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GROWTH AND DEVELOPMENT OF THE ORGANISM AND IMMUNOPHYSIOLOGICAL INDICES OF BLOOD OF MALE F_2 RATS, AFFECTED BY DIFFERENT DOSES OF NANOGERMANIUM CITRATE

R. S. Fedoruk [1], U. I. Tesarivska [2], M. I. Khrabko [1], M. M. Tsap [1]

[1] Institute of Animal Biology, NAAS
38, V. Stusa Str., Lviv, Ukraine, 79034
[2] State Scientific Research Control Institute of Veterinary Medicinal Products and Feed Additives
11, Donetska Str., Lviv, Ukraine, 79000

E-mail: ecology@inenbiol.com.ua

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Aim. To study age-related changes in the bodyweight, internal organs, and immunophysiological indices of blood for male F_2 rats, affected by different doses of nanogermanium citrate. **Methods.** Physiological, immunological, clinical, colorimetric, and statistical methods were applied; automatic veterinary analyzer Orphee Mythic 18 Vet (Germany) was used to determine hematological indices. **Results.** The decade-wise dynamics of changes in the bodyweight, the weight of heart, liver, kidneys, testicles, lungs, spleen, and weight coefficients of these organs at the age of 2–3 and 4–5 months demonstrated that there were intergroup dose-dependent and age-related differences in the intensity of growth and development of the organism of male rats. The highest gain of bodyweight was registered for male rates, which received 10 μg Ge/kg of bodyweight 30 days after weaning, this tendency was kept for the lower level of these indices in groups III and IV at the effect of 20 and 200 μg Ge. On days 97, 107, and 117, males of group II and IV demonstrated non-reliably lower indices of bodyweight, whereas in group III their values did not differ much from those for the control. Reliably higher indices of weight of liver and spleen were demonstrated along with their weight coefficients for younger males, but they were lower at the age of 4–5 months compared to the control. The blood of 2–3 m.o. group II males had reliably lower content of hemoglobin and hematocrit index as well as the tendency to the decrease in the number of erythrocytes. At the age of 4–5 months, there was an evident decrease in the number of leukocytes and their forms – lymphocytes, monocytes, and granulocytes, which was more expressed in the blood of group II and IV males compared to the control. At the impact of nanogermanium citrate (NGeC), the blood of males of experimental groups of both age periods demonstrated higher content of immunoglobulins, circulating immune complexes, hexoses, bound to proteins, and ceruloplasmin (except for group II animals at the age of 4 months). The effect of applied doses of NGeC inhibited the input of acute phase proteins into the blood which was evident in the reliably lower content of mean mass molecules at the age of 2–3 months with its staying at the level of the control group of animals at the age of 4– months. **Conclusions.** The biological effect of NGeC in the doses of 10, 20, and 200 μg Ge/kg of bodyweight was demonstrated in differently directed changes in the intensity of organism growth and development, specific internal organs, and hematological indices, which was more expressed for animals, receiving 10 μg Ge, as well as in the differences in immunophysiological indices of blood of males of the experimental groups of both age periods, which indicated the activation of immunobiological reactivity of their organism at the age of 2–3 and 4–5 months.

Keywords: nanogermanium citrate, rats, blood, immunophysiological indices, organism growth and development.
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INTRODUCTION

The multidirectional effect of organic Ge compounds is the reason for a wide spectrum of studies on their biological action [1–4]. It was determined that long-term application of GeO_2 in the dose of 0.9 mg/kg per day with feeds conditioned the bodyweight gain of young

rats. However, the increase in the dose up to 8.4 mg/kg per day reduced growth intensity of rats during the first period of application compared to the previous dose [1]. The publications of other authors [5] demonstrated that feeding rats with sodium germanate starting from their birth until natural death did not cause any changes in growth indices, but the duration of their life changed slightly. It has been proven that Ge gets absorbed in the organism fast regardless of the administration mode and may be bound to proteins of blood serum. In the venous blood, Ge is mainly localized in red blood cells, whereas in arterial blood it gets dissolved in plasma [1]. At the effect of sublethal (100–150 mg/kg) doses of GeO_2, the blood of rats and rabbits demonstrated the decrease in the content of hemoglobin and the tendency to the reduced number of erythrocytes and leukocytes [1, 6]. This indicates uneven biological effect of different doses of mineral Ge compounds.

