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THE INDICES OF THYROID SYSTEM AND METABOLISM OF RATS UNDER THE INFLUENCE OF NANOCOMPOSITION BASED ON IODINE AND CITRATE

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The search for a new biologically effective iodine compounds that may have practical use and do not cause toxic effects is relevant. The aim of the research was to examine the influence of nanocomposition based on citric acid – iodine helated compex (I_2Citr) on the indices of thyroid system activity and metabolism in the blood serum of young male rats. Rats of the experimental groups received I_2Citr with water daily for 40 days at a dose of 24 μg I/kg (1st experimental group) and 240 μg I/kg (2nd experimental group) of the body weight. The level of hormones and antibodies in the blood serum was determined with ELISA. Reduced Tg level and triacylglycerols content and elevated AntiTg, AntiTPO levels and Ca and albumin content in the serum of rats in both groups compared to control were observed. It was found that in the serum of rats in the 2nd experimental group the levels of T3, T4 and urea, as well as the activity of alkaline phosphatase and AlAT were reduced. No changes in the coefficients of the rats internal organs mass were detected under the action of I_2Citr except for the spleen mass, which decreased by about 40% in the rats of 1st group and by 33% – in rats of 2nd group as compared to control. The data obtained indicate modulating influence of I_2Citr in a studied doses on thyroid activity without causing toxic effects on animal organism.

K e y w o r d s: nanocomposition basede on iodine and citrate, thyroid hormones, metabolic indices, internal organs mass of rats.

Significant fluctuations in the intake of iodine in organisms of humans and animals can cause thyroid dysfunction with the development of iodine deficiency diseases, or the toxic influence of its excess [1, 2]. In Ukraine, the number of thyroid diseases has almost doubled in recent decades, which is associated with the lack of proper systemic prevention of iodine deficiency [3]. Therefore, in human nourishment and animal nutrition, salt, mineral, and organic iodine compounds are used to be added to water, various components of food and feed ration [4-7].

It is proved that the chemical form of iodine additive had a significant influence on the level of its assimilation and biological activity in the organism [3, 7, 8]. In particular, iodates and iodides, ethylenediamine dihydroiodide (EDDI), iodinated casein, pentacalcium orthoperiodate, and others are approved for application in the EU [3, 5, 6, 9]. Iodine-containing yeast, seaweed, biotechnological preparations of microbiological synthesis are also used in animal nutrition [9-11]. However, such additives are not of wide practical application due to the weak normative basis for their dosage, interaction with other minerals and vitamins, low level of solubility of certain iodine-containing compounds [11, 12]. Therefore, the search for new biologically effective iodine compounds, including those produced on the basis of nanotechnologies and suitable for use in human nutrition [3-5] and animal feeding [6, 13],

are conducted. A new organic compound of iodine, where iodine is coordinated by carbohydrates and polypeptides, was shown to improve hematological and certain biochemical parameters in rats and dogs. The objective of this study was to evaluate the impact of a developed compound of iodine and citrate on male rats. To assess its effectiveness, we determined the indices of the pituitary-thyroid system and metabolic activities in the blood. The influence of the new compound of iodine coordinated by carbohydrates and polypeptides on the body of rats and dogs in the form of oral treatment with the drug for 30 days was studied. The established maximum tolerated dose of 2,000 mg/kg led to a decrease in the rate of body weight gain in male and female rats. Changes in hematological and certain biochemical parameters in rats were observed at a dosing of 1,000 and 2,000 mg/kg [11]. The purpose of the research was to determine the influence of a developed organic compound of iodine and citrate nanocomposition, made by nanotechnology, on the activity of the pituitary-thyroid system and metabolic processes in the blood of young male rats.

Materials and Methods

Conditions for animal keeping. The research was performed on young males of white laboratory rats, divided into control and two exposed (1^{st}, 2^{nd}) groups, having six animals in each. Males of control and exposed groups were kept in a vivarium of the State Scientific-Research Control Institute of Veterinary Preparations and Feed Additives (SSRCI VP and FA) certified according to current sanitary and veterinary requirements [14] on a ration of granulated combined feed with free access to drinking water. All animal experimental were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Method and dosing of iodine and citrate nanocomposition. Nanocomposition of iodine and citrate (I_2Citr) was made in two stages of Ltd "Nanomaterials and nanotechnologies", Kyiv from crystalline iodine and citric acid classification "ch. p." – chemically pure and deionized water. I_2Citr was characterized by the following indicators: iodine content – 0.1 wt. %, citric acid – 0.7 wt. %, water – the rest [4].

