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FEATURES OF NEURO-ENDOCRINE AND IMMUNE REACTIONS TO VARIOUS WATER-SALT LOADS IN FEMALE RATS

Olena A Gozhenko[1], Yuriy V Zavidnyuk[1,2], Mykhaylo M Korda[2], Igor R Mysula[2], Ivan M Klishch[2], Walery Zukow[3], Igor L Popovych[4]

[1]Ukrainian Scientific Research Institute of Transport Medicine, Odesa, Ukraine, olena.gozhenko@gmail.com
[2]IY Horbachevs'kyi State Medical University, Ternopil', Ukraine, zavidnyukyv@tdmu.edu.ua
[3]Nicolaus Copernicus University, Torun', Poland, w.zukow@.wp.pl
[4]OO Bohomolets' Institute of Physiology, Kyiv, Ukraine, i.popovych@biph.kiev.ua

Abstract

Background. Previously, we have shown that the weekly load of rats with water-salt solutions of different chemical compositions causes both general and specific reactions of the parameters of metabolism. The purpose of this study is to identify under these conditions specific neuroendocrine and immune responses. **Materials and methods.** Experiment was performed on 58 healthy female Wistar rats 240-290 g divided into 6 groups. Animals of the first group remained intact, using tap water from drinking ad libitum. Instead, the other rats received the same tap water as well as waters Sophiya, Naftussya, Gertsa and its artificial salt analogue through the probe at a dose of 1,5 mL/100 g of body mass for 6 days. The day after the completion of the drinking course in all rats some neuroendocrine and immune parameters were registered. **Results.** The method of discriminant analysis revealed 29 parameters of the neuroendocrine-immune complex (10 of them reflect the neuroendocrine regulation, 4 thymus mass and thymocytogram elements, 5 elements of splenocytogram, 10 elements of immunocytogram and leukocytogram of blood and parameters of phagocytosis), according to which the reaction on various water-salt loads are identified with an accuracy of 98.3%. **Conclusion.** The peculiarities of the reactions of the parameters of the neuroendocrine-immune complex are due to the content of water in sulfate, bicarbonate and magnesium, as well as organic carbon and nitrogen.

Key words. Water-salt loads, neuroendocrine and immune parameters, female rats.

INTRODUCTION

In the previous article we reported that screening registered parameters of water-salt, nitrous and lipid metabolism as well as the neuroendocrine-immune complex found 42 among them who in rats subjected to various water-salt loads, significantly different from that of intact rats, but on average the same group of animals that received liquids with different mineralization and chemical composition [19,20]. The purpose of next article was to find out the features of the reactions of the parameters of metabolism. The method of discriminant analysis revealed 33 variables (among them 8 refer to plasma/erythrocytes electrolytes, 7 to electrolytes of urine, to other metabolic parameters of plasma 5 and urine 9, as well as glomerular filtration, canalicular reabsorption, diurese and urine osmolarity), the totality of which the metabolic reactions to various water-salt loads are identified with an accuracy of 98,3%. The features of the reactions of the parameters of metabolism are due to the content in waters NaCl, SO_4^{2-} as well as organic carbon and nitrogen [6]. The purpose of this article is to find out the features of the reactions of the parameters of neuroendocrine regulation and immunity, which interact closely within the framework of the triune complex [9,12-14,16-18].

MATERIAL AND METHODS

Experiment was performed on 58 healthy female Wistar rats 240-290 g divided into 6 groups. Animals of the first group remained intact, using tap water from drinking ad libitum. Rats of the second (control) group for 6 days injected a daily (single tap) water through the probe at a dose of 1,5 mL/100 g of body mass. In the third group (reference for the organic component) was given daily drinking of animals with water Naftussya from the Truskavets' layer, in the fourth group (reference to the salt component) the rats were watered with the water Sophiya of the Truskavets' field. The rats of the main group received the native water from the Gertsa field, and the second control group its artificial salt analogue. The chemical composition of the applied waters (according to Truskavetsian Hydrogeological Regime-operational Station data) is given in Table 9 (see further).

The day after the completion of the drinking course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for leukocytogram analysis. Then they assessed the state of autonomous regulation. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: moda (Mo), amplitude of the moda (AMo) and variational swing (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [1].

Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine and testosterone (by the ELISA); as well as electrolytes: calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (by flamming photometry) both in plasma and in daily urine. urine.

The analyzes were carried out according to the instructions described in the manual [5]. The analyzers “Tecan” (Oesterreich), “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets and flamming spectrophotometer “СФ-47”.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyrin by coefficient $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonin by coefficient $(Cau•Pu/Cap•Pp)^{0,25}$ as well as mineralocorticoid by coefficient $(Nap/Kp)^{0,5}$, based on their classical effects and recommendations by IL Popovych [9,16].

In the blood, the parameters of immunity were determined according to the tests of the 1st and 2nd levels of the WHO, as described in the manual [10]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by M Jondal et al [7], their theophylline-resistant (T-helper) and theophyllin-susceptible (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline by S Limatibul et al [11]; the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by C Bianco [2]. Natural killers were identified as large granules contain lymphocytes.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocyte index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [3,4].

After decapitation, the spleen, thymus and adrenal glands were removed from the animals. Immune organs weighed and made smears-imprints for counting splenocytogram and thymocytogram [3]. For them, as well as leukocytogram, Shannon’s entropy was calculated [9,16]. In the adrenal glands after weighing, the thickness of glomerular, fascicular and reticular zones was measured under a microscope [3].

Digital material is statistically processed on a computer using the software package "Statistica 5.5".

RESULTS AND DISCUSION

Given a significant number of registered parameters, a discriminant analysis was immediately applied. The program forward stepwise [8] included in the model 29 variables (Table 1). Among them, 10 reflects **neuroendocrine regulation**, 4 **thymus mass and timocytogram** elements, 5 elements of **splenocytogram**, 10 reflects elements of **immunocytogram and leukocytogram** of blood and parameters of phagocytosis. Instead, other variables were out of the model.

Table 1. Summary of Stepwise Analysis
Step 29, N of vars in model: 29; Grouping: 6 grps
Wilks' Lambda: 0,0013; approx. $F_{(145)}$=2,4; $p<10^{-6}$

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Microbian Count of Neutrophils, Bacteras/Phagocyte	5,4	10^{-3}	,659	5,4	10^{-3}
Monocytes Blood, %	4,1	,004	,471	4,7	10^{-4}
Moda HRV, msec	3,0	,018	,362	4,1	10^{-5}
Lymphocytes Spleen, %	3,0	,020	,278	3,9	10^{-6}
Entropy of Leukocytogram	2,6	,038	,219	3,6	10^{-6}
Stub Neutrophils Blood, %	3,1	,017	,165	3,6	10^{-6}
B-Lymphocytes Blood, %	2,6	,037	,128	3,5	10^{-6}
Basophils Blood, %	2,3	,057	,102	3,4	10^{-6}
Adrenals Mass Index, %	2,1	,080	,082	3,3	10^{-6}
$(Cau•Pu/Pp•Cap)^{0.25}$ as Calcitonin Activity	2,3	,059	,065	3,3	10^{-6}
Triiodothyronin, nM/L	2,1	,080	,051	3,2	10^{-6}
Neutrophils Spleen, %	2,2	,071	,041	3,2	10^{-6}
0-Lymphocytes Blood, %	1,7	,150	,033	3,1	10^{-6}
Leukocytes Blood, 10^9/L	2,0	,105	,027	3,1	10^{-6}
$(Nap/Kp)^{0.5}$ as Mineralocorticoid Activity	1,9	,119	,021	3,1	10^{-6}
Corticosterone, nM/L	1,5	,229	,018	3,0	10^{-6}
Thymus Mass Index, %	1,3	,269	,015	2,9	10^{-6}
Testosterone, nM/L	1,3	,293	,013	2,8	10^{-6}
Eosinophils Blood, %	1,5	,216	,010	2,8	10^{-6}
Macrophages Spleen, %	1,1	,357	,009	2,7	10^{-6}
Entropy of Splenocytogram	1,7	,158	,007	2,7	10^{-6}
$(Cap•Pu/Pp•Cau)^{0.25}$ as Parathyrin Activity	1,2	,330	,006	2,6	10^{-6}
Killing Index of Neutrophils, %	1,7	,155	,005	2,6	10^{-6}
Lymphocytes Thymus, %	1,2	,335	,004	2,6	10^{-6}
Variative Swing HRV as Vagal Tone, msec	1,3	,299	,003	2,6	10^{-6}
Eosinophils Spleen, %	1,6	,196	,002	2,6	10^{-6}
Plasmocytes Thymus, %	1,1	,369	,002	2,5	10^{-6}
Reticular Zone of Adrenal Cortex, μM	1,0	,418	,002	2,5	10^{-6}
Lymphoblastes Thymus, %	1,3	,304	,001	2,4	10^{-6}

