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PATHOPHYSIOLOGICAL MECHANISMS OF THYROID GLAND HORMONAL DYSREGULATION DURING EXPERIMENTAL THERMAL EXPOSURE

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The purpose of the study was to study the concentration of pituitary and iodine-containing hormones as well as hormones secreted by the parathyroid gland and the adrenal cortex in the dynamics of skin burns in rats under physiological 0.9 % NaCl solution administration. Blood plasma levels of thyroid-stimulating hormone, triiodothyronine, thyroxin, luteinizing hormone, follicle-stimulating hormone, aldosterone, corticosterone, testosterone and parathyroid hormone were determined 1, 3, 7, 14, 21 and 30 days after thermal skin burns in 138 white Wistar rats. The entire dysfunction of axis of hypothalamic-pituitary-thyroid regulation of a biological organism was established. A simultaneous secretory activity changes were shown in several functional regulatory axis – the pituitary-parathyroid gland, the pituitary-adrenal gland and the pituitary-gonads. Authors showed the primarily inhibition and disorganization of the morphological intraglandular organization in the initial time intervals after a skin burn and some spontaneous compensation after 7–14 days of the post-burn period was confirmed in a change of thyroid gland itself and other important endocrine elements secretory activity. NaCl administration is insufficient for therapeutic purposes and it is necessary to develop the effective pathogenetical scheme of thyroid gland functional state correction in skin burns.

Key words: thyroid gland, burning injury, thyroid-stimulating hormone, triiodothyronine, thyroxin, hormonal dysregulation.

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ПАТОФІЗІОЛОГІЧНІ МЕХАНІЗМИ ГОРМОНАЛЬНОЇ ДИЗРЕГУЛЯЦІЇ ЩИТОВИДНОЇ ЗАЛОЗИ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ТЕРМІЧНОМУ ВПЛИВІ

Метою роботи було дослідження концентрації гіпофізарних, йод-вмісних гормонів, гормонів паращитоподібної залози та кори наднирників у динаміці опікової травми шкіри щурів при введенні фізіологічного 0,9 % розчину NaCl. Через 1, 3, 7, 14, 21 і 30 діб після термічних опіків шкіри у плазмі крові 138 білих щурів лінії Вістар визначали рівень тиреотропного гормону, трийодтироніну, тироксину, лютеїнізуючого та фолікулостимулюючого гормону, альдостерону, кортикостерону, тестостерону і паратгормону. Встановлено повну дисфункцію вісі гіпоталамо-гіпофізарно-тиреоїдної регуляції біологічного організму. Виявлено одночасні зміни секреторної активності кількох функціональних регуляторних осей – гіпофіз-паращитоподібна залоза, гіпофіз-надниркова залоза та гіпофіз-статеві залози. Автори показали первинне гальмування і дезорганізацію морфологічної внутрішньозалозистої організації в початкові проміжки часу після опіку шкіри і деяку спонтанну компенсацію через 7–14 днів післяопікового періоду, що було підтверджено зміною секреторної активності щитоподібної залози та інших важливих ендокринних органів. Введення NaCl є недостатнім з лікувальною метою, і необхідно розробити ефективну патогенетичну схему корекції функціонального стану щитоподібної залози при опіках шкіри.

Ключові слова: щитоподібна залоза, опікова травма, тиреотропний гормон, трийодтиронін, тироксин, гормональна дизрегуляція.

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The urgency of burn injury problem is determined by frequent thermal lesions of various age groups of patients, by their treatment complexity and duration, long-term periods of disability and relatively high mortality [14]. According to WHO, thermal injuries account for 6 % of peacetime injuries [12]. The number of burn victims is increasing worldwide especially in industrialized countries. Among the causes of deaths in various injuries, burns account for 20 % in children and 28 % in people over 65 years old [3]. Thermal burns among all burn injuries are the most common [3, 12].

One should stress that burn damage is one of the most common and severe diseases in people, the second belongs to traffic injuries. Depending on the area and depth of the lesion a burn wound causes multiple and long-lasting disturbances in homeostasis, which cause dysfunction of organs and systems [3, 5, 13].

