

SUBMICROSCOPIC CHANGES IN HEMOCAPILLARIES OF ADRENAL GLAND AND PANCREATIC ISLETS OF LANGERHANS IN CASES OF IMMOBILIZATION STRESS WITH UNDERLYING HYPOTHYROIDISM

I. M. Klishch¹, *A. V. Dovbush¹, H. O. Havryliuk-Skiba², O. P. Andriyishyn¹
1 – I. HORBACHEVSKY TERNOPIIL NATIONAL MEDICAL UNIVERSITY, TERNOPIIL, UKRAINE
2 – BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

Background. Today, among the increasing number of various diseases, stress is a trigger in the development of diseases and mortality among the population.

Objective. To establish submicroscopic changes in the hemocapillaries of the islets of Langerhans of the pancreas and adrenal glands in the presence of immobilization stress with underlying hypothyroidism.

Methods. The experiment was carried out on 20 mature white male rats. Hypothyroidism was modeled by daily per os injection with a probe of the pharmacopoeial mercazole thyreostatics at a dose of 25 mg/kg for 21 days. Acute immobilization stress was modeled by tying the experimental rats in a supine position in 4 limbs without restricting head mobility for 3 hours. The study was conducted 2 hours later (anxiety stage) after the end of the stress impact. The material was collected for electron microscopy and tissue processing was performed according to generally accepted methods.

Results. Electron microscopic examination of the blood capillaries of the islets of Langerhans in the pancreas and the hemocapillaries of the adrenal cortex after the action of the stress factor in cases of hypothyroidism showed slightly enlarged lumens and moderate perivascular edema. The basement membrane was moderately thickened but clearly contoured. Endotheliocyte nuclei were mostly hypertrophied, euchromatin was uniformly located in the nucleoplasm, and nucleoli were defined. The karyolemma was clearly contoured, the number of nuclear pores was small, and the perinuclear lumen was locally widened. Endoplasmic reticulum with moderately expanded and short tubules, moderately thickened cisterns of the Golgi complex were observed in the cytoplasm of endotheliocytes. There were two types of mitochondria: hypertrophied with an electron-light matrix, single cristae, and with clear visible cristae and a moderately electron-dense matrix. In addition to the capillaries mentioned above, there were also single narrowed microvessels that had perivascular edema and vaguely contoured fenestrae.

Conclusions. When modeling a stressor in cases of hypothyroidism, structural changes of all components of the walls of blood capillaries of the pancreas and adrenal glands take place.

KEYWORDS: stress, pancreas, adrenal gland, submicroscopic changes, hemocapillaries.

Introduction

Today, among the increasing number of various diseases, stress is a trigger in the development of diseases and mortality among the population. Stress is a non-specific reaction of the body in response to a very strong effect or stimulus from the environment, which can significantly affect the body increasing the chances of development of pathological conditions and diseases [1, 2, 3, 4]. It is established that the thyroid gland hormones have a significant influence on the functions of the body as a whole; therefore, their dysfunction – hypothyroidism (reduced production of thyroid hormones) is one of the main endocrine pathology [5, 6]. The

*Corresponding author: Andriy Dovbush, Associate Professor, Department of Histology and Embryology, I. Horbachevsky Ternopil National Medical University, Ternopil, 46002, Ukraine. E-mail: dovbush@tdmu.edu.ua

cells of the endocrine part of the pancreas and endocrinocytes of the adrenal glands produce a complex of hormones that regulate most of the vital functions of the body, provide metabolic processes responsible for maintaining body homeostasis in general and stressful conditions in particular. As a result of the action of the stress factor, a structural rearrangement of both the components of the organs and their microcirculatory bed take place; the blood-filled, dilated vessels and remodeling of the hemocapillary wall are the signs of microcirculation disorders. In recent decades, the negative effect of the stress factor on various human organs has already been proven. However, the state of the hemocapillaries of the endocrine glands in cases of immobilization stress with underlying hypothyroidism is studied insuf-

ficiently and necessitates detailed research [5, 6, 7, 8, 9].

Therefore, the aim of our work was to establish submicroscopic changes in the hemocapillaries of the islets of Langerhans of the pancreas and adrenal glands in cases of immobilization stress with underlying hypothyroidism.

