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Ultrastructural features of the adrenal glands during the chronic hypoxia

Samire Mammadhasan Yagubova

Azerbaijan Medical University, Department of Pathological Anatomy, Baku

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Abstract
The objective of this study is to study the morphofunctional properties of ultrastructural changes in the cellular and extracellular structures of the adrenal glands during the chronic hypobaric hypoxia. The materials of the study were 30 male white rats weighing 180-200 grams. The results of electron microscopic studies show the presence of several structural dystrophic-dezorganization changes in the adrenal glands of animals with the chronic hypobaric hypoxia model are noted. The absence of serious changes in the pathohystological picture of cell structures in the ultrathin preparations taken from the gland tissues is characterized by acceleration of compensatory-adaptive processes, development of reparative-regeneration. On the last day of the study, the regeneration processes occurring in the adrenal gland parenchyma and stroma, the restoration of tissue structures and cell organelles, most of the morphometric indicators similar to the control group, in general, indicate the adaptation of the organism to the hypoxia.

Keywords: Ultrastructure, hypobaric hypoxia, adrenocorticocytes, adrenal cortex, adrenal medulla

Introduction

The response of the organism to various factors of the external environment and the processes of adaptation are regulated by the endocrine system, in particular, the hypothalamo-hypophyseal-adrenal system (HHAS); adrenocorticotropic hormone (ACTH), which activates the synthesis of corticosteroids is very important [1,2]. The cause of the occurrence of «long-term» adaptive processes during the repeated exposure to external environment factors, in particular «stress» factors, is «urgent» adaptive processes that leave the deep «traces» in the body [2,3].

Stress factors, on the other hand, have a systemic effect on the morphology of the endocrine system organs, lead to dysfunction of various organs and disruption of homeostasis in the body [4,5]. During acute hypoxia, which is one of the main stress factors, various changes occur in the level of cellular and extracellular structures in the organs and tissues of the body, and chronic hypoxia leads to the formation of compensatory phase of these factors and, as a result, to the restoration of lost functions of organs [6,7]. All processes occurring at this time reflect the morphofunctional state of the organs. Research work in this direction is of great importance in terms of features of morphofunctional changes in the body, their evaluation and forecast in terms of hypoxia, especially in conditions of long-term hypoxia [7,8].

Although numerous research studies have been devoted to the study of changes in the structures of the tissues of the adrenal glands that regulate the level of basal cell metabolism of the organism, a number of questions in the literature are still not covered, contradictory and unanswered.

The objective of this study is to study the morphofunctional properties of ultrastructural changes in the cellular and extracellular matrix of the adrenal glands during the chronic hypobaric hypoxia.

Material and Methods

Animal research was carried out in Pharmacology and Experimental Surgery departments, and the Electron Microscopy Laboratory of the Scientific Research Center of Azerbaijan Medical University. The design of experiment was approved by the ethical committee (Protocol No. 31 of the Ethics Rules Commission and Bioethics Committee under the Ministry of Health of the Republic of Azerbaijan on 21.04.2008.). Total of 30 normal male white outbred rats weighing 180-200 grams, were used in the study. Experimental animals were kept in standard vivarium conditions with food provided ad libitum.

***Coresponding Author:** Samire Mammadhasan Yagubova, Azerbaijan Medical University, Department of Pathological Anatomy, Baku
E-mail: syagubova.71@gmail.com

The animals were divided into 2 groups: control and experimental.

The experimental animals were subdivided into 2 equal subgroups with 10 animals in each group (subgroup 1 and subgroup 2) with duration of experiment for 15 and 30 days accordingly. The animals included into subgroup I were kept in specially hypobaric chamber with pressure at an altitude of 2000-3000 meters above sea level and 5 times a week for 2 hours, rats of subgroup II were kept in specially hypobaric chamber with pressure of 2000-3000 meters above sea level every 2 hours at 10:00-15:00 hours, which is considered the lightest time of day. Particles of natron lime ($Ca(OH)_2$ 81%+NaOH 3,4%+H_2O 15,6%) were applied to absorb CO_2.

Experiment and control animals were euthanized by decapitation under anaesthesia by injection of 2-2,5% of thiopental-sodium. Thyroid and adrenal glands were sampled and analyzed using histological, electron microscopic and morphometric methods.

Tissue processing for electron microscopy was done as recommended [9]. Semithin (1-2 μm) and ultrathin (70-100 nm) sections of the embedded tissue were obtained on the ultratome LGB-III, Leica EM OC7. Semithin sections were stained 0,5% methylene blue, conterstained with 1% Azur II, mounted and viewed under light microscope «Olympus BX-41».