Iodine nanoparticles were obtained in the form of their aqueous colloidal solution by ablation of iodine crystals in deionized water by high voltage electric current – up to 105 V/m in the first stage. The energy of the electric current pulses exceeded the energy of iodine sublimation. This generated spark discharges with explosive dispersion of iodine crystals into nanoparticles. The formed nanoparticles in deionized water are cooled and form a colloidal solution of iodine nanoparticles. The size of iodine nanoparticles in colloidal solution before the formation of the chelate complex with citric acid was 5-20 nm. Control determination of the concentration and size of nanoparticles of colloidal iodine solution was performed using a laser beam and the Tyndall effect.

In the second stage, citric acid was added to the colloidal solution of iodine nanoparticles to obtain a chelate complex – nanocomposition of iodine and citrate with pH of 1.79. No free iodine nanoparticles remained in the synthesized chelate complex, as they bind completely to the added citrate.

Iodine and citrate nanocomposition in the form of a chelated complex of iodine nanoparticles with citric acid [4] was added to rats' exposed group drinking bowls with a daily amount of water in estimated doses 24 μg I/kg of body weight (1^{st} exposed group) and 240 μg I/kg of body weight (2_{nd} exposed group). Doses of I_2Citr were selected from the literature [3, 8], taking into account the daily requirement of iodine and its intake by the organism. I_2Citr was given to the rats daily for 40 days to determine the absolute and relative indicators of the dynamics of changes in animals' body weight compared to the preparatory period and the control group.

Biological material for research. On the 40^{th} day of the animals treating with the I_2Citr, the decapitation of rats was performed after immobilization in a laboratory desiccator filled with CO_2 according to the bioethical requirements for the treatment of animals used in scientific studies [15]. In animals of the control and exposed groups, blood samples were obtained by cardiac puncture after euthanasia followed by decapitation, and the following internal organs were dissected: heart, lungs, thymus, liver, spleen, kidneys, and testicles.

Research methods, indicators, and devices. Indicators of the mass of internal organs (g) and the coefficients of their masses were determined by the ratio of the mass of the organ (g) to the body weight (kg). The level of thyroxine (free T4, pmol/l) was determined in the blood serum obtained after its treatment and centrifugation at 2500 rpm [14]. Triiodothyronine (free T3, pmol/l), thyroglobulin (Tg, ng/

ml), thyroid-stimulating hormone (TSH, μIU/ml), antibodies against thyroglobulin (Anti Tg, IU/ml), antibodies against thyroperoxidase (Anti TPO, IU/ml) were determined by the enzyme-linked immunosorbent assay on a STAT analyzer FAX-3000. Determination of these indicators was performed using "Thyroid IFA-T" (RF) sets. The content of albumin (g/l), creatinine, triacylglycerols (TAG), urea, cholesterol, Ca, P, all expressed in mmol/l (mM), the activity of alanine aminotransferase (AlAT), aspartate aminotransferase (AsAT), and alkaline phosphatase (AP), expressed in ncat/L, were also determined in the serum. Research of these indicators was performed on a biochemical analyzer "Humalizer-2000" using the recommended sets of reagents for the device.

Statistical analysis. The statistical processing of the obtained results was performed using the one-way ANOVA test. Data are presented as mean ± SD. Results were considered significant when $P < 0.05$.

Results and Discussion

The use of I_2Citr had an influence on the biosynthetic activity of the thyroid gland (TG) of rats of both exposed groups. However, more expressed changes in biochemical indicators were found in the blood of the 2nd group of rats. In particular, in the blood of the 2nd group of rats, a significant decrease in the concentration of free thyroid hormones T3 ($P < 0.01$) and T4 ($P < 0.05$), and also thyroglobulin (Tg) ($P < 0.01$) was found, however, there was an increase ($P < 0.05$) of antibodies to Tg (Fig. 1).

Tg content ($P < 0.001$) lower than in the control group and improbable T3 and T4 were also observed in the blood of rats of the 1st exposed group. The concentration of antibodies to thyroid peroxidase increased in the blood of rats under the action of both doses 24 and 240 μg I/kg. Similar changes were observed in the content of TSH in the blood of rats of exposed groups under the action of both concentrations of iodine.