Methods

The experimental part was carried out on 20 mature white male rats with a body weight of 200-250 g, which were kept in the vivarium of I. Horbachevsky Ternopil National Medical University. The object of the study was the pancreas and adrenal glands. Hypothyroidism was modeled by daily per os injection with a probe of the pharmacopoeial mercazole thyreostatics (Health, Ukraine) at a dose of 25 mg/kg for 21 days. The completeness of hypothyroidism was monitored by measuring the concentration of triiodothyronine and thyroxine in blood serum, as well as by the dynamics of the animals' weight and their motor activity. Acute immobilization stress (AIS) was modeled by tying the experimental rats in a supine position in 4 limbs without limiting the mobility of the head for 3 hours. The study was conducted 2 hours later (anxiety stage) after the end of the stress impact [10, 11]. All studies on animals were carried out in compliance with the international rules and principles of the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" (Strasbourg, France, 1986) and the Law of Ukraine No. 3447-IV "On Protection of Animals from ill-treatment", dated 21.02.2006.

For electron microscopy, pieces of the pancreas and adrenal glands were fixed in a 2.5% glutaraldehyde solution with an active reaction medium of pH 7.3-7.4. After fixation, they were washed with a buffer solution for 30 minutes. Postfixation was carried out with a 1% osmium tetroxide solution in the Millonig's buffer for 60 minutes; after that it was dehydrated in alcohols and propylene oxide and embedded into a mixture of epoxy resins. Sections were made on the LMB 4801 A ultramicrotome contrasted with uranyl acetate, lead citrate according to the Reynolds method and studied using an electron microscope PEM-125K [12].

Results

During the electron microscopic study in the blood capillaries of the pancreatic islets of Langerhans 2 hours after the end of the action

of the stressor factor in cases of hypothyroidism, the ultrastructural rearrangement of the hemocapillary wall was insignificant. Moderate perivascular edema of fenestrated hemocapillaries was observed. Their lumens were slightly widened, they showed aggregation of erythrocytes and leukocytes. The basement membrane was moderately thickened, but clearly contoured; pericytes with compacted nuclei were adjacent to it. The plasmolemas of endotheliocytes were not clearly defined, and some areas of the luminal surface contained cytoplasmic outgrowths, fenestrae were defined in the peripheral cytoplasmic areas, some were vaguely contoured. Endotheliocyte nuclei were mostly hypertrophied, of a round shape; euchromatin was uniformly located in the nucleoplasm, but heterochromatin was marginally accumulated, and nucleoli were identified. The karyolemma was clearly contoured, the number of nuclear pores was small, the perinuclear lumen was locally widened. In the cytoplasm of endotheliocytes, there were areas of lightening, swollen mitochondria with a compacted matrix and clear cristae, slightly expanded tubules of the endoplasmic reticulum with a moderate number of ribosomes; and moderately thickened cisterns of the Golgi complex were observed. The number of micropinocytotic vesicles decreased in the cytoplasmic areas (Fig. 1).

Electron microscopic studies of the hemocapillaries of the adrenal cortex in 2 hours after the action of the stress factor in cases of hypothyroidism showed structural changes in the walls of microvessels. The lumens of some hemocapillaries were slightly dilated, filled with blood, in addition to erythrocyte stasis, a few leukocytes were also present. Platelets sometimes were attached to the luminal surface of the endothelium. There were no signs of diapedesis of the formed blood elements into the perivascular space. The nuclei of some endothelial cells were of elongated shape, the inner and outer nuclear membranes were clearly contoured, but they formed deep intussusceptions in some areas. The karyoplasm was mostly electron-bright due to the predominance of euchromatin in it, but there were also clumps of heterochromatin, which was mostly present in the area of the karyolemma. Nucleoli were small or absent. The endoplasmic reticulum was represented by expanded and short tubules. The number of pinocytotic vesicles and free ribosomes in the cytoplasm was insignificant and moderately distributed in the cytoplasm. Some mitochondria located near the nucleus