The chemical synthesis of organic Ge compounds and comprehensive study of their properties demonstrate promising future for the application of these complexes in biology, medicine, veterinary practice, and animal breeding, as they are notable for immunostimulating, hepato- and membrane-protective, cardiotropic and neurotropic activity [7–10]. Obtaining of Ge carboxylates using nanotechnology methods opens new possibilities for wide application of these non-toxic compounds in the mentioned industries. However, the investigation of biological effects of the application of organic Ge compounds, obtained by nanotechnologies, has started only in recent 2–3 years. In our previous experimental works, we have noted reliably expressed dose-dependent biological effect of nanogermanium citrate on the intensity of growth and development of females of generations F_0 and F_1 and their reproductive abilities [11, 12]. The continuation of the long-term experiment allowed us to obtain the second generation offspring and to study the biological effect of the applied doses of nanogermanium citrate on the ontogenetic development of the organism of male F_2 rats, and to establish the changes in the indices of their immunophysiological state.

The investigations were aimed at the study of the effect of different doses of nanogermanium citrate on the dynamics of changes in the bodyweight of male F_2 rats after weaning, the development of their internal organs and immunophysiological indices of blood.

MATERIALS AND METHODS

The study was conducted at the Institute of Animal Biology, NAAS, and the State Scientific Research Control Institute of Veterinary Medicinal Products and Feed Additives, using white male laboratory rats F_2, divided into four groups by the analogue principle during the weaning period at the age of 37 days, with the bodyweight of 93–105 g. Group I animals (control group) were fed with balanced standard diet, granulated compound feed, and unlimited water supply during the whole period of investigations. The animals of experimental groups II–IV were fed with standard compound feed and water with the addition of nanogermanium citrate (NGeC), produced by the nanotechnological method [13, 14] in the following amounts (μg Ge/kg of bodyweight): II group – 10; III – 20; IV – 200. Aqueous solution of NGeC in the concentration of 1.2 g/c. dm, pH 1.30, was obtained from "Nanomaterials and nanotechnologies" LLC (Ukraine). The intake of NGeC into the organism of male F_2 rats of the experimental groups lasted during the lactation period of female rats F_1 until 4–5 months old. On the 60–90th and 120–150th days of life, 5–9 male rats from each group were decapitated after narcosis and immobilization using CO_2 in compliance with the bioethical norms [15]. During this period, the blood samples were taken for the investigation by the methods, described below in accordance with the reference book [16] and the following internal organs were extracted: liver, kidneys, lungs, thymus, spleen, heart, testes, which were used to determine the weight and weight coefficients. The number of leukocytes, lymphocytes, monocytes, granulocytes, erythrocytes, platelets, hematocrit count, and hemoglobin content was determined using the hematological analyzer Orphee Mythic 18 Vet (Germany); the content of immunoglobulins (Ig) was defined by the nephelometric method; the content of mean mass molecules (MMM), circulating immune complexes (CIC), hexoses, bound to proteins, sialic acids, and ceruloplasmin was defined by the methods, described in the reference books [16].

The obtained digital material was processed by the variation statistics method using Student's *t*-criterion. Arithmetic mean values (M) and deviations from arithmetic mean values (± *m*) were calculated. The changes were deemed reliable at $P \leq 0.05$. The calculations were performed in Excel program.

RESULTS AND DISCUSSION

The analysis of the results obtained indicates some differences in the biological impact of NGeC doses, applied to rats, in particular, regarding the growth dynamics of males F_2 (Table 1). The bodyweight gain on the 30th day after weaning was the highest (240.2 %) for group II males, fed with 10 μg Ge, which exceeded the

gain for control group animals by 9.5 % (67th day). The bodyweight of the latter increased 2.03-fold for the same period, amounting to 202.7 %. During the same period, the application of higher Ge doses to group III and IV males resulted in lower indices of growth intensity compared both to the control group (by 4.1 and 9.8 %) and experimental group II (by 12.4 and 17.6 % respectively). In two months after weaning (97th day), the relative growth intensity was higher for group II and III males (268.6 and 272.0 %) compared to the control (262.3 %), but the bodyweight was lower in experimental groups II and IV (94.7 and 86.1 % respectively), and it was 100.7 % from the control for group III animals. The mentioned tendency was preserved on the 107th and 117th days, which indicates the most expressed positive impact of NGeC in the dose of 10 μg/kg of bodyweight on the growth of young male F_2 rats only during the first 2 months of their life. However, feeding with NGeC until the 137th day conditioned the increase in the bodyweight of male rates of group III for the same period by 18.4 %, and that in the growth intensity for 100 days – by 69 % compared to the control.

The analysis of the indices of growth and development of internal organs of animals in terms of their weight and weight coefficients indicates considerable differences between males of the control and experimental groups at the age of 2–3 months (Table 2). In particular, group II males of this age had lower indices of the weight of lungs, heart, kidneys, testes, as well as coefficients of their bodies at the background of higher indices of these values for liver and spleen compared to the control group. The mentioned indices for the animals of group IV did not have any significant differences for heart, liver, and spleen compared to the control group, but the weight and weight coefficient for lungs and testes increased reliably with the decrease ($P < 0.05$) of the weight coefficient for kidneys.

