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PHYSIOLOGICAL REACTIVITY AND ANTIOXIDANT DEFENSE SYSTEM OF THE ANIMAL ORGANISM INDUCED BY GERMANIUM, CHROMIUM, AND SELENIUM "NANOAQUACITRATES"

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Aim. To study the state of physiological reactivity of the organism and blood antioxidant defense system of female rats and their offspring under prolonged feeding with "nanoaquacitrates" of germanium, chromium, and selenium. **Methods.** To accomplish the stated objective we carried out physiological and biochemical studies, using colorimetric methods to study the content of glycoproteins and separate monosaccharides of their carbohydrate components. The state of antioxidant defense system was studied by the intensity of lipid peroxidation that was also determined using the colorimetric methods based on the content of lipid hydroperoxides and TBA-active products in blood. **Results.** The intergroup differences in the content of glycoproteins and their carbohydrate components in the blood of female rats and their offspring of both experimental groups have been observed compared against the control. This may indicate approximately the same integrated biological effect of chromium, selenium, germanium in the second group, fed with chromium and selenium citrates along with the drinking water, calculated as 50 μg per 1 kg of body weight (b.w.) for Cr and Se, and germanium citrate – 15 μg Ge per 1 kg of b.w. The first group served as a control; and the third one was fed with chromium and germanium citrates, calculated as 50 μg per 1 kg of b.w. for Cr, and germanium citrate – 15 μg Ge per 1 kg of b.w., which results in the activation of the physiological reactivity system. However, the integrated physiological effect of citrate compounds of the three elements – chromium, selenium, and germanium – on the rats of the second group was more significant, as confirmed by a high probability of differences compared against the control group. The complex of microelements, used in these quantities, demonstrates significant antioxidant activity, which is typical for each microelement. **Conclusions.** Feeding mature, 4–8-month-old, rats (feeding started at 4 months and lasted till the end of the 8th month) and young, 0–4-month-old, rats (feeding started at birth and lasted for 4 months) with "nanoaquacitrates" of germanium, chromium, and selenium causes an increase of physiological reactivity in their bodies, which is evidenced as follows, 1) the increase in the ceruloplasmin content in the blood of mothers and infant rats, in haptoglobin and sialic acids – only in mothers, and in protein-bound hexoses – in infant rats; 2) a reduction in the intensity of lipid peroxidation in the blood of mature females and young rats with lower content of lipid hydroperoxides and TBA-active products in the blood of the animals from experiment groups, which is more significant in females; 3) identical orientation of the integrated physiological effect of chromium, selenium, germanium, and the combination of chromium and germanium on the organism of female and young rats which causes general activation of reactivity of the organism and its antioxidant system that is more vivid for females.

Key words: "nanoaquacitrates" of germanium, chromium, selenium, serum glycoproteins, lipid peroxidation, rats.

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INTRODUCTION

Germanium is the least studied microelement among the three used, but the current information about its properties demonstrates promising future of its application in veterinary science, cattle breeding and medicine. In particular, as an immunomodulator Ge stimulates the