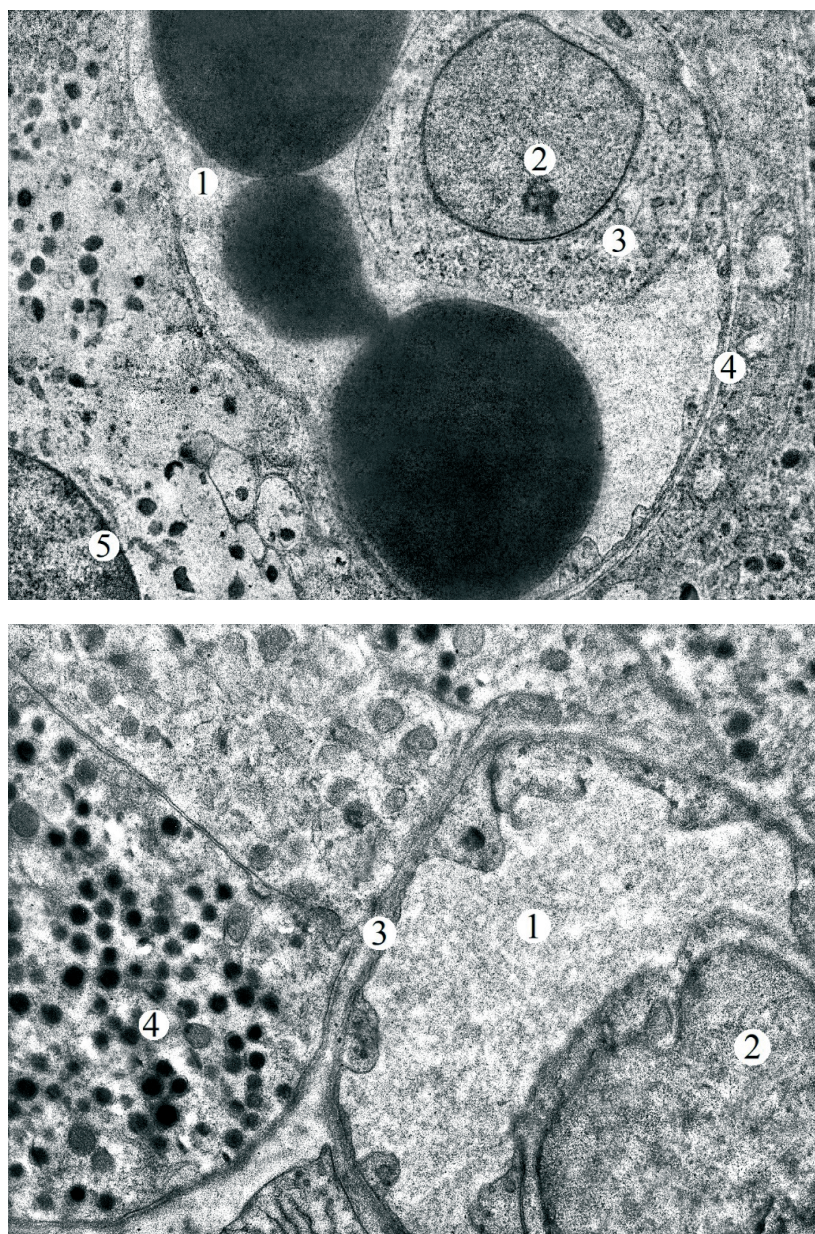


Fig. 1. Ultrastructural changes in the hemocapillary of the rat pancreas in 2 hours (anxiety stage) after the end of the exposure to a stressor in cases of hypothyroidism.
 A. 1 - capillary lumen with erythrocytes, 2 - endotheliocyte nucleus, 3 - endotheliocyte cytoplasm, 4 - basement membrane, 5 - endocrinocyte fragment. TEM $\times 12,000$.
 B. 1 - lumen of a capillary, 2 - endotheliocyte, 3 - basement membrane, 4 - fragment of an endocrinocyte. TEM $\times 14,000$.

had signs of swelling, were hypertrophied, with an electron-light matrix and single cristae. However, there were also mitochondria with clear visible cristae and a matrix of moderate electron density.

The Golgi complex was formed by fragmented, shortened cisternae and vacuoles. Peripheral areas of the cytoplasm had both thinned and sometimes locally thickened zones; fenestrae were clearly defined. The basement

membrane of blood capillaries contained both locally thickened and thinned areas, moderate perivascular edema was observed in some places. Pericytes were found around the hemocapillaries; their processes were located in the splits of the basal membrane. The cytoplasm of pericytes contained indistinct tubules of the endoplasmic reticulum and cisterns of the Golgi complex; mitochondria were of a round-oval shape, ribosomes were single.