The endocrine system having a wide range of regulatory hormonal influences on various organs and systems plays a leading role in the development of organisms’ responses to extreme stimulus influences. At the same time the total endocrine balance developed in the stressful conditions but not individual hormones does determine the protective and compensatory processes character, manifestation and adequacy thus providing the processes of both adaptation and resistance in the whole organism [1, 4]. Endocrine dysregulation at the initial stages of the pathological process manifested by significant metabolic disorders which direction and severity are directly related to endogenous hormones level seems to be one of the essential questions of significant importance in the field of scientific understanding of burn shock pathogenesis. However, the data available are sparse and often contradictory [1, 10, 13]. It is not clear al least which of the named changes should be considered as adaptive and which ones as pathological [1, 3].

Hence, it seems to be reasonable to study the burn injury influence on the thyroid gland - an organ that plays an important role in the endocrine system functional activity [5, 8], which provides a wide range of hormonal effects on various organs and systems [7]. Thyroid gland functional activity change leads to hormonal imbalance development which affects the physiological processes manifestation [11] together with its target organs intracellular metabolism and adaptation processes [8].

Additionally, it should be taken into consideration that thyroid gland under the influence of temperature factors of threshold and suprathreshold intensity, taking into account large-scale duplicative feedback mechanisms, thyroid hormones wide range of physiological activity, structural and functional organization and morpho-functional peculiarities, is considered to be one of the leading subject to thermal shock [7, 10, 12]. Thyroid gland is considered to be highly sensitive to adverse environmental factors influence, in particular burn injuries [6].

Therefore, it seems important to us to the study the thyroid and related endocrinal glands hormonal activity in terms of development of their potential dysfunction and/or hyperfunction in conditions of experimental skin burns. Moreover, it’s interesting to investigate the dysfunction at all levels of regulation of the hierarchical endocrine system – hypothalamic-pituitary-thyroid-parathyroid-adrenal relationships.

The purpose of the study was to investigate the concentration of pituitary and iodine-containing hormones as well as hormones secreted by the parathyroid gland and the adrenal cortex in the dynamics of skin burns in rats under physiological 0.9 % NaCl solution administration.

Materials and Methods. Experimental studies were performed on 138 white male Wistar rats weighing 180–220 g. The animals were kept in standard vivarium conditions. Experimental animals keeping and manipulation was done in accordance with the “General Ethical Principles of Animal Experiments” adopted by the Fifth National Congress on Bioethics (Kyiv, 2013) 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 Odesa National Medical University (Prot. N17-C from 12.11.2021).

Thermal skin burns of 2-3 degrees were modeled by four copper plates (each surface area equal to 13.86 cm^2) applying to pre-depilated side surfaces of the rats’ body for 10 sec, these rats were preheated for 6 min in water with a temperature of 100°C [9]. The total area of skin lesions was 21-23 %. Rats were infused with 0.9 % NaCl solution into the inferior vena cava throughout the first 7 days of a trial. Animals were euthanized by decapitation (after 1, 3, 7, 14, 21 and 30 days). Shaving, venous catheterization, skin burns and decapitation of rats were performed under propofol (i.v., 60 mg/kg) anesthesia.

Using enzyme immunoassay method the blood plasma level of the following hormones were determined using reagent kits: thyroid-stimulating hormone (TSH) - TTH-EIA-5296 (“DRG Diagnostics HmbH”, Geermany), triiodothyronine (T_3), thyroxin (T_4), luteinizing hormone (LH), follicle-stimulating hormone (FSH), aldosterone, corticosterone and testosterone (ELISA-BEST “Vector-Best”, RF), parathyroid hormone (“Diasoure PTH ELISA”, Belgium) according to the manuals attached on the analyzer “StatFox2100” (USA).

The data obtained were calculated statistically using one-way variant ANOVA parametric criterion accompanied by a post-hoc Newman-Keuls test. The minimum statistical probability was determined at $p<0.05$.

Results of the study and their discussion. TSH content in the rats’ blood plasma on the 1st day after the burn (group N2) was 91.9 % higher than the corresponding index in intact rats, the concentration of triiodothyronine and thyroxine was equal to 1.97±0.17 nmol/l and 17.8±2.1 nmol/l, respectively which was found to be 2.37 times and 2 times less comparing with the same indexes in the blood of rats of the control group (in all cases $p<0.001$, table 1). The indexes investigated of TSH and iodine-containing hormones in the group of rats with burns injected with NaCl solution only on the 1st day (group N3) did not differ significantly ($p>0.05$) from similar indexes in the group of rats with burns (group N2) (table 1).

