage, according to this theory, develops in two stages: in the first – under the influence of

thrombin (primary coagulation damage), and in the second – as a result of a sharp disorganization of their trophic (secondary dystrophic damage). The cause of cell trophic disturbances, in turn, is: a) denaturation of intracellular proteins, enzymes and regulators, with a decrease in their biological activity; b) reduction of neurohumoral trophic influence on cells, on the one hand, as a result of non-perception of these influences by coagulation-damaged receptors of cells, and on the other hand, as a result of impaired passage of trophic impulses along coagulation-damaged internal nerve fibers. As a result, the nervous system cannot send and cells cannot perceive neurotrophic influences. We found similar ultrastructural damage to the gums during the ultrastructural examination of the gums of patients with periodontitis without clinical manifestations of inflammation. In particular, in the gingival stroma, there are microthrombi in the capillaries, mainly in the form of a homogeneous protein mass of increased electron density. There were also bundles of fibrin fibers or erythrocyte-platelet-fibrin microthrombi. Significant damage to nerve fibers and their synapses was recorded. They appeared in the form of damage to the myelin and non-myelin fibers of the gums, which undoubtedly created dystrophic effects on the periodontal tissues [12].

Precipitates, coagulates or continuous cytogel were found in the cells, next to which we found damage to membrane and non-membrane organelles. Frequent damage to the integrity of tissue basophils with their degranulation into the intercellular space was revealed. The main sign of damage to membrane structures (mitochondria, endoplasmic reticulum, Golgi complex, karyotheca and cytomembrane) was the loosening (loss of contour) of their membranes. The basement membrane is loosened and in many places there is a violation of lilosity. A number of areas of the surface layers of the epithelium are formed by flat-shaped electron-dense and electron-light cells, which are more typical of cells of the granular layer than of the stratum corneum, and there are also areas that often completely lack cells of the stratum corneum [12].

So, at the ultrastructural level, disseminated microthrombosis, mucoid swelling and fibrinoid transformation of the intermediate connective tissue and coagulation-dystrophic changes of periodontal tissues and cells were revealed. Therefore, taking into account the results of the studies highlighted in the literature [3,12,14,15] and similar lesions of the gums found at the ultrastructural level, presented by us, as a result of studies, on animals, have signs of coagulation-dystrophic damage caused by generalized thrombinogenesis, and by its the essence is actually a process of decompensated enhanced biocoagulation (cyto-histo-hemocoagulation).

CONCLUSIONS

Coagulative damage to the ultrastructural elements of the periodontal nervous system is one of the important factors in the pathogenesis of dystrophic periodontal damage. Under these conditions, trophic disturbances occur, similar to those that occur when the integrity of the nerve is disturbed (neurotrophic mechanism of dystrophy).

Under conditions of predominance of thrombinogenesis (thrombin action), irreversible changes in cells and nerve fibers can occur, and therefore, the physiological process turns into a pathological one with irreversible damage to the structures of cells and nerve fibers of the periodontium.

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CONFLICT OF INTEREST
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* Contribution: A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval.