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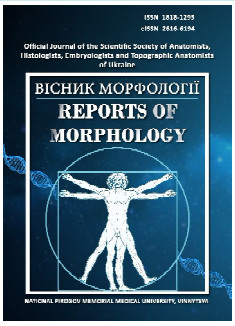

Morphological changes in the white rats' thyroid gland 14 days after simulated thermal trauma of the skin on the background of the administration of 0.9 % NaCl solution

Tiron O.I.
Odessa National Medical University, Odessa, Ukraine

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CORRESPONDING AUTHOR
e-mail: ichekina.o@ukr.net
Tiron O.I.

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According to the World Health Organization (WHO), there are about 11 million burns in the world each year, of which 180,000 are fatal. One of the systemic consequences of burn injuries is the so-called burn-associated hypermetabolism, the clinical picture of which includes immunological dysfunction, loss of bone mineral density, endocrine disorders, including thyroid dysfunction. The aim of the study was to establish histological and ultrastructural changes of the thyroid gland of experimental animals 14 days after the simulated thermal trauma of the skin on the background of using first 7 days 0.9 % NaCl solution. At the optical and ultrastructural levels of the study 14 days after thermal skin burn on the background of 0.9 % NaCl solution in the thyroid gland of experimental animals found: follicles - mostly large, overstretched (epithelium flat, with desquamation); cytoplasm of thyrocytes is poor in organelles, part of mitochondria with damaged cristae and enlightened matrix, available electron-dense lysosomes, apical surface smoothed; there is swelling of the capsule and trabeculae, leukocyte infiltration; components of the arterial and venous bed are dilated, there is a deformation of the lumen of blood vessels; hemocapillaries are dilated with numerous erythrocytes and perivascular edema. The detected changes cause a violation of the secretory cycle of thyrocytes, transendothelial metabolism and lead to an imbalance in the production and excretion of thyroid hormones.
Keywords: *thyroid gland, structural changes, skin burn, 0.9 % NaCl solution.*

Introduction

Despite the modernization of working and living conditions in the XXI century, burn injuries and their long-term consequences remain a pressing problem in modern medicine and create a significant burden on the health care system in Ukraine and around the world. Thus, according to the World Health Organization (WHO), about 11 million burns are registered worldwide each year, of which 180,000 are fatal [11, 20]. At the same time, 90 % of burn injuries occur in developing countries [5, 17]. According to the American Burn Association, the majority of burn injuries (41 %) are thermal burns [1].

Modern combustiology recognizes that, due to the body's systemic response, severe skin burns to some extent affect the condition of all human organs. This is confirmed by the fact that during the autopsy of the dead due to severe burns in more than 70 % of cases the direct cause of death is multiple organ failure [9].

One of the systemic consequences of burn injuries is the so-called burn-associated hypermetabolism, the clinical picture of which includes immunological dysfunction, loss of bone mineral density, endocrine disorders, including thyroid dysfunction [8]. A series of our studies is devoted to the study of morpho-functional changes of the latter in different periods after thermal skin burns. One of the most important steps in providing medical care to patients with burns is fluid resuscitation, the main purpose of which is to maintain adequate perfusion of distant organs. Discussions are still underway to determine the "drug of choice" for the treatment of burn shock, which is usually accompanied by massive loss of protein, electrolytes and plasma [10, 12, 15, 19].

Thus, the urgent task is not only to describe the morphological changes of the thyroid gland on the background of burn injury, but also to find the optimal solution for infusion therapy, the use of which would minimize the impact of burn shock on the functioning of

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this endocrine gland.

The aim of the study was to establish histological and ultrastructural changes of the thyroid gland of experimental animals 14 days after the simulated thermal trauma of the skin on the background of using first 7 days 0.9% NaCl solution.

