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O.I. Tiron, A.V. Stetsenko, O.I. Yatsyna[1], L.M. Zayats[2], A.O. Kolotvin, K.S. Shumilina
Odessa National Medical University, Odessa; [1]National Cancer Institute, Kyiv
[2]Ivano-Frankovsk National Medical University, Ivano-Frankovsk

THE MORPHOLOGICAL CHANGES OF THE WHITE RATS' THYROID GLAND 21 DAYS AFTER EXPERIMENTAL THERMAL BURN INJURY UNDER NaCl SYSTEMIC ADMINISTRATION

e-mail: chekina.o@ukr.net

The histological features of the experimental animals' thyroid gland 21 days after modelled burn injury under NaCl systemic administration can be described as adaptive and compensatory processes in the follicular wall, vascular and stromal components. Most of the thyroid follicles were round or oval in shape, some of them were overstretched and filled with dense colloid. The follicular epithelial cells were flattened, contained pyknotic nuclei surrounded by thin layer of cytoplasm. In some regions it was observed the desquamation of the thyrocytes within the follicular lumen. The electron microscopic examination of the gland 21 days after modelled burn confirmed the changes that had been previously established by the analysis of histological specimens. The flattened thyrocytes contained osmiophilic nuclei with heterochromatin. Their cytoplasm was electron lucent and contained organelles exhibiting the signs of destruction. The microvilli were observed on the apical domain of thyrocytes. Along with the follicles with altered histological structure (which indicates an imbalance in the synthesis and excretion of hormones) there were observed the follicles with intact wall and unaffected blood supply. The ultrastructure of the latter indicates active protein-synthetizing and protein-secreting processes.
Keywords: burn injury, thyroid gland, light microscopy, electron microscopy.

О.І. Тірон, А.В. Стеценко, О.І. Яцина, Л.М. Заяць, А.О. Колотвін, К.С. Шуміліна

МОРФОЛОГІЧНІ ЗМІНИ ЩИТОПОДІБНОЇ ЗАЛОЗИ БІЛИХ ЩУРІВ ЧЕРЕЗ 21 ДОБУ ПІСЛЯ ЕКСПЕРИМЕНТАЛЬНОГО ТЕРМІЧНОГО ОПІКУ ПРИ СИСТЕМНОМУ ВВЕДЕННІ NACL

Гістологічні особливості щитовидної залози піддослідних тварин через 21 день після змодельованого опікового ушкодження при системному застосуванні NaCl можна охарактеризувати як адаптаційні та компенсаторні процеси в стінці фолікула, судинному та стромальному компонентах. Більшість фолікулів щитовидної залози були круглої або овальної форми, деякі з них були перерозтягнутими і заповнені щільним колоїдом. Клітини фолікулярного епітелію були сплощені, містили пікнотичні ядра, оточені тонким шаром цитоплазми. У деяких регіонах спостерігали десквамацію тироцитів у просвіті фолікула. Електронно-мікроскопічне дослідження залози через 21 день після змодельованого опіку підтвердило зміни, які раніше були встановлені аналізом гістологічних препаратів. Сплощені тиреоцити містили осміофільні ядра з гетерохроматином. Їх цитоплазма була електронно прозорою і містила органели з ознаками руйнування. Мікроворсинки спостерігали на апікальній області тироцитів. Поряд із фолікулами зі зміненою гістологічною структурою (що свідчить про дисбаланс у синтезі та виведенні гормонів) спостерігалися фолікули з інтактною стінкою та неушкодженим кровопостачанням. Ультраструктура останнього свідчить про активні білково-синтезуючі та білково-секреторні процеси.
Ключові слова: опікова травма, щитовидна залоза, світлова мікроскопія, електронна мікроскопія.

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It is widely known that severe burn immediately subjects the human body to extreme stress which leads to a series of immunological, neuroendocrine and metabolic reactions [11, 13]. Although the initial response to severe burn is typically characterized by hypometabolism, this state quickly (72–96 hrs) changes to hypermetabolism [4, 7]. The post-burn hypermetabolism is believed to be caused by the sustained sympathetic tone and may last up to 36 months after the initial injury [8]. One of the manifestations of the hypermetabolic stage is thyroid dysfunction [9, 12]. However, to the best of our knowledge, the scientific data regarding the structural and functional changes of the thyroid gland after burn injury is limited and inconclusive. Moreover, to evaluate the role of the thyroid gland in the development of post-burn metabolic reactions the examination of the gland needs to be conducted in different terms after injury. The current study is a part of our research on the morphological changes of the white rats' thyroid structure in different terms after experimental thermal burn injury under 0.9 % NaCl systemic administration.

The purpose of the study was to determine the microscopic and ultramicroscopic changes in the thyroid structure of the experimental animals 21 days after modeled skin burn under NaCl systemic administration.

Materials and methods. 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) and 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 (Prot. N1 from 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 sec, 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 and 21 days). Shaving, venous catheterization, skin burns and decapitation of rats were performed under propofol (i.v., 60 mg/kg) anesthesia.

The collection of biological material for microscopic examination was performed according to the generally accepted method [2]. The thyroid gland samples were fixed with 10 % neutral formalin solution, then dehydrated by passing through increasing concentrations of alcohol and embedded into paraffin blocks. The obtained sections, 5–6 μm thick, were stained with Hematoxylin-Eosin [2]. The histological sections were examined under the MIKROmed SEO SCAN light microscope, the photomicrographs were taken with the Vision CCD Camera with an image output system for histological specimens. The thyroid gland samples collected for electron microscopic examination were fixed with 2.5 % glutaraldehyde solution, then post-fixed with 1 % osmium tetroxide prepared with phosphate buffer. Further processing was performed according to the generally accepted method [2]. Ultrathin sections made with ultramicrotome LKB–3 were contrasted with uranyl acetate, and lead citrate according to the Reynolds method [1] and examined under the electron microscope PEM–125K.

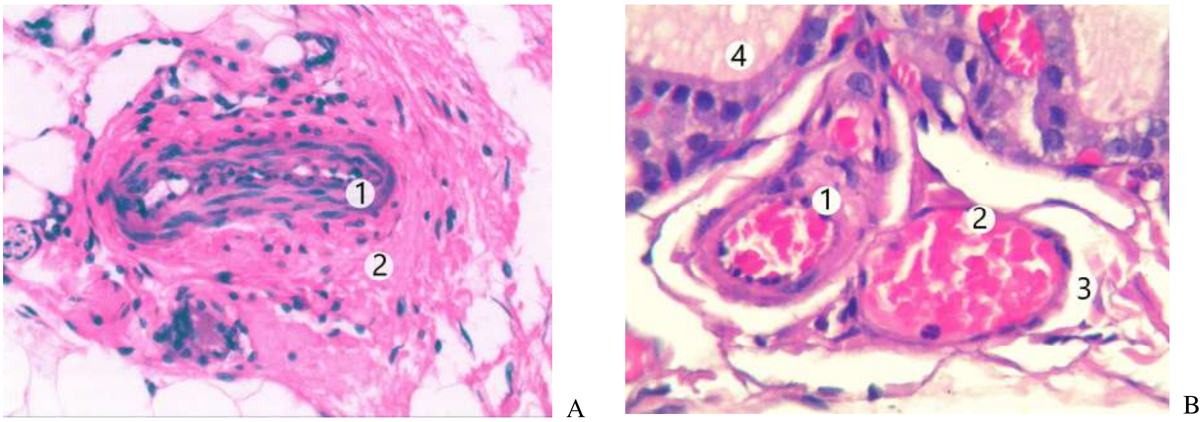


Fig. 1. Histological changes of the thyroid blood vessels 21 days after modeled burn injury under 0.9 % NaCl systemic administration. A – 1 – tunica media of the artery; 2 – tunica adventitia with infiltration and signs of fibrosis. Hematoxylin-Eosin staining. x 400. B – 1 – arteriole and 2 – venule with 3 – perivascular edema, 4 – region of the follicle. Hematoxylin-Eosin staining. x 400.

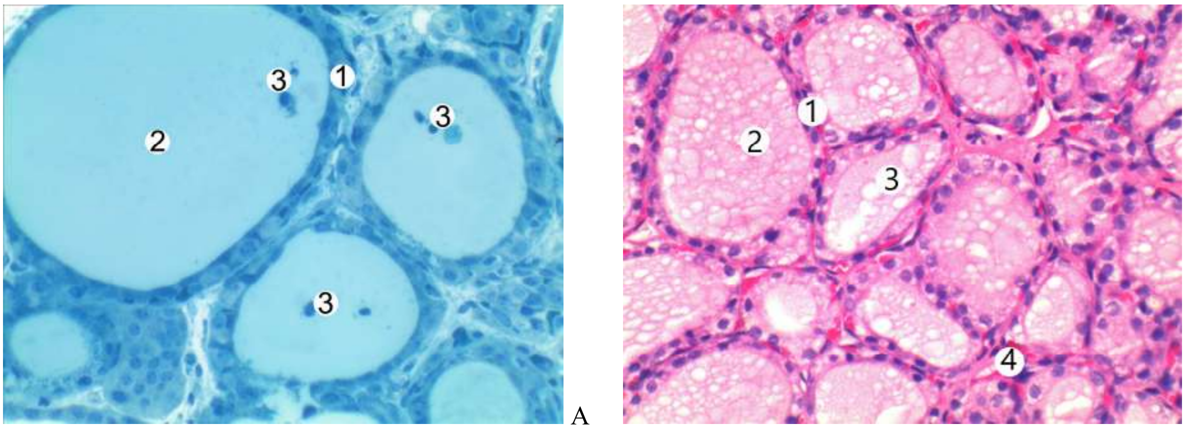
All research was conducted under the agreement on scientific cooperation between the research center of National Pirogov Memorial Medical University, Vinnytsya and the Department of Histology, Cytology and Embryology of Odessa National Medical University (from 01.01.2018), as well as between the Department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

Results of the study and their discussion. The histological features of the experimental animals' thyroid gland 21 days after modeled burn injury under NaCl systemic administration can be described as adaptive and compensatory processes in the follicular wall, vascular and stromal components.

The connective tissue of thyroid follicles were edematous and exhibited lymphocytic infiltration. It was observed the spasm of the thyroid blood vessels, the vascular wall was thickened and exhibited an altered structure (fig. 1 A).

In the tunica media of the arteries it was observed the edema of smooth muscle cells. The tunica adventitia contained the regions of defibering and leukocytic infiltration. The thyroid arterioles, capillaries and venules were excessively blood-filled with occasionally altered endothelial layer. The perivascular edema was observed around several blood vessels of different diameter (fig. 1 B). There were also seen moderately blood-filled vessels without any changes of microscopic structure.

Most of the thyroid follicles of the experimental animals 21 days after modeled burn injury under 0.9 % NaCl systemic administration were round or oval in shape, some of them were overstretched and filled with dense colloid. The follicular epithelial cells were flattened, contained pyknotic nuclei surrounded by thin layer of cytoplasm. In some regions it was observed the desquamation of the thyrocytes within the follicular lumen (fig. 2 A).



A B

Fig. 2. Morphological changes of the thyroid blood vessels 21 days after modeled burn injury under 0.9 % NaCl systemic administration. **A –** 1 – thyrocytes and 2 – large follicles' colloid 3 – desquamated thyrocytes within follicular lumen. Semithin section. Methylene blue staining. x 400. **B –** 1 – thyrocytes, 2 – colloid, 3 – vacuoles of resorption, 4 – blood capillaries. Hematoxylin-Eosin staining. x 200.