activity of natural-killer T-cells, increases the production of gamma-interferon, and enhances the activity of anti-tumor immunity in case of carcinogenesis [1–3]. In addition, Ge improves cell oxygenation and cell metabolism. Due to its ability of transferring electrons, this element decreases the activity of peroxidation processes, increases the level of reduced glutathione, the activity of glutathione peroxidase and superoxide dismutase. The most promising way of using germanium is its medical application as an oncoprotector in the antitumor therapy [2]. However, there are literature data, proving that organic and mineral compounds of germanium, obtained via chemical synthesis, are characterized by the toxic impact due to the presence of inorganic admixtures of this element (germanium dioxide, germanium tetrachloride) which are predecessors in the synthesis of its organic forms [4, 5]. Therefore, it is urgent to obtain germanium in the bioavailable form, without any toxic effect. The achievements in the nanotechnology field, in particular, the elaboration of electric discharge nanotechnology, allowed synthesizing the carboxylates of microelements, including chemically pure citrates of germanium, chromium, and selenium in Ukraine [9]. The results of previous studies on the effect of carboxylates of some microelements on the animal organisms confirm the positive effect of the "nanoaquacitrates" of germanium, chromium, and selenium on the physiological and biochemical processes. It was established that the biotic microelements in the form of "nanoaquacitrates" stimulate the activity of the antioxidant and immune system in a better way; they also improve protein, mineral, and vitamin profile of the blood and enhance the disintoxication processes in the organism of animals [6–8]. In particular, selenium is a powerful antioxidant with protective functions. It is a part of glutathione peroxidase, participates in reduction-oxidation and antioxidation processes, the breathing of cells and in the synthesis of specific functional proteins, containing selenides. In addition, selenium enhances the immune defense of the organism and promotes life duration prolongation. This element activates cellular, humoral, and phagocytic components of innate immunity and promotes the increase in non-specific resistance of the organism [6, 7]. The literature data demonstrate that selenium and germanium have synergistic effect and enhance the properties of one another, due to which the combined application of these elements may enhance the functional activity of both immune and antioxidant systems considerably. The interaction of germanium and other elements has not been studied sufficiently, so it is urgent to study the effect of organic compounds of germanium on the organism of humans and animals in combination with other essential microelements. There are good prospects for the study of the interaction between germanium and chromium, the biological effect of which is directed towards enhancing the effect of insulin, the normalization of carbohydrate, lipid and protein metabolism as well as general regulation of metabolism.

The aim of this work was to study the state of physiological reactivity of the organism and blood antioxidant defense system of female rats and their offspring in conditions of prolonged feeding with "nanoaquacitrates" of germanium, chromium, and selenium.

MATERIALS AND METHODS

The solutions of germanium, chromium, and selenium citrates, obtained by the nanotechnology method [9] in the Ukrainian State Research Institute of Nanobiotechnologies and Resource Preservation (Kyiv), were used in the work.

The experiments were performed in the vivarium of the Institute of Animal Biology, NAAS, using three groups of white laboratory rat females and their offspring. The groups of females were formed by the analogue principle at the age of 4 months; 4 pregnant animals per group were left after the mating which was conducted a month since the beginning of the experiment. Feeding females of rats from experiment groups II and III with the solutions of "nanoaquacitrates" was conducted for one month prior to the impregnation and up to three months thereafter, which included the periods of pregnancy and feeding of the offspring. The animals of group I (control group) were kept on balanced standard ratio with granulated combined feed during the whole study period with unrestricted drinking of water. The animals of group II received the ratio using the scheme of the control group, were fed with chromium and selenium citrates along with the drinking water, calculated as 50 μg per 1 kg of body weight (b.w.) for Cr and Se, and as for germanium citrate – 15 μg Ge per 1 kg of b.w. During this period the rats of group III received the ratio which included feeding with citrates of Cr and Ge only in accordance with the scheme of group II. The young rats of experiment groups I (control), II and III were left in the conditions, in which their mothers from the corresponding groups were kept and fed, for up to four months; they were fed with Cr, Se and Ge or Cr and Ge in accordance to the mother's group along with the mother's milk during the suckling period; during the

post-weaning period they were fed with these citrates in water in the amount, given to the females.

To obtain the blood samples, the euthanasia of 4 females from each group was conducted with brief ether anesthesia after 4 months of feeding with citrates, and that of five young females – at the age of 4 months. The manipulations were conducted with respect to the norms of humane treatment of laboratory animals, with the consideration of common bioethical norms and in accordance with international provisions on experimental works [10]. The content of glycoproteins and products of lipid peroxide oxidation (LPO) (TBA-active products, lipid hydroperoxides (LHP)) in the blood was evaluated [11]. The digital material obtained was processed by the method of variation statistics using the Student's criterion. The arithmetic mean values (M) and the deviations of arithmetic mean values ($\pm m$) were calculated. The changes were deemed probable at $P < 0.05$. The estimates were made using MS Excel program.

