ABSTRACT
Diabetes mellitus (DM) leads to a significant deterioration in the quality of life as a result of chronic complications associated primarily with angiopathy. In the article the ultrastructural changes in the secretory epithelium and state of microcirculatory bed of parotid and submandibular glands of white rats with experimental streptozotocin-induced diabetes duration 1, 2 and 3 months have been studied. Ultramicroscopic study of submandibular gland with diabetes showed the presence of significant destructive changes in the endothelium of the capillaries in the early stages of observation, which became more prominent with longer duration of hyperglycemia. Changes of microcirculation caused impairment transport of oxygen and nutrients to glandulocytes, leading to disruption of synthesis and excretion of secrets. Destructive changes of mucocytes and serocytes submicroscopic organization indicate violations of the functional activity of acinar cells. Electron microscopic research of parotid gland in experimental diabetes mellitus also found a violation of microcirculation that significantly worsened secretion. Reorganization of secretory epitheliocytes were accompanied by damage of nuclei and cytoplasmic organelles that gradually decreased synthetic processes in serocytes and excretion of secret.

KEYWORDS: Diabetes mellitus, salivary glands, blood vessels.

INTRODUCTION
According to official data by the end of 2012 the number of diabetics in Ukraine reached 1 303 157 people - about 2.9% of the population. Statistical data about the increase in the incidence of diabetes mellitus in the world testify that the number of patients with diabetes mellitus (DM) will double every 15 years. In obedience to generalized data of WHO and the International Diabetes Federation (IDF) the prevalence of DM in the world in 2014 reached 8.3% of the population.[5] The development of complications of diabetes mellitus leads to a significant impairment in the quality of life. Xerostomia, associated with violating of salivation is one of the early symptoms of diabetes mellitus. The functional activity of digestive glands, including salivary glands, depends mainly on the stimulation of the autonomic nervous system and the hormonal regulation. At the same time we know that endocrine disorders cause a significant impact on the structure and function of salivary glands. In the scientific literature, the study of the salivary glands[2,3,6] and microcirculatory bed in norm and various pathologies received considerable attention.[7,8] The angiopathy plays an important role in the pathogenesis of DM complications development. However the ultrastructural changes of major salivary glands hemocapilares and secretory cells of parotid and submandibular salivary glands acini in DM weren’t described enough, which is why our studies are important.

MATERIALS AND METHODS
The study had been conducted at 72 mature white male rats weighing 180-200 g. The experiments fully compliant with the requirements set out in the «General principles of animal experimentation», approved at the National Congress on Bioethics (Kiev, Ukraine, 2001) and consistent with the provisions of the «European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes» (Council of Europe, Strasburg, 1986).

Insulin-dependent form of diabetes mellitus in rats had been modeled by the single intraperitoneal introduction of streptozotocin («Sigma», USA) at the dose 60 mg/kg. Euthanasia of animals has been conducted by...
intraperitoneal administration of sodium thiopental (25 mg / kg) in 1, 2 and 3 months after the start of the experiment, then sampling of biological material was conducted.

For electron microscopic studies small pieces submandibular and parotid glands were took and fixed with 2.5 – 3 % solution of glutaraldehyde, postfixed in 1 % osmium tetra oxide solution in phosphate buffer (pH 7.2 - 7.4). Specimens were then dehydrated in alcohol and propylene oxide and embedded in a mixture of epoxy resins with araldite, cut into ultrathin sections with an ultramicrotome, and stained with uranyl acetate and lead citrate (Reynolds, 1963).[1, 10] The material was examined and photographed in the electron microscope PEM - 125K at the Laboratory of Electron Microscopy, Histology and Embryology Department, Ternopil State Medical University.

RESULTS
Electron microscopic study of the parotid and submandibular glands of rats showed the presence of significant changes in acinar cells even in the early stages of observation. In 1 month of experimental DM submicroscopic organization of acinar cells was changed. In epithelioctyes of mucosal acini were available nuclei delimited with irregular contours karyolema and perinuclear spaces with irregularly thickened areas. Karyoplasm of many nuclei had increased electron density, nucleoli were observed rarely. In the basal part of the cytoplasm were available unevenly dilated ducts of the granular endoplasmic reticulum (GER), in perinuclear area detected thickened tubules and increased vesicles and vacuoles of Golgi complex (GC). Some of mitochondria were hypertrophied, with light matrix and damaged cristae. Also, found a few small organelles with osmiophilic matrix and badly contoured cristae. Secretory granules in the apical part of these cells formed different sized clusters (Fig. 1).

In mixed secretory departments were detected similar changes of nuclei and organelles of serocytes like changes of mucocytes. Their cytosol has the greater electron density, it included a round, osmiophilic granules, also were available microvesicles and vacuoles.