Ultrathin sections 70-100 nm thick were stained with 2% uranium-acetate solution and 0,6% pure lead-citrate and studied under JEM-1400 transmission electron microscope (JEOL-Japan) with 80-120kv voltage.

Morphometric parameters were analysed statistically using Statistica10 (StatSoft. Inc.) software and the Mann-Whitney U-test was performed.

Results

According to electromicroscopic studies, in animals included in the control group, the nuclei of adrenocorticocytes of the glomerular zone of the adrenal gland are rounded and located mainly in the center of the cell, and the nuclei are outside the center. The membrane of the nucleus and the nucleolus are clearly visible, made by granular components. In the cytoplasm of adrenocorticocytes of the glomerular zone, mainly under the capsule and on the central part a large amount of lysosomes and a small amount lysosomes with of smooth edges on the border with the fascicular zone are identified. The edges of the mitochondria of the cells of the glomerular zone are denticular, the cristae have a lamellar structure.

The number of ribosomes and lipid vesicles in the cytoplasm of adrenocytes of the fascicular zone is low. They are unevenly distributed in the cytoplasm. A small amount of lysosomes are mainly located around the Golgi complex. It should be noted that the number of lisosomes in the deep layers of the fascicular zone and near the reticular zone is very small. Spongiocytes of the fascicular zone have large mitochondria with curved surfaces compared with the cells of the glomerular zone, and vesicular cristae with vacuoles appear in the transverse section, and the vacuoles are associated with the inner membrane of the mitochondria.

On the 15th day of the experiment, the histological examination of samples taken from the adrenal gland of animals showed that there were no serious changes. However, even if the general cellular structure of the gland cells is restored, dystrophic changes and even destroyed cell complex are noticeable in some adrenocytes. The quantitative indicators obtained as a result of the study also did not undergo any different changes compared to the control group (table 1).

Table 1. Morphometric parameters of the nuclei of the adrenal gland (M±m), (min-max) in norm and in chronic hypoxia

	Glomerular zone (Max±min)			Fascicular zone (Max±min)			Reticular zone (Max±min)			Adrenal medulla (Max±min)		
	N	Days of experiment		N	Deys of experiment		N	Deys of experiment		N	Deys of experiment	
		15nd day	30 day		15nd day	30 day		15nd day	30 day		15nd day	30 day
Diameter M±m	4.44±0.27	4.54±0.27**	4.48±0.27*	5.55±0.20	5.64±0.19**	5.58±0.20*	4.45±0.17	4.63±0.17	4.87±0.16	5.47±0.19	5.56±0.19**	5.50±0.19*
Min-max	2.66-5.82	2.73-5.91	2.64-5.8 6	4.62-6.35	4.79-6.42	4.64-6.39	3.77-5.26	3.97-5.47	4.19-5.75	4.66-6.35	4.73-6.42	4.69-6.39
Square M±m	46.0±3.83	46.9±3.89**	46.2±3.87*	34.2±1.87	34.1±1.88	34.4±1.89 *	23.9±2.16	24.9±2.20 **	24.0±2.17	30.9±1.47	31.9±1.48**	31.1±1.46*
Min-ma	33.53-68.45	34.12-70.23	33.63-69.01	23.47-41.64	24.14-42.23	23.62-41.78	15.47-36.43	16.34-37.65	15.68-36.55	23.47-37.63	24.14-38.56	23.62-37.87

*Note: N-control group; n-number of animals; M±m: M-average indicator of variation, m-standard error, * - p<0.05, * * - p<0.01

The color of the adrenal gland is restored, its consistency is soft, normal, the capsule is thickened, and in some parts foldings are observed. Adrenal cortex and adrenal medulla are visually distinguished, the adrenal cortex is darker than the adrenal medulla.

On the 15th day of the experiment, the adrenocytes of the adrenal cortex and medulla have a homogeneous structure. The contours of the nuclei of the adrenal cortex is smooth, the basal membrane and nuclear chromatin are clearly visible, the nuclei are in the center of the cell. Slight hypertrophy and hyperplasia of cytoplasmic organelles – lysosomes, as well as endoplasmic reticulum and Golgi complex is detected. The mitochondria have changed their shape: they have changed their elongated form to rounded, and have cristae with the septum and unclear contours.

Complete restoration of the structure of the majority of adrenocytes of the adrenal medulla is noted (figure 1).

