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# LIGHT- AND ELECTRON-MICROSCOPIC CHARACTERISTICS OF CHANGES IN THE THYROID GLAND DURING CHRONIC HYPOXIA

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**ABSTRACT** — The investigation aims were to study the ultrastructural changes in the cellular and extracellular matrices of the thyroid gland in the norm and chronic hypoxia.
Materials and methods: 40 male white rats were divided into a test and control group. Animals of the control (I) group (n=20) were not subjected to intervention, in the test group (n=20) a model of chronic hypoxia was created - the animals were kept in a special ventilation barochamber daily for 2 hours 5 times a week. Anatomical, histological, histochemical, electron microscopic and morphometric methods were used. Morphometric parameters were calculated by use of program Statistica 10 (StatSoft.Inc.) (statistical processing was performed using W — Wilcoxon test (paired samples) with the control group).
Results: Pathomorphological picture of the thyroid gland of rats subjected to hypobaric hypoxia, on the 15th day of the study was characterized by the acceleration of adaptive processes in gland cells and the development of reparative regeneration. Small structural changes in thyrocytes observed on the 15th day of the experiment show that the gland cells are not completely restored, adaptive processes do not occur completely. The area (12.21±1.43) and the diameter (6.25±0.48) of the nuclei are relatively increased compared to the control group.
Analysis of samples from the thyroid gland of animals adapted to chronic hypoxia on the 30th day of the study shows that the gland cells adapted to the new conditions and completely restored their normal structure and size. The morphometric parameters of the thyroid gland were relatively close to the norm (normal cell diameter (5.96±0.38) and the area of the nuclei (12.11±1.43)).
Conclusion: The results of the studies showed that under the influence of prolonged hyperbaric hypoxia, the body's resistance to it, especially the thyroid gland, increases, and it adapts to hypoxic conditions, and this is accompanied by the processes of structural reorganization of organs. Thyrocytes of the thyroid gland adapt earlier to chronic hypoxia and respond to this by hyperplasia and hypertrophy and differentiation of thyrocytes.

**KEYWORDS** — thyroid gland, chronic hypoxia, thyrocytes, follicles.

## INTRODUCTION

Hypoxia is a process depending on the degree and duration of hypoxic effect, leading to alterative changes in cells, especially to a complex of metabolic disorders, in which energy processes play a key role. Hypoxia develops in conditions of oxygen deficiency in the environment, as well as as a result of various pathologies associated with impaired oxygen transport function of the blood. Oxygen deficiency, which causes structural, functional and metabolic insufficiency of cells, in all its manifestations ultimately leads to a decrease in the supply of oxygen to the tissues. And this leads to the appearance of morphofunctional changes in the organs of not only the respiratory, nervous and cardiovascular systems but also the endocrine system, especially in the thyroid gland [1-3]. At the same time, the thyroid gland plays a key role in regulating the functions of various organs and systems in the processes of vital activity of the body, as well as in the formation of adaptive reactions against adverse environmental factors [4–6].

Some scientists note that the hypoxic condition caused by intensive physical work, as well as appearing in high-altitude conditions, plays an important role in the occurrence of some morphological changes and thyroid dysfunction [7–9]. The authors show that the state of periodic hypoxia, which occurs to one degree or another, is a common phenomenon for many forms of labor, sports and military activities [10–14].

Despite all the studies of pathomorphological changes in the thyroid gland, as the main mechanism of adaptation processes in the body during hypoxia of various origins, the analysis of the literature data gives us reason to re-conduct a broad comparative analysis of these changes in the formation of adaptation mechanisms.

*The objective of this study* was to investigate the features of the ultrastructural organization of cellular and extracellular components of the thyroid gland in normal and chronic hypobaric hypoxia.

## MATERIALS AND METHODS

Materials of the study were 40 healthy white male rats weighing 180–200 grams, divided into 2 groups. Animals of the control group (n=20) were not subjected to intervention, animals included in the hypoxia group (Group II) (n=20) were subdivided into 2 equal subgroups (subgroup 1 and subgroup 2) with the duration of the experiment for 15 and 30 days accordingly and were kept in a special barochamber with a temperature of 19–20° C, atmospheric pressure equal to the pressure at an altitude of 2000–3000 m above sea level, 5 times a week for 2 hours every day. Particles of natron lime ($Ca(OH)_2$ 81%+NaOH 3,4%+$H_2O$ 15,6%) were applied to absorb $CO_2$. All experiments were carried out within 10:00–15:00 hours, which is considered the lightest time of the day.