In the submandibular gland were established changes of blood capillaries. They had both a narrow (arterial site) and wide (venous sites) lumens. The nuclear part of endothehium of the first one was low, with ellipse shape nuclei, surrounded by a narrow rim of cytoplasm. Perinuclear spaces of different thickness, nuclear pores poorly detected. In the paranuclear area of cytoplasm were noted little organelles. Endothelial cytoplasmic areas in some areas looked thickened, swollen with few little pinocytosis vesicles (Fig. 2). Luminal part of endothelial cells membrane formed cytoplasmic protrusions, invaginations, reflecting violation of trans capillary exchange.

Figure 2. Ultrastructure of capillary of the submandibular gland in 1 month of experimental diabetes mellitus.

Lumen with erythrocytes (1) narrow cytoplasmic region (2), basement membrane (3), and perivascular space (4). x 12 000.

Perivascular spaces around the vessels of microcirculatory bed were dilated. There prevailed transparent amorphous component, with some loosely arranged fibrous structures.

Electron microscopic study of submandibular gland conducted in 2 months of experimental DM found that changes in the acinar cells were more prominent compared to the previous term of experiment. Mucocytes were characterized by small, pyknotic nuclei with osmiophilic karyoplasm. Electron density of karyoplasm was significant, nucleoli were not observed. Nuclear envelope was irregular, perinuclear spaces unevenly thickened by protrusions of outer nuclear membrane (Fig. 3).

Figure 1. Ultrastructure of mucocyte of submandibular gland in 1 month of experimental diabetes mellitus.

The nucleus (1) and cytoplasm (2), granular endoplasmic reticulum (3) secretory granules (4). x 14 000.
The lumen of the capillary with erythrocytes (1), swollen cytoplasmic region of endotheliocyte (2), basement membrane (3). x 12 000.

Endothelial cells were delimited with unclear plasmolemma, cytoplasmic protrusions cytoplasmic and microvilli on their luminal surface were isolated. Electron dence dark delicate areas endothelial cytoplasm include isolated organelles and pinocytosis vesicles. Uneven thickness of the basement membrane in some areas was not clearly contoured. Venous areas of capillaries were more extensive, filled with blood. Their walls were thickened by edema of endothelial cytoplasm. Basement membrane was not clear, homogeneous, sometimes damaged. Around the vessel observed irregularly thickened perivascular spaces.

In 3 months of experimental DM found that, changes of secretory cells were like the changes of epitheliocytes in previous term experiment. Most nuclei of mucose cells had electrondence karyoplasm, uneven contours of karyolema and poorly expressed or locally thickened perinuclear spaces. Nucleoli in karyoplasm rarely detected. Such ultrastructural state of nucleus could mean about karyopkynosis.

Basal part of the cytoplasm included irregularly thickened or fragmented GER ducts and cisterns of GC. In addition, large, secondary lysosomes with different electron density and locally damaged cytoplasm could be identified. Most mitochondria were hypertrophied, with light damaged cristae and matrix. In apical areas of such mucocytes accumulation of electron light, various sizes secretory granules observed.

In serocytes were available nuclei with invaginations of karyolema and locally enlarged parts of perinuclear space, their karyoplasm included osmiophilic areas of heterochromatin. In the apical cytoplasm’s areas of acinar cells were few secretory granules but many small vacuoles. There were flat or unevenly dilated ducts of GER and GC cisterns. Most mitochondria were hypertrophied, with electron transparent matrix and few cristae (Fig. 5).

Osmiophilic nucleus (1), tubules of GER (2), cisterns of GC (3), secretory granules (4). x 11 000

In the basal part of the cytoplasm were observed irregularly thickened tubules of GER, as well as small cisterns of GC and large vacuoles. Some of mitochondria were hypertrophied, with a light matrix and damaged cristae. Secretory granules of different sizes had a light content, creating congestion in the apical parts of cytoplasm (see. Fig. 3).

Peripheral to the acinar cells plasmolemma were located destructively altered myoepitheliocytes. In these cells were observed prolonged small nuclei, short or fragmented processes, and in their cytoplasm were damaged organelles.

Ultrastructural state of serocytes of mixed type acini was similar to the previous period of observation. The nuclei of the cells were small, with osmiophilic karyoplasm. The cytoplasm included round granules, vacuoles, and microvesicles. In this term of the experiment in submandibular gland violations of ultrastructure of the capillaries were found. There were wide and feel with blood vessels and wall of these vessels were altered. In the endothelium of the capillaries were available regions of cytoplasm with different electron density. Light, thickened, swollen areas had little organelles and they were significantly damaged (Fig. 4).

Figure 3. Ultrastructure of submandibular gland mucocytes in 2 months of experimental diabetes mellitus.

Figure 4. Ultrastructural changes of submandibular glands blood capillaries in 2 months of experimental diabetes mellitus.

Figure 5. Ultrastructure of serocyte in submandibular glands acini in 3 months of experimental diabetes mellitus.
The nucleus (1), tubules of GER (2), mitochondria (3) and secretory granules (4). x 12 000.

