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MORPHOLOGICAL CHARACTERISTICS OF THE PINEAL GLAND OF NEWBORN RATS UNDER CONDITIONS OF CHRONIC PRENATAL HYPOXIA

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Prenatal ontogeny is a period of high sensitivity to the influence of stress factors. Prenatal hypoxia is one of the most common and clinically significant stress factors that cause mortality and disability among newborns. Prenatal hypoxia during critical periods of brain formation leads to pathomorphological changes in brain structures, accompanied by behavioral disorders and learning abilities, neurological disorders, and disruption of adaptation processes. Since the pineal gland is actively involved in adaptation processes in response to stress factors, the aim of our study was to study the morphological and functional changes in the pineal gland of newborn rats after exposure to chronic prenatal hypoxia.

Experimental studies were conducted on the offspring of female Wistar rats of both sexes. Experimental animals were divided into 2 groups: control and experimental. The first (control) group was formed by 6 newborn animals from females that were in normal vivarium conditions without the influence of additional factors. The second group was formed by 6 newborn animals from female rats, which were injected subcutaneously with a sodium nitrite solution at a dose of 50 mg/kg every day from the 16th to the 21st day of pregnancy.

Prenatal hypoxia is accompanied by morphological changes in the parenchyma and vascular bed of the pineal gland. It was established that in the peripheral regions of the parenchyma, stromal components, light cells with vacuolated cytoplasm, and glial cells were identified. The central zone of the parenchyma is formed by a mass of undifferentiated immature pinealocytes. n the central zones of the pineal gland, we also found areas with signs of focal and complete lysis of pinealocytes, manifested by discoloration of the nuclear substance and nucleolus and the formation of nuclear "shadows" with their subsequent dissolution. Microscopic examination of the blood bed of the pineal gland revealed marked signs of extra-organ blood circulation disorder in the form of venous hyperemia, plasmatization, and spasm of arterial vessels. The detected pathomorphological changes indicate a decrease in the functional activity of the pineal gland, which leads to a violation of adaptation mechanisms.

Key words: *rats, pineal gland, pinealocytes, prenatal hypoxia, blood circulation.*

Connection of the publication with planned research works.

The present work is part of the research topic of the Department of Anatomy, Clinical Anatomy, Operative Surgery, Pathomorphology and Forensic Medicine of the Petro Mohyla Black Sea National University of the Ministry of Education and Culture of Ukraine «Role of ecologically hazardous factors in the development of diseases of civilization», state registration number 0120U002026.

Introduction.

Prenatal ontogeny is a period of high sensitivity to the influence of stress factors, so any stressor can lead to changes in morpho-functional, biochemical indicators, behavioral and cognitive functions of the body. The most common and clinically significant stress factor to which the embryo can be exposed is hypoxia. Prenatal hypoxia during critical periods of brain formation leads to significant pathological changes at various stages of postnatal life in the development of cognitive functions, neurological disorders, behavioral disorders and learning abilities, which correlate with morphological changes in brain structures involved in learning and memory. which is the most common cause of death and disability [1, 2, 3 ,4]. Prenatal hypoxia also leads to pathologies of the neuroendocrine system, which are accompanied by a violation of the processes of synthesis and secretion of neurohormones, dysfunction of the pineal gland, and a decrease in the adaptation potential [2, 5, 6]. Therefore, the problem of prenatal hypoxia and its remote consequences is extremely relevant, as it largely determines the state of health and quality of life of a person in the postnatal ontogenesis [7, 8]. However, the analysis of literary sources shows that the most studied are changes in the hippocampus and cerebral cortex under conditions of prenatal hypoxia, because these structures are responsible for the formation of behavior and learning abilities [7, 8]. At the same time, changes in the morphology of the pineal gland, which is one of the main organs that ensures adaptation to the action of a stress factorn newborn animals through a rapidly occurring non-specific hormonal reaction i, have not been studied enough, which is due to the small size of the pineal gland and its topographic features [9]. In the literature, there is only isolated information about the influence of prenatal hypoxia on the morphological state of the pineal gland of adult rats [10], which prompted us to conduct our own research.

The aim of the study.

To find out the morphofunctional changes of the pineal gland of newborn rats after exposure of chronic prenatal hypoxia.

Object and methods of research.