By the 15th and 30th days of the experiment, the rats of both groups (groups I and II) were euthanized by decapitation, accordingly. At the same time, all painful procedures were performed under surface ether anesthesia and general anesthesia using a 2–2,5% solution of thiopental sodium (100 mg/kg) injected into the peritoneal cavity of animals. After decapitation of the animals, the thyroid gland was completely extracted, materials were taken for anatomical, histological, histochemical, electron microscopic and morphometric studies.

The preparations were first macroscopically evaluated, and then the pieces of the thyroid gland were fixed with a 2,5% solution of glutaraldehyde in a 0,1 M phosphate buffer (pH 7.4) and 2% paraformaldehyde, post-fixed (2 hours) in a solution of 1% osmic acid made in the same buffer, dehydrated, respectively, in solutions of 50°, 70°, 80°, 90° ethyl alcohol (30 minutes), in solutions of 96° and 100° ethyl alcohol (1 hour) and blocks of Araldite-epon and spur resins were made from them [15].

Semithin (1–2 μm) and ultrathin (70–100 nm) sections (by LKB-III, Leica EM UC7 ultratoms) were made from the obtained blocks, which were colored in various ways (methylene blue with fuchsin, uranium-acetate and pure lead citrate). Semithin section viewed under the 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). Morphometric parameters were analyzed statistically using Statistica 10 (StatSoft Inc., USA) software and the Mann-Whitney U-test was performed.

The animal research was carried out in the Department of Pharmacology, Experimental Surgery and the Electron Microscopy Laboratory of the Scientific Research Center of AMU based on ethical principles approved by the Commission on Ethics Rules under the Ministry of Health of the Republic of Azerbaijan on 21.04.2008 (Protocol No. 31) and used following the requirements of the Bioethics Committee.

## RESULTS

Macroscopically, the thyroid gland is clearly distinguished in front of the trachea under the larynx. In histological preparations, the connective tissue capsule covering the thyroid gland, thin trabeculae extending from the capsules into the thickness of the gland, as well as the lobules of the organ separated from each other by trabeculae are visible.

In histological preparations, small follicles consisting of cylindrical cells predominate in the central part of the gland, and large follicles consisting of cuboidal cells predominate in the peripheral part compared to the central part. A colloid in the form of a viscous liquid evenly fills the lumen of the follicles. The cytoplasm of thyrocytes stained with methylene blue with fuchsin and round nuclei, the well-developed interlobular, interfollicular connective tissue of the thyroid gland and blood capillaries are well visualized.

According to electron microscopic studies in the thyroid gland of rats included in the control group, thyrocytes are located in one layer on the basement membrane. There is a close connection between follicular thyrocytes through well-developed numerous lamellae. Round nuclei are located in the center of cells, the membrane of the nucleus and the nucleolus are well identified. Microvilli, on the apical surface of thyrocytes facing the follicle lumen, are observed.

The study of ultrathin sections showed that the basal surface of thyrocytes is folded, which increases the contact of thyrocytes with the perifollicular cavities. On the lateral surfaces of thyrocytes, finger-like protrusions entering into the corresponding indentations of the lateral surfaces of neighboring cells are well distinguished, Thyrocytes have well-developed organelles-ribosomes, mitochondria, lysosomes, endoplasmic reticulum and Golgi complex. A few ribosomes and lysosomes are unevenly distributed in the cytoplasm and are mainly located around the Golgi complex (Fig. 1).

The pathomorphological picture of the thyroid gland of rats subjected to hypobaric hypoxia by the 15th day of the experiment is characterized by an acceleration of adaptive processes in thyrocytes and the development of regeneration.

Under chronic exposure to hypoxia, the structure of the thyroid gland did not undergo significant changes. The capsule of the gland is thickened, but not deformed, although the boundary between the central and peripheral parts of the gland is not clear.

Mild structural changes in thyrocytes observed by the 15th day of the experiment show that the thyrocytes are not completely restored, the adaptation processes are slow. Microscopically, the cytoplasm is weakly oxyphilic, foamy in some cells, pale stained with hematoxylin-