The obtained data indicate the inhibiting impact of the low dose of NGeC on the growth and development of lungs, heart, kidneys, and testes, but the stimulating impact on such parenchymatous organs as liver and spleen for young male F_2 rats. The established difference in the impact of NGeC on different organs in the mentioned dose may be conditioned by higher intensity of the growth of group II animals at the age of 2 months and structural-functional specificities of these organs and their capability to synthesize the immune complexes. These results indicate that Ge dose of 200 μg may have a stronger stimulating effect on the development of organs of breathing and reproduction of males with the inhibition of the excretion function.

Table 1. The growth intensity of young rats F_2 after weaning, while fed with different doses of germanium citrate ($n = 4–9$)

Growth period, day	Group						
	I – control	II – 10 μg	% from the control	II – 20 μg	% from the control	IV – 200 μg	% from the control
37	100.4 ± 1.97	92.8 ± 4.1	92.4	97.5 ± 1.63	97.1	104.8 ± 4.0	104.4
47	136.7 ± 3.84	133.5 ± 4.06	97.7	157.9 ± 4.59	115.5	125.9 ± 4.91	92.1
57	184.6 ± 5.15	173.6 ± 6.05	94.0	174.1 ± 3.85	94.3	155.0 ± 4.38	84.0
67	203.5 ± 8.60	222.9 ± 11.70	109.5	195.2 ± 5.01	95.9	183.6 ± 4.30	90.2
Gain for 30 days, %	202.7	240.2		200.2		175.2	
77	230.4 ± 6.70	225.7 ± 7.27	98.0	215.7 ± 6.40	93.6	195.0 ± 4.58	84.6
87	251.1 ± 11.27	244.3 ± 5.61	97.3	244.0 ± 7.16	97.2	220.0 ± 5.43	87.6
97	263.3 ± 11.06	249.3 ± 7.35	94.7	265.2 ± 5.91	100.7	226.8 ± 6.58	86.1
Gain for 60 days, %	262.3	268.6		272.0		216.4	
107	278.3 ± 11.61	267.9 ± 8.16	96.3	282.9 ± 6.78	101.7	255.0 ± 7.36	91.6
117	288.3 ± 10.77	285.0 ± 9.88	98.9	285.3 ± 6.31	99.0	275.0 ± 6.18	95.4
127	306.3 ± 12.56	–	–	312.8 ± 16.71	102.1	293.2 ± 5.53	95.7
137	315.6 ± 13.21	–	–	373.7 ± 17.37	118.4	307.7 ± 5.32	97.5
Gain for 100 days, %	314.3	–		383.3		293.6	

The estimation of growth and development of internal organs of males at the age of 4–5 months indicates some differences in the indices of their weight and weight coefficients in the experimental groups compared to the control group (Table 3). In particular, group II males had lower indices of the weight of spleen ($P < 0.05$), as well as liver and its weight coefficient ($P < 0.05$), and group III animals – those of the weight of lungs ($P < 0.05$), heart ($P < 0.01$) and liver ($P < 0.05$) compared to the control.

The mentioned differences may partially be conditioned by the difference in the age of animals of these groups as control group males were killed at the age of 135 days, experimental group II animals – 130, III – 120, and IV – 147 days. This may be confirmed by the weight coefficients of internal organs which, except for liver in group II and testes in group III, had approximately the same level of these indices in the control group. However, higher indices of the weight of liver and spleen and their weight coefficients for group II males at the age of two months with their decrease at the age of five months may testify to some stimulating effect of NGeC in the dose of 10 μg/kg on the growth and development of these organs in young animals and the decrease of its effect for adult rats. Group IV males had a reliable decrease in the weight of spleen and its weight coefficient ($P < 0.01$).

The analysis of hematological indices of rats at the age of 2–3 months indicates the absence of reliably expressed action of NGeC in all the applied doses on the hemostasis system (Table 4). However, the blood of group II males had reliably lower indices of hemoglobin and hematocrit level and unreliably lower ones for erythrocytes which may be conditioned by the inhibiting effect of Ge in the dose of 10 μg on hemoglobin synthesis and the inclusion of Fe, as well as less expressed effect on erythropoiesis which was noted by other authors at the impact of inorganic compounds of Ge [1, 5].