Materials and methods

All research was conducted under the agreement on scientific cooperation between the research center of National Pirogov Memorial Medical University, Vinnytsya and the Department of Histology, Cytology and Embryology of Odessa National Medical University (from 01.01.2018), as well as between the Department of Histology and Embryology of Ternopil National Medical University named after I. Gorbachevsky and the Department of Histology, Cytology and Embryology of Odessa National Medical University (from 01.01.2019).

Experimental studies were conducted on 90 white male rats weighing 160-180 g (obtained from the vivarium of the Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine), conducted on the basis of the Research Center of National Pirogov Memorial Medical University, Vinnytsya. The keeping and manipulation of animals was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), and was guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes. (Strasbourg, 1985), guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine on "Preclinical studies of drugs" (2001), as well as rules of humane treatment of experimental animals and conditions approved by the **Committee on Bioethics** of National Pirogov Memorial Medical University, Vinnytsya (Minutes № 1 dated 14.01.2010).

Thermal skin burns of 2-3 degrees were performed by applying four copper plates (each with a surface area of 13.86 cm^2) to pre-depilated side surfaces of the body of rats for 10 seconds, which were preheated for 6 minutes in water with a temperature of 100°C [6]. The total area of skin lesions was 21-23 %. The first 7 days, rats were infused with 0.9 % NaCl solution into the inferior vena cava. Animals were removed from the experiment by decapitation (after 1, 3, 7, 14, 21 and 30 days). Shaving, venous catheterization, skin burns, and decapitation of rats were performed under intravenous propofol anesthesia (60 mg/kg body weight).

Collection of material for microscopic examinations was performed according to generally accepted methods [7]. Pieces of the thyroid gland were fixed in 10 % neutral formalin solution, dehydrated in alcohols of increasing concentration, poured into paraffin blocks. The sections, 5-6 μm thick, were stained with hematoxylin-eosin [7]. Histological specimens were studied using a MIROMED SEO SCAN light microscope and photo-documented using a Vision CCD Camera with a histological specimen image output system.

For electron microscopic examinations, pieces of the thyroid gland were removed, fixed in 2.5 % glutaraldehyde solution, and fixed with 1 % osmium tetraoxide solution on phosphate buffer. Further processing was performed according to the generally accepted method [7]. Semi-thin sections were stained with methylene blue. Ultrathin sections made on an LKB-3 ultramicrotome were contrasted with uranyl acetate, lead citrate according to the Reynolds method [14] and studied under a PEM-125K electron microscope.

Results

14 days after skin burn on the background of the introduction of 0.9 % NaCl solution at the microscopic level in the thyroid gland of experimental animals, significant changes in its structural elements were observed. Swollen trabeculae departed from the slightly thickened connective tissue capsule, which went deep into the organ, dividing it into lobes. In the layers, sometimes with leukocyte infiltration, loose connective tissue, there were numerous vessels that supply blood to the body. It should be noted that the components of the arterial and venous channels were dilated, there was a significant blood supply, protrusion of endothelial cells into the lumen, deformation of the lumen, which is often associated with changes in the media (Fig. 1).

The lobe itself consisted of follicles of different sizes and shapes. Among small, medium and large round and oval follicles, large ones were dominant. They looked significantly overstretched, limited by thinned, squamous epithelium and filled with homogeneous oxyphilic colloid. The epithelial cells were intensely basophilic, largely due to elongated, pyknotic nuclei with large amounts of