18 Wistar rats were involved in the experimental study, of which 6 adult females and 12 newborn animals of both sexes. The model of chronic hemic nitrite-induced prenatal hypoxia was used to conduct the study. The experimental animals were divided into 2 groups: control and experimental. The first (control) group was formed by 6 newborn animals from three female rats

(2 animals from each female), which were in normal vivarium conditions without the influence of additional factors. The second group was formed by 6 newborn animals from three female rats (two animals from each female), which were injected subcutaneously with sodium nitrite solution daily from the 16th to the 21st day of pregnancy, which corresponds to the third trimester of human pregnancy. A solution of sodium nitrite was administered at a dose of 50 mg/kg, which, according to literature sources, causes hypoxia of medium severity [8].

The material for histological examination was collected on the 1st day of postnatal ontogenesis at 19.00. After the decapitation procedure was completed, the brains of the animals were removed and fixed in a 10% solution of neutral formalin. After isolated of the pineal gland, the material was enclosed in paraffin blocks using standard methods, from which sections with a thickness of 5 µm were made on a rotary microtome of the semiautomatic type «Microm» (Germany) and stained with hematoxylin-eosin according to the standard method. The obtained histological preparations of the pineal gland were studied under the magnification of binoculars lens ×10, objective lens ×20, ×40 of the microscope of the brand «Carl Zeiss» (Germany). Photo documentation of the research results was made using a Canon digital SLR camera.

During the histological study of pineal preparations, the number of pinealocytes in the field of view of the microscope (objective ×20, binocular ×10) was counted by analogy with Goryaev's counting chamber. Morphometric measurements were carried out using an eyepiece screw type micrometer MOV 1-16 with an objective magnification (×40). The large and small diameters of the nucleus and cytoplasm were measured. Processing of the obtained research results was carried out using the methods of variational statistics, namely the Student's t-criterion. The difference was considered reliable when the numerical parameters between the groups differed at a value of at least $p<0.05$. Statistical calculations were performed on a personal computer using the standard software «STATISTICA 6» for computer equipment with the Windows operating system.

All stages of research, manipulative interventions and euthanasia of animals were carried out in accordance with the provisions of the «General Ethical Principles of Animal Experiments» adopted by the VII National Congress on Bioethics in 2019 and in accordance with other international agreements and current national legislation in the field of medical and biological research.

Research results and their discussion.

Microscopic examination of the pineal gland of newborn rats in the control group revealed that it had a crescent shape **(fig. 1)**.

On the periphery, the pineal gland is surrounded by a thin capsule, which is formed by connective tissue containing thin reticular fibers. In newborn rats, the volume of connective tissue is not significant and the division of the organ into lobules was not clearly detected. It is also necessary to note the fact that the fibers of the connective tissue were evenly located in the parenchyma of the pineal gland.

The parenchyma of the pineal gland of newborn rats was mainly represented by round pineal cells with

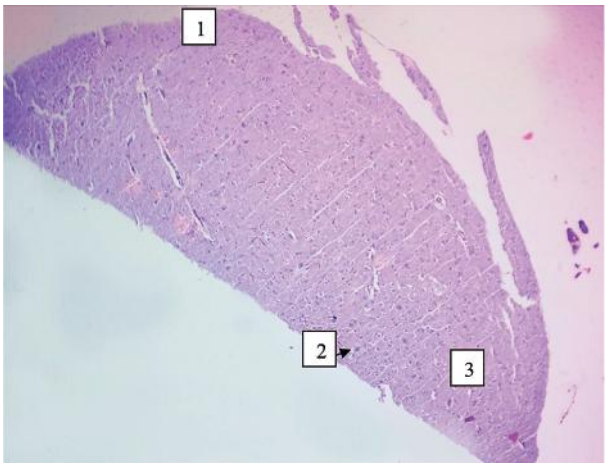

Figure 1 – The shape of the pineal gland of newborn rats of the control group. Staining with hematoxylin and eosin. Magnification: ×100. Symbols: 1 – capsule; 2 – pinealocytes with vacuolated cytoplasm; 3 – apex of the pineal gland.