Table 1

Thyroid-stimulating hormone, triiodothyronine and thyroxin blood plasma levels in rats after skin burn damage and 0.9 % NaCl solution administration

N	Experimental groups	Levels of the hormones investigated (M±m)		
		TSH, mIU/l	T_3, nmol/l	T_4, nmol/l
	Day 1			
1	Control (intact rats), n=9	1.24±0.13	1.97±0.17	17.8±2.1
2	Rats with a burn, n=7	2.38±0.21***	0.83±0.09***	8.9±0.9***
3	Rats with a burn + 0.9 % NaCl, n=7	2.41±0.23***	0.87±0.11***	9.1±0.9***
	Day 3			
1	Control, n=9	1.18±0.11	2.01±0.18	17.4±1.9
2	Rats with a burn, n=7	2.44±0.24***	0.89±0.11***	8.6±0.8***
3	Rats with a burn + 0.9 % NaCl, n=7	2.49±0.21***	0.88±0.13***	8.9±0.9***
	Day 7			
1	Control, n=9	1.21±0.12	2.03±0.19	17.7±2.1
2	Rats with a burn, n=7	1.97±0.19**	1.16±0.13**	11.4±1.3*
3	Rats with a burn + 0.9 % NaCl, n=7	2.19±0.19**	1.03±0.12**	9.8±1.0*
	Day 14			
1	Control, n=9	1.26±0.14	1.98±0.19	17.3±1.8
2	Rats with a burn, n=7	1.71±0.17*	1.31±0.16**	12.3±1.3*
3	Rats with a burn + 0.9 % NaCl, n=7	1.66±0.17*	1.36±0.18*	11.7±1.2*
	Day 21			
1	Control, n=9	1.23±0.13	2.04±0.16	18.1±1.9
2	Rats with a burn, n=7	1.51±0.15	1.31±0.16**	12.3±1.3*
3	Rats with a burn + 0.9 % NaCl, n=7	1.44±0.16	1.47±0.17*	13.5±1.4
	Day 30			
1	Control, n=9	1.18±0.14	1.96±0.16	17.9±2.1
2	Rats with a burn, n=7	1.39±0.14	1.43±0.14*	13.4±1.3*
3	Rats with a burn + 0.9 % NaCl, n=7	1.37±0.16	1.39±0.13*	13.7±1.4

Note: * – p<0.05, ** – p<0.01, *** – p<0.001 – the significant differences of the investigated indexes compared with the analogous data in control rats (ANOVA test).

TSH concentration in the blood of rats with a burn on the 3rd day of the trial reached 2.44±0.24 mIU/l which was 2.07 times higher than that of control measurements (p<0.001). Both T_3 and T_4 levels were 2.26 and 2.02 times less than similar indexes in intact rats (p<0.001). The content of the studied hormones in the blood of rats with a burn and NaCl injection was identical to the corresponding indexes in group N2 (p>0.05).

TSH content in the blood of rats during the 7th and 14th days of the trial remained significantly higher compared with this index in the control group of rats (by 62.8 % and by 35.7 %, respectively, p<0.05) and the concentration of triiodothyronine (by 75 % and by 51.1 %, respectively, p<0.01) and thyroxin (by 55.3 % and by 40.7 %, respectively, p<0.05) significantly exceeded the same control data. Investigated hormones levels during these intervals of the experiment in the group of rats with burns and forthcoming NaCl injected for 7 days did not differ significantly from such data in rats of group N2 (p>0.05).

On the 21st day of the trial the TSH content in the group N2 rats blood exceeded the same control indexes by 22.8 % (p>0.05). The triiodothyronine and thyroxine concentration were equal to 1.31±0.16 nmol/l and 12.3±1.3 nmol/l, respectively, which was 55.7 % (p<0.01) and 47.2 % (p<0.05) less compared to such control measurements. The level of the studied hormones in the group N3 blood was also comparable to similar indexes in rats with burns without treatment (p>0.05). We recorded similar measurements on the 30th day of the trial.