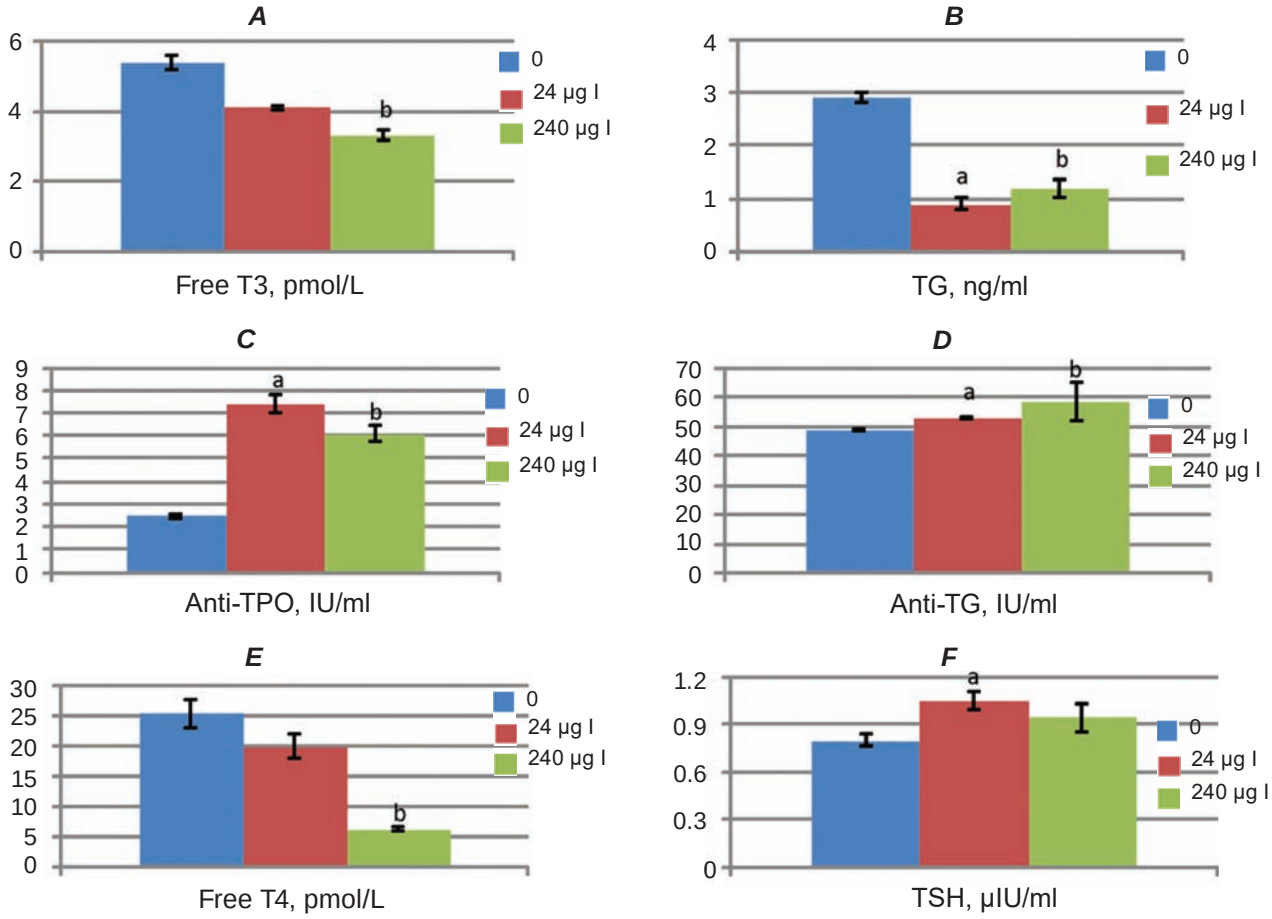

Fig. 1. The content of hormones in the blood serum of rats under the action of different concentrations iodine and citrate nanocomposition, n = 6: a – probable differences between C (0) and 1st (24 μg I/kg) groups, $P < 0.05–0.001$; b – probable differences between C (0) and 2nd (240 μg I/kg) groups, $P < 0.05–0.001$

Obviously, the activation of the pituitary gland for the synthesis of this hormone is due to a decrease in thyroid function under the action of I_2Citr, characterized by a decrease in thyroglobulin release and biosynthesis and secretion of T4 and T3.