At present, there are different views on the mechanisms of Ge compounds impacting the hemopoiesis. In particular, the increase in the number of erythrocytes at the action of GeO_2 has been noted, which has been explained by the authors by the dehydration of the organism rather than by the stimulating effect on spleen and bone marrow. According to the data of other au-

Table 2. The weight of internal organs and their weight coefficients for male rats F_2, 2–5 m.o., fed with different doses of germanium citrate ($M \pm m$, $n = 4–9$)

Organ	Group		
	I – control	Experimental, μg Ge/kg of bodyweight	
		II – 10	IV – 200
Weight of the organ (g) / weight coefficient (g/kg)			
Lungs	1.17 ± 0.08	0.76 ± 0.05**	1.54 ± 0.08**
	5.5 ± 0.29	4.64 ± 0.19**	7.09 ± 0.53**
Heart	0.74 ± 0.03	0.50 ± 0.03***	0.74 ± 0.02
	3.5 ± 0.11	3.08 ± 0.20	3.26 ± 0.05
Liver	6.84 ± 0.42	8.13 ± 0.29*	7.08 ± 0.31
	32.1 ± 0.67	50.35 ± 3.39***	30.74 ± 0.84
Spleen	0.72 ± 0.06	0.87 ± 0.07	0.75 ± 0.01
	3.36 ± 0.20	5.32 ± 0.37**	3.33 ± 0.08
Kidneys	1.60 ± 0.09	1.08 ± 0.07**	1.49 ± 0.05
	7.53 ± 0.22	6.56 ± 0.25*	6.62 ± 0.28*
Testes	2.63 ± 0.12	1.90 ± 0.09***	3.88 ± 0.32**
	12.48 ± 0.78	11.65 ± 0.39	17.24 ± 1.69*

Note. In this table and the following ones, the difference is statistically reliable compared to the control (I) group *$p \leq 0.05$; **$p \leq 0.01$, ***$p \leq 0.001$.

thors, the effect of Ge on hemopoiesis is mediated by its impact on the metabolism of Fe, Cu, Se, and other elements [2, 3].

The application of higher (20 and 200 μg) doses of NGeC to male F_2 rats did not enhance the depressing effect on the number of leukocytes, erythrocytes, and hemoglobin. On the contrary, there was an evident increase in the absolute and relative number of granulocytes in the blood of males in the experimental groups with the reliable difference at the effect of 200 μg Ge in group IV.

The reliably lower number of leukocytes and lymphocytes was registered in the blood of 4–5 m.o. males in groups II and IV, while that of monocytes was found only in group II (Table 5). However, other blood indices of males in the experimental groups did not demonstrate reliable intergroup changes regarding the control. This may indicate that the organism of older males in the experimental groups produced adaptive hematopoietic mechanisms concerning the long-term effect of NGeC in the applied doses, which stabilize the morphological composition of red blood of rats at the level of physiological optimum.

More expressed effect of the applied Ge doses was noted for the indices of immunophysiological condition of the organism. In particular, the blood of males in the experimental groups had higher content of Ig both at the age of 2–3 and 4–5 months with reliable values of differences in groups III and IV (Table 6).

The effect of the applied Ge doses enhanced the intake of immune and glycoprotein complexes into the peripheral blood of animals in the experimental groups with the increase in the content of circulating immune complexes, hexoses, bound to proteins, and ceruloplasmin, except for animals of group II at the age of 4–5 months. At the same time, at the age of 2–3 months the proteins of the acute phase of blood reacted with the reliable decrease in the number of mean mass molecules with the preservation of this tendency at the age of 4–5 months for males in groups III and IV. At the background of these intergroup and age-related differences, there was a weakly observed tendency to the increase in the content of sialic acids in the blood of males in groups II and IV at the age of 2–3 months, and groups II and III – at the age of 4–5 months. Long-term feeding males of group IV with 200 μg Ge conditioned reli-

Table 3. The weight of internal organs and their weight coefficients for male F_2 rats, 4–5 m.o., fed with different doses of germanium citrate ($M \pm m$, $n = 4–9$)