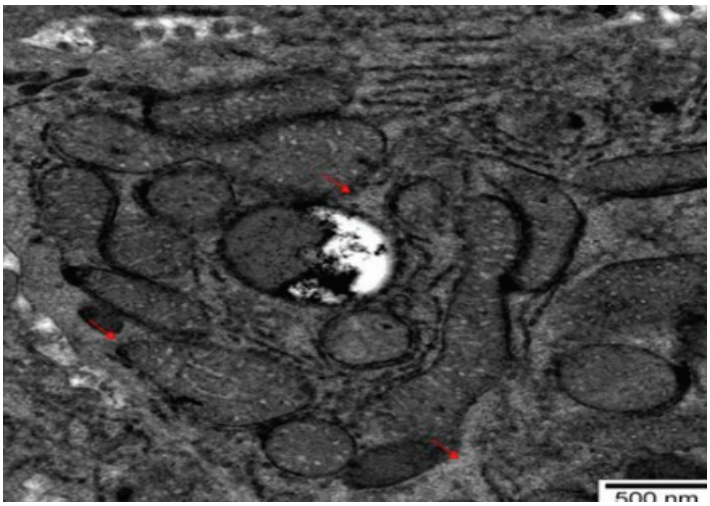

Figure 1. 15th day of hypoxia. Electron micrograph of structures of the adrenal gland. TEM. The explanation is given in the text. Stain: uranium acetate and plumbum citrate. Scale: 500 nm.

Thus, the cytoplasm and nuclei of the cells are transparent, the basal membrane is congested, the fading of the nuclear chromatin is clearly visible, the presence of a small amount of fat droplets in the cytoplasm are considered a sign of fat dystrophy. Electron micrographs show that the inner connective tissue layer, consisting of collagen fibers, fibroblast and fibrocytes, is located between the reticular zone and the adrenal medulla. For epinephrine (adrenal) cells, it is characteristic that the nuclei are located in the center (centric), and for norepinephrine – excentric. In addition, noradrenocytes are distinguished by more dense secretory granules. Restoration of the walls of the capillaries occurs. This recovery process is provided by collagen synthesis of fibroblasts. It is possible to clearly see the restored layers and narrowing of the capillaries (figure 2, marked with a red arrow).

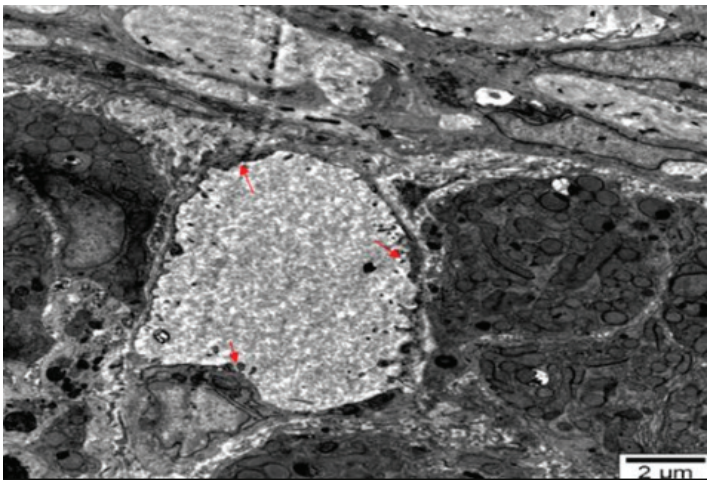

Figure 2. 15th day of hypoxia. Electron micrograph of structures of the adrenal gland. TEM. The explanation is given in the text. Stain: uranium acetate and plumbum citrate. Scale: 2 µm.

On the 30th day of hypoxic effect, acute morphological changes in the structure of the adrenal gland are not oobserved, the histological picture of individual zones of the gland are characterized by the restoration of adrenocytes characteristic for the normal structure of the gland.

During the macroscopic examination of the adrenal gland, the structure of the gland is similar to the norm, the color, the connective tissue capsule covering the gland and the consystency are completely restored, but the capsule is thickened.

Electron microscopic examination provided at the end of the study showed that, the process of recovery of the adrenal gland tissue was completed. Ultrastructural changes that occur under the influence of hypoxia – dystrophy and dezorganization of the cells of the gland parenchyma and stroma are not detected, the process of restoration of cell sizes is clearly visible (table 1). It is associated with the regeneration – adaptation and restoration of morphological structure of adrenocytes – the main cellular elements of the adrenal parenchyma (figure 3, marked with a red arrow).