Let's dwell on the individual components of the neuroendocrine-immune complex. As we see in Table. 2, almost all of the registered parameters of neuroendocrine regulation were detected with regard to the specificity of the balneoreaction, namely: a vagal tone, a humoral channel of regulation, levels in the plasma of triiodothyronine, testosterone and corticosterone, calcitonin, parathyrin and mineralocorticoid activities, assessed by subordinates of their influence on the parameters of exchange of electrolytes, as well as the mass of the adrenal glands and the thickness of the reticular zone of their cortex. And only the thickness of the glomerular and fascicular zone of cortex of the adrenal glands, as well as the sympathetic tone, were beyond the discriminant model.

Table 2. Discriminant Function Analysis Summary for Neuroendocrine variables

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Par-tial Λ	F-re-mo-ve	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftu ssya (9)
Variative Swing HRV as Vagal Tone, msec	2,1	,594	3,3	,021	,061	53 1,00 0,00	41 0,77 -0,29	53 1,01 +0,01	48 0,91 -0,12	63 1,19 +0,24	31 0,59 -0,53
Moda HRV as Humoral Channel, msec	3,0	,417	6,7	10^{-3}	,038	124 1,00 0,00	109 0,88 -1,03	119 0,96 -0,34	112 0,90 -0,82	124 1,00 0,00	112 0,90 -0,82
Triiodothyronine, nM/L	2,2	,565	3,7	,013	,015	2,14 1,00 0,00	2,09 0,98 -0,09	2,63 1,23 +0,86	2,09 0,98 -0,08	2,44 1,14 +0,52	2,26 1,06 +0,22
$(Cau•Pu/Pp•Cap)^{0,25}$ as Calcitonin Activity	1,9	,659	2,5	,060	,236	3,14 1,00 0,00	3,66 1,17 +0,41	3,10 0,99 -0,03	4,03 1,28 +0,70	4,00 1,27 +0,67	4,08 1,30 +0,73
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyrin Activity	1,8	,722	1,8	,141	,450	3,30 1,00 0,00	2,90 0,88 -0,83	2,89 0,88 -0,84	3,20 0,97 -0,20	3,52 1,07 +0,45	2,94 0,89 -0,74
Testosterone, nM/L	2,1	,593	3,3	,021	,388	3,93 1,00 0,00	5,98 1,52 +1,92	5,53 1,41 +1,49	3,98 1,01 +0,05	4,76 1,21 +0,78	4,11 1,05 +0,17
Adrenals Mass Index, mg/100 g Body Mass	1,6	,799	1,2	,337	,561	25,2 1,00 0,00	28,1 1,11 +0,42	26,9 1,07 +0,25	26,6 1,06 +0,21	24,7 0,98 -0,08	29,8 1,18 +0,68
Reticular Zone of Adrenal Cortex, µM	1,7	,743	1,7	,182	,332	43 1,00 0,00	42 0,98 -0,10	42 0,98 -0,10	42 0,98 -0,09	46 1,08 +0,44	45 1,05 +0,28
Corticosterone, nM/ L	1,7	,739	1,7	,175	,366	467 1,00 0,00	373 0,80 -0,52	379 0,81 -0,49	397 0,85 -0,39	450 0,97 -0,09	619 1,33 +0,85
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	1,4	,889	0,6	,700	,466	5,57 1,00 0,00	6,07 1,09 +0,44	6,55 1,17 +0,87	6,25 1,12 +0,60	5,87 1,05 +0,27	5,86 1,05 +0,26

Variables currently not in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Par-tial Λ	F to en-ter	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftu ssya (9)
AMo HRV as Sympathetic tone, %	5,5	,973	,16	,955	,369	56 1,00 0,00	69 1,23 +0,75	58 1,04 +0,14	60 1,07 +0,23	48 0,86 -0,47	69 1,25 +0,79
Glomerulary Zone of Adrenals, µM	5,4	,959	,25	,905	,581	193 1,00 0,00	187 0,97 -0,14	170 0,88 -0,52	198 1,03 +0,11	186 0,96 -0,17	192 0,99 -0,04
Fasciculary Zone of Adrenals, µM	5,5	,977	,14	,964	,342	391 1,00 0,00	386 0,99 -0,06	470 1,20 +0,92	371 0,95 -0,22	431 1,10 +0,46	394 1,01 +0,04

Note. In the case of each variable, the first line displays the actual average for different loads (L), the second one is its ratio with the average norm (L/N) taken for 1, the third is the Z-value: Z=(L/N-1)/Cv.

Regarding the central organ of immunity, the thymus, its mass, more precisely the mass index, and the relative content in the thymocytogram of lymphoid elements: lymphocytes, lymphoblasts and plasmocytes (Table 3), was revealed as a recognizable, while the reticulo-endothelial elements of thymocytogram and its entropy appeared beyond the discriminant model.

Table 3. Discriminant Function Analysis Summary for Thymic variables

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Partial Λ	F-remove	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftussya (9)
Thymus Mass Index, mg/100 g Body Mass	2,3	,540	4,1	,008	,249	28 1,00 0,00	30 1,06 +0,14	25 0,88 -0,31	29 1,03 +0,07	28 0,98 -0,04	30 1,06 +0,14
Lymphocytes Thymus, %	1,8	,722	1,8	,141	,247	70,3 1,00 0,00	69,0 0,98 -0,54	68,7 0,98 -0,69	68,6 0,98 -0,73	69,9 0,99 -0,15	69,4 0,99 -0,36
Lymphoblastes Thymus, %	1,6	,789	1,3	,304	,408	7,40 1,00 0,00	7,22 0,98 -0,21	6,90 0,93 -0,59	7,18 0,97 -0,26	6,50 0,88 -1,07	7,56 1,02 +0,18
Plasmocytes Thymus, %	1,6	,802	1,2	,344	,395	1,80 1,00 0,00	2,11 1,17 +0,25	2,20 1,22 +0,51	2,09 1,16 +0,37	1,50 0,83 -0,38	1,89 1,05 +0,11