large, dark, intensely basophilic nuclei filled with varying amounts of functionally inactive heterochromatin. It was established that in some cells the content of heterochromatin was so high that it masked the nucleolus. In other cells, a basophilic nucleolus can be seen due to a decrease in heterochromatin content. Pinealocytes with a lighted nucleus were located without certain regularity both in the central zones of the parenchyma and on the periphery. It was established that in some cells the nucleus occupied almost the entire volume of the cell, and the cytoplasm surrounded the nucleus with a narrow rim. In other cells, the volume of the cytoplasm exceeded the volume of the nucleus. Such a different degree of vacuolization indicates a different functional state of the cells. It should be noted that pinealocytes were located in the parenchyma with a low density **(fig. 2)**. It was determined that the number of pineal cells in one field of view of the microscope was 174.6±10.3 cells. The sizes of the cells varied within very wide limits, which also indicates a different state of functional activity. Thus, the average area of the smallest cells was 21.76±0.81 µm^2, and the largest – 39.92±0.65 µm^2. The average size of the vast majority of

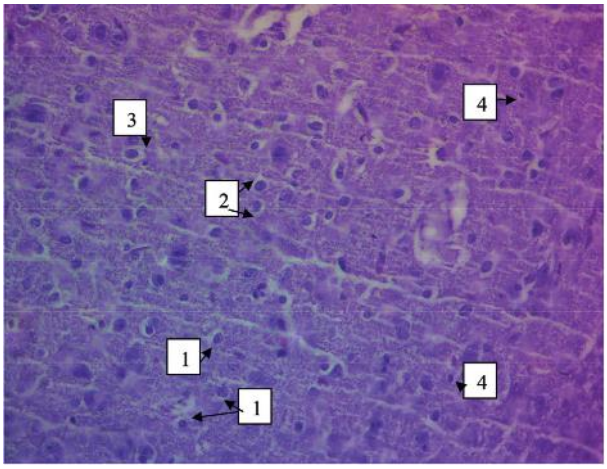

Figure 2 – A fragment of the parenchyma of the pineal gland of newborn rats of the control group. Staining with hematoxylin and eosin. Magnification: ×200. Symbols: 1 – pinealocytes with a lighted nucleus; 2 – pinealocytes with a bulky nucleus and a narrow rim of cytoplasm; 3 – pinealocytes with vacuolated cytoplasm; 4- small pinealocytes.

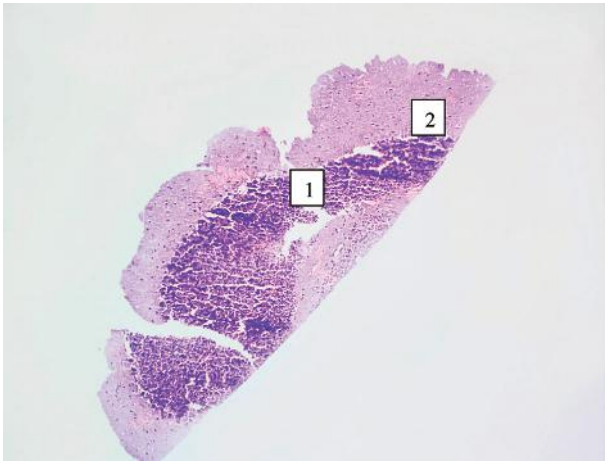

Figure 3 – The shape of the pineal gland of newborn rats under conditions of chronic prenatal hypoxia. Staining with hematoxylin and eosin. Magnification: ×100. Symbols: 1 – the central part of the parenchyma; 2 – peripheral zone of parenchyma.

cells ranged from 26 to 32 μm^2. The increase in the size of the cells occurred due to an increase in the average area of the cytoplasm. In small cells, the main volume was occupied by the nucleus.

During the study of blood vessels, both directly adjacent to the capsule of the pineal gland and intraorgan, we did not detect any changes in the venous and arterial segments of blood circulation, as well as no violations of rheological processes in these vessels. The lumens of arterial vessels were evenly filled with groups of intact erythrocytes. It should be noted that the pinealocytes that accompanied the intraorgan blood vessels are characterized by a slightly increased volume of cytoplasm, which may indicate the flow of cytoplasmic contents into the vascular bed. It was established that the nuclei of such pinealocytes acquired a pale basophilic appearance, which indicates an increase in their functional activity.