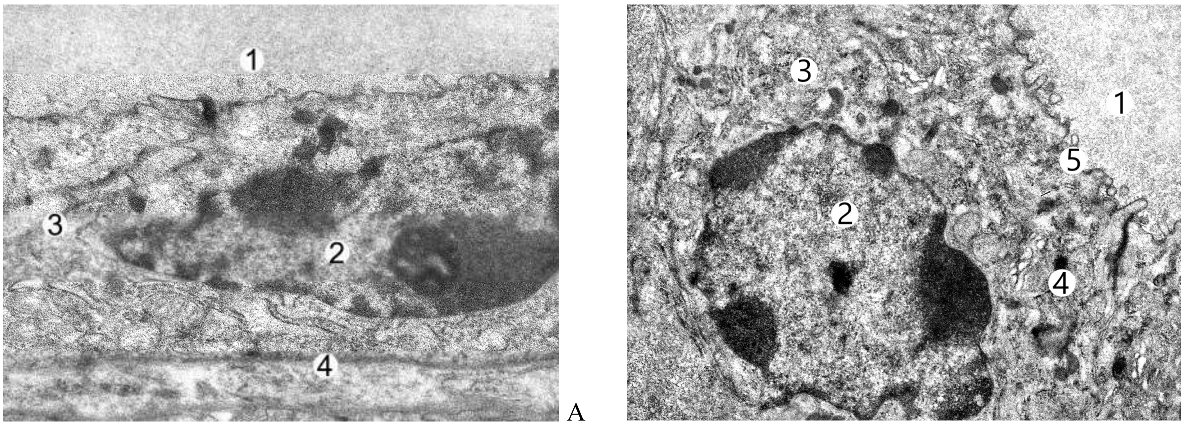
Among the large follicles there were also found the follicles containing cuboid thyrocytes. Some of the latter were characterized by epithelial cells exhibiting the signs of edema and destruction, rarefied cytoplasm and vacuole-like structures. Such follicles contained rare colloid with the signs of resorption (fig. 2 B).

Other follicles were lined with low columnar thyrocytes containing moderately oxyphilic cytoplasm without the signs of destruction. Their colloid was homogenous, containing occasional vacuoles of resorption.

The electron microscopic examination of the experimental animals' thyroid gland 21 days after modeled burn injury under 0.9 % NaCl systemic administration confirmed the changes that had been previously established by the analysis of histological specimens. The flattened thyrocytes contained osmiophilic nuclei with heterochromatin. Their cytoplasm was electron lucent and contained organelles exhibiting the signs of destruction; most of the rough Endoplasmic reticulum (rER) canaliculi and mitochondria remained intact. The microvilli were observed on the apical domain of thyrocytes (fig. 3 A).

The ultramicroscopic changes of the cytoplasm of some low columnar thyrocytes were characterized by intracellular edema, vacuolization of the organelles, specifically rER, Golgi complex and mitochondria. The nuclei of such epithelial cells exhibited mild nuclear envelope invaginations with adjusting heterochromatin. The ultrastructure of most of the cuboidal thyrocytes was characterized by the unaltered protein-synthesizing organelles, and presence of lysosomes and microvilli on the cells' apical domain. The nuclei of such cells contained strongly osmiophilic nucleoli and marginally located heterochromatin (fig. 3 B).

The ultrastructural changes of thyroid blood capillaries 21 days after modeled burn injury under 0.9 % NaCl systemic administration were polymorphic. Some of the capillaries had altered endothelial layer and basal lamina. The endothelial cells' nuclei contained predominantly heterochromatin. Their electron dense cytoplasm was poor in organelles. The micropinocytotic vesicles and projections of the luminal surface were occasional which indicates the slowdown of the transendothelial transport (fig. 3 C).