Variables currently not in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Partial Λ	F to enter	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftussya (9)
Reticulocytes Thymus, %	5,0	,890	,74	,572	,117	4,70 1,00 0,00	4,89 1,04 +0,11	4,90 1,04 +0,12	4,82 1,03 +0,07	4,63 0,98 -0,04	4,56 0,97 -0,08
Endotheliocytes Thymus, %	5,2	,922	,50	,733	,254	2,60 1,00 0,00	2,67 1,03 +0,07	2,10 0,81 -0,52	2,91 1,12 +0,32	2,38 0,91 -0,23	3,11 1,20 +0,53
Epitheliocytes Thymus, %	5,3	,944	,36	,836	,047	8,80 1,00 0,00	9,00 1,02 +0,10	10,0 1,14 +0,60	9,55 1,08 +0,37	9,63 1,09 +0,41	9,00 1,02 +0,10
Macrophages Thymus, %	5,5	,973	,17	,953	,407	2,70 1,00 0,00	3,22 1,19 +0,39	3,10 +1,15 +0,30	3,00 1,11 +0,22	3,50 1,30 +0,60	2,44 0,91 -0,19
Hassal's corpuscles Thymus, %	5,2	,924	,49	,743	,425	1,70 1,00 0,00	1,89 1,11 +0,38	2,15 1,26 +0,84	1,91 1,12 +0,39	1,94 1,14 +0,44	2,00 1,18 +0,56
Entropy of Thymocytogram, •10^{3}	5,6	,996	,03	,999	,068	439 1,00 0,00	456 1,04 +0,60	458 1,04 +0,68	460 1,05 +0,74	442 1,01 +0,11	450 1,03 0,40

Among the elements of the splenocytogram, the discriminant model included lymphocytes, eosinophils, neutrophils/microphages and monocytes/macrophages, as well as its entropy, while the mass of the spleen and the contents of the splenocytogram of the two lymphoid (lymphoblasts and plasmocytes) and phagocyting (fibroblasts and reticulocytes) elements outside the model (Table 4).

Among the registered immune parameters of the blood, the overall content of leukocytes and the relative content in the leukocytogram of its minor elements: basophils, eosinophils,

rodenuclear neutrophils and monocytes, as well as its entropy, were revealed. The second subgroup is composed of indicators of intensity and completeness of phagocytosis of neutrophils, and the third is the content in the immunocytogram of blood B- and 0-lymphocytes (Table 5). Outside the discriminant model, the major elements of the leukocytogram: segmental neutrophils and general lymphocytes were, as well as most elements of the immunocytogram: natural killers and T-killers and T-helper lymphocytes, as well as its entropy. The same applies to the activity of phagocytosis of neutrophils/microphages and monocytes/macrophages and the intensity of phagocytosis of the latter.

Table 4. Discriminant Function Analysis Summary for Splenic variables

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Par-tial Λ	F-re-mo-ve	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftu ssya (9)
Lymphocytes Spleen, %	2,2	,575	3,6	,015	,054	48,7 1,00 0,00	49,0 1,01 +0,11	47,7 0,98 -0,37	49,4 1,01 +0,24	48,5 1,00 -0,07	46,0 0,94 -0,99
Eosinophils Spleen, %	1,9	,677	2,3	,078	,152	1,50 1,00 0,00	1,30 0,87 -0,19	1,60 1,07 +0,09	1,27 0,85 -0,21	1,50 1,00 0,00	1,67 1,11 +0,15
Neutrophils Spleen, %	2,7	,473	5,4	,002	,215	13,0 1,00 0,00	11,8 0,91 -0,85	12,7 0,98 -0,21	13,2 1,01 +0,13	12,7 0,98 -0,18	14,1 1,09 +0,78
Macrophages Spleen, %	2,3	,553	3,9	,010	,115	7,90 1,00 0,00	8,80 1,11 +0,56	8,30 1,05 +0,25	8,55 1,08 +0,40	7,25 0,92 -0,41	9,00 1,14 +0,69
Entropy of Splenocytogram •10^3	1,8	,691	2,1	,095	,058	613 1,00 0,00	605 0,99 -0,30	615 1,00 +0,09	606 0,99 -0,29	610 1,00 -0,09	627 1,02 +0,57

Variables currently not in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Par-tial Λ	F to en-ter	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftu ssya (9)
Spleen Mass Index, mg/100 g Body Mass	5,2	,925	,49	,744	,404	312 1,00 0,00	269 0,86 -0,43	304 0,97 -0,08	289 0,93 -0,23	275 0,88 -0,37	316 1,01 +0,05
Lymphoblastes Spleen, %	5,6	,993	,04	,996	,354	3,90 1,00 0,00	4,00 1,03 +0,08	3,70 0,95 -0,17	4,45 1,14 +0,46	4,50 1,15 +0,50	3,78 0,97 +0,10
Plasmocytes Spleen, %	5,2	,922	,50	,733	,254	2,50 1,00 0,00	2,00 0,80 -0,32	1,60 0,64 -0,57	1,91 0,76 -0,37	1,75 0,70 -0,47	2,00 0,80 -0,32
Fibroblastes Spleen, %	5,5	,977	,14	,964	,469	8,20 1,00 0,00	7,90 0,96 -0,14	8,60 1,05 +0,19	7,09 0,86 -0,53	8,50 1,04 +0,14	8,00 0,98 -0,10
Reticulocytes Spleen, %	5,0	,890	,74	,572	,117	14,3 1,00 0,00	15,2 1,06 +0,48	15,8 1,10 +0,79	14,2 0,99 -0,06	15,3 1,07 +0,50	15,5 1,08 +0,61

Table 5. Discriminant Function Analysis Summary for immune variables of Blood

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Par-tial Λ	F-re-mo-ve	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftu ssya (9)
Leukocytes Blood, 10^9/L	1,7	,761	1,5	,224	,374	12,7 1,00 0,00	12,7 1,01 +0,01	10,7 0,84 -0,33	12,9 1,02 +0,03	8,70 0,69 -0,67	11,4 0,90 -0,22
Basophils Blood, %	1,9	,652	2,6	,054	,286	0,30 1,00 0,00	0,20 0,67 -0,21	0,60 2,00 +0,62	0,45 1,52 +0,32	0,13 0,42 -0,36	0,11 0,37 -0,39
Eosinophils Blood, %	1,7	,755	1,6	,211	,477	4,60 1,00 0,00	3,60 0,78 -0,33	3,30 0,72 -0,43	3,64 0,79 -0,32	3,50 0,76 -0,37	3,00 0,65 -0,54
Stub Neutrophils Blood, %	1,9	,654	2,5	,056	,097	3,60 1,00 0,00	3,00 0,83 -0,56	3,90 1,08 +0,28	3,18 0,88 -0,39	2,50 0,69 -1,02	3,00 0,83 -0,56
Monocytes Blood, %	3,3	,388	7,6	10^{-3}	,034	4,80 1,00 0,00	3,40 0,71 -0,47	7,00 1,46 +0,73	3,27 0,68 -0,51	5,38 1,12 +0,19	4,78 1,00 -0,01
Entropy of Leukocytogram •10^3	2,5	,499	4,8	,003	,035	310 1,00 0,00	292 0,94 -0,42	338 1,09 +0,63	310 1,00 -0,01	299 0,96 -0,25	307 0,99 -0,08
Microbian Count of Neutrophils, Bacteras/Phagocyte	3,1	,415	6,8	10^{-3}	,021	8,60 1,00 0,00	8,30 0,97 -0,16	6,40 0,74 -1,16	8,45 0,98 -0,08	7,25 0,84 -0,71	7,78 0,90 -0,43
Killing Index of Neutrophils, %	1,7	,731	1,8	,158	,404	50,7 1,00 0,00	50,2 0,99 -0,08	55,2 1,09 +0,70	54,8 1,08 +0,64	52,6 1,04 +0,30	53,6 1,06 +0,45
B-Lymphocytes Blood, %	2,3	,552	3,9	,010	,108	16,0 1,00 0,00	16,7 1,04 +0,24	17,9 1,12 +0,65	15,1 0,94 -0,31	14,75 0,92 -0,42	14,2 0,89 -0,60
0-Lymphocytes Blood, %	2,5	,500	4,8	,003	,085	20,9 1,00 0,00	21,0 1,00 +0,02	16,6 0,79 -0,87	24,0 1,15 +0,63	22,2 1,06 +0,26	24,6 1,18 +0,75