Our measurements proved that the concentration of gonadotropic hormones – FSH and LH – was equal to 4.68±0.43 IU/l and 3.71±0.36 IU/l, respectively, which was 1.68 times and 1.69 times higher in the rat's blood 1 day after a skin burn injury comparing the same data registered in the control rats (in all cases p<0.05). The parathyroid hormone concentration in group N2 rats was 1.52 times higher than the same control index (p<0.05).

Adrenal cortex hormones - aldosterone and corticosterone – blood plasma concentration in rats after skin burning was also significantly higher (4.75 times and 1.5 times, respectively) than similar data in control observations (p<0.05). Corticosterone level was 1.44 times lower compared to the same control index (p<0.05) (table 2).

Table 2

Gonadotropic hormones, parathyroid and adrenal cortex hormones blood plasma levels in rats after skin burn damage and 0.9 % NaCl solution administration

N	Experimental groups,	Levels of the hormones investigated (M±m)					
		FSH, IU/l	LH, IU/L	Parathyroid hormone, pg/lл	Aldosterone, nmol/l	Corticosterone, nmol/l	Testosterone, nmol/l
	Day 1						
1	Control (intact rats), n=9	2.79±0.26	2.19±0.19	17.2±1.6	1.12±0.09	321.7±29.4	24.4±2.2
2	Rats with a burn, n=7	4.68±0.43*	3.71±0.36*	26.1±2.4*	5.32±0.47***	483.1±46.4*	16.9±1.3*
3	Rats with a burn + 0.9 % NaCl, n=7	4.72±0.44*	3.66±0.37*	26.4±2.5*	5.17±0.48***	476.7±44.8*	16.7±1.4*
	Day 3						
1	Control, n=9	2.76±0.26	2.17±0.18	17.4±1.7	1.17±0.11	319.8±30.2	24.7±2.1
2	Rats with a burn, n=7	4.84±0.47*	3.91±0.37*	24.9±2.4*	5.44±0.48***	491.7±47.6*	15.6±1.4*
3	Rats with a burn + 0.9 % NaCl, n=7	4.64±0.46*	3.77±0.36*	22.7±2.2*	5.03±0.44***	476.1±45.6*	17.1±1.4*
	Day 7						
1	Control, n=9	2.81±0.27	2.21±0.19	17.1±1.7	1.16±0.11	317.2±27.7	24.7±2.3
2	Rats with a burn, n=7	4.68±0.44*	3.59±0.36*	23.3±2.2*	4.76±0.46***	469.1±44.7*	16.9±1.6*
3	Rats with a burn + 0.9 % NaCl, n=7	4.47±0.43*	3.31±0.33*	22.4±2.3*	4.26±0.41***	454.2±43.8*	16.7±1.6*
	Day 14						
1	Control, n=9	2.83±0.27	2.16±0.17	16.6±176	1.13±0.09	324.5±30.1	25.1±2.4
2	Rats with a burn, n=7	3.89±0.37*	3.17±0.31*	22.8±2.1*	3.21±0.31***	443.7±42.1*	18.7±1.6*
3	Rats with a burn + 0.9 % NaCl, n=7	3.64±0.31*	3.26±0.33*	21.1±2.3*	2.97±0.26***	451.1±43.6*	19.2±1.7*
	Day 21						
1	Control, n=9	2.82±0.23	2.16±0.17	16.7±1.6	1.19±0.12	320.6±28.9	25.1±2.4
2	Rats with a burn, n=7	2.76±0.26	2.12±0.18	23.1±2.2*	2.57±0.27***	419.2±37.8*	19.4±1.7*
3	Rats with a burn + 0.9 % NaCl, n=7	2.81±0.29	2.23±0.17	21.6±2.2*	2.21±0.21**	408.6±35.1*	19.9±1.8
	Day 30						
1	Control, n=9	2.77±0.27	2.26±0.22	17.1±1.7	1.16±0.11	316.4±27.9	24.1±2.1
2	Rats with a burn, n=7	2.18±0.23	1.78±0.18	19.8±1.8	1.93±0.17**	403.4±34.9*	21.2±2.1
3	Rats with a burn + 0.9 % NaCl, n=7	2.27±0.24	1.86±0.19	20.2±1.9	1.72±0.17**	384.1±36.3*	21.9±2.2

Note: * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ – the significant differences of the investigated indexes compared with the analogous data in control rats (ANOVA test).