Osmiophilic cell nuclei were of an oval or round shape. In the cortex of the adrenal glands, in addition to capillaries with an enlarged lumen and the presence of clear fenestrae in the endothelium, there were also single narrowed microvessels with perivascular edema and vaguely contoured fenestrae. The nuclei of the endothelial cells of these hemocapillaries were of a chimeric shape; the membranes of the karyolemma formed numerous intussuscep-

tions. The electron-light cytoplasm of such endotheliocytes contained rare destructively altered organelles: some mitochondria were enlarged, with signs of swelling, the tubules of the endoplasmic reticulum were shortened and expanded, and the cisterns of the Golgi complex were rare. The basement membrane was indistinct and contained areas of uneven thickness. Pericytes also showed signs of edema (Fig. 2).

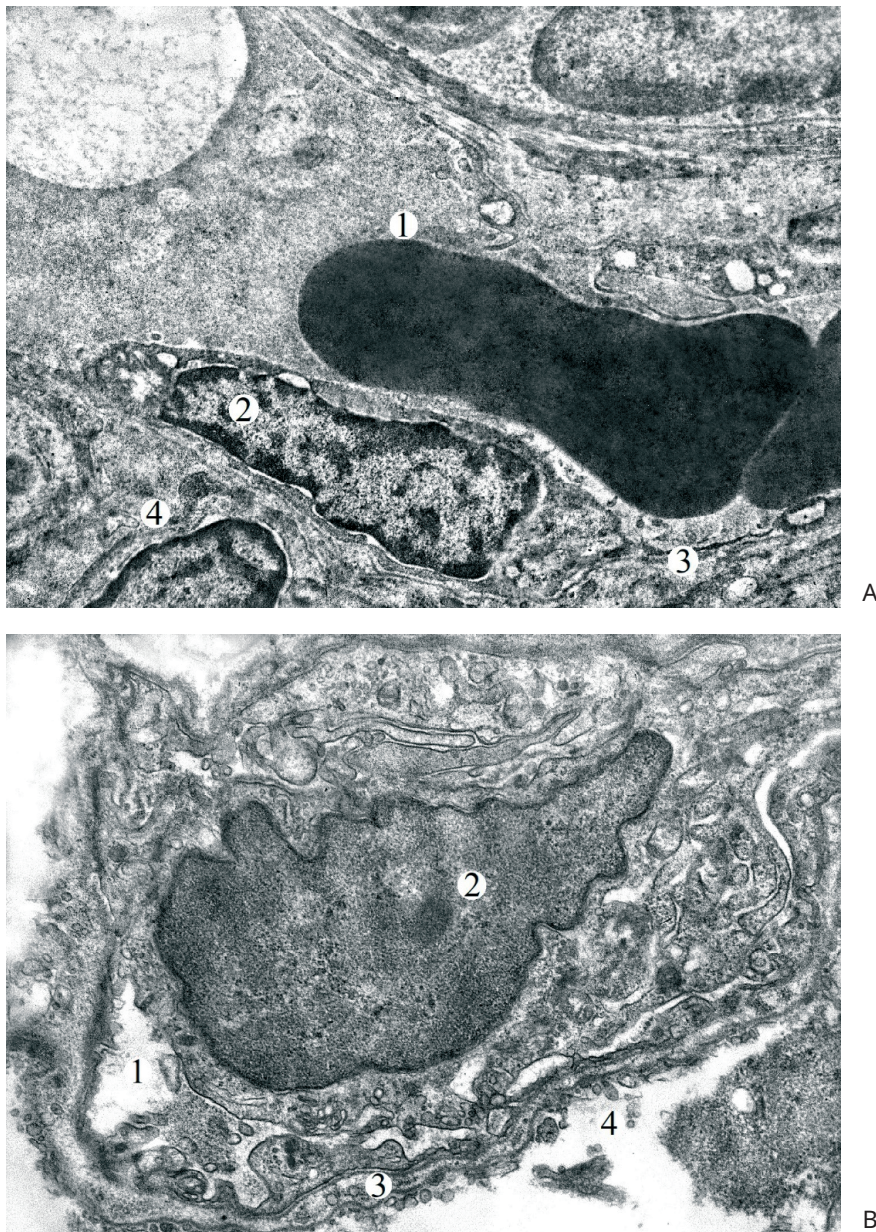


Fig. 2. Ultrastructural changes in hemocapillaries of the zona fasciculata of the rat adrenal cortex in 2 hours (anxiety stage) after exposure to a stressor in cases of hypothyroidism.
 A. 1 – capillary lumen with erythrocytes, 2 – endotheliocyte nucleus, 3 – basement membrane, 4 – a fragment of an endocrinocyte. TEM $\times 12,000$.
 B. 1 – narrow capillary lumen, 2 – endotheliocyte nucleus, 3 – basement membrane, 4 – perivascular edema. TEM $\times 16,000$.

Discussion

The study of hemocapillaries of the endocrine part of the pancreas and adrenal cortex in cases of immobilization stress with underlying hypothyroidism showed some similar changes in the structure of the walls of microvessels. Thus, the presence of a moderate perivascular edema, erythrocyte aggregation in the lumen of fenestrated hemocapillaries, a thickened basement membrane, and the presence of swollen mitochondria in the cytoplasm of endotheliocytes showed typical changes characteristic of the organ response in the stage of anxiety after stress factor exposure. However, unlike the pancreas, the adrenal glands were characterized by the presence of capillaries with both widened and narrowed lumens, which clearly indicated the significance of these glands in the body response to stress and the release of hormones into the blood.

According to some studies [13] the use of only short-term immobilization is partially consistent with our results as a stress factor does not cause negative changes in the rats' body. However, other authors [2] believe that the body of rats begins responding to stress in the early stages and later enters the adaptation state. Similar research for morphological changes [3] established that the use of additional stressful factors (for example, a change in the temperature regime) were manifested by cardinal changes in the organs; however, the impact of the additional stress factor turned out to be a more damaging factor than the increased immobilization load (the longer and more diverse the stress, the more significant changes in internal organs) that is consistent with the results of our research.