RESULTS AND DISCUSSION

The studies on the content of glycoproteins and some monosaccharides of their carbohydrate components in the blood of female rats established unequal intergroup differences in their levels. In particular, the content of seroglycoids and hexoses, bound to proteins, is reliably decreased in the blood of female rats (Table 1) whereas the level of ceruloplasmin, haptoglobin and sialic acids increases ($P < 0.05$; $P < 0.01$). As ceruloplasmin and haptoglobin also play a significant part in the functioning of the antioxidant system of the organism, the complex of microelements, used in groups II and III, might have apparent antioxidant activity which is a characteristic feature of each microelement of the admixture used [12–15]. The mentioned inter-group differences in the content of glycoproteins and their carbohydrate components in the blood of female rats of both experiment groups compared against the control may indicate the same direction of the biological activity of the complex application of chromium, selenium, germanium in group II and that of chromium and germanium in group III. This effect may be greatly conditioned by the activity of citrates of chromium and germanium which causes the activation of the immunobiological reactivity of the organism, the results obtained are in good agreement with the literature data on the properties of these microelements [14–17].

However, the integrated physiological effect of citrate compounds of the three elements – chromium, selenium, and germanium – in the rats of the second group was more significant, as confirmed by a high probability of differences compared against the control group. The animals of group III had the lowest probability level for hexoses, bound to proteins, ceruloplas-

Table 1. The content of glycoproteins and some monosaccharides of their carbohydrate components in the blood of female rats, fed with citrates of chromium, selenium and germanium ($M \pm m$, $n = 4$)

Group	Seroglycoids, g/l	Hexoses, bound to proteins, g/l	Ceruloplasmin, c.u.	Haptoglobin, g/l	Sialic acids, c.u.
I	0.201 ± 0.002	2.29 ± 0.085	237.7 ± 7.88	1.23 ± 0.090	54.0 ± 1.53
II	0.173 ± 0.005*	1.77 ± 0.059**	393.3 ± 5.49***	1.70 ± 0.065**	62.7 ± 1.45**
III	0.185 ± 0.010	1.79 ± 0.120*	302.3 ± 6.89**	1.56 ± 0.130	63.0 ± 2.08*

Note. The difference in this and the following Tables is statistically reliable compared against group I: *P < 0.05; **P < 0.01; ***P < 0.001.

Table 2. The content of glycoproteins and some monosaccharides of their carbohydrate components in the blood of the offspring of female rats, fed with citrates of chromium, selenium and germanium ($M \pm m$, $n = 5$)

Group	Seroglycoids, g/l	Hexoses, bound to proteins, g/l	Ceruloplasmin, c.u.	Haptoglobin, g/l	Sialic acids, c.u.
I	0.149 ± 0.002	2.01 ± 0.085	274.2 ± 14.94	1.84 ± 0.07	66.0 ± 3.62
II	0.141 ± 0.006	2.36 ± 0.060*	368.4 ± 13.19***	2.20 ± 0.05*	49.8 ± 2.78*
III	0.143 ± 0.005	1.90 ± 0.080	336.2 ± 11.02*	2.21 ± 0.05**	53.6 ± 4.02

min and sialic acids ($P < 0.05$) and unreliable differences – for seroglycoids and haptoglobin.

The analysis of the study results for the content of glycoproteins in the blood of the offspring indicates some differences in the impact of chromium, selenium and germanium on the immunobiological reactivity of their organism compared against their mothers (Table 2). The trend of changes, similar to those of female rats, regarding a higher level of ceruloplasmin ($P < 0.001$; $P < 0.05$) and haptoglobin ($P < 0.05$; $P < 0.01$) as well as seroglycoids was registered in the blood of young rats of groups II and III, but the decrease is unreliable. At the same time the level of hexoses, bound to proteins, and sialic acids in blood has an opposite direction for the young rats of experiment groups. The intergroup differences, revealed in the content of glycoproteins in the blood of young rats compared to their mothers, may indicate some specificities of physiological and biochemical mechanisms of the effect of chromium, selenium and germanium on the mature organism of the females and young rats who received the citrates of these microelements along with their mothers' milk and drinking water in the period of intense growth and development. It may be determined by the physiological differences in the functioning of the adaptation and antioxidant systems of females in the lactation period and the feeding of young rats as well as by the physiological reactivity of the organism, where glycoproteins take an active part, and the compounds used stimulate the mentioned functions [14, 16–18].