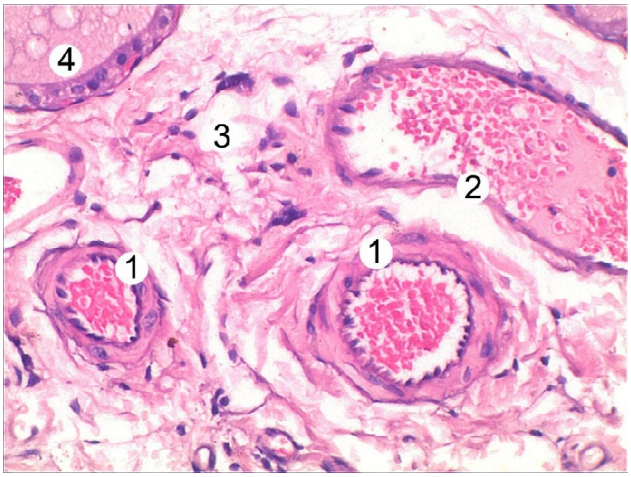

Fig. 1. Histological changes in the thyroid gland of the animal 14 days after skin burn on the background of the introduction of 0.9 % NaCl solution. 1 - arterioles 2 - venules, 3 - swollen interparticle loose connective tissue, 4 - a fragment of the follicle. Staining with hematoxylin and eosin. Magnification x400.

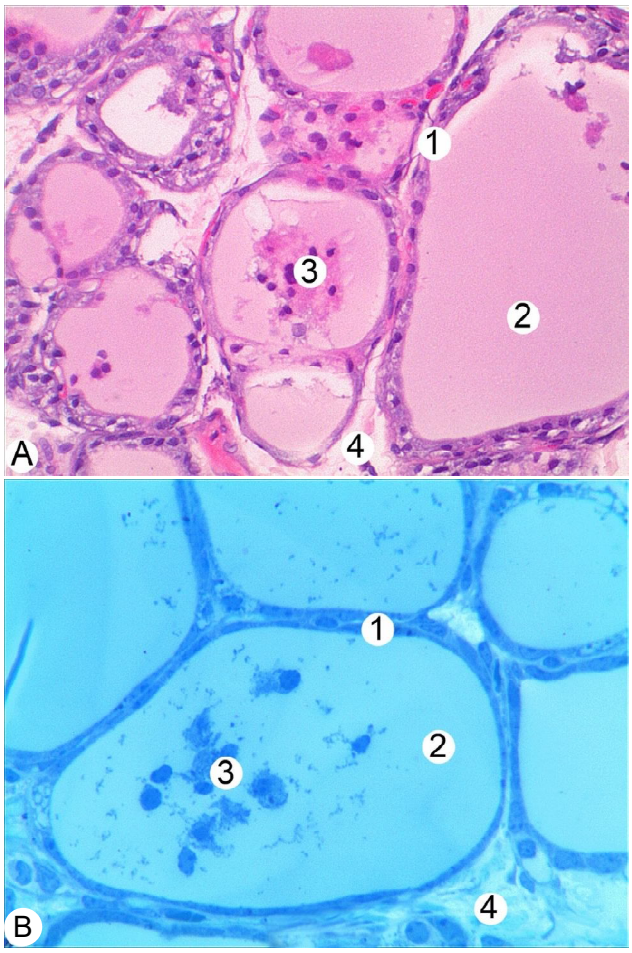

Fig. 2. Microscopic condition of the animal's thyroid gland 14 days after skin burn on the background of 0.9 % NaCl solution. 1 - thyrocytes and 2 - colloid of large follicles, 3 - desquamated thyrocytes in the lumen of the follicle, 4 - perifollicular connective tissue. A - Staining with hematoxylin and eosin. Magnification x 400. B - Semi-thin slice. Methylene blue color. Magnification x400.

heterochromatin. In some follicles at this time of the experiment were desquamated thyrocytes in the colloid (Fig. 2A, 2B).

Medium and small follicles, which did not predominate numerically, were covered with cubic epithelium with central nuclei and light cytoplasm. The lumen of such follicles was filled with thyroglobulin, which had a dispersed appearance. Resorptive vacuoles were observed in some follicles (Fig. 3).

Significant changes in the components of the hemomicrocirculatory tract were found 14 days after skin burns on the background of 0.9 % NaCl solution in the swollen interfollicular connective tissue of the thyroid gland. Numerous erythrocytes were present in the unevenly dilated capillaries, and perivascular edema was present around them. The nuclei of the endothelial cells lining these hemocapillaries are dense, intensely basophilic. Adventitia of most arterioles and venules is infiltrated by leukocyte cells. They were also characterized by plethora and unequal lumen diameter along the length of the vessel, in addition, there was detachment of the endothelium from the basement membrane (Fig. 4).