The analysis of other biochemical indices of the blood shows the expressed biological action of iodine at a dose of 240 μg I/kg (Table 1). In particular, lower urea, AlAT activity (between the control group and the 2nd exposed group; 1st and 2nd exposed groups, $P < 0.05$) were found in the blood serum of rats. However, the albumin content increased significantly in the blood serum of rats of both exposed groups.

Changes in concentrations of TAG, cholesterol, Ca and AP were slightly different (Table 1) from previous indicators in the blood of rats of exposed groups compared to the control group (Table 2).

The influence of the used doses of I_2Citr caused an increase in cholesterol content in the blood serum of rats of 1st and 2nd exposed groups ($P < 0.001$) compared to the decrease of TAG ($P < 0.05$). This may be explained by different intensities of the action of the used doses of I_2Citr on the synthesis and use of these classes of lipids in the metabolic processes of the rat organism, which is analyzed in monographs [2, 3]. The blood serum of rats of the exposed groups also had a higher content of Ca ($P < 0.001$), which confirms the presence of a synergistic connection between iodine and Ca [2, 3]. However, the activity of AP in the blood of animals of the exposed groups was lower, which is more expressed in the 2nd exposed group ($P < 0.001$). The content of organic P in the blood of rats of the exposed groups relative to the control group probably did not change.

The analysis of the rats` body weight and mass of the internal organs indicated a minor influence of the used doses of I_2Citr on the studied indicators. No significant changes in body weight, mass of heart, lungs, thymus, liver, kidneys and testes in rats of exposed groups compared to the control group were observed (Fig. 2). However, there was a decrease in the indicators of spleen mass in animals of the exposed groups ($P < 0.001$) by 40% (1st exposed group) and 33% (2nd exposed group) (Fig. 2, *E*).

The high probability of changes in the mass of the spleen indicates an expressed inhibitory influence of the used doses of I_2Citr, on the development of the structure, and possibly the depositing capacity of this organ. This is confirmed by the research results on hypersensitivity to the iodine and the action of thyroid enzymes, catalyzing metabolic reactions in the cells of lymphopoiesis organs, including the spleen [3, 16]. BBDR-HPT axis model simulations show a steep dose-response relationship between dietary intake of iodide and serum T4 and TSH when

Table 1. Indicators of protein metabolism in the blood serum of rats under the action of different concentrations iodine and citrate nanocomposition, (mean ± SD, n = 6)

Dose of iodine, μg I/kg b.w.	Creatinine, mmol/l	Urea, mmol/l	AlAT, ncat/l	AsAT, ncat/l	Albumin, g/l
0-C	63.20 ± 2.44	8.50 ± 0.23	47.40 ± 6.93	140.5 ± 24.38	29.1 ± 2.66
24-1st	60.3 ± 1.3[a]	8.3 ± 1.32	50.0 ± 4.18[c]	140.9 ± 25.84	37.3 ± 2.41[a]
240-2nd	61.30 ± 2.96	7.20 ± 0.78[b]	39.0 ± 6.07[b,c]	123.9 ± 23.78	33.1 ± 0.98[b]

Note: [a] groups C and 1st, $P < 0.05$–0.01; [b] groups C and 2nd, $P < 0.05$; [c] groups 1st and 2nd, $P < 0.05$

Table 2. Indicators of lipid and mineral metabolism in the blood serum of rats under the action of different concentrations iodine and citrate nanocomposition, ((mean ± SD, n = 6)

Dose of iodine, μg I/kg b.w.	Triacyl glycerols, mmol/l	Cholesterol, mmol/l	Alkaline phosphatase, ncat/l	Calcium, mmol/l	Inorganic phosphorus, mmol/l
0-C	0.76 ± 0.20	1.23 ± 0.16	202.2 ± 16.63	1.80 ± 0.22	2.60 ± 0.32
24-1st	0.57 ± 0.15[a]	2.13 ± 0.31[a]	158.00 ± 45.98	2.49 ± 0.15[a]	2.8 ± 0.2
240-2nd	0.59 ± 0.07[b]	1.99 ± 0.24[a]	132.40 ± 9.92[b]	2.38 ± 0.12[b]	2.60 ± 0.29

Note: [a] groups C and 1st, $P < 0.05$–0.001; [b] groups C and 2nd, $P < 0.05$–0.001