Variables currently not in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Par-tial Λ	F to en-ter	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Gertsa (11)	Salt Anal G (8)	Naftu ssya (9)
Phagocytose Index of Neutrophils, %	5,4	,959	,25	,905	,581	69,5 1,00 0,00	70,9 1,02 +0,32	66,9 0,96 -0,60	71,2 1,02 +0,39	69,1 0,99 -0,09	69,0 0,99 -0,12
Phagocytose Index of Monocytes, %	5,2	,925	,49	,744	,404	2,90 1,00 0,00	2,95 1,02 +0,07	2,70 0,93 -0,29	2,55 0,88 -0,51	3,50 1,21 +0,86	2,83 0,98 -0,10
Microbian Count of Monocytes, Bacteras/Phagocyte	5,5	,973	,16	,955	,369	4,98 1,00 0,00	4,02 0,81 -0,51	5,84 1,17 +0,46	3,63 0,73 -0,72	5,23 1,05 +0,13	4,38 0,88 -0,32
Segmented Neutrophils Blood, %	5,6	,993	,04	,996	,354	26,0 1,00 0,00	26,4 1,02 +0,06	26,1 1,00 +0,01	32,7 +1,26 +0,99	25,0 0,96 -0,15	27,1 1,04 +0,16
Panlymphocytes Blood, %	5,5	,977	,14	,964	,342	60,7 1,00 0,00	63,4 1,04 +0,29	59,1 0,97 -0,17	56,5 0,93 -0,44	63,5 1,05 +0,30	62,0 1,02 +0,14

Natural Killer Lymphocytes Blood, %	5,3	,944	,36	,836	,047	15,6 1,00 0,00	14,6 0,94 -0,35	17,7 1,13 +0,74	14,8 0,94 -0,31	16,7 1,07 +0,38	15,9 1,02 +0,09
T-cytolytic Lymphocytes Blood, %	5,2	,924	,49	,743	,425	16,0 1,00 0,00	16,6 1,04 +0,25	16,6 1,04 +0,25	15,7 0,98 -0,11	15,9 0,99 -0,05	14,6 0,91 -0,60
T-helper Lymphocytes Blood, %	5,5	,973	,17	,953	,407	31,5 1,00 0,00	31,1 0,99 -0,13	31,2 0,99 -0,10	30,5 0,97 -0,34	30,5 0,97 -0,32	30,8 0,98 -0,23
Entropy of Immunocytogram $\bullet 10^3$	5,6	,996	,03	,999	,068	472 1,00 0,00	468 0,99 -0,60	468 0,99 -0,64	469 0,99 -0,45	474 1,00 +0,25	467 0,99 -0,74

The dividing information contained in 29 variables is condensed in 5 canonical discriminant roots (Tables 6 and 7). The first root contains 49,8% of discriminative opportunities, the second 21,1%, the third 10,7%, the fourth 9,6%, the fifth 8.8%.

Table 6. Chi-Square Tests with Successive Roots Removed

	Eigen-value	Cano-nical R	Wilks' Λ	Chi-Square	Degree freadom	p-level
0	8,41	,945	,001	263	145	10^{-6}
1	3,56	,884	,012	175	112	,0001
2	1,81	,802	,054	115	81	,008
3	1,64	,788	,153	74	52	,024
4	1,48	,773	,403	36	25	,074

Table 7 presents standardized and raw coefficients and constants for discriminant variables.

Table 7. Standardized and Raw Coefficients and Constants for Canonical Variables

Variables currently in the model	Standardized Coefficients					Raw Coefficients				
	Root 1	Root 2	Root 3	Root 4	Root 5	Root 1	Root 2	Root 3	Root 4	Root 5
Microb Count Neut	-5,27	-,186	,414	1,990	-,826	-4,589	-,162	,360	1,731	-,719
Monocytes Blood	4,064	-,701	1,556	,663	-1,177	1,951	-,337	,747	,318	-,565
Moda HRV	3,344	-2,46	-,440	,326	-,709	,165	-,122	-,022	,016	-,035
Lymphocytes Splen	-2,43	-,625	1,806	-,259	-,580	-1,041	-,268	,775	-,111	-,249
Entropy Leukocyto	-4,02	,204	-,236	-,137	-,070	-,132	,067	-,078	-,045	-,023
Stub Neutroph Bloo	1,767	,649	,548	-,270	-,576	1,581	,581	,490	-,241	-,515
B-Lymphocyt Blood	2,068	,047	-,231	-,480	-,528	,684	,016	-,076	-,159	-,175
Basophils Blood	-,695	,510	,851	-,270	-,337	-1,413	1,036	1,730	-,548	-,685
Adrenals Mass Ind	-,049	,098	-,072	-,638	-,392	-,012	,023	-,017	-,152	-,093
$(Cau•Pu/Pp•Cap)^{0,25}$	-,708	-,961	-,198	,167	,622	-,592	-,803	-,165	,140	,520
Triiodothyronine	-5,39	-,787	-,427	1,480	,958	-14,94	-2,184	-1,185	4,105	2,658
Neutrophils Spleen	-1,28	-,930	,452	-,373	-,412	-,648	-,472	,230	-,189	-,209
0-Lymphocyt Blood	2,400	-,895	-,368	-,196	,068	,340	-,127	-,052	-,028	,010
Leukocytes Blood	-,567	-,647	-,019	,190	-,070	-,124	-,141	-,004	,041	-,015
$(Nap/Kp)^{0,5}$	-,059	-,008	,371	-,420	,250	-,091	-,012	,572	-,647	,385
Corticosterone	,155	-,720	,265	-,602	-,167	,001	-,004	,002	-,004	-,001
Thymus Mass Index	1,252	-,116	-,794	,155	-,159	,156	-,014	-,099	,0196	-,020
Testosterone	,425	,863	-,583	,370	-,017	,214	,434	-,293	,186	-,009
Eosinophils Blood	,376	,068	-,405	,658	-,118	,196	,035	-,211	,343	-,061
Macrophage Spleen	,563	-1,964	,251	-,621	-,708	,313	-1,090	,139	-,345	-,393
Entropy Splenocyto	-1,51	-1,169	1,819	-,176	-,331	-,082	-,063	,098	-,009	-,018
$(Cap•Pu/Pp•Cau)^{0,25}$	,125	-,650	,539	,318	,201	,220	-1,144	,949	,559	,354
Killing Ind Neutrop	-,246	-,326	,765	,145	,480	-,038	-,051	,120	,023	,075
Lymphocyt Thymus	,944	,292	-,582	,235	-,121	,363	,112	-,224	,090	-,046
Variat Swing HRV	-2,18	1,399	,917	-,308	,693	-,046	,030	,020	-,007	,015
Eosinophils Spleen	1,302	-,482	-,692	,418	-,095	1,540	-,570	-,818	,494	-,113
Plasmocyt Thymus	,524	-,182	-,437	-,357	-,220	,631	-,219	-,526	-,429	-,265
Reticular ZAdrCort	-,753	,552	,036	,211	,061	-,069	,051	,003	,019	,006
Lymphoblast Thym	,357	-,568	-,418	-,206	-,144	,361	-,575	-,423	-,209	-,146
					Constants	138,9	91,44	-88,05	-6,47	39,07
					Discriminant Properties, %	49,8	21,1	10,7	9,6	8,8

After calculating for each animal the magnitudes of discriminant roots as the sum of products of raw coefficients to the individual values of discriminant variables together with the constant, it becomes possible to visualize the localization of each rat in the information space of the roots.

On the plane of the first two roots, which contain 71% of the discriminant information, there is a clear separation along the axis of the major root of the cluster of rats louded by Gertsa water from other clusters (Fig. 1 and 2).