Schur MB [14], in the study of the effect of experimental hypothyroidism on the microcirculatory bed in the retina of rats, describes the presence of typical dilated hemocapillaries of vascular walls with aggregation of erythrocytes and platelets in the lumen of vessels with destructively altered nuclei of endotheliocytes that had deep invaginations of karyolemmas and densely packed adjacent heterochromatin. Hemocapillaries with a narrowed lumen and perivascular edema were also present in the retina that is consistent with our research and proves development of destructive changes

caused by experimental pathology of the thyroid gland. Similar changes were evidenced in the research [15] of hemocapillaries of the lateral pterygoid muscle in hypothyroidism. Thus, the most significant manifestation of changes in the structure of hemocapillary walls were edematous and dystrophic changes in the dynamics of the experiment. Thus, deformation of the lumen of the most capillaries was observed; the basement membrane was disintegrated and thickened; the perivascular area was expanded and vacuolated, that is also partially consistent with our research.

Conclusions

The study showed that when simulating a stressor in cases of hypothyroidism, structural changes of all components of the walls of blood capillaries of the pancreas and adrenal glands took place. However, the blood capillaries in the adrenal glands underwent more intensive changes compare to the capillaries of the pancreatic islets, which indicated the significance of these glands in the organ response to stress. The changes had adaptive-compensatory and partially alterative disturbances, which were manifested by blood filling or narrowing of their lumens as a result of slight swelling of the capillary wall components. The nuclei changed their shape, the content of heterochromatin in the karyoplasm increased, and the perinuclear space expanded locally. Clearing of the cytoplasm with slightly changed organelles, decreased number of fenestrae in the cytoplasmic areas, and uneven thickening of the basement membrane were established. The detected changes indicate disturbed microcirculation in the organ, which probably led to dysfunction of the organs and the body as a whole.

Conflict of Interests

The authors declare no conflict of interest.

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Author's Contributions

Ivan Klishch – conceptualization, writing – reviewing and editing; *Andriy Dovbush* – investigation, writing – original draft; *Halyna Havryliuk-Skiba* – methodology, formal analysis; *Oleksandra Andriyishyn* – investigation, data duration.