During the histological examination of the pineal glands of the experimental animals after the introduction of sodium nitrite solution, significant deviations in the morphology of the pineal gland were observed compared to animals of the control group. The

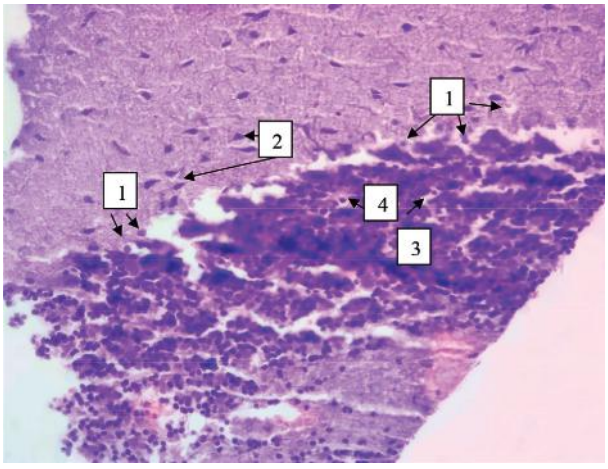

Figure 4 – A fragment of the parenchyma of the pineal gland of newborn rats under conditions of chronic prenatal hypoxia. Staining with hematoxylin and eosin. Magnification: ×200. Symbols: 1 – light pinealocytes of the peripheral region; 2 – glial cells; 3 – mass of undifferentiated cells in the central region of the parenchyma; 4 – single light pinealocytes of the central zone of the parenchyma.

shape of the pineal gland of this group of animals was crescent-shaped, but with uneven jagged edges and a non-solid capsule. Stromal components were located mainly on the periphery of the organ, and in the center there were large masses of cells with a small amount of connective tissue spaces **(fig. 3)**.

It is necessary to note the peculiarities of the location of pineal cells in the parenchyma of newborn animals that were under conditions of chronic prenatal hypoxia. t was revealed that the central zone of the parenchyma is formed by closely adjacent undifferentiated immature pinealocytes, which have a rounded small nucleus and a small volume of cytoplasm and intensively perceive staining with basic dyes and single light cells with a lightened nucleus and vacuolated cytoplasm **(fig. 3)**. In the peripheral regions of the parenchyma, light-colored cells with a lighted rounded nucleus and vacuolated cytoplasm and glial cells were identified, which are located either singly or in groups of 4-5 cells, forming a kind of glial fields **(fig. 4)**. It should be noted that vacuolated pineal cells were located in the deep layers of the peripheral region at the border with the central zone. It was established that in the central regions of the parenchyma, the number of cells in one field of view of the microscope at a total magnification of ×200 was 1405.6±58.7, and in the peripheral regions it was 112.6±4.5. The increase in the density of the location of pinealocytes is explained by the fact that in the center there was a large mass of small, non-vacuolated cells with a small volume of cytoplasm, and on the periphery – reticular fibers and light pinealocytes with varying degrees of cytoplasmic vacuolization.

It should be noted that in the central zones of the pineal gland we found areas with a sparse arrangement of cells and signs of focal and complete lysis of pinealocytes, manifested by discoloration of the nuclear substance and nucleolus and the formation of «shadows» of nuclei and their subsequent dissolution **(fig. 5)**. In some cells, vacuolization of the cytoplasm led to disruption of the integrity of the plasmolemma and even its disappearance [10]. Focally, we found isolated small cysts and accumulation of fluid as a result of complete cell lysis. According to the literature, the presence of vacuolated cells and cysts indicates a delay in hormone secretion [11].

The described state of pineal cells indicates their death as a result of increased functional activity and a decrease in the synthesis and secretion of melatonin, which is the result of a decrease in the number of active cells. The identified morphological features are the response of the pineal gland to the influence of stress factors in the form of prenatal hypoxia and indicate the growth of dystrophic and dystrophic-necrotic changes.