Organ	Group			
	I – control	Experimental, μg Ge/kg of bodyweight		
		II – 10	III – 20	IV – 200
Weight of the organ (g) / weight coefficient (g/kg)				
Lungs	1.55 ± 0.09	1.35 ± 0.09	1.30 ± 0.06*	1.36 ± 0.08
	5.39 ± 0.30	5.05 ± 0.28	5.52 ± 0.23	4.71 ± 0.21
Thymus	0.62 ± 0.03	0.54 ± 0.04	–	–
	2.10 ± 0.07	2.03 ± 0.15		
Heart	0.88 ± 0.01	0.85 ± 0.03	0.72 ± 0.04**	0.92 ± 0.03
	3.06 ± 0.08	3.20 ± 0.08	3.06 ± 0.11	3.18 ± 0.09
Liver	8.97 ± 0.46	6.94 ± 0.45**	7.59 ± 0.39*	7.92 ± 0.26
	31.2 ± 1.80	25.9 ± 1.01*	32.8 ± 2.73	27.46 ± 0.76
Spleen	1.08 ± 0.08	0.81 ± 0.05*	0.90 ± 0.04	0.75 ± 0.04**
	3.80 ± 0.36	3.02 ± 0.11	3.84 ± 0.19	2.61 ± 0.14**
Kidneys	1.78 ± 0.08	1.65 ± 0.07	1.64 ± 0.04	1.97 ± 0.04
	6.20 ± 0.30	6.19 ± 0.19	6.95 ± 0.19	6.83 ± 0.14
Testes	3.05 ± 0.05	2.90 ± 0.05	2.96 ± 0.13	2.99 ± 0.10
	10.61 ± 0.39	10.96 ± 0.43	12.57 ± 0.48**	10.38 ± 0.31

ably higher content of these compounds in the blood of animals at the age of 4 months, which testified to a direct connection to a high concentration of circulating immune complexes and hexoses, bound to proteins.

CONCLUSIONS

Thus, the biological effect of NGeC in the doses of 10, 20, and 200 μg Ge/kg of bodyweight is notable for differently directed changes in the intensity of organism growth and development, specific internal organs, and hematological indices, which was more expressed for animals, receiving 10 μg Ge, as well as in the differences in immunophysiological indices of blood of males of the experimental groups of both age periods. This indicates the activation of immunobiological reactivity of their organism at the age of 2–3 and 4–5 months and gives grounds for the conclusions, stated below.

The doses of NGeC, applied with water (10, 20, and 200 μg/kg of bodyweight) to male F_2 rats, conditioned uneven effect on the growth and development of their organism, more expressed stimulating effect on the bodyweight gain within 30 days after weaning, as well as on the bodyweight gain of liver and spleen, and their weight coefficients, which was noted for group II males, receiving 10 μg Ge/kg of bodyweight.

Lower content of hemoglobin, hematocrit indices and the number of erythrocytes was registered in the blood

Table 4. The hematological indices for male F_2 rats, 2–3 m.o., fed with different doses of germanium citrate ($M \pm m$, $n = 4–6$)

Index	Measurement units	Group			
		I – control	Experimental, μg Ge/kg of bodyweight		
			II – 10	III – 20	IV – 200
Leukocytes	10^9/l	7.20 ± 0.63	5.98 ± 0.80	10.08 ± 1.99	6.92 ± 1.37
Lymphocytes	10^9/l	5.38 ± 0.38	4.05 ± 0.63	7.02 ± 1.30	4.63 ± 0.98
Monocytes	10^9/l	1.05 ± 0.10	0.98 ± 0.14	1.24 ± 0.19	0.80 ± 0.12
Granulocytes	10^9/l	0.75 ± 0.22	0.95 ± 0.21	1.80 ± 0.51	1.48 ± 0.39
Lymphocytes	%	74.8 ± 3.53	67.1 ± 4.09	71.9 ± 2.03	67.1 ± 2.55
Monocytes	%	15.4 ± 1.11	15.9 ± 1.12	11.9 ± 0.79*	12.2 ± 1.20
Granulocytes	%	9.8 ± 2.53	16.8 ± 2.68	16.2 ± 2.35	20.7 ± 2.62*
Erythrocytes	10^{12}/l	7.45 ± 0.41	6.74 ± 0.25	7.38 ± 0.26	7.65 ± 0.07
Hemoglobin	g/l	156.8 ± 6.13	130.8 ± 3.90**	148.4 ± 5.35	153.0 ± 2.19
Hematocrit	l/l	0.443 ± 0.025	0.371 ± 0.014*	0.414 ± 0.014	0.432 ± 0.014
Platelets	10^9/l	448.8 ± 22.84	604.0 ± 65.43	393.8 ± 24.32	383.6 ± 80.09

Table 5. The hematological indices for male F_2 rats, 4–5 m.o., fed with different doses of germanium citrate ($M \pm m$, $n = 4–6$)