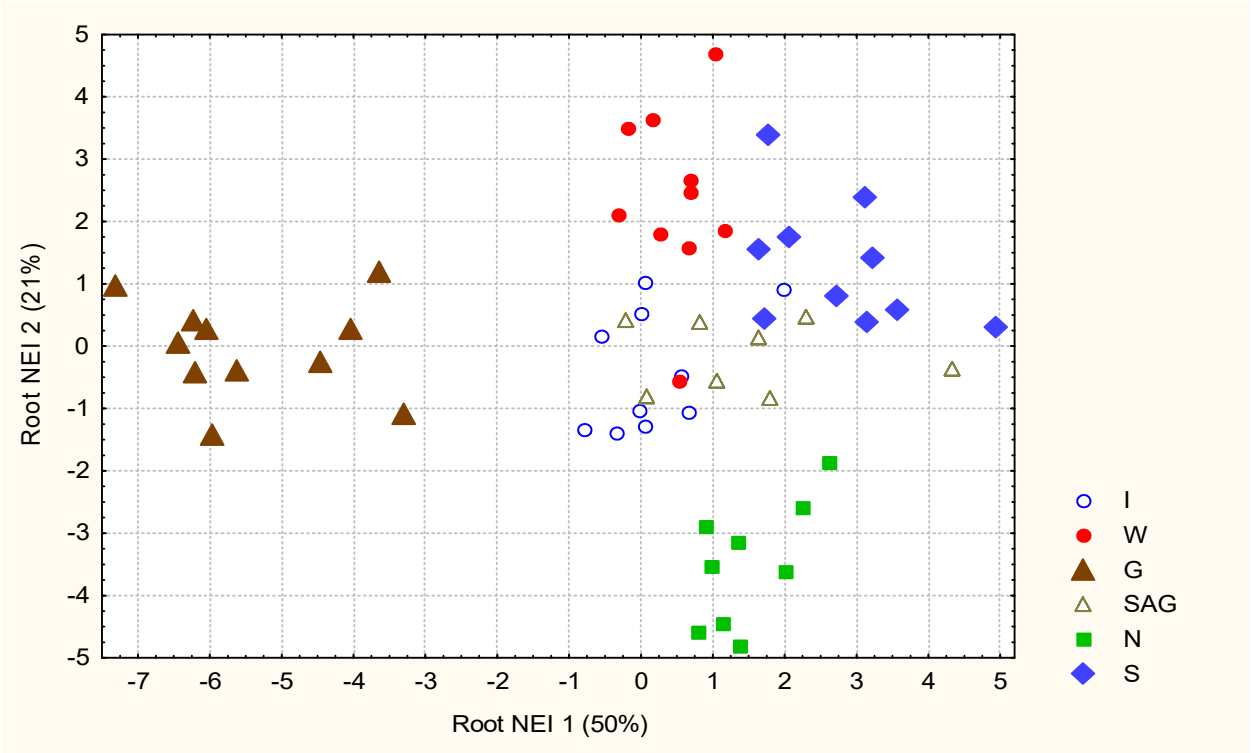


Fig. 1. Individual values of the first and second roots of the parameters of neuro-endocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Gertsa (G) and its artificial salt analogue (SAG)

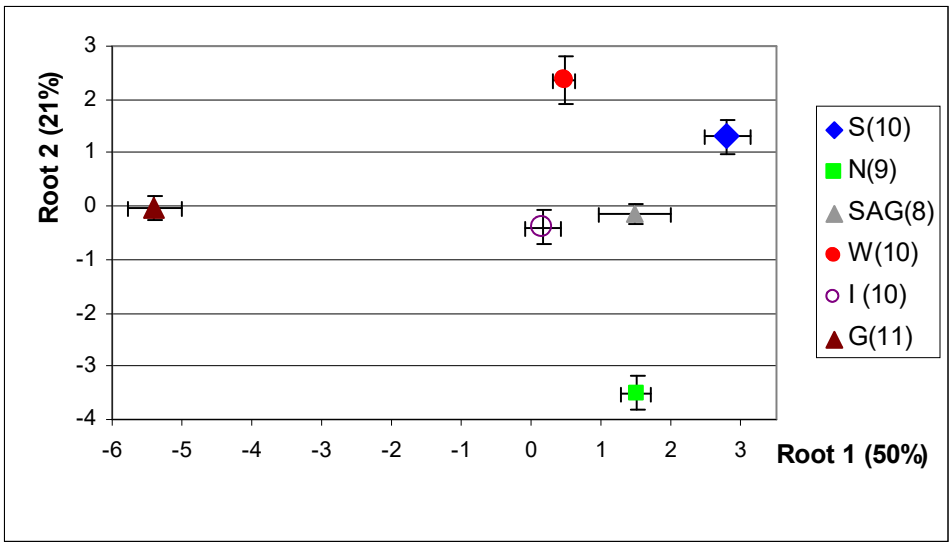


Fig. 2. Means of the first and second roots of the parameters of neuroendocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Gertsa (G) and its artificial salt analogue (SAG)

Such an extreme localization of this cluster along the axis of the first root (centroid: -5,39) reflects the minimum values of the parameters correlated with the root positively (the content of monocytes in the blood, the eosinophils in the spleen and the level of plasma in triiodothyronine), in conjunction with the maximum values of negative correlated with the

root parameters (microbial number of neutrophils, content of leukocytes and 0-lymphocytes in blood and calcitonin activity) (Table 8).

Table 8. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Variables for Groups

Variables currently in the model	R 1	R 2	R 3	G	I	W	N	SAG	S
Root 1 (49,8%)				**-5,39**	+0,16	+0,47	+1,49	+1,47	**+2,79**
Monocytes Blood, %	**,17**	-,01	,32	**3,27**	4,80	3,40	4,78	5,38	**7,00**
Triiodothyronin, nM/L	**,14**	,01	,27	**2,09**	2,14	2,09	2,26	2,44	**2,63**
Eosinophils Spleen, %	**,05**	-,06	,04	**1,27**	1,50	1,30	1,67	1,50	***1,64***
Microbian Count Neutr, Bact/Phagoc	**-,16**	-,04	-,28	***8,5***	8,6	8,3	7,8	7,3	**6,4**
0-Lymphocytes Blood, %	**-,08**	-,13	-,14	***24,0***	20,9	21,0	24,6	22,2	**16,6**
Leukocytes Blood, 10^9/L	**-,07**	,03	-,07	**12,9**	12,7	12,7	11,4	8,7	10,7
$(Cau•Pu/Pp•Cap)^{0,25}$ as Calcitonin A	**-,06**	-,09	-,12	***4,03***	3,14	3,66	4,08	4,00	**3,10**
Root 2 (21,1%)				-0,03	-0,39	**+2,38**	**-3,50**	-0,14	+1,30
Corticosterone, nM/L	,04	**-,21**	-,06	397	467	**373**	**619**	450	379
Neutrophils Spleen, %	-,02	**-,18**	,06	13,2	13,0	**11,8**	**14,1**	12,8	12,7
Entropy of Splenocytogram	,08	**-,17**	,02	0,606	0,613	**0,605**	**0,627**	0,610	0,615
Lymphoblastes Thymus, %	-,02	**-,08**	-,09	7,18	7,40	***7,22***	**7,56**	6,50	6,90
Reticular Zone Adrenals Cortex, µM	,02	**-,05**	-,02	42,0	42,8	**42,0**	***45,0***	46,3	42,0
B-Lymphocytes Blood, %	,05	**,18**	,10	15,1	16,0	**16,7**	**14,2**	14,8	17,9
Lymphocytes Spleen, %	-,10	**,18**	,01	49,4	48,7	**49,0**	**46,0**	48,5	47,7
Testosterone, nM/L	,07	**,15**	-,08	4,0	3,9	**6,0**	***4,1***	4,8	5,5
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Act	-,01	**,12**	,152	6,25	5,57	***6,07***	**5,86**	5,87	6,55
MxDMn HRV as Vagal tone, msec	,00	**,05**	,10	48	53	***41***	**31**	63	53
Eosinophils Blood, %	-,02	**,03**	,02	3,64	4,60	***3,60***	**3,00**	3,50	3,30
Root 3 (10,7%)				+0,53	+0,45	**-2,16**	-0,92	+0,11	**+1,86**
Entropy of Leukocytogram	,04	,01	**,33**	0,310	0,310	**0,295**	0,307	0,299	**0,338**
Basophils Blood, %	-,03	,08	**,22**	0,45	0,30	***0,20***	0,11	0,13	**0,60**
Stub Neutrophils Blood, %	,02	,05	**,19**	3,18	3,60	***3,00***	3,00	2,50	**3,90**
Killing Index of Neutrophils, %	-,02	-,04	**,16**	54,8	50,7	**50,2**	53,6	52,6	**55,2**
Thymus Mass Index, mg/100 g BM	-,04	-,04	**-,15**	29	28,5	**30**	30	28	**25**
Root 4 (9,6%)				-0,47	**+1,74**	-0,37	-1,08	**+1,75**	-1,27
Moda HRV, msec	,04	-,01	,14	112	**124**	108	112	**124**	119
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyrin A	-,04	-,04	,07	3,20	**3,30**	2,90	2,94	**3,52**	2,89
Lymphocytes Thymus, %	,03	-,05	-,03	68,5	**70,3**	69,0	69,4	**69,9**	68,7
Adrenals Mass Index, %	,02	-,07	-,15	0,266	**0,252**	0,281	0,298	**0,247**	0,269
Macrophages Spleen, %	-,02	-,02	-,11	8,55	**7,90**	8,80	9,00	**7,25**	8,30
Plasmocytes Thymus, %	-,02	,06	,02	2,09	**1,80**	2,11	1,89	**1,50**	2,20
Root 5 (8,8%)				+0,34	**-1,85**	-0,23	-0,06	**+2,26**	-0,06