During the microscopic examination of the blood vessels of the pineal gland, in all observations, pronounced signs of a disorder of extra-organ blood circulation were revealed in the form of sharp full blood vessels of the venous type with the phenomena of erythrocyte adhesion. It was found that arterial vessels of various calibers, which were surrounded by similar venous elements, often turned out to be ischemic. It was established that there were practically no formed elements of blood in their lumens. It is necessary to note morphological changes in the structures of the vascular wall of arterioles. First of all, there is a eaction on the part

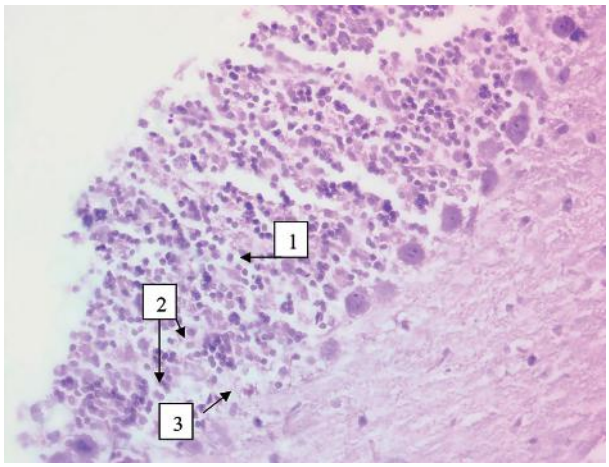

Figure 5 – A fragment of pineal gland parenchyma of newborn rats with signs of pinealocyte lysis under conditions of chronic intrauterine hypoxia. Staining with hematoxylin and eosin. Magnification: ×200. Symbols: 1 – light vacuolated pinealocytes; 2 – «shadows» of pinealocyte nuclei; 3 – focal lysis of pinealocytes.

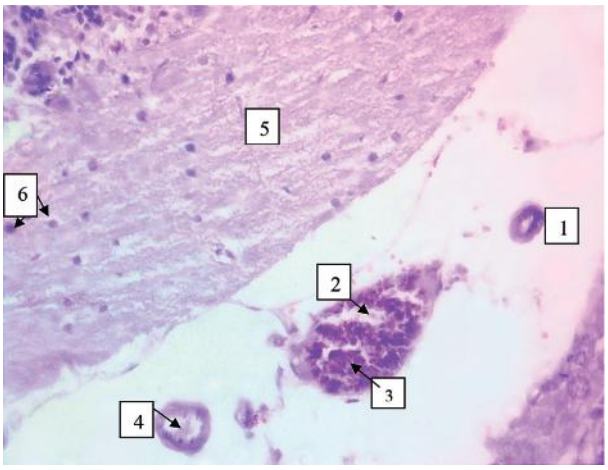

Figure 6 – Photomicrograph of extra-organ blood vessels of newborn rats under conditions of prenatal hypoxia. Staining with hematoxylin and eosin. Magnification: ×200. Symbols: 1 – spasmodic vessel of the arterial type; 2 – lumen of the vein; 3 – adhesion of erythrocytes; 4 – lysis of erythrocytes in the lumen of the arteriole; 5 – peripheral zone of the parenchyma of the pineal gland. 6 – pineal cells.

of endotheliocytes. They appeared hypertrophied with enlarged nuclei. Along with this, spasm phenomena are characteristic of the muscular membrane of the inner wall of a blood vessel **(fig. 6)**. In arteriolar-type vessels, erythrocyte lysis was often noted, which appeared on histological specimen s in the form of a pale pink homogeneous mass. It was established that such an erythrocyte mass occupied the central zone of the lumen of a blood vessel.

Thus, the morphological manifestations of the remodeling of the vascular bed in the form of venous hyperemia and plasmatization of arterial vessels indicate hypoxia of the pineal tissue, and as a result, a decrease in functional activity, which leads to a violation of adaptation mechanisms in conditions of prenatal hypoxia [10].

Conclusions.

The influence of chronic prenatal hypoxia caused pronounced morphological and functional changes in the parenchyma and vascular bed of the pineal gland of newborn rats, manifested in a change in the location of pineal cells, an increase in the number of undifferentiated immature pinealocytes, an increase in the density of the location of cells, the appearance of areas with signs of focal lysis of pinealocytes, venous hyperemia and plasmatization arterial vessels. The identified morphological features indicate an increase in dystrophic-necrotic changes in the cellular elements of the pineal gland, an imbalance in the processes of synthesis and secretion of metabolites of the pineal gland, and as a result, a decrease in its functional activity and a violation of adaptation mechanisms in newborn rats.

Prospects for further research.

In the future, it is planned to investigate the possibility of correcting pathological changes of the pineal gland under conditions of prenatal hypoxia by introducing exogenous melatonin.