At the ultrastructural level, 14 days after skin burn on the background of 0.9 % NaCl solution, the wall of large follicles was lined with a single layer of squamous epithelium, the boundaries between the cells of which were poorly contoured. The apical surface of thyrocytes of these follicles is smoothed, with several microvilli, which indicated their low functional activity. The basal part of the plasmalemma was adjacent to the unevenly thickened basement membrane. The elongated forms of the nucleus are filled with karyoplasm, in which the accumulations of heterochromatin were placed marginally, and euchromatin occupied a more central position. One, and in some

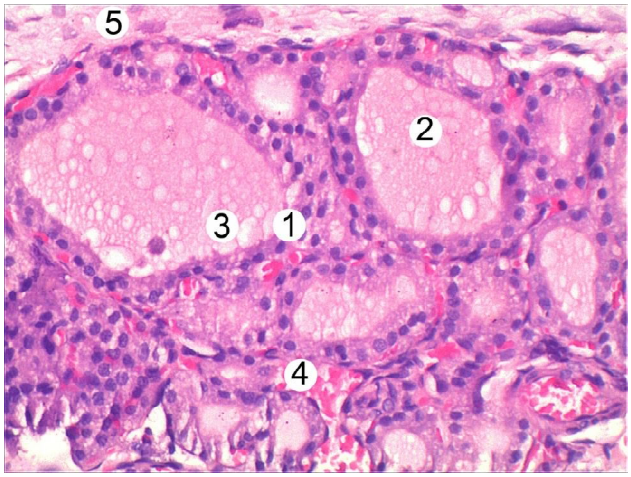

Fig. 3. Medium and small follicles of the thyroid gland of the animal 14 days after skin burn on the background of the introduction of 0.9 % NaCl solution. 1 - thyrocytes, 2 - colloid, 3 - resorption vacuoles, 4 - vessels, 5 - connective tissue. Staining with hematoxylin and eosin. Magnification x200.

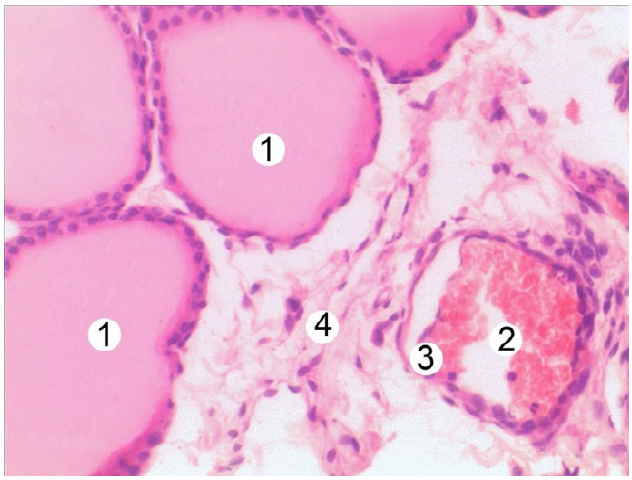

Fig. 4. Histological changes in the thyroid gland of the animal 14 days after skin burn on the background of 0.9 % NaCl solution. 1 - follicles, 2 - venules with 3 - endothelial detachment, 4 - swollen connective tissue. Staining with hematoxylin and eosin. Magnification x400.