All investigated hormone values in the blood of rats with skin burns injected with NaCl differed from the same indexes in group N2 rats by 0.8–2.9 % ($p>0.05$).

Similar dynamics of FSH, LH, parathyroid hormone, aldosterone, corticosterone and testosterone content was observed during the 14 days of the study. FSH and LH blood concentration in group N2 rats on the 21st day of the trial differed from the corresponding control measurements by 2.2 % and 1.9 % ($p>0.05$). The parathyroid hormone level was 38.3 % higher than that in the control ($p<0.05$). Aldosterone level in blood of group N2 rats was 2.16 times higher than the control data ($p<0.001$). The content of corticosterone and testosterone in the blood of rats with a burn differed by 30.8 % and 22.7 % pertaining the control parameters ($p<0.05$). The concentrations of all studied hormones in the blood of rats with burns injected by NaCl during the first 7 days of the experiment did not differ significantly from the same indexes in group N2 rats ($p>0.05$). The same dynamics of studied hormones concentration was observed in the blood of rats of all the experimental groups on the 30th day of the trial.

Thus, we studied thyroid hormones concentration dynamics as well as hormones of pituitary origin and some functionally related endocrine glands throughout 30 days of the post-burn period. The entire dysfunction of hierarchical axis of hypothalamic-pituitary-thyroid regulation of a biological organism was established. We also studied the parathyroid gland and the adrenal cortex hormones dynamics to have the complete picture of hormonal stress dysregulation.

A simultaneous secretory activity changes were shown in several functional regulatory axis - the pituitary-parathyroid gland, the pituitary-adrenal gland and the pituitary-gonads.

Hence, our data confirm the previously published results of thyroid tissue morphological studies within 30 days after the thermal damaging effects application [15]. Indeed, the primarily inhibition and disorganization of the morphological intraglandular organization in the initial time intervals after a skin burn and some spontaneous compensation after 7–14 days of the post-burn period was confirmed in a change of thyroid gland itself and other important endocrine elements secretory activity.

It is important that confirming the morphological data our results convincingly indicate a functional shift in the thyroid gland hormonal activity towards the hypothyroidism development during the first 7 days after the burn. The low level of triiodothyronine and thyroxine is explained by morphological disorders which resulted to iodine-containing thyroid hormones inability to enter the bloodstream through the basement membrane of thyrocytes [15] due to impaired microcirculation and thyroid interstitial tissue swelling and a base metabolism intensity increase.

A more expressed thyroxin concentration decrease might be explained, in our opinion, by its greatest functional activity as the main thyroid gland producer, changes in D_1 and D_2-iodothyronine deiodinases enzymatic activity in peripheral tissues [7] and, possibly, by iodine metabolism autonomous regulation continuation by thyroid parenchyma follicular cells [14].

TSH hypersecretion we consider to be associated with hypothyroidism, since in this case, according to the mechanism of negative feed-back, the pituitary gland makes all attempts to normalize the thyroid gland functional activity decrease induced by burn exposure. At the same time the parathyroid gland hypersecretion is also understandable, since both the thyroid and parathyroid glands secretory activity has a reciprocal mechanism of interaction [8].

Adrenal cortex hyperfunction is aimed to compensate the alterating burn exposure sequences. It is vital to understand that iodine-containing hormones are capable to exert anti-stress activity by themselves. They also stimulate the glucocorticoids secretion and enhance their oxidation and intrahepatic conjugation. Therefore, the glucocorticoids content increase as a result of skin thermal burn seems logical which can be explained by the secondary activation of one of the critical components of the organisms' anti-stress system which has to limit the effects of a pathological thermal stress reaction [1, 4].