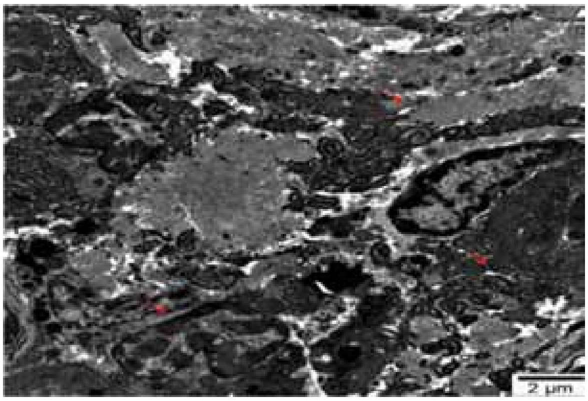

*Fig. 1. TEM. Control group: the structure of the thyroid gland. Stain: uranium-acetate and pure lead citrate. Scale: 2 μm*

eosin. Although the morphological structure of the stroma of the gland corresponds to the norm, the destruction of collagen and reticulin fibers is detected, signs of local metachromasia are noted. Despite perivascular and pericellular edema, diapedesis hemorrhage and hyperemic foci are not observed during microscopic examination. Capillaries also do not differ from ordinary capillaries in diameter and structure of the walls. But in some histological preparations, the diameter of the capillaries is wide and the contours are fuzzy.

By the 15th day of the experiment, the electronograms clearly show that a small amount of lipid droplets is found in the cytoplasm of thyroid cells, and glycogen grains are not detected due to hypoxia. During the regeneration of the central part of the gland, hypertrophy, and hyperplasia of cell organelles are observed.

The contours of the cytoplasm and nuclei are clear, the nucleus is located in the center of the cell, and the lysosomes, ribosomes, endoplasmic network, and Golgi complex are located around it. The area of the nuclei (12.21±1.43) and the diameter (6.25±0.48) is relatively increased in comparison with the control group animals (Table 1).

There is paleness of the nuclear chromatin of thyrocytes in the peripheral zone, a relative thinning of the nuclear and basement membranes, and weak mitochondrial hyperplasia, clear boundaries of the basement membrane, and complete cell recovery in epithelial cells. Although the same analogous changes are found in the thyrocytes of the central zone, the morphologically cuboidal epithelium becomes rarer under the influence of hypoxia, and a small number of lipid droplets in the cytoplasm is also observed.

Stratification in the basement membranes of the capillaries, vascularizing follicles, hypertrophy of endothelial cells, and narrowing of the lumen of capillaries are revealed. In addition, due to hypoxia, there is an increase in the number of fibroblasts around the capillaries, filling the interstitial substance with collagen and fibrous tissue. This, in turn, leads to a narrowing of the capillary lumen and a deepening of hypoxia.

The analysis of samples taken from the thyroid gland of animals adapted to chronic hypoxia by the 30th day of the experiment shows that the gland cells adapted to the new conditions and completely restored their normal structure and size, there was a restructuring of the parenchyma and stroma of the gland tissues.

Macroscopically, the thyroid gland differs little from the thyroid gland of the control group animals. The capsule covering the glands, lobules, septa separating them, as well as the boundaries between the central and peripheral zones of the gland are visualized (Fig. 2). Although the structure of the thyroid gland in histological preparations taken from animals on the last day of chronic hypoxia is close to the norm, it is possible to see the development of fatty and hydropic degeneration in some cells after hypoxia. But these changes are focally observed in some cells.

The follicles and thyrocytes of the gland have completely restored the structure as a result of proliferation and differentiation, the colloid is transparent and dense, evenly fills the lumen of the follicles. On histological preparations, attention is drawn to the develop-

***Table 1.** Morphometric parameters of thyroid cells in the norm and chronic hypoxia (M±m), (min-max)*

| | Thyroid gland | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Diameter of cells | | | Diameter of nuclei | | | Area of nuclei | | |
| | N | Duration of the experiment (days) | | N | Duration of the experiment (days) | | N | Duration of the experiment (days) | |
| | | 15th day | 30th day | | 15th day | 30'h day | | 15th day | 30th day |
| N | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| MM±m | 5,94±0,38 | 6,06±0,37 | 5,96±0,38 | 6,04±0,49 | 6,25±0,48 | 6,09±0,48 | 12,08±1,43 | 12,21±1,43 | 12,11±1,43 |
| $M_{min-max}$ | 3,95-7,67 | 4,12-7,79 | 3,98-7,69 | 3,59-7,92 | 3,73-8,13 | 3,61-7,97 | 3,91-20,01 | 4,02-20,12 | 3,97-20,03 |

*** Note:** N — control group; n — number of animals; M±m: M — the average variation, m — standard error.*

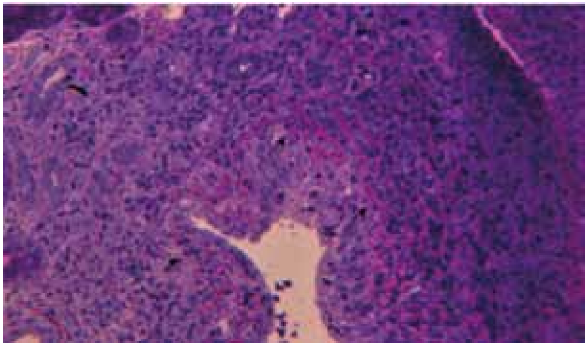

*Fig. 2. Semi-thin section. The structure of the thyroid gland on the 30th day of hypoxia. Stain: methylene blue with fuchsin. Magnification: ×40*

ment of fibrous tissue among the follicles, an increase in the number of fibroblasts, as well as capillaries.