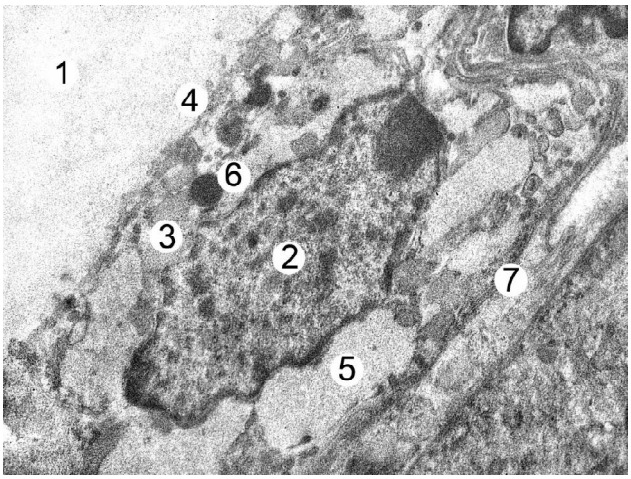

Fig. 5. Ultrastructure of the wall of the follicle of the thyroid gland of the animal 14 days after skin burn on the background of the introduction of 0.9 % NaCl solution. 1 - lumen of the follicle, 2 - nucleus and 3 - cytoplasm of thyrocyte, 4 - single microvilli on the apical surface of cells, 5 - vacuolated tubules of granular endoplasmic reticulum, 6 - lysosome, 7 - basement membrane. Electronogram. Magnification x13 000.

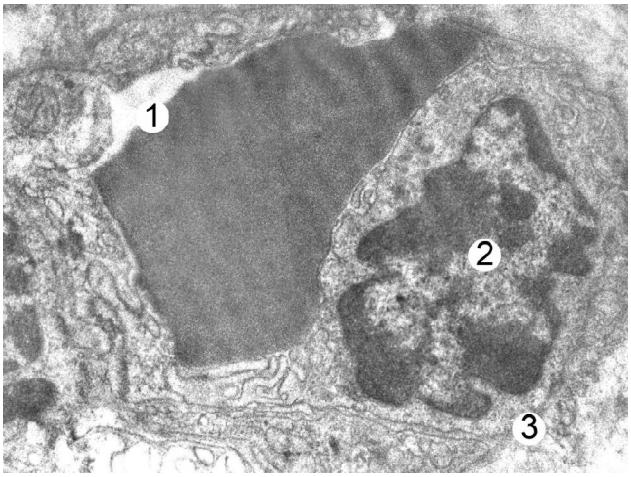

Fig. 6. Submicroscopic organization of the hemocapillary of the thyroid gland of the animal 14 days after skin burn on the background of the introduction of 0.9 % NaCl solution. 1 - capillary lumen with erythrocytes, 2 - endothelial cell nucleus, 3 - capillary basement membrane. Electronogram. Magnification x12 000.

thyrocytes, two nucleoli were visible. Nuclear pores are poorly expressed due to the electron-tight fit of heterochromatin to the karyolemma. The cytoplasm of squamous follicular epitheliocytes is poor in organelles. There was a small number of tubules of the granular endoplasmic reticulum with a small number of ribosomal granules on their membranes. In some thyrocytes, the components of the granular endoplasmic reticulum reached very large sizes, were expanded. The Golgi tanks were also characterized by variability in lumen size. Electron-dense lysosomes, mostly round in shape and of different sizes, were located in groups in the cytoplasm of follicular epitheliocytes. There were few mitochondria. They have damaged cristae and enlightened matrix, some of them resembled vacuoles due to significant destruction of the inner membrane (Fig. 5).

Significant changes in hemocapillaries at the submicroscopic level were observed 14 days after skin burn on the background of 0.9 % NaCl solution administration. Most of them were characterized by wide lumens, densely filled with shaped elements, mainly erythrocytes. However, there were also significantly narrowed capillaries with a slit lumen. Capillary wall endotheliocytes contained osmophilic nuclei with a small nucleolus and a predominance of heterochromatin in the karyoplasm. Their karyolemma had numerous intussusception. The cytoplasm of endothelial cells was significantly swollen, but with a small number of pinocytic microbubbles, indicating a low level of transendothelial metabolism. Organelles are few, mostly found mitochondria (Fig. 6).