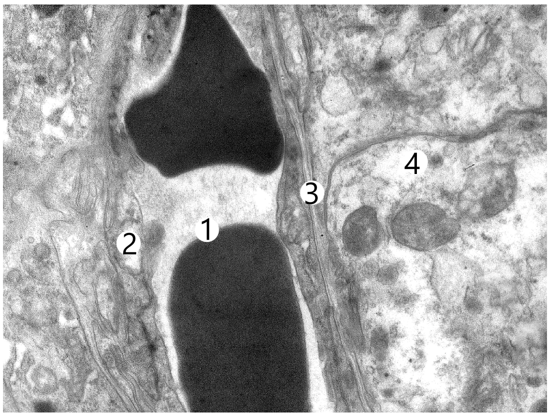


Fig. 3. Thyroid follicular wall ultrastructure 21 days after modeled burn injury under 0.9 % NaCl systemic administration. **A –** 1 – follicular lumen, 2 – nucleus і 3 – cytoplasm of the thyrocyte, 4 – basal lamina. Electron micrograph. x 14 000. **B –** Submicroscopic picture. 1 – follicular lumen, 2 – nucleus and 3 – cytoplasm of the thyrocyte, 4 – lysosome, 5 – microvilli on the apical domain. Electron micrograph. x 13 000. **C –** Ultramicroscopic structure of the thyroid blood capillary. 1 – capillary lumen with erythrocytes, 2 – cytoplasm of the endothelial cell, 3 – capillary basal lamina, 4 – thyrocyte. Electron micrograph. x 12 000.

The basal lamina was unevenly thickened along the perimeters of blood capillaries, occasionally indistinctly contoured. The lumen of such capillaries contains erythrocytes exhibiting the signs of stasis. However, some of the capillaries were lined with endothelium containing well-structured nuclei with electron lucent karyoplasm and nucleolus. The cytoplasm of the latter contained numerous micropinocytotic vesicles which indicates an active transendothelial metabolism.

Twenty-one days after modeled burn injury under 0.9 % NaCl systemic administration the thyroid stroma contained fibroblasts whose ultrastructure indicated their functional activity: abundance of euchromatin in the nucleus, numerous hypertrophic eER canaliculi, evident Golgi complex cisterns and mitochondria. Collagen fibrils were observed in the intercellular matrix surrounding the fibroblasts.

Our results on pathomorphologic changes in the thyroid gland of experimental animals 21 days after modeled burn injury actually corresponds to our previous data [14, 15] and do not contradict to the results in other studies [3, 5, 10]. Those results are reflecting that within 2-24 hrs after the burn, the main pathological changes in the thyroid gland correspond to the first phase of the lesion **–** the phase of post-traumatic depression. This phase is characterized by widespread enlargement of the gland follicles. 14 days after the burn we observed the maximum degree of destructive changes in the thyroid gland [15]. And now one could suppose the evidence of the signs of exhaustion which were registered in the morphological examination of the walls of thyroid follicles, their microstructure and walls composition. It should be evident that all registered thyroid micro- and ultra-microchanges cause its secretory cycle disturbance together with transendothelial metabolism failure and lead to thyroid hormones synthesis, storage and excretion imbalance. Despite all these evedencies, the histological features of the experimental animals' thyroid gland 21 days after modeled burn injury under NaCl systemic administration can be described as adaptive and compensatory processes in the follicular wall, vascular and stromal components.

Conclusion

Twenty-one days after modeled burn injury under 0.9 % NaCl systemic administration the experimental animals' thyroid gland exhibited the signs of destruction, as well as compensatory-adaptive changes. Such changes were observed in the stromal, parenchymal and vascular components of the organ. Along with the follicles with altered histological structure (which indicates an imbalance in the synthesis and excretion of hormones) there were observed the follicles with intact wall and unaffected blood supply. The ultrastructure of the latter indicates active protein-synthetizing and protein-secreting processes.

Prospects for furthers researches include a comprehensive experimental investigation of pathomorphological correlates of burn injury of the macroorganism and the thyroid gland with its microenvironment to elucidate the pathogenetic mechanisms of this injury and the concomitant damage to organs and tissues induced by them. This is necessary for the development of a detailed scheme of pathogenetic pharmacological correction of burn injury of the thyroid gland.

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