The pointed hyperaldosteronemia as a result of a skin burning can be explained from a fundamental position by the critical loss of body fluid. The adrenal cortex bundle layer increased secretory activity and revealed hypercorticosteronemia might be explained, in our opinion, from several positions: firstly, the organism tries to cope with the hyperthermic factor powerful influence. And the named thermal effect, secondly, has a direct stress altering character to manage with which the adrenal cortex hormones are efficient.

The pituitary-testicular system secretory activity change was shown in experimental conditions. At the same time the gonadotropic hormones level increases and testosterone concentration decreases which, firstly, demonstrates the regulatory relationships preservation inside the pituitary-gonadal axis during thermal induced hypothyroid functional state and, secondly, demonstrates a well-known pattern of androgens reduced bioavailability, transport capacity and efficacy in hypothyroidism. In this case our mass of actual results does not contradict the known data [8], they confirm the validity of the used burn model to reproduce thyroid dysfunction and its hypofunctional condition.

According to the time parameters of the studied hormone concentrations, we see that the severity of compensatory-adaptive reactions caused by skin burns is maximal during 7-14 days of the post-burn period. Our data convincingly show the maximal destructive and decompensatory hormonal disturbances during the first 7 days of the experimental skin burn.

One could observe a tendency of hormonal regulatory activity normalization by the 21^{st} day of research which, however, remains as a trend up to the 30^{th} day of a trial. Thus, we note that our actual data somewhat differ from the morphological results in which the maximal degree of destructive changes in the thyroid parenchyma falls on the first 14 days of the pathological burn process, and the gland's compensatory activity optimum falls on the 21^{st} day of a trial. In our opinion, such discrepancies in the essence of the burn process dynamics inside the thyroid gland are natural, they are understandable and are explained by different temporal aspects of intraorganic both morphological and functional changes. Such time-dependent morphological and functional differences are considered to be quite natural in post-traumatic and post-stress conditions [2].

The inconclusive hormonal results in groups of animals with physiological saline injections turned out to be ineffective in an attempt to stimulate the thyroid gland endocrine activity and indicated the NaCl administration insufficiency for therapeutic purposes and the necessity to develop and test the efficacy of other pathogenetical scheme for thyroid gland functional state correction in skin burns. From this point of view we suppose to stress that our data indicate the reasonability of pharmacological drugs using for this purpose that have pituitary, thyroid, parathyroid and adrenal orientation. Our logical premises have, among other things, some clinical evidence [10].

We are convinced that biochemical hormonal studies results in the thyroid gland burn injury being significant continued to be insufficient without their confirmation by lymphoid tissue morphometric investigation, without investigation of the “lipoperoxidation-antioxidant defense” functional system activity changes in the blood and, for instance, in homogenates of vital organs responsible for the adaptative changes implementation, without studying the prostaglandin, catecholamine and other components of stress-limiting systems activity dynamics [1, 2, 4, 5, 13], as well as some other studies which will provide more evidences in favor the studied pathological condition effective pharmacocorrection taking into account its pathogenetic mechanisms.

Conclusions

1. The entire dysfunction of hierarchical axis of hypothalamic-pituitary-thyroid regulation of a biological organism was established throughout 30 days of the post-burn period.

2. A simultaneous secretory activity changes were shown in several functional regulatory axis – the pituitary-parathyroid gland, the pituitary-adrenal gland and the pituitary-gonads.

3. The primarily inhibition and disorganization of the morphological intraglandular organization in the initial time intervals after a skin burn and some spontaneous compensation after 7–14 days of the post-burn period was confirmed in a change of thyroid gland itself and other important endocrine elements secretory activity.

4. Our results convincingly indicate a functional shift in the thyroid gland hormonal activity towards the hypothyroidism development characterized by iodide thyroid hormones concentration decrease and TSH level increase during the first 7 days after the burn. Both parathyroid gland and adrenal cortex hyperfunction is aimed to compensate the alterating burn exposure sequences.

5. NaCl administration is insufficient for therapeutic purposes and it is necessary to develop the effective pathogenetical scheme of thyroid gland functional state correction in skin burns.

Prospects for furthers research include a comprehensive experimental investigation of other pathogenetical mechanisms of thyroid dysfunction in conditions of burn disease. This will allow to develop pathogenetically substantiated therapy aimed to thyroid gland functional activity improvement in the post-burn period.

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