Discussion

Referring to the data of our previous studies [18], we can trace the morphological changes of thyroid components in 1, 7 and 14 days after thermal burns on the background of the first 7 days of 0.9 % NaCl solution at optical and electron microscopic levels (Table 1).

Therefore, we see that 1 day after thermal trauma of the skin (ie in the stage of burn shock) on the background of the introduction of 0.9 % NaCl solution in the thyroid gland, there are reactive adjuvant-compensatory changes and initial manifestations of destruction. These changes in vascular, stromal and parenchymal components of the body correspond to the state of "stress", which is characterized by intensification of metabolic processes.

7 days after the simulated burn injury on the background of the introduction of 0.9 % NaCl solution in the structural elements of the thyroid gland is already dominated by the phenomena of destruction and alteration, which may be accompanied by clinically significant deterioration of functional activity of the organ.

Increased destructive changes are observed after 14 days of observation, as evidenced primarily by desquamation of thyrocytes, significant reduction and destruction of their organelles, the disappearance of microvilli, as well as edema of the connective tissue of the thyroid gland.

Unfortunately, relatively little attention has been paid to the study of the thyroid response to thermal skin burns, and therefore the body of available scientific data on this issue is limited.

Functional changes in the thyroid gland after severe burns were studied by determining the level of thyroid hormones in the blood, determining the uptake of radioactive iodine by the thyroid gland and describing pathological changes in the gland. However, the results of such studies contain contradictions.

The following are the data and conclusions of studies

Table 1. Morphological changes in the components of the thyroid gland at 1, 7 and 14 days after thermal burns on the background of the introduction of 0.9 % NaCl solution at the optical and electron microscopic level.

Indicators	Term of observation		
	1 day	Day 7	Day 14
The size of the follicles	Mostly medium in size.	Different. In the center of the lobe - small (highly prismatic epithelium), on the periphery - large (epithelium flat, with the phenomena of desquamation).	Mostly large, overstretched (epithelium flat, with the phenomena of desquamation).
Thyrocytes	Low-prismatic form, resorption vacuoles are present. Mitochondria are swollen, with symptoms of cristae discomplexation. Lysosomes and phagosomes that cleave colloids on the apical surface. Available microvilli.	Cytoplasmic edema, destruction of organelles, mitochondria are few, their cristae are reduced. On the apical surface - a few vesicles and lysosomes. Single microvilli.	The cytoplasm is poor in organelles, few mitochondria with damaged cristae and an enlightened matrix. Available electron-dense lysosomes. The apical surface is smoothed.
Connective tissue	Swelling of loose connective tissue, intercellular edema.	Swelling of loose connective tissue, intercellular edema.	Capsule and trabeculae edema, leukocyte infiltration.
Vessels	Hemocapillaries without signs of excessive blood supply, endothelial cytoplasm without signs of edema.	Blood supply to arteries and veins, endothelial destruction, marginal leukocytes. Blood filling of hemocapillaries, stasis and sludge of erythrocytes, deformation of endothelial cells.	Extended components of the arterial and venous bed, deformation of the vascular lumen. Dilated hemocapillaries with numerous erythrocytes and perivascular edema.

in which pathological changes of the thyroid gland after severe burns were studied using light and electron microscopy, as well as peroxidase histochemistry [2, 3, 4, 13, 16].

At the optical level, the authors observed ubiquitous expansion of follicles at 2, 12, 24 hours and 3 days after burn, with follicular epithelial cells were markedly flattened, cubic or flat. This contradicts our results somewhat, as we observed mostly large follicles covered with squamous epithelium only on the 14th day of observation. In the apical part of follicular cells, the authors noted a meager amount of cytoplasm with a significantly reduced number of PAS-positive granules, colloid droplets were virtually absent. In addition, a large number of blisters and furrows (cracks) appeared in the apical cytoplasm within 12 hours to 3 days after the burn.