Less distinct from other cluster of rats loaded with Sofiya water, which is localized in the right extreme zone of the first root (centroid: +2,79). Such a localization almost reciprocally reflects the maximum values of parameters that are related to the root directly, and the minimum values of inverse related parameters.

Given the specificity of the chemical composition of the waters of Gertsa and Sofiya (Table 9), it seems that the maximum effect on the listed endocrine and immune parameters of Gertsa water is carried out by organic carbon (Fig. 3) and trace elements (H_3BO_3, Br^-, J^-), the content of which is maximum among the liquids used.

Table 9. The chemical composition of the applied mineral waters

	Daily Water	Sofiya	Gertsa	Salt analog	Naftussya
Electrolytes, mM/L					
Na^+	0,5	156	196,7	196,7	0,6
Cl^-	3,4	142	205	205	1,0
HCO_3^-	**2,9**	7,5	5,6	5,6	**8,2**
Ca^{2+}	3,4	5,3	3,40	3,40	2,9
Mg^{2+}	**0,5**	**4,3**	3,44	3,44	2,3
K^+	0,4	0,3	0,4	0,4	0,3
SO_4^{2-}	1,2	**13,1**	0,1	0,1	1,0
Trace elementes, mg/L					
H_2SiO_3	5	4,43	9,88	0	9,5
H_3BO_3	0,25	8,39	**42,76**	0	0,200
Br^-	8,3	6,7	**21,17**	0	0,034
J^-	0,025	1,29	**6,62**	0	0,004
F^-	0,95	0,52	0,57	0	0,160
Organic substances, mg/L					
C org	5,0	5,5	**34**	0	12,8
N org	0,02	**0,80**	0,14	0	0,33

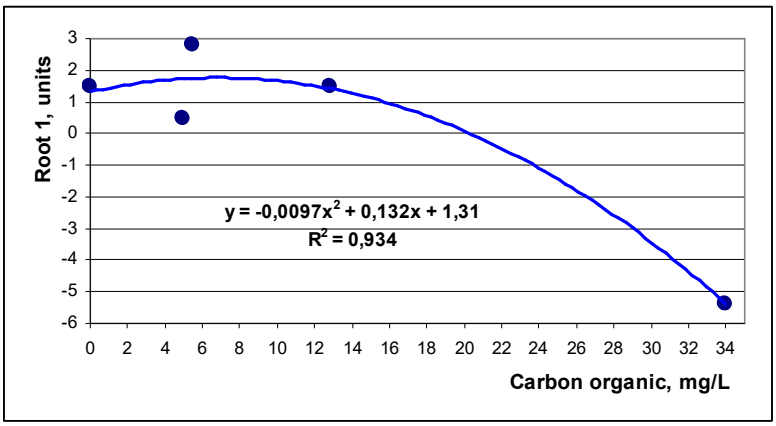


Fig. 3. Relationship between the concentration of organic carbon in water and its influence on the parameters condensed in the first canonical discriminant root

Water Sofiya realizes its opposite maximum effect on these parameters by sulfate (Fig. 4) and organic nitrogen, the content of which is also maximal.

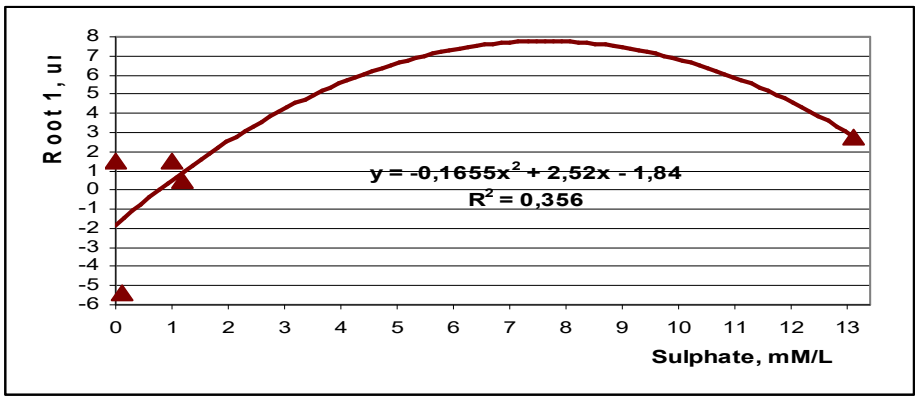


Fig. 4. Relationship between concentration in water of sulfate and its influence on parameters condensed in the first canonical discriminant root

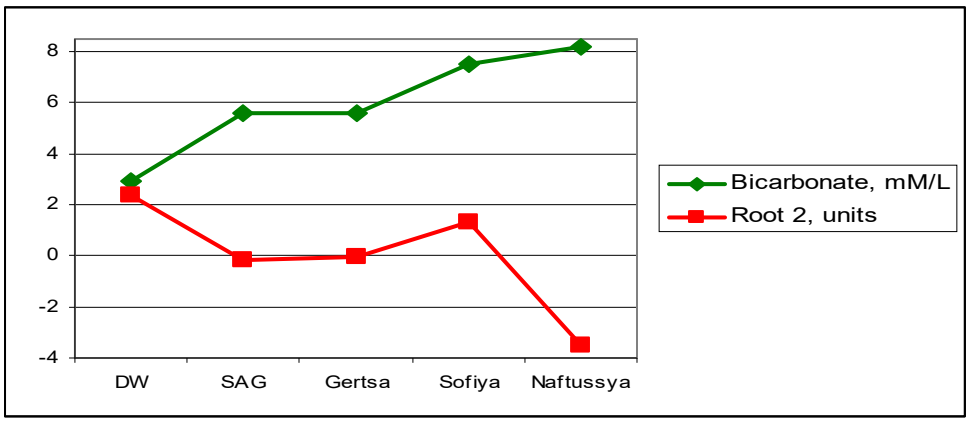
Other clusters occupy intermediate positions along the axis of the first root, without a clear distinction.