Index	Measurement units	Group			
		I – control	Experimental, μg Ge/kg of bodyweight		
			II – 10	III – 20	IV – 200
Leukocytes	10^9/l	10.13 ± 2.14	4.72 ± 0.52*	7.64 ± 0.64	4.76 ± 0.55*
Lymphocytes	10^9/l	6.86 ± 1.21	3.76 ± 0.36*	5.21 ± 0.53	3.12 ± 0.36*
Monocytes	10^9/l	1.66 ± 0.43	0.62 ± 0.12*	1.45 ± 0.20	1.06 ± 0.15
Granulocytes	10^9/l	1.60 ± 0.51	0.34 ± 0.09*	0.98 ± 0.21	0.58 ± 0.13
Erythrocytes	10^{12}/l	5.97 ± 0.96	7.00 ± 0.57	6.48 ± 0.48	5.64 ± 0.58
Hemoglobin	g/l	144.0 ± 13.23	132.0 ± 9.94	129.4 ± 11.61	131.8 ± 6.59
Hematocrit	l/l	0.346 ± 0.052	0.360 ± 0.027	0.361 ± 0.033	0.331 ± 0.035
Platelets	10^9/l	602.3 ± 31.02	561.7 ± 91.00	645.8 ± 132.22	666.3 ± 122.18

Table 6. The content of immune and glycoprotein complexes in the blood of male F_2 rats, 2–3 and 4–5 m.o., fed with different doses of germanium citrate ($M \pm m$, $n = 4–6$)

Index	Month	Group			
		I – control	Experimental, μg Ge/kg of bodyweight		
			II – 10	III – 20	IV – 200
Immunoglobulins, g/l	2	6.5 ± 0.34	7.7 ± 0.47	9.8 ± 0.70**	10.4 ± 0.74**
	4	4.9 ± 0.47	5.6 ± 0.51	8.5 ± 0.47***	5.8 ± 0.30
Mean mass molecules, CU	2	0.35 ± 0.003	0.31 ± 0.001***	0.24 ± 0.002***	0.25 ± 0.004***
	4	0.19 ± 0.011	0.21 ± 0.005	0.18 ± 0.004	0.17 ± 0.003
Circulating immune complexes, mmol/l	2	42.8 ± 1.80	124.2 ± 2.64***	81.6 ± 3.65***	72.0 ± 2.97***
	4	61.4 ± 2.50	113.7 ± 1.23***	76.0 ± 2.77*	100.9 ± 1.71***
Hexoses, bound to proteins, g/l	2	5.19 ± 0.06	5.42 ± 0.06*	5.43 ± 0.08*	5.47 ± 0.06*
	4	5.35 ± 0.07	5.43 ± 0.07	6.14 ± 0.09***	6.15 ± 0.08***
Sialic acids, CU	2	206.3 ± 5.66	215.7 ± 3.03	208.8 ± 6.35	217.8 ± 6.26
	4	224.2 ± 4.12	237.8 ± 3.81	233.0 ± 4.34	240.7 ± 3.18*
Ceruloplasmin, CU	2	314.0 ± 4.18	350.2 ± 6.06**	336.8 ± 6.64*	357.7 ± 4.67***
	4	386.5 ± 7.97	377.8 ± 6.91	401.8 ± 6.96	416.2 ± 7.63*

of group II males at the age of 2–3 months, whereas the blood of animals of groups II and IV at the age of 4–5 months had a decreased number of leukocytes at the expense of lymphocytes, monocytes, and granulocytes compared to the control group.

The effect of the applied doses of Ge citrate on the immunophysiological reactivity of male F_2 rats was demonstrated in lower ($P < 0.001$) content of mean mass molecules in the blood of young animals and its increase for immune complexes globulins, circulating immune complexes, hexoses, bound to proteins, and ceruloplasmin (except for group II animals at the age of 4 months) in the rats of both age periods.

Ріст і розвиток організму та імунофізіологічні показники крові самців щурів F_2 за дії різних доз цитрату наногерманію

Р. С. Федорук [1], У. І. Тесарівська [2],
М. І. Храбко [1], М. М. Цап [1]

e-mail: ecology@inenbiol.com.ua

[1] Інститут біології тварин НААН
Вул. В. Стуса, 38, Львів, Україна, 79034
[2] Державний контрольний НДІ ветпрепаратів
та кормових добавок
Вул. Донецька, 11, Львів, Україна, 79000