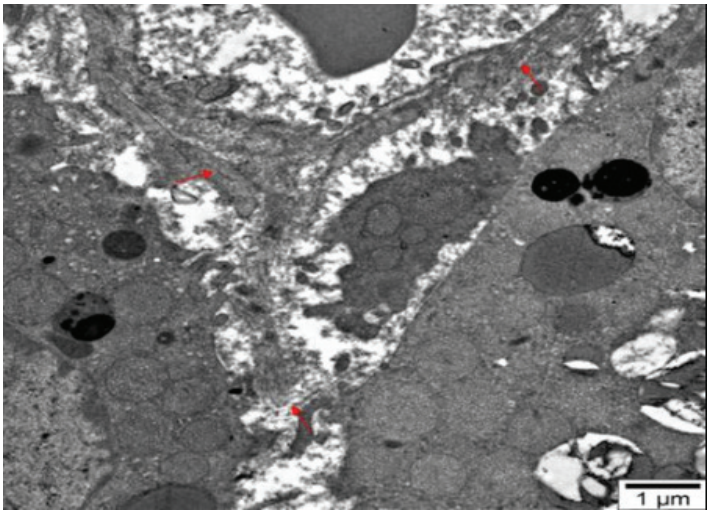

Figure 3. 30th day of hypoxia. Electron micrograph of structures of the adrenal gland. TEM. The explanation is given in the text. Stain: uranium acetate and plumbum citrate. Scale: 1 µm.

In the electron micrographs of ultrathin sections, the plasmolemma is thickened, the number of organelles are decreased, nucleus and nuclear chromatine is faded, the cristae of mitochondria is smoothed, the endoplasmic reticulum was relatively small, and the Golgi complex was not visible (figure 4, marked with a red arrow).

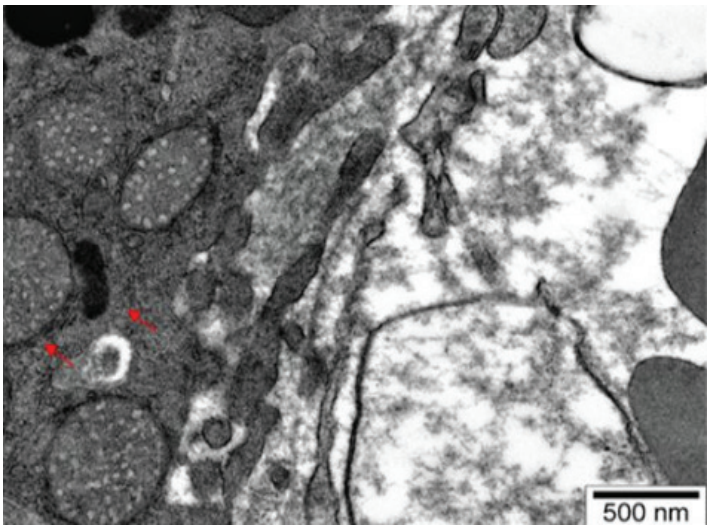

Figure 4. 30th day of hypoxia. Electron micrograph of structures of the adrenal gland. TEM. The explanation is given in the text. Stain: uranium acetate and plumbum citrate. Scale: 500 µm.

The presence of a very small amount of glycogen grains in the cytoplasm confirms that during a prolonged hypoxia the cell energy is depleted, and oxygen is an indicator of hypoxia. The development of connective tissue between cells, the basal membrane of the capillaries is clearly monitored (figure 5, marked with a red arrow).

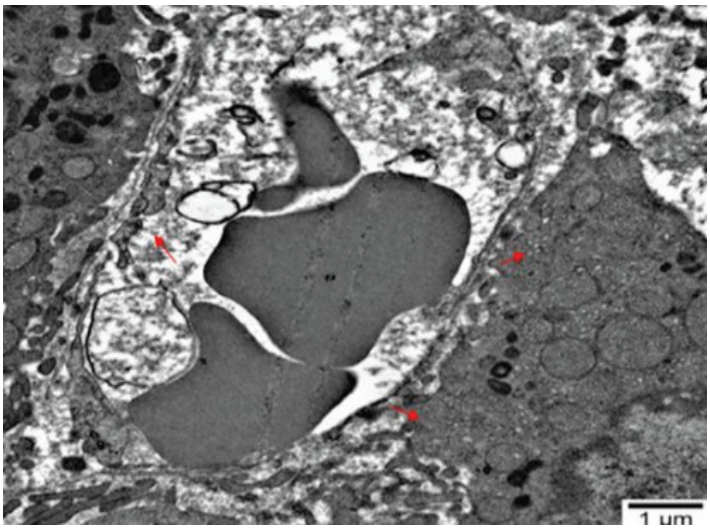

Figure 5. 30th day of hypoxia. Electron micrograph of structures of the adrenal gland. TEM. The explanation is given in the text. Stain: uranium acetate and plumbum citrate. Scale: 1 μm.