Table 3. The intensity indices of the lipid peroxidation in the blood of female rats, fed with chromium, selenium and germanium ($M \pm m$, $n = 4$)

Group	Lipid hydroperoxides, OU/ml	TBA-active products, nmol/ml
I	6.20 ± 0.12	1.85 ± 0.07
II	5.33 ± 0.33	1.56 ± 0.04*
III	5.18 ± 0.32*	1.65 ± 0.05

Table 4. The intensity indices of the lipid peroxidation in the blood of young rats, fed with chromium, selenium and germanium ($M \pm m$, $n = 5$)

Group	Lipid hydroperoxides, OU/ml	TBA-active products, nmol/ml
I	6.90 ± 0.04	2.25 ± 0.07
II	6.15 ± 0.09*	2.07 ± 0.03
III	6.24 ± 0.09*	2.16 ± 0.05

Another confirmation of the apparent biological effect of citrates of chromium, selenium and germanium is probable intergroup differences in LPO indices in the blood of female rats (Table 3). The analysis of these data testifies to the antioxidant activity of chromium, selenium and germanium in the organism of rats, which is accompanied with the decrease in LHP content in the blood of female rats of groups II and III by 14.0 and 16.5 % respectively, and the decrease of TBA-active products in group II by 15.7 and group III – by 10.9 %.

It is remarkable that probable lower content of these metabolites was also registered in the blood of the offspring of female rats (Table 4). For instance, LHP concentration in the blood of young rats of group II was lower by 10.9 ($P < 0.05$), and group III − by 9.6 %, compared against the control. The content of TBA-active products in the blood of animals in the experiment groups testifies only to the tendency towards the lower content without any probable differences. Our previous studies on the effect of chromium nanocitrate on the intensity of LPO process revealed insignificant change in the concentration of LHP and TBA-active products in the blood of rats and cows [6, 7]. The current experiment established reliable decrease in LPO products in the blood of female rats and their offspring both for the integrated use of selenium and germanium and for germanium only. In its turn it may confirm both the positive effect of germanium on the prooxidant-antioxidant balance in the organism of animals and possible synergistic effect on the antioxidant properties of chromium, similar results were also obtained by other authors while studying the antioxidant properties of germanium compounds [20–22].

Therefore, the results obtained demonstrate equal direction of the antioxidant activity of chromium, selenium and germanium in the organism of female rats and their offspring, but this effect was more evident in the organism of mothers-rats in terms of the probability of intergroup differences. The differences in the effect of citrates of chromium, selenium and germanium may be conditioned both by the specificities of the physiological state of females (lactation) and young rats (intensive growth) and the period of their effect which included the antenatal development for young rats.

CONCLUSIONS

Feeding mature females and young rats with "nano-aquacitrates" of germanium, chromium, and selenium causes an increase of physiological reactivity in their bodies, which is evidenced as follows, 1) the increase in the ceruloplasmin content in the blood of mothers and infant rats, in haptoglobin and sialic acids – only in mothers, and in protein-bound hexoses – in infant rats only; 2) a reduction in the intensity of LPO processes in the blood of mature females and young rats with lower content of LHP and TBA-active products in the blood of the animals from research groups, which is more significant in females; 3) identical orientation of the integrated physiological effect of chromium, selenium, germanium, and the combination of chromium and germanium on the organism of female and young rats which causes general activation of reactivity of the organism and its antioxidant system that is more vivid for females.

Фізіологічна реактивність і система антиоксидантного захисту організму щурів за дії «наноаквацитратів» германію, хрому, селену