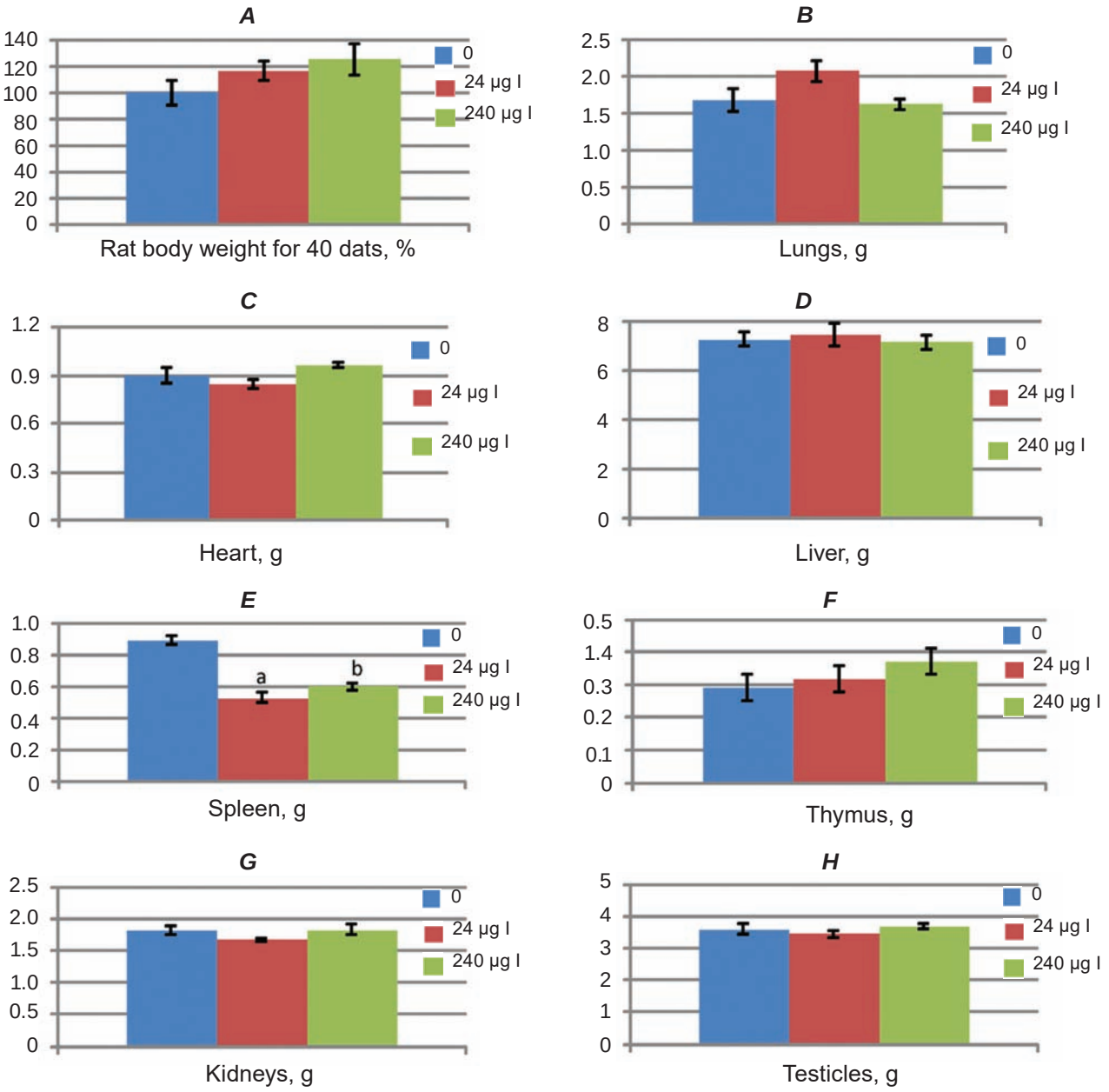

Fig. 2. Body weight and mass of the internal organs of rats under the action of different concentrations iodine and citrate nanocomposition, n = 6: a – probable differences between C (0) and 1st (24 μg I/kg) groups, P < 0.001; b – probable differences between C (0) and 2nd (240 μg I/kg) groups, P < 0.001

dietary iodide intake becomes insufficient (less than 2 μg/day). This model can be linked to physiologically based pharmacokinetic models for thyroid-active chemicals to evaluate and predict dose-dependent HPT axis alterations based on hypothesized modes of action [17].