The increase in these indicators reaches a maximum on the 15th day of the experiment, the next stage is approaching the norm, and the morphological structure of the gland not differs from the norm. Morphological changes occur in the vascular beds of the fascicular and reticular zones, high activity of cells is ensured in these zones, which are involved in the formation of adaptive reactions and regulation of exchange processes in the body during the hypoxia.

On the 30th day of hypoxic effect, regeneration processes in the parenchyma and the stroma of the adrenal gland, restoration of tissue structures indicates the adaptation of the organism to hypoxia in general.

Discussion

The results of the studies showed that morphometric parameters of the adrenal parenchyma increase from the 5th day of hypoxia and are characterized by increased blood supply to the adrenal glands, which occurs both by the dilatation of capillaries and by an increase in their density. The increase in these parameters reaches a maximum on the 15th day of the experiment, the next stage is approaching the norm, and the morphological structure of the gland not differs from the norm. Morphological changes occur in the vascular beds of the fascicular and reticular zones, high activity of cells is ensured in these zones, which are involved in the formation of adaptive reactions and regulation of metabolism in the body during the hypoxia.

Thus, the results of electron microscopic studies show the presence of several structural dystrophic-dezorganization changes in the adrenal glands of animals with the chronic hypobaric hypoxia model are noted. The absence of serious changes in the pathohystological picture of cell structures in the ultrathin sections taken from the gland tissues is characterized by acceleration of compensatory-adaptive processes, development of reparative-regeneration. On the last day of the study, the regeneration processes occurring in the adrenal gland parenchyma and stroma, the restoration of tissue structures and cell organelles, most of the morphometric parameters similar to the control group, in general, indicate the adaptation of the organism to the hypoxia.

Conclusion

Adaptation to hypoxia increases the reserve capacity and the development of the body's resistance to physical stress [5].

An essential role in adaptation to hypoxia of any genesis belongs to the endocrine system and, first of all, to the pituitary, adrenal, and thyroid glands [2]. At the same time, it is important to analyze the ongoing rearrangements not in one, separately taken gland, but in their relationship, since any adaptive process is an interdependent integration of different parts of the endocrine system [5].

Taking into account the phase structure of the formation of tissue mechanisms of adaptation to hypoxia and the unequal participation of endocrine glands in this, a comparative study of changes in tissue structures in the adrenal glands at different stages of adaptation of the body to hypoxia is relevant [4].

The adrenal glands in embryogenesis develop from different germ leaves: the cortex is of mesoderm origin, medulla - ectoderm origin and is derived from nerve crest cells (11). The cortical substance belongs to the interrenal, and the medullar substance to the adrenal system. In this regard, the adrenal glands are both an endocrine organ and a highly specialized part of the sympathetic nervous system (12).

It should be noted that the adrenal glands play an important role in the early adaptation of the body to hypoxia. Long-term hypoxia increases the body's resistance to it, mainly the organs of the hypothalamic-pituitary-adrenal system, and they adapt to hypoxic conditions regulated by the processes of structural reorganization of organs (13).

The increase the morphometric parameters reaches a maximum on the 15th day of the experiment, the next stage is approaching the norm. Morphological changes occur in the vascular bed of the fascicular and reticular zones, high activity of cells is ensured in these zones, which are involved in the formation of adaptive reactions and regulation of metabolizm in the body during the influence of hypoxia. On the 30th day of hypoxic effect, regeneration processes in the parenchyma and stroma of the adrenal gland, restoration of tissue structures indicate adaptation of the organism to hypoxia in general. Our results coincide with the results of Zerkalova IO.F. [4,13].

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Ethical approval
Before the study, permissions were obtained from the local ethical committee.

Samire Mammadhasan Yagubova ORCID: 0000-0002-6794-7798

References

1. Frodl T. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans / T.Frodl, V.O'Keane // Neurobiology of disease, 2013;52:24-37.