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Мета. Вивчити стан фізіологічної реактивності організму та антиоксидантного захисту крові матерів і приплоду щурів за умов тривалого випоювання «наноаквацитратів» германію, хрому і селену. **Методи.** Для фізіолого-біохімічних досліджень використано колориметричні методи визначення вмісту глікопротеїнів та окремих моноцукрів їхніх вуглеводних компонентів; для вивчення інтенсивності процесів пероксидації ліпідів цими ж методами визначали вміст гідропероксидів ліпідів і ТБК-активних продуктів у крові. **Результати.** Зафіксовані міжгрупові розбіжності у вмісті глікопротеїнів і їхніх вуглеводних компонентів у крові як самок, так і приплоду щурів обох дослідних груп порівняно з контролем можуть вказувати на приблизно однаковий комплексний біологічний вплив хрому, селену, германію в II та хрому і германію − в III групах, що зумовлює підвищення фізіологічної реактивності їхніх організмів. Однак комплексна фізіологічна дія цитратної сполуки трьох елементів – хрому, селену і германію у щурів II групи була більш виражена, що підтверджується вищою вірогідністю різниць показників стосовно контролю. Використаний комплекс мікроелементів у застосованих кількостях обумовлює виражену антиоксидантну активність, яка є характерною для кожного окремого мікроелементу. **Висновки.** Випоювання «наноаквацитратів» германію, хрому і селену самицям і молодим щурам спричиняє посилення фізіологічної реактивності та антиоксидантного захисту їхніх організмів, що позначається на 1) підвищенні вмісту в крові самок-матерів і щуренят церулоплазміну, а також лише у самок − гаптоглобіну і сіалових кислот і лише у щуренят − гексоз, зв'язаних з білками; 2) зменшенні інтенсивності пероксидного окиснення ліпідів у крові статевозрілих самок і молодих щурів з нижчим вмістом гідропероксидів ліпідів і ТБК-активних продуктів у крові тварин дослідних груп, що більше виражено у самок; 3) аналогічній спрямованості комплексної фізіологічної дії хрому, селену і германію, а також хрому з германієм на організм самок і молодих щурів щодо активації реактивності організму та його антиоксидантної системи, яка, однак, більше проявляється у самок.

Ключові слова: «наноаквацитрати» германію, хрому, селену, глікопротеїни крові, пероксидація ліпідів, щури.

Физиологическая реактивность и система антиоксидантной защиты организма крыс при действии «наноаквацитратов» германия, хрома, селена

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Цель. Изучить состояние физиологической реактивности организма и антиоксидантной защиты крови матерей и приплода крыс в условиях длительного выпаивания «наноаквацитратов» германия, хрома и селена. **Методы.** Для физиолого-биохимических исследований использованы колориметрические методы определения содержания гликопротеинов и отдельных моносахаридов их углеводных компонентов; для изучения интенсивности процессов пероксидации липидов этими же методами определяли содержание гидроперекисей липидов и ТБК-активных продуктов в крови. **Результаты.** Отмеченные межгрупповые различия содержания гликопротеинов и их углеводных компонентов в крови как самок, так и приплода крыс обеих опытных групп по сравнению с контролем могут указывать на примерно одинаковое комплексное биологическое влияние хрома, селена, германия во II и хрома и германия − в III группах, что способствует активации физиологической реактивности их организма. Однако комплексное физиологическое действие цитратов трех элементов – хрома, селена и германия у крыс II группы оказалось более выражено, что подтверждается более высокой достоверностью различий показателей относительно контроля. Использованный комплекс микроэлементов в примененных количествах обусловливает выраженную антиоксидантную активность, что является характерным для

каждого микроэлемента. **Выводы.** Выпаивание «наноаквацитратов» германия, хрома и селена самкам и молодым крысам определяет усиление физиологической реактивности и антиоксидантной защиты их организмов, что проявляется в 1) повышении содержания в крови самок-матерей и крысят церулоплазмина, только у самок − гаптоглобина и сиаловых кислот и только у крысят − гексоз, связанных с белками; 2) уменьшении интенсивности перекисного окисления липидов в крови самок и крысят с более низким содержанием гидроперекисей липидов и ТБК-активных продуктов в крови животных опытных групп, которое более выражено у самок; 3) в аналогичной направленности комплексного физиологического действия хрома, селена и германия, а также хрома с германием на организм самок и молодых крыс, что сопровождается активацией реактивности организма и его антиоксидантной системы, больше проявляющейся у самок.

Ключевые слова: «наноаквацитраты» германия, хрома, селена, гликопротеины крови, перекисное окисление липидов, крысы.

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