Electron microscopically, hyperplasia of cytoplasmic organelles, thickening of the plasmalemma and basement membrane, their boundaries, as well as the contours of nuclei and nucleoli located in the center of cells and nuclear chromatin can be observed. Noticeable microvilli on the apical surface of thyrocytes, and protrusions on the lateral surfaces reflect the complete restoration of thyrocytes. The normal cell diameter (5.96±0.38) and the area of the nuclei (12.11±1.43), as well as the noticeable restoration of cristae in the mitochondria, once again shows the supply of oxygen to the cells (Table 1). The narrowing of the capillary lumen attracts attention, but the structure of all three layers of the basement membrane of the capillary loops is close to normal histological structures (Fig. 3). By the 30th day of the experiment, the morphometric parameters of the thyroid gland were also relatively close to the norm.

Thus, the results of studies have shown that as a result of short-term and long-term exposure to hypobaric hypoxia, various morphological changes occur in the cells of the thyroid gland. So, if the analysis of histological and ultra-thin sections at an early stage of the experiment showed the effect of acute hypoxia, then in the cells of the thyroid gland, as well as in the intercellular spaces, there is the formation of edema, stagnation, as well as cell destruction, but at the end of the study there is a recovery process at a noticeable level, there is a process of restoration of follicles, thyrocytes, cytoplasmic organelles and an increase in their size and number. The manifestation of this result at the ultrastructural level can be assessed as the restoration and adaptation of thyroid cells to hypoxic conditions.

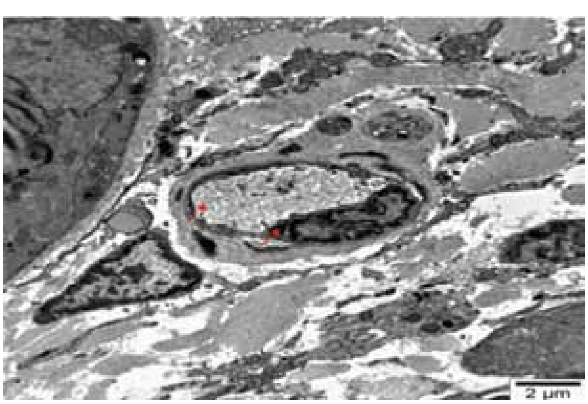


*Fig. 3. TEM. 30th day of hypoxia. Stain: uranium-acetate and pure lead citrate. Scale: 2 µm*

## DISSCUSSION

Under the influence of hypoxia, especially chronic hypoxia, the reactivity and adaptation tension of the pituitary-thyroid, pituitary-adrenal and simpato-adrenal systems increases. As a result of hypoxia, the signs of follicular destruction in the thyroid gland tissue are clearly noticeable [2]. During the experiment, the presence of resorption vacuoles in the colloid, desquamation of some thyrocytes into the follicular cavity and changes in the ratio of follicle-colloid relationship were noted [16]. According to most authors, the process of reconstruction of the thyroid gland takes place at this time. Due to these processes, lymphatic drainage weakens, as a result of which edema develops and the proportion of interstitial tissue increases [8]. Thus, the authors concluded that the sanogenic level of hypoxia can increase the functional activity and the processes of physiologic regeneration of the thyroid gland in adult animals [4, 6, 16]. According to the results of our study, under the influence of chronic hypoxia, significant structural changes occur in the cells of the thyroid gland, and these changes result in the reconstruction of the structure of the gland. Our results once again confirm the views of these authors.

## CONCLUSION

To sum up it should be noted that the thyroid gland plays an important role in the early adaptation of the body to hypoxia. With prolonged hypoxia, the body's resistance to it increases, mainly the thyroid gland adapts to hypoxic conditions, which is accompanied by the processes of structural restructuring of organs. On the other hand, it should be noted that adaptive changes occur not only depending on the type, nature, duration, and intensity of hypoxia but also morphofunctional features of organs. The structure of the thyroid gland adapts first of all to prolonged hypoxia and responds to this with hyperplasia and hypertrophy and differentiation of thyrocytes. The manifestation of this result at the ultrastructural level, as we noted above, maybe due to the adaptation of thyroid tissues to new conditions.

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