Мета. Вивчити вікові зміни маси тіла, внутрішніх органів та імунофізіологічних показників крові самців щурів F_2 за дії різних доз цитрату наногерманію. **Методи.** Застосовано фізіологічні, імунологічні, клінічні, колориметричні і статистичні методи, для визначення гематологічних показників використано автоматичний ветеринарний аналізатор Orphee Mythic 18 Vet (Німеччина). **Результати.** Встановлено міжгрупові дозозалежні та вікові відмінності в інтенсивності росту та розвитку організму самців за щодекадною динамікою змін маси тіла, а також маси серця, печінки, нирок, тестикулів, легень, селезінки і коефіцієнтів мас цих органів у віці 2–3 і 4–5 місяців. Відзначено вищий приріст маси тіла у самців, які отримували 10 мкг Ge/кг маси тіла через 30 діб після відлучення, зі збереженням тенденції до нижчого рівня цих показників у III і IV групах за дії 20 і 200 мкг Ge. На 97-, 107- і 117-ту доби життя у самців II і IV груп відзначено не вірогідно нижчі показники маси тіла, у той час як у III групі їх величини суттєво не відрізнялися від контролю. Показано вірогідно вищі значення маси печінки і селезінки, а також коефіцієнтів їхніх мас у самців молодшого віку, але нижчі – у віці 4–5 місяців порівняно з контролем. У крові самців II групи у віці 2–3 місяці спостерігались вірогідно нижчий вміст гемоглобіну та показник гематокриту, а також тенденція до зменшення кількості еритроцитів. У віці 4–5 місяців відмічено зменшення кількості лейкоцитів та їх форм – лімфоцитів, моноцитів і гранулоцитів, що більше виражено у крові самців II і IV груп стосовно контролю. У крові самців дослідних груп обох вікових періодів за дії наногерманію цитрату (HGeЦ) відзначено вищий вміст імуноглобулінів, циркулюючих імунних комплексів, гексоз, зв'язаних з білками, і церулоплазміну (крім тварин II групи у віці 4 місяці). Дія застосованих доз HGeЦ пригнічувала надходження в кров білків гострої фази, що проявлялось вірогідно нижчим вмістом молекул середньої маси у

віці 2–3 місяці зі збереженням його на рівні контрольної групи тварин у віці 4–5 місяці. **Висновки.** Біологічна дія HGeЦ у дозах 10, 20 і 200 мкг Ge/кг маси тіла проявлялася у неоднаково спрямованих змінах в інтенсивності росту і розвитку організму, окремих внутрішніх органів та гематологічних показників, що більше виражено у тварин, які отримували 10 мкг Ge, а також відмінностями імунофізіологічних показників крові у самців дослідних груп обох вікових періодів, що вказує на активацію імунобіологічної реактивності їхнього організму у віці 2–3 і 4–5 місяців.

Ключові слова: цитрат наногерманію, щури, кров, імунофізіологічні показники, ріст і розвиток організму.

Рост и развитие организма и иммунофизиологические показатели крови самцов крыс F_2 при действии различных доз цитрата наногермания

Р. С. Федорук [1], У. И. Тесаривская [2],
М. И. Храбко [1], М. М. Цап [1]

e-mail: ecology@inenbiol.com.ua

[1] Институт биологии животных НААН
Ул. В. Стуса, 38, Львов, Украина, 79034
[2] Государственный контрольный НИИ ветеринарных препаратов и кормовых добавок
Ул. Донецкая, 11, Львов, Украина, 79000

Цель. Изучить возрастные изменения массы тела, внутренних органов и иммунофизиологических показателей крови самцов крыс F_2 при действии различных доз цитрата наногермания. **Методы.** Применены физиологические, иммунологические, клинические, колориметрические и статистические методы, для определения гематологических показателей использовано автоматический ветеринарный анализатор Orphee Mythic 18 Vet (Германия). **Результаты.** Установлены межгрупповые дозозависимые и возрастные различия в интенсивности роста и развития организма самцов по ежедекадной динамике изменений массы тела, а также массы сердца, печени, почек, тестикулов, легких, селезенки и коэффициентов масс этих органов в возрасте 2–3 и 4–5 месяцев. Отмечен больший прирост массы тела у самцов, получавших 10 мкг Ge/кг массы тела через 30 сут после отлучения, с сохранением тенденции к более низкому уровню этих показателей в III и IV группах при действии 20 и 200 мкг Ge. На 97-, 107- и 117-е сутки жизни у самцов II и IV групп отмечены не достоверно низшие показатели массы тела, тогда как в III группе их величины существенно не отличались от контроля. Показаны достоверно высшие значения массы печени и селезенки, а также коэффициентов их масс у самцов младшего возраста, но низшие – в возрасте 4–5 месяцев по сравнению с контролем. В крови самцов II группы в возрасте 2–3 месяца наблюдались достоверно низшее содержание гемоглобина и показатель гематокрита, а также тенденция к уменьшению количества эритроцитов. В возрасте 4–5 месяцев отмечено уменьшение количества лейкоцитов и их форм – лимфоцитов, моноцитов и гранулоцитов, что более выражено в крови самцов II и IV групп относительно контроля. В крови самцов опытных групп обоих возрастных периодов при действии наногермания цитрата (HGeЦ) отмечено высшее содержание иммуноглобулинов, циркулирующих иммунных комплексов, связанных с белками гексоз и церулоплазмина (кроме животных II группы в возрасте 4 месяца). Действие примененных доз HGeЦ угнетало поступление в кровь белков острой фазы, что проявлялось достоверно низшим содержанием молекул средней массы в возрасте 2–3 месяца с сохранением его на уровне контрольной группы животных в возрасте 4–5 месяца. **Выводы.** Биологическое действие HGeЦ в дозах 10, 20 и 200 мкг Ge/кг массы тела проявлялось в разнонаправленных изменениях интенсивности роста и развития организма, отдельных внутренних органов и гематологических показателей, что более выражено у животных, которые получали 10 мкг Ge, а также различиями иммунофизиологических показателей крови у самцов опытных групп обоих возрастных периодов, что указывает на активацию иммунобиологической реактивности их организма в возрасте 2–3 и 4–5 месяцев.