СУБМІКРОСКОПІЧНІ ЗМІНИ ГЕМОКАПІЛЯРІВ НАДНИРКОВОЇ ЗАЛОЗИ ТА ОСТРІВЦІВ ЛАНГЕРГАНСА ПІДШЛУНКОВОЇ ЗАЛОЗИ ЗА УМОВ ІММОБІЛІЗАЦІЙНОГО СТРЕСУ НА ТЛІ ГІПОТИРЕОЗУ

I. М. Кліщ¹, А. В. Довбуш¹, Г. О. Гаврилюк-Скиба², О. П. Андрієшин¹

1 – ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО
МОЗ УКРАЇНИ, ТЕРНОПІЛЬ, УКРАЇНА

2 – НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ О. О. БОГОМОЛЬЦЯ, КИЇВ, УКРАЇНА

Вступ. У сучасному світі серед зростаючої кількості різноманітних захворювань, стрес є пусковим механізмом у розвитку захворювань та смертності серед населення.

Мета. встановлення субмікроскопічних змін гемокapілярів острівців Лангерганса підшлункової залози та надниркових залоз за умов іммобілізаційного стресу на тлі гіпотиреозу.

Методи. Експеримент проведений на 20 статевозрілих білих щурах самцях. Гіпотиреоз моделювали щоденним введенням *per os* фармакопейного тиреостатика мерказолілу у дозі 25 мг/кг протягом 21-ї доби. Гострий іммобілізаційний стрес моделювали шляхом прив'язування піддослідних щурів у положенні на спині за 4 кінцівки без обмеження рухливості голови тривалістю 3 години. Дослідження проводили через 2 години (стадія тривоги) після завершення дії стресорного фактора. Для електронної мікроскопії забір матеріалу та його проведення здійснювали згідно загальноприйнятих методик.

Результати. При електронномікроскопічному дослідженні кровоносних капілярів острівців Лангерганса підшлункової залози та гемокapілярів кори надниркових залоз після дії фактору стресу на тлі гіпотиреозу спостерігались незначно розширені просвіти та помірний периваскулярний набряк. Базальна мембрана помірно потовщена, але чітко контурована. Ядра ендотеліоцитів переважно гіперторофовані, в нуклеоплазмі рівномірно розташований еухроматин, визначалися ядерця. Каріолема чітко контурована, кількість ядерних пор невелика, перинуклеарний просвіт локально розширений. В цитоплазмі ендотеліоцитів спостерігали ендоплазматичну сітку з помірно розширеними та короткими канальцями, помірно потовщеними цистернами комплексу Гольджі. Наявні два типи мітохондрій: гіперторофовані з електроннопросвітленим матриксом, поодинокими кристами та з чіткими кристами і матриксом помірної електронної щільності. Крім вище згаданих капілярів були також поодинокі звужені мікросудини, що мали периваскулярний набряк та нечітко контуровані фенестри.

Висновки. При моделюванні стресорного фактора на тлі гіпотиреозу зміни відбуваються у всіх структурних компонентах стінок кровоносних капілярів підшлункової та надниркових залоз.

КЛЮЧОВІ СЛОВА: стрес; підшлункова залоза; надниркова залоза; субмікроскопічні зміни; гемокapіляри.

Information about the authors

Ivan M. Klishch, Professor of Department of Functional and Laboratory Diagnostics, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

<https://orcid.org/0000-0001-6226-4296>, e-mail: klishch@tdmu.edu.ua

Andriy V. Dovbush, Associate Professor of Department of Histology and Embryology I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

<https://orcid.org/0000-0003-2246-6218>, e-mail: dovbush@tdmu.edu.ua

Halyna O. Havryliuk-Skiba, Senior Lecturer of Department of Histology and Embryology Bogomolets National Medical University, Kyiv, Ukraine.

<https://orcid.org/0000-0003-1121-7689>, e-mail: galinags712@gmail.com

Oleksandra P. Andriyishyn, Associate Professor of Department of Histology and Embryology I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

<https://orcid.org/0000-0001-7253-7761>, e-mail: andriyishyn@tdmu.edu.ua

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