The defined organo-systemic differences in the action of the used doses of I_2Citr may indicate the peculiarities of the regulatory influence of this compound on physiological development and hematodeponizing ability of a spleen in male rats. In addition, it is worth noting the increase in thymus mass in rats of the 1st exposed group by 10.3% and in the 2nd exposed group by 27.6% (Fig. 2, *F*). This may indicate a tendency to higher metabolic and proliferative activity with decreasing age involution of this organ in animals of exposed groups under the regulatory influence of the used doses of I_2Citr citrate

on these processes. Thymus cells have been shown to be metabolically sensitive to iodine and thyroid hormone levels [2, 16].

The analysis of the coefficients of the masses of the internal organs of rats confirms the tendency to change of their mass indicators, ($P < 0.001$) decrease of which is expressed only for the spleen: in the 1st exposed group – by 42%, and in the 2nd exposed group – by 39% compared to the control group (Fig. 3)

There are differences in biochemical and physiological indicators of rats during the period of action of I_2Citr indicating certain features of the course of metabolic reactions in some organs and systems, which were noted for some iodine compounds by other authors [11, 18, 19]. The general direction of changes in the biochemical indices of the metabolic activity of the pituitary-thyroid system is preserved in the blood of rats under the action of both doses of iodine. However, the probability of such changes is more expressed for a dose of 240 μg I/kg, as well it was observed under the action of high levels of iodine in some researches [17, 18]. It is known that excess iodine, the physiological level of which in rats is 12–18 μg/per individual, inhibits the synthesis and reduces the level of T3 and T4 in the blood [2, 3, 20]. According to these publications, the rat organism uses 1.4 μg of iodine per day for the synthesis of thyroid hormones in thyrocytes, with the participation of thyroglobulin and thyroperoxidase. The research [20] has shown that repeated administration of KI at 1 mg/kg/24 h does not cause modification of thyroid hormones level but leads to a reversible modification of the expression of genes involved in the synthesis and secretion of thyroid hormones. An important role in the mechanisms of decreasing or increasing the concentration of T3, T4, thyroglobulin, antibodies to thyroglobulin and Anti-TPO belongs to thyrocytes, which under physiological conditions are stable with low ability to both proliferation and apoptosis. However, their mitotic activity is significantly increased both under deficiency and excess iodine in the organism [2, 3, 18, 19].

It is possible that the doses of I_2Citr used in our research exceeded the physiological needs of the organism. Therefore, the thyroid gland of rats inhibited the oxidation of the iodine-anion and its binding to tyrosine residues Tg and their dimerization with a decrease in the biosynthesis of both Tg and T4 and T3. However, a probable decrease in the level of Tg, as an autocrine regulator of metabolic processes in the thyroid gland, apparently, also caused a decrease in the synthesis of T4 and T3 in rats of the exposed groups, which is more expressed under the action of 240 μg I/kg. Under such conditions, the synthesis of thyroid hormones and their inclusion in Tg is inhibited. The synthesis of this iodine-containing protein is regulated by transcription factors that control the synthesis of TPO and TSH [17, 20]. An important and preventive role of iodine is the protection of the thyroid gland from radiation exposure [16, 21]. In exposed model research, the maximum tolerable dose of the new organic iodine compound in which iodine is coordinated by carbohydrates and polypeptides was established [20]. The level of negative biochemical effects of this complex in dogs is a dose of 22.8 mg I/kg, which is equivalent to 12.3 mg I/kg of humans [11].

In publications [1, 3, 21] is reported that an excess of iodine in the organism slows down the mechanism of oxidation and organization of iodide mediated by TPO-thyrocytes. As a heme-containing enzyme-glycoprotein, TPO catalyzes the oxidation of the iodide anion and the condensation of iodinated tyrosine derivatives only in the presence of H_2O_2, the Ca^{2+} dependent transmembrane system (Thox) is involved in the formation of hydrogen peroxide. Higher levels of Anti-TPO ($P < 0.001$) and Ca content ($P < 0.001$) on the background of decreased AP in the blood of rats of the exposed groups may indicate a hyper stimulatory action of the used doses of I_2Citr on the synthesis and activity of TPO in thyroid gland thyrocytes, the reaction of the immune system and the earnings of Anti-TPO into the peripheral blood.