Ключевые слова: цитрат наногермания, крысы, кровь, иммунофизиологические показатели, рост и развитие организма.

REFERENCES

1. *Lukevics EJ, Gar TK, Ignatovich LM, Mironov VF*. The biological activity of germanium compounds. Riga, Zinatne. 1990;191 p.
2. *Kresyun VI, Shemonayeva KF, Vidavska AG*. Pharmacological characterization of germanium compounds. *Clinical Pharmacy*. 2004;**8**(4):64–8.
3. *Seifullina II, Martsinko EE, Afanasenko EV*. Design and synthesis of new homo- and heterometal coordination compounds of germanium (IV) for preparation of low toxic drugs with a wide therapeutic action. *Bulletin of the Odessa National University. Chemistry*. 2015; **20**(4):6–17.
4. *Stadnyk AM, Byts GA, Stadnyk OA*. The biological role of germanium in animals and humans. *Scientific Bulletin of Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky*. 2006;**8**(2):174–85.
5. *Schroeder HA, Kanisawa M, Frost DV, Mitchener M.* Germanium, tin and arsenic in rats: Effects on growth, survival, pathological lesions and life span. *J Nutr*. 1968;**96**(1):37–45.
6. *Hatano M, Ishimura K, Fuchigami K, Ito I, Hongo Y, Hosokawa Y, Azuma I.* Toxicological studies on germanium dioxide (GeO_2). (1) Acute, subacute, chro-

nic toxicity and successive irritation to the eye. Pharmacometrics [Oyo Yakuri]. 1981;21(5):773–796.
7. *Kudrin AV, Skalny AV, Zhavoronkov AA, Skalnaya MG, Gromova OA*. Immunopharmacology microelements. Moscow, Publ. KMC. 2000;537 p.
8. *Sakhanda IV*. Preparations of germanium and their application in medicine. Ukrayinskyy naukovo-medychnyy molodizhnyy zhurnal.2014;(4):83–6.
9. *Thayer JS*. Germanium compounds in biological systems. *Rev Silicon Germanium Tin Lead Compounds*. 1985;**8** (2–3):133–55.
10. *Lin CH, Chen TJ, Hsieh YL, Jiang SJ, Chen SS*. Kinetics of germanium dioxide in rats. *Toxicology*. 1999;**132** (2–3):147–53.
11. *Dolaychuk OP, Fedoruk RS, Kovalchuk II, Kropyvka SI*. Physiological and biochemical processes in the organisms of rats that were fed with different amounts of germanium citrate. *Animal biology*. 2015;**17**(2):50–6.
12. *Fedoruk RS, Dolaychuk OP, Kovalchuk II, Tsap MM*. Reactions of physiological systems in the organisms of rats to feeding with low and high doses of germanium "nanoaquacitrate". *Agric Sci Pract*. 2015;**2**(3):15–21.
13. *Pat*. of Ukraine No. 38391. N a 200810939. 2009. Method for metal carboxylates obtaining "Nanotechnology of obtaining metal carboxylates". Kosinov MV, Kaplunenko VG. Appl. 08.09.2008; publ. 12.01.2009. Bull. N 1:5 p.
14. *Borisevich VB, Kaplunenko VG, Kosinov NV, Borisevich VB*, *et al*. Nanomaterials in biology. Fundamentals of nanoveterinary science. K.: WA Avicenna. 2010; 416 p.
15. *European* convention for the protection of vertebrate animals used for experimental and other scientific purposes: Council of Europe. European Treaty Series – No. 123. Strasbourg. 1986;53 p.
16. *Vlizlo VV*. Laboratory methods of research in biology, stockbreeding and veterinary medicine: a guide. Lviv, Spolom. 2012;764 p.