2. Zarechnova NN, Slynko EN. Influence of mountain hypoxia on endocrine system organs at insufficiency of adrenal and pancreatic hormones. Bulletin Med Technol Electronic Journal, 2018;4:3-10.

3. Erik R.Swenson, Peter Bartsch, High Altitude / Human Adaptation to Hypoxia, Springer, New York, 2014;495

4. Калюжная Л.И., Тарарак Т.Я., Калюжный И.Т. Эндокринные механизмы адаптации организма к условиям высокогорья: в кн.: Гипоксия. Адаптация, патогенез, клиника // под ред. Ю.Л. Шевченко. СПб.: ООО «Элби-СПб», 2000, с.235-265. [Kalyuzhnaya L.I., Tararak T.YA., Kalyuzhnyy I.T. Ehndokrinnye mekhanizmy adaptatsii organizma k usloviyam vysokogor'ya: v kn.: Gipoksiya. Adaptatsiya, patogenez, klinika // pod red. YU.L. Shevchenko. SPb.: OOO «Ehlbi-SPb», 2000, s.235–265. (Endocrine mechanisms of adaptation to the high altitude [Text]: in the book: Hypoxia. Adaptation, pathogenesis, clinic // Under the editorship of J.L.Shevchenko / Kalyuzhnaya, L.I., Tararak T.I., Kalyuzhny I.T. SPb: LLC «ELBI-SPb», 2000235-65.

5. Садыкова Г.С., Джунусова Г.С. Функциональные особенности эндокринных систем у жителей высокогорья // Международный журнал прикладных и фундаментальных исследований, 2016, № 4-5, с.943-947. [Sadykova G.S., Dzhunusova G.S. Funktsional'nye osobennosti ehndokrinnykh sistem u zhiteley vysokogor'ya // Mezhdunarodnyy zhurnal prikladnykh i fundamental'nykh issledovaniy, 2016, № 4-5, s.943-947. (Functional features of endocrine systems in the inhabitants of the highlands // International journal of applied and fundamental research, 2016;4-5.943-7.

6. Giovanni Vitale, Stefano Salvioli, Claudio Franceschi / Oxidative stress and the ageing endocrine system // Nature Reviews Endocrinology, 2013;9:228-40.

7. Hueston CM, Deak T. The inflamed axis: the interaction between stress, hormones, and the expression of inflammatory-related genes within key structures comprising the hypothalamic-pituitary-adrenal axis. Physiol. Behav. 2014;124:77-91.

8. Woods DR, Davison A, Stacey M, et al. The cortisol response to hypobaric hypoxia at rest and post-exercise. Hormone and Metabolic Research. 2012;44:302–5.

9. Weekly B.S. Electron microscopy for beginners. M.: Мир, 1975, 324c. (Electron-microscopy. Methods and Protocols. Edited by John Kuo. USA, Totowa, New Jersey: Humana Press Inc. 2007;608:85.

10. Ахундов Р.А. Гипоксия и антигипоксанты / Баку,«Aspoliqraf», 2010, 328c. Axundov R.A. Hipoksiya i antihipoksantı /Baku, «Aspoliqraf», 2010, 328 s. (Hypoxia and antihypoxants /Baku, «Aspoligraph», 2010, 328 pp.

11. Kuo BR. Erickson Regional differences in neural crest morphogenesis. Cell Adh Mİg. 2010;4:567-85.

12. Фадеев В.В. Надпочечниковая недостаточность (клиника, диагностика, лечение): рекомендации для врачей / В.В.Фадеев. М.: Медпрактика, 2008, 44с. [Fadeev V.V., Nadpochechnikovaya nedostatochnost' (klinika, diagnostika, lechenie): rekomendatsii dlya vrachey / V.V.Fadeev. M.: Medpraktika, 2008, 44s.(Adrenal insufficiency (clinic, diagnosis, treatment): recommendations for doctors / V.V.Fadeev. M.: Medpraktika, 2008

13. Зеркалова Ю.Ф. Морфофункциональная характеристика некоторых эндокринных желез при гипоксии различного генеза // дис. ... кандидата медицинских наук, Санкт-Петербург, 2004, 23с. Zerkalova YU.F. Morfofunktsional′naya kharakteristika nekotorykh ehndokrinnykh zhelez pri gipoksii razlichnogo geneza // Dis... kandidata meditsinskikh nauk, Sankt-Peterburg, 2004, 23s. (Morphofunctional characteristics of some endocrine glands in hypoxia of different genesis //: dis. ... candidate of medical Sciences, St. Petersburg, 2004, 23p..