In contrast, along the axis of the second root, a cluster of rats loaded with Naftussya water (centroid: -3,50) is clearly distinguished (Figs. 1 and 2). Such a localization of the cluster reflects the minimum values for parameters correlating with the second root positively (the level of corticosterone, the thickness of the reticular cortex of the adrenal glands, the content of the lymphoblasts in the thymus and neutrophils in the spleen, and the entropy of the splenocytogram) in conjunction with the maximum values of the negative correlates with the root parameters (vagal tonus, mineralocorticoid activity, plasma testosterone level, B-lymphocytes content in the blood and total lymphocytes in the spleen) (Table 8).

Rats loaded with daily water (centroid: +2,38) occupy the polar position along the axis of the second root. This reflects the diametrically opposite levels of the listed neuroendocrine-immune parameters. Other clusters occupy intermediate positions, with the centroids of Gertsa water and its salt analogue practically equal (-0,03 and -0,14, respectively).

When comparing the parameters of the chemical composition, it was found that cluster ranking by average values of the second root coincides well with the ranking of liquids by content of bicarbonate in them (Fig. 5).

In particular, it is the maximum in the water of Naftussya (8,2 mM/L) and the minimum in the daily water (2,9 mM/L), and in other fluids intermediate (7,5 mM/L in water Sophiya and 5,6 mM/L in Gertsa water and its salt analogue). That is, there are grounds for the assumption that the influence of the applied fluids on the neuroendocrine-immune parameters associated with the second root through bicarbonate is realized.



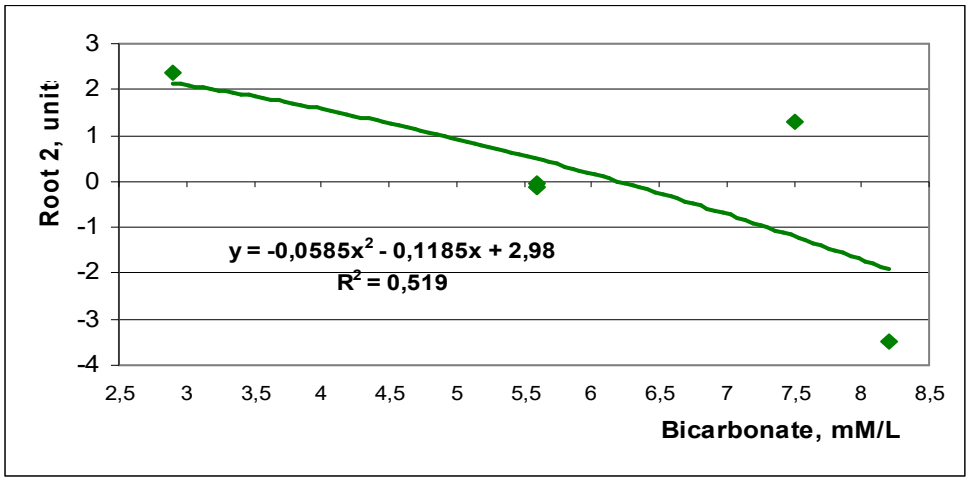


Fig. 5. Relationship between the concentration in water of bicarbonate and its influence on the parameters condensed in the second canonical discriminant root

Along the axis of the third root (Figures 6 and 7), the cluster of rats loaded with water Sofiya (centroid: +1,80) and daily water from the tap (centroid: -2,16) occupy the polar positions.

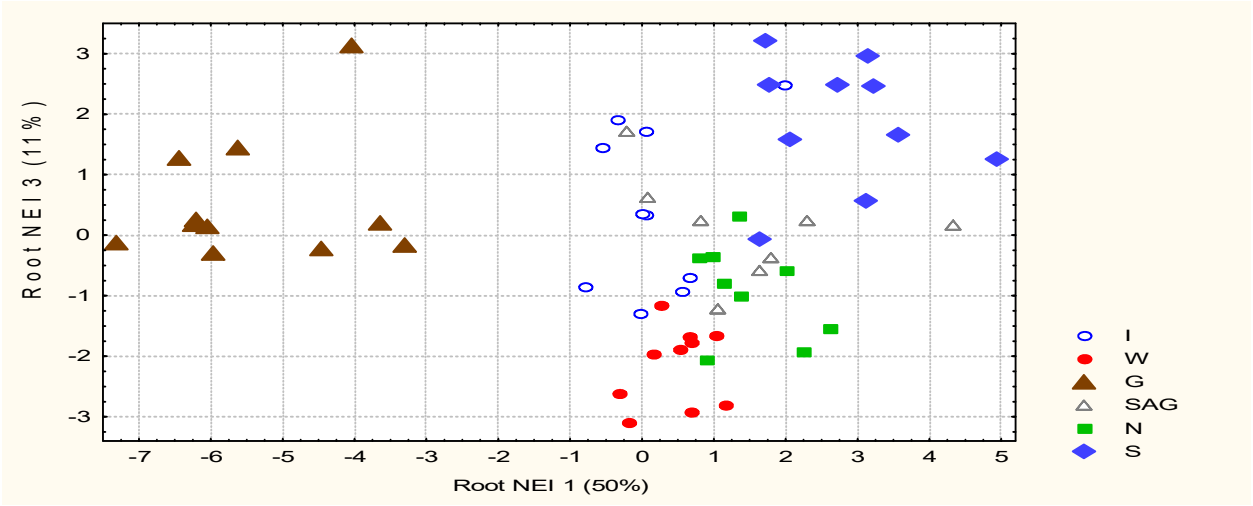


Fig. 6. Individual values of the first and third roots of the parameters of neuro-endocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Gertsa (G) and its artificial salt analogue (SAG)

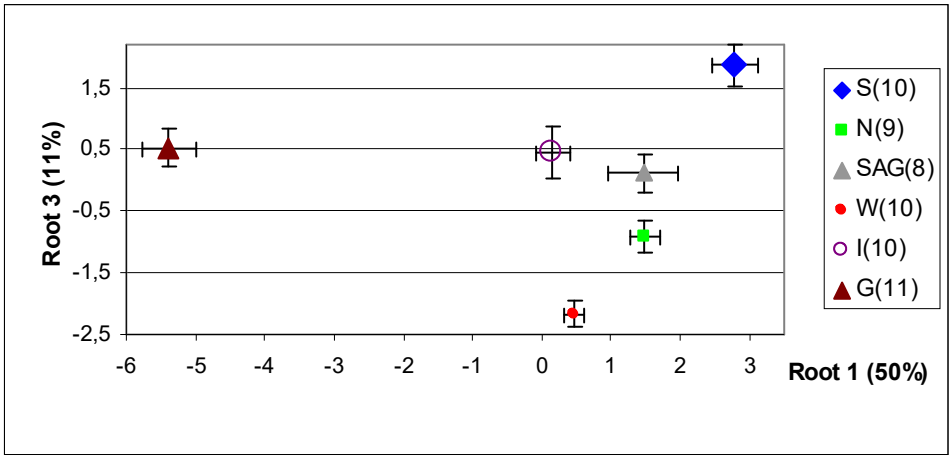
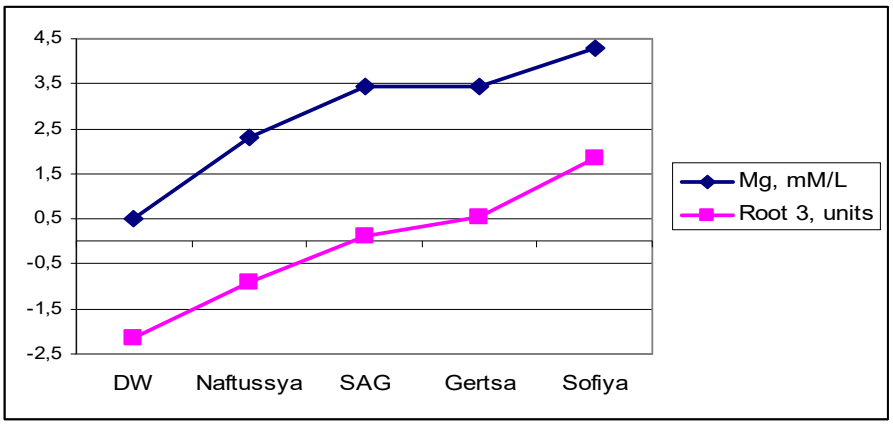


Fig. 7. Means of the first and third roots of the parameters of neuroendocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Gertsa (G) and its artificial salt analogue (SAG)

This reflects the maximum values of the entropy of the leukocytogram, the contents of the basophils and the rodenuclear neutrophils in it, and the completeness of phagocytosis of neutrophils/microphages, in combination with the minimum thymus mass in the rats of the first cluster, while the opposite values of these immune parameters for the latter cluster. Other clusters occupy intermediate positions. And in this case, centroids of Gertsa water and its salt analogue are almost equal (+0,53 and +0,11 respectively).