In contrast to our data, the authors of previous studies note the gradual restoration of the structure of the thyroid gland in 3 days after the burn. They note that in the apical cytoplasm of follicular cells, a large number of PAS-positive drops of colloid were formed from 10 to 15 days after the burn. The colloid in the lumen of the follicle was depleted, which is a sign of increased hormone secretion.

Also in previous studies it was noted that the cells of the follicular epithelium gradually returned to normal in the period from 3 to 6 days after the burn. Signs of organelle damage were significantly reduced. A characteristic feature of this stage was an increase in the number of lysosomes, multivesicular cells and autophagosomes, the appearance of polyribosomes in the cytoplasm, an increase in the nucleus of euchromatin, the appearance of a large nucleolus. There were signs of active functioning of follicular epithelial cells in the period from 10 to 15 days after thermal burns. The main morphological manifestations were the formation of numerous pseudopodia and colloid droplets, the appearance of well-developed microvilli, as well as the accumulation of large numbers of lysosomes and mitochondria in the cytoplasm of follicular cells of the apical surface; expansion of ER; the presence of a well-developed Golgi complex; euchromatin and giant nucleolus; increase in the number of fenestrae in the endothelium of the hemocapillary wall.

The results obtained by us also do not confirm the above, because 14 days after the burn we observed the maximum degree of destructive changes in the thyroid gland.

Conclusions

1. 14 days after thermal skin burn on the background of the first 7 days of 0.9 % NaCl solution in the thyroid gland of experimental animals, destructive changes in vascular walls, especially microcirculatory tract, thinning and destructuring of the follicle wall, with desquamation of epitheliocytes and its predominance lobules of large follicles was found.

2. The detected changes cause a violation of the secretory cycle of thyrocytes, transendothelial metabolism and lead to an imbalance in the production and excretion of thyroid hormones.

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МОРФОЛОГІЧНІ ЗМІНИ ЩИТОПОДІБНОЇ ЗАЛОЗИ БІЛИХ ЩУРІВ ЧЕРЕЗ 14 ДІБ ЗА УМОВ ЗМОДЕЛЬОВАНОЇ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ НА ФОНІ ВВЕДЕННЯ 0,9 % РОЗЧИНУ NACL

Тірон О.І.

За даними Всесвітньої організації охорони здоров'я (ВООЗ), у світі щороку реєструють близько 11 мільйонів опікових травм, з яких 180000 випадків є фатальними. Одним із системних наслідків опікових травм є так званий опік-асоційований гіперметаболізм, клінічна картина якого включає імунологічну дисфункцію, втрату мінеральної щільності кісткової тканини, ендокринні порушення, серед яких - дисфункція щитоподібної залози. Мета дослідження - встановити гістологічні та ультраструктурні зміни щитоподібної залози експериментальних тварин через 14 діб після змодельованої термічної травми шкіри на фоні введення впродовж перших 7 діб 0,9 % розчину NaCl. На світлооптичному та ультруструктурному рівнях дослідження через 14 діб після термічного опіку шкіри на фоні введення 0,9 % розчину NaCl у щитоподібній залозі піддослідних тварин встановлено: фолікули - переважно великі, перерозтягнуті (епітелій плаский, з явищами десквамації); цитоплазма тироцитів бідна на органели, частина мітохондрій з пошкодженими кристами та просвітленим матриксом, наявні електронно щільні лізосоми, апікальна поверхня згладжена; спостерігається набряк капсули та трабекул, лейкоцитарна інфільтрація; компоненти артеріального та венозного русла розширені, спостерігається деформація просвіту судин; гемокапіляри розширені з чисельними еритроцитами та периваскулярним набряком. Виявлені зміни спричиняють порушення секреторного циклу тироцитів, трансендотеліального обміну та ведуть до дисбалансу в утворенні та виведенні тиреоїдних гормонів.

Ключові слова: *щитоподібна залоза, структурні зміни, опік шкіри, 0,9 % розчин NaCl.*