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МОРФОЛОГІЧНА ХАРАКТЕРИСТИКА ЕПІФІЗУ НОВОНАРОДЖЕНИХ ЩУРІВ ЗА УМОВ ХРОНІЧНОЇ ПРЕНАТАЛЬНОЇ ГІПОКСІЇ

Пшиченко В. В., Черно В. С., Чеботар Л. Д., Ларичева О.М., Петрова О.І.

Резюме. Вплив пренатальної гіпоксії на ембріон у критичні періоди формування органів центральної нервової системи призводить до значних патологічних змін у розвитку когнітивних функцій, неврологічних розладів, порушення поведінки та здібностей до навчання, що проявляються на різних етапах постнатального життя і є найпоширенішою причиною смертності та інвалідності. Пренатальна гіпоксія призводить і до патологій нейроендокринної системи, що супроводжуються порушенням процесів синтезу та секреції нейрогормонів, дисфункцією епіфіза та зниженням адаптаційного потенціалу. Оскільки епіфіз активно залучається до адаптаційних процесів і забезпечує підтримання гомеостазу, метою дослідження було вивчення морфо функціональних змін епіфізу новонароджених щурів після впливу хронічної пренатальної гіпоксії.

Експериментальні дослідження проводили на потомстві самиць щурів лінії Вістар обох статей. Для проведення досліджень використовувалася модель хронічної гемічної нітрит-індукованої пренатальної гіпоксії. Піддослідні тварини були розподілені на 2 групи: контрольну і дослідну. Перша (контрольна) група сформована 6 новонародженими тваринами від самок, які перебували у звичайних умовах віварію без впливу додаткових факторів. Друга (дослідна) група сформована 6 новонародженими тваринами від самок щурів яким щодня підшкірно вводився розчин нітриту натрію з 16 до 21 доби вагітності, що відповідає третьому триместру вагітності людини. Розчин нітриту натрію вводили у дозі 50 мг/кг, що спричиняє гіпоксію середнього ступеню важкості.

Пренатальна гіпоксія супроводжується морфологічними змінами паренхіми та екстраорганного судинного русла епіфізу. Встановлено, що у периферичних регіонах паренхіми визначалися стромальні компоненти, світлі клітини з вакуолізованою цитоплазмою та гліальні клітини. Центральна зона паренхіми утворена масою недиференційованих незрілих пінеалоцитів. У центральних зонах епіфізу нами були виявлені ділянки з ознаками вогнищевого та повного лізису пінеалоцитів, що проявлялися знебарвленням ядерної субстанції і ядерця та утворенням «тіней» ядер з послідуючим їхнім розчиненням. При мікроскопічному дослідженні кровоносного русла епіфізу виявлені виражені ознаки розладу екстраорганного кровообігу у вигляді венозної гіперемії, плазматизації та спазму артеріальних судин. У судинах артеріолярного типу відзначалися явища лізису еритроцитів. Виявлені морфологічні зміни свідчать про дисбаланс процесів синтезу і секреції метаболітів епіфізу, зниження функціональної активності епіфізу, що призводить до порушення механізмів адаптації у новонароджених тварин.

Ключові слова: щури, епіфіз, пінеалоцити, пренатальна гіпоксія, кровообіг.

MORPHOLOGICAL CHARACTERISTICS OF THE PINEAL GLAND OF NEWBORN RATS UNDER CONDITIONS OF CHRONIC PRENATAL HYPOXIA

Pshychenko V. V., Cherno V. S., Chebotar L. D., Larycheva O. M. Petrova O. I.

Abstract. The influence of prenatal hypoxia on the embryo during the critical periods of the formation of the organs of the central nervous system leads to significant pathological changes in the development of cognitive functions, neurological disorders, impaired behavior and learning abilities, which are manifested at various stages of postnatal life and is the most common cause of mortality and disability. Prenatal hypoxia also leads to pathologies of the neuroendocrine system, which are accompanied by a violation of the processes of synthesis and secretion of neurohormones, dysfunction of the pineal gland, and a decrease in adaptation potential. Since the pineal gland is actively involved in adaptation processes and ensures the maintenance of homeostasis, the purpose of the study was to study the morpho-functional changes in the pineal gland of newborn rats after exposure to chronic prenatal hypoxia.