The regulatory role of TSH in metabolic processes, which depends on the level of T3 and T4, was developed by an increase in the concentration of thyrotropin in the blood of animals of exposed groups under the action of both 24 and 240 μg I/kg ($P < 0.05$). It can be assumed that the inhibition of synthesis and secretion of T4 and T3 in rats of the exposed groups under applied doses of I_2Citr intensified the secretion of TSH. However, these indicators show a more expressed negative connection of endocrine regulation of metabolic processes in the pituitary-thyroid system under the action of the highest dose of I_2Citr. This is confirmed by a probable decrease in the content of T4 and T3 in the serum of rats of the 2nd exposed group. The excessive iodine in the blood causes a high degree iodination of Tg,

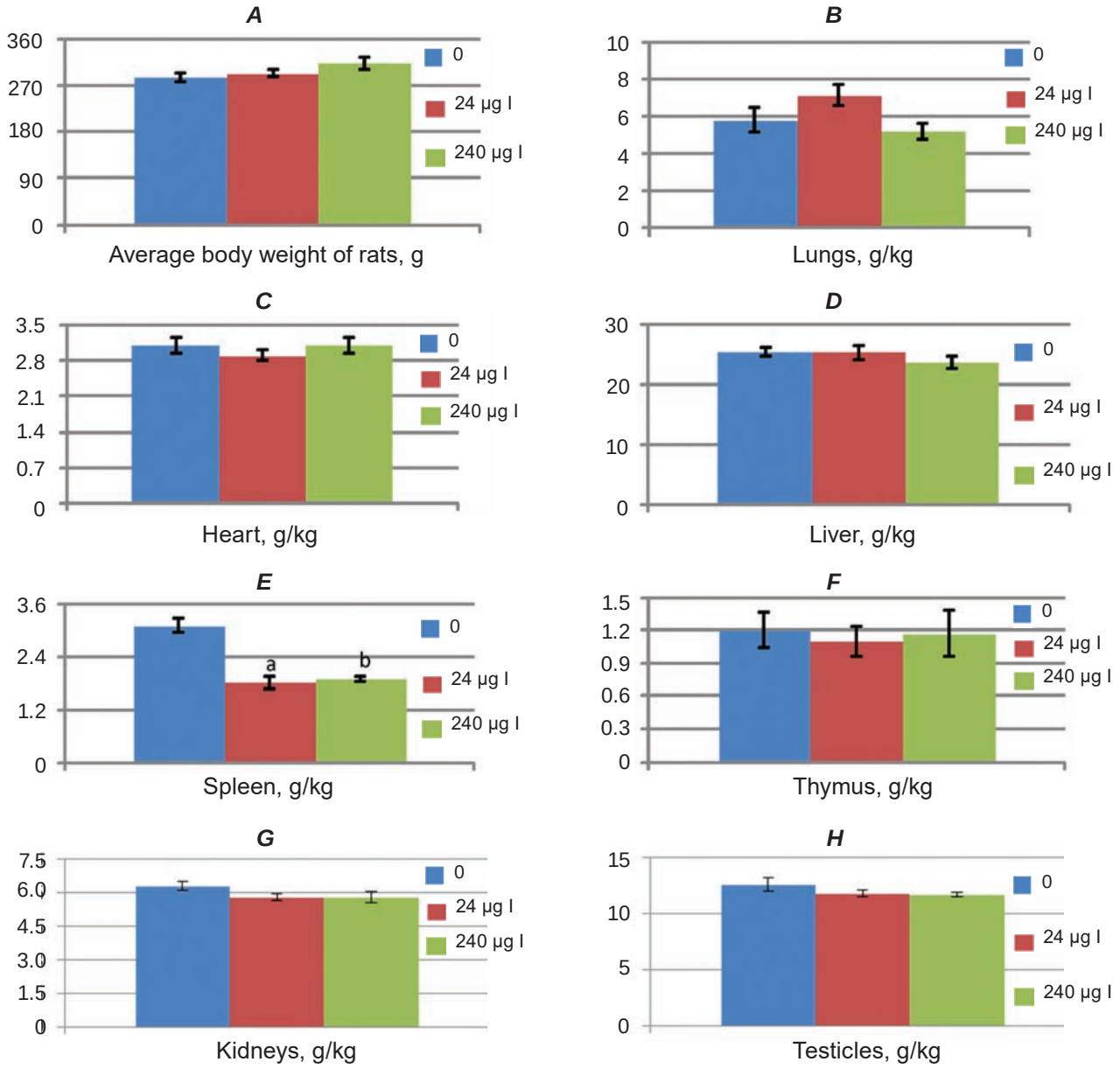

Fig. 3. The average body weight and mass coefficients of the internal organs of rats under the action of different concentrations of iodine and citrate nanocomposition, n = 6: a – probable differences between C (0) and 1st (24 μg I/kg) groups, P < 0.001; b – probable differences between C (0) and 2nd (240 μg I/kg) groups, P < 0.001

triggering autoimmune reactions and thyroiditis development [16].