At this period of the experiment in the submandibular gland of experimental animals kept changing of microcirculatory bed. Capillary with extended lumen and damaged structural components of the wall been observed.

In endothelial cells were swollen area with electron light cytoplasm. There were found damaged individual organelles in such cells. Basal membrane had unclear contours, in some areas were destroyed. Perivascular spaces were irregularly enlarged, swollen, electron transparent.

Some blood capillaries had narrow lumens filled with red blood cells in the form of sludge effect. The capillary wall was greatly thined, narrow areas had little cytoplasmic organelles and pinocytosis vesicles. In endothelial cells were prolonged nuclei with irregular contours of karyolema and osmiophilic karyoplasm. In paranuclear areas observed damaged organelles. Basement membrane was narrow, osmiophilic, clearly contoured. Perivascular spaces were regular, sometimes swollen.

Electron microscopic study of parotid gland in experimental diabetes mellitus found that changes in the components of the microcirculatory bed were similar to changes in the submandibular gland in all terms of observation.

In the salivary glands of experimental animals were available dilated capillaries with blood filled lumen and damaged structural components of the vascular wall. The thickness of basement membrane of capillars was uneven, sometimes homogeneous, clearly contoured.

Endothelial cells have areas of cytoplasm with different electron density. Thick swollen endothelial cells included light cytoplasm with destructively altered organelles and their density was low. On the membranes of short ducts of endoplasmic reticulum was a low number of ribosomes. Small mitochondria included light matrix and significantly damaged cristae.

There were also blood capillaries with thin, structureless areas of endothelial cells cytoplasm, which electron density was higher. Were found the oval shaped nuclei of the cells, in their karyoplasm heterochromatin, osmiophilic areas dominated that illustrated karyopycnosis signs. This state of endothelial nuclei testified to their low functional activity. (Fig. 6). In 3 months of experimental diabetes mellitus in perivascular spaces were set sclerotic changes.

Figure 6. Ultrastructural changes of blood capillaries of parotid gland in 2 months of experimental diabetes mellitus.

Dilated lumen with erythrocytes (1), the nucleus (2), cytoplasm (3) endothelial basement membrane (4) and perivascular space (5). x 10 000.

The study of secretory parts of parotid gland found that changes in acinar cells increased depending on the duration of hyperglycemia. Ultrastructural reorganization of serocytes in 1 month in experimental diabetes mellitus manifested with destruction of their nuclei and organelles.

In karyoplasm was found a significant content osmiophilic clumps of heterochromatin, nucleoli were small, compact or non observed. Perinuclear spaces were locally increased by protrusions of outer nuclear membrane, nuclear pores were few.

In the cytoplasm of acinar endotheliocytes were noted irregular thickening of HER tubuls, their partial fragmentation, on the membranes surface were few ribosomes. Cisterns of Golgi complex were thickened, it vacuoles and vesicles were increased. Mitochondria were hypertrophied, with light matrix and damaged cristae. In the apical cytoplasm area were few secretory granules and they had a round shape, with different size and osmiophilic.

Ultramicroscopic research of parotid gland in later period of observation - 2 and 3 months of experimental diabetes mellitus found increase of the degree of acinar cells destruction and the changes in these terms were the same type.

Nuclei in most secretory cells were located in the basal part, contours of nuclear membranes were sometimes unclear and perinuclear spaces - locally increased. In karyoplasm nucleoli weren’t found, but were available clumps of heterochromatin.
Figure 7. Ultrastructural changes of serocyte of parotid glands acinus in 2 months of experimental diabetes mellitus.

The nucleus with clumps of heterochromatin (1) osmiophilic cytoplasm (2) GER tubules (3), damaged mitochondria (4), secretory granules. x 14 000

In the basal part of the cytoplasm of glandulocytes observed expanded GER tubules and perinuclearily - thickened GC cisterns, near which were available vacuoles and vesicles. Some mitochondria were with signs of swelling, with light matrix and damaged cristae. Secretory granules in these cells were few, different in size, located at GC and in the apical area of the cytoplasm (Fig. 7).

CONCLUSIONS

Thus, ultrastructural changes of submandibular glands microcirculatory bed in experimental diabetes mellitus indicate disorders of histohematic barrier and transcapillary exchange.

In the long term in experimental hyperglycemia blood capillaries in the submandibular gland established as much or moderately altered vessels. Reorganisation of vascular wall’s structural components significantly impairment transport of oxygen and nutrients causing destructive changes of acinar cells ultrastructure and disruption of secretion.

Electron microscope study of parotid gland in experimental diabetes also found microcirculatory disorders that significantly violate the secretion. Reorganization of serocytes in dynamics of the experiment was accompanied by damage of nuclear and cytoplasmic organelles that gradually worsens synthetic processes in acini.

REFERENCES