Experimental studies were conducted on the offspring of female Wistar rats of both sexes. The model of chronic hemic nitrite-induced prenatal hypoxia was used for the research. Experimental animals were divided into 2 groups: control and experimental. The first (control) group was formed by 6 newborn animals from females that were in normal vivarium conditions without the influence of additional factors. The second (experimental) group was formed by 6 newborn animals from female rats that were injected with sodium nitrite solution subcutaneously every day from the 16th to the 21st day of pregnancy, which corresponds to the third trimester of human pregnancy. Sodium nitrite solution was administered at a dose of 50 mg/kg, which causes moderate hypoxia.

Prenatal hypoxia is accompanied by morphological changes in the parenchyma and extra-organ vascular bed of the pineal gland. It was established that in the peripheral regions of the parenchyma, stromal components, light cells with vacuolated cytoplasm, and glial cells were identified. The central zone of the parenchyma is formed by a mass of undifferentiated immature pinealocytes. In the central zones of the pineal gland, we found areas with signs of focal and complete lysis of pinealocytes, manifested by discoloration of the nuclear substance and nucleolus and the formation of nuclear «shadows» with their subsequent dissolution. Microscopic examination of the blood vessels of the pineal gland revealed marked signs of extra-organ blood circulation disorder in the form of venous hyperemia, plasmatization, and spasm of arterial vessels. Phenomena of erythrocyte lysis were observed in arteriolar vessels. The identified morphological changes indicate an imbalance in the processes of synthesis and secretion of metabolites of the pineal gland, a decrease in the functional activity of the pineal gland, which leads to a violation of adaptation mechanisms in newborn animals.

Key words: rats, pineal gland, pinealocytes, prenatal hypoxia, blood circulation.

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Conflict of interest:
The Authors declare no conflict of interest.

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TYPICAL TOPOGRAPHIC-ANATOMICAL CHARACTERISTICS OF THE STRUCTURE OF THE SUBMANDIBULAR VEGETATIVE NODE IN ELDERLY AND OLD PEOPLE

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At the current stage of the development of morphology and clinical medicine, the interest in vegetative nodes, which are complex peripheral nerve centers connected to the central nervous system and are trophic and communication and distribution centers, does not decrease.

In this regard, it becomes clear that without the doctor's knowledge of the features of the topography and individual structure of the submandibular node (SMN) and its connections with neighboring nerves, it is not possible to provide significant help in understanding the pathogenesis and clinical manifestations of diseases of the node, developing new ones and optimizing already existing methods of treatment.

Before starting the main part of our study, we determined the shape of the head (skull) and face – head and face indices. In the future, we used anatomical preparation of the SMN after cosmetic access to it. The sizes of the node were measured using a millimeter ruler. Photography of the prepared SMN was carried out using a digital camera.

Based on the results of the research, we determined the main topographical positions of the SMN; revealed variants of the external structure of SMNs by frequency of occurrence and their sizes; the extreme types of the structure of the SMNs are established and it is shown in which forms of the head and face they occur; the topography of the branched connections of the SMN – adductor roots: tympanic-lingual, sympathic and efferent roots has been clarified.

The revealed dependence of the topographic-anatomical variability of the human SMN on the shape of the skull will help clinicians to justify the choice of the node blockade method in various pathological conditions.

Key words: *man, submandibular vegetative node (ganglion), topographic-anatomical structure, elderly and old age.*

Connection of the publication with planned research works.

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Introduction.

At the current stage of the development of morphology and clinical medicine, the interest in vegetative nodes, which are complex peripheral nerve centers connected to the central nervous system and are trophic and communication and distribution centers, does not decrease [1, 2, 3, 4].

In this regard, it becomes clear that without the doctor’s knowledge of the features of the topography and individual structure of the SMN and its connections with neighboring nerves, it is not possible to provide significant assistance in understanding the pathogenesis and clinical manifestations of node diseases, developing new and optimizing existing treatment methods.

In addition, the clinical picture of the SMN syndrome is represented by severe pain and senostopathic phenomena that affect the mental state of patients, which can cause cancerophobia. Blockade of regional submandibular nodes is recommended to remove these phenomena and stop pain in the tongue area [5, 6, 7].

Interest in such changes as neurodegeneration in the structures of the nervous system in the elderly remains relevant even today [8].