Metabolism of iodine in the thyroid gland is associated with certain classes of lipids involved in the oxidation of the iodine-anion on the membrane of thyrocytes, and also with the inclusion of iodine to Tg with the formation of iodolactones and iodoaldehydes [3, 9, 17]. Lower TAG content ($P < 0.05$) and increased cholesterol ($P < 0.001 – P < 0.05$) in the blood of rats of the exposed groups may indicate increased use of TAG, but the reduction of cholesterol in the oxidation of iodide anion and metabolism of iodine processes in the thyroid gland under the action of the used concentrations of I_2Citr. This hypothesis is confirmed by a probably higher ($P < 0.001$) Ca content and a decrease in the level of AP in the blood of rats under the action of I_2Citr. The optimization of metabolic processes in the organism of rats of exposed groups indicates a probable decrease in the level of urea and AlAT activity in their

blood. It was proved that organic iodine compounds in the gastrointestinal tract are exposed to proteolytic enzymes with the formation of iodotyrosine. The amount of these enzymes is directly proportional to iodine deficiency in the organism. Another protective ability of the organism against excess iodized amino acids is by means of liver transferases. The specified mechanism ensures iodine intake into the organism from organic compounds within the limits of need [2, 3, 18, 20]. Therefore, the used doses of iodine in the form of citrate made by the method of nanotechnology, cause multidirectional changes in the activity of the pituitary-thyroid system and the course of metabolic processes in rats, which can be considered as an intermediate exposed result for further search of other doses of this organic compound of iodine in order to adjust the metabolism and function of the thyroid gland and for the selection of most effective compositions between different organic complexes of iodine.

Conclusions. The treatment of male rats with iodine and citrate nanocomposition made by nanotechnology for 40 days at doses of 24 and 240 μg I/kg caused a corrective influence on the pituitary-thyroid system and metabolic processes in the blood, namely:

– intensification of the inhibitory effect of the pituitary gland on the thyroid system with an increase in TSH and Anti-TPO levels; and a decrease in Tg, T4 and T3, urea levels, and alkaline phosphatase and AlAt activities under the action of iodine and citrate nanocomposition at dose 240 μg I/kg;

– a regulatory display of lipid metabolism with increasing cholesterol and Ca; a decrease of triacylglycerols in the blood of rats of both exposed groups and hemodeposition function of the spleen with a decrease of its mass and mass coefficients of these animals.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПОКАЗНИКИ ТИРЕОЇДНОЇ СИСТЕМИ ТА МЕТАБОЛІЗМУ В ЩУРІВ ЗА ДІЇ НАНОКОМПОЗИЦІЇ НА ОСНОВІ ЙОДУ ТА ЦИТРАТУ

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Пошук нових біологічно ефективних сполук йоду, які не спричинюють токсичних ефектів і можуть мати практичне застосування є актуальним. Метою дослідження було оцінити вплив нанокомпозиції на основі хелатного комплексу йоду з лимонною кислотою (I_2Citr) на активність тиреоїдної системи та на показники метаболізму в крові молодих самців щурів. Щури експериментальних груп отримували I_2Citr з водою щодня протягом 40 днів у дозі 24 мкг I/кг (1-а група) та 240 мкг I/кг (2-а група). Рівень гормонів та антитіл у сироватці крові визначали за допомогою ІЕА. Виявлено зниження рівня Tg та вмісту триацилгліцеролів, підвищення рівнів AntiTg, AntiTPO та вмісту альбуміну і Ca у сироватці крові щурів обох груп порівняно з контролем. Показано зниження рівнів T3, T4, сечовини, а також активності лужної фосфатази та AlAT у сироватці щурів 2-ої групи. За дії I_2Citr не виявлено змін у коефіцієнтах маси внутрішніх органів щурів за винятком маси селезінки, яка зменшилася приблизно на 40% в 1-ій та на 33% в 2-ій групах порівняно з контролем. Одержані дані свідчать про модулювальний вплив I_2Citr у досліджуваних дозах на активність щитоподібної залози без спричинення токсичних ефектів на організм тварин.

Ключові слова: нанокомпозиція на основі йоду та цитрату, гормони щитоподібної залози, показники метаболізму, маса внутрішніх органів щурів.

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