When comparing the magnitudes of centroids with the chemical composition parameters, their almost linear relationship with the concentration of magnesium (Fig. 8) was found, suggesting a hypothesis about its main role in the effects of applied water loads on the listed immune parameters.



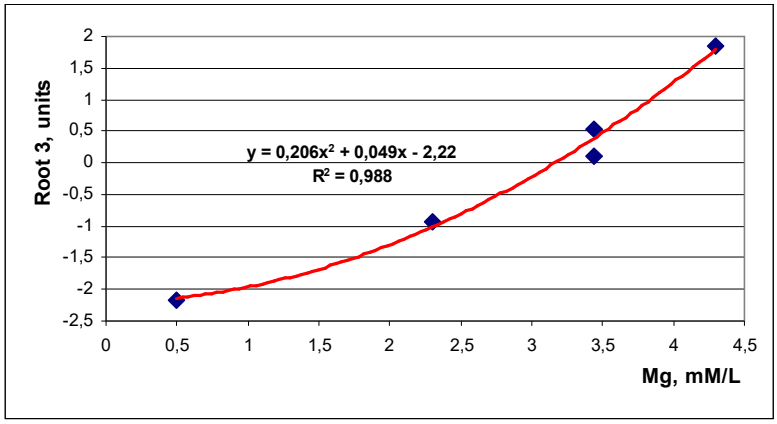


Fig. 8. Relationship between the concentration of magnesium in water and its effect on immune parameters condensed in the third canonical discriminant root

The top positions along the axis of the fourth root are placed in clusters of intact rats and loaded with the salt analogue of Gertsa water, while other clusters are located in the lower zone of the axis (Fig. 9).

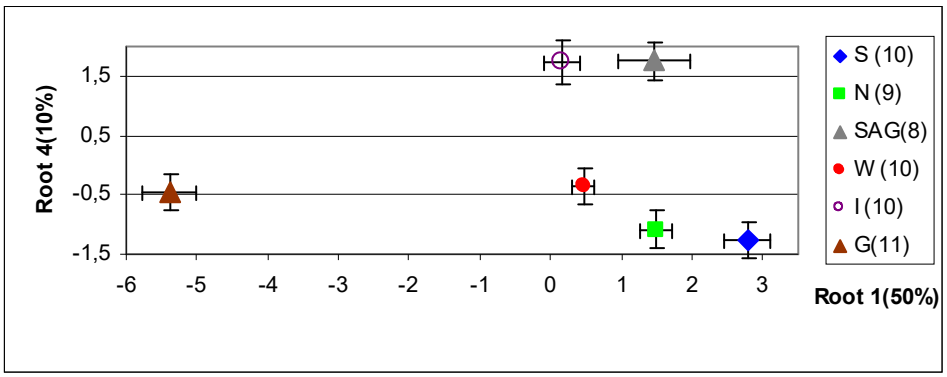


Fig. 9. Means of the first and fourth roots of the parameters of neuroendocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Gertsa (G) and its artificial salt analogue (SAG)

Such a localization reflects a decrease in some parameters (the mode of HRV, parathyrin activity, the content of lymphocytes in the thymus) and the increase of other (adrenal mass, the content of macrophages in the spleen and plasmacytes in the thymus) in response to the loading of different fluids, whereas the salt analogue of Hertsa water reaction of these parameters missing.

In general, in the information space of discriminatory roots, all six clusters are clearly delimited with one another, that is, they differ from each other by the constellation of 29 parameters of the neuroendocrine-immune complex, which is documented by calculating the squared Mahalanobis distances between them (Table 10).

Table 10. Squared Mahalanobis Distances, F-values (df=29) and p-levels between groups

Groups	I	SAG	W	N	S	G
Intact rats (I)	0 F p	21 1,31 ,249	24 1,73 ,088	27 1,84 ,066	27 1,92 ,054	45 3,42 ,002
Salt Analogue of Gertsa (SAG)	21 1,31 ,249	0 F p	26 1,62 ,114	29 1,70 ,093	24 1,50 ,159	62 4,09 10^{-3}
Daily Water (W)	24 1,73 ,088	26 1,62 ,114	0 F p	42 2,83 ,006	26 1,88 ,059	53 4,01 10^{-3}
Water Naftussya (N)	27 1,84 ,066	29 1,70 ,093	42 2,83 ,006	0 F p	36 2,45 ,014	69 4,89 10^{-4}
Water Sofiya (S)	27 1,92 ,054	24 1,50 ,159	26 1,88 ,059	36	0 F p	79 5,99 10^{-4}
Water Gertsa (G)	45 3,42 ,002	62 4,09 10^{-3}	53 4,01 10^{-3}	69 4,89 10^{-4}	79 5,99 10^{-4}	0 F p

The application of the classifying functions (Table 11) enables the retrospective identification of the five clusters unmistakably, and the intact cluster with a single error (Table 12).

Table 11. Coefficients and Constants for Classification Functions

Variables currently in the model	I p=,172	W p=,172	G p=,190	SAG p=,138	N p=,155	S p=,172
Microbian Count, Bac/Phag	887	880	907	878	875	8689
Monocytes Blood, %	-144	-148	-157	-144	-143	-140
Moda HRV as Humoral channel, msec	-9,467	-9,786	-10,541	-9,415	-8,947	-9,380
Lymphocytes Spleen, %	427	423	432	424	425	424,5
Entropy of Leukocytogram	14,302	14,306	15,042	14,123	14,125	13,964
Stub Neutrophils Blood, %	-227,3	-226,8	-236,4	-227,3	-227,9	-221,6
B-Lymphocytes Blood, %	-50,51	-50,00	-54,34	-50,30	-49,41	-48,62
Basophils Blood, %	225,2	223,2	233,3	220,2	218,1	226,1
Adrenals Mass Index, mg/100 g Body Mass	2,687	2,963	2,890	2,297	2,883	2,961
$(Cau•Pu/Pp•Cap)^{0,25}$ as Calcitonine Activ	103,8	102,4	107,6	105,1	106,3	101,2
Triiodothyronin, nM/L	2879	2867	2958	2870	2861	2827
Neutrophils Spleen, %	185,7	183,7	189,1	183,8	186,2	183,7
0-Lymphocytes Blood, %	-25,35	-25,39	-27,21	-24,88	-24,34	-24,65
Leukocytes Blood, 10^9/L	11,95	11,42	12,46	11,69	12,09	11,23
$(Nap/Kp)^{0.5}$ as Mineralocorticoid Activity	139,3	139,7	142,1	140,5	140,9	142,5
Corticosterone, nM/L	,294	,284	,293	,289	,315	,300
Thymus Mass Index, mg/100 g Body Mass	-,0258	-,0256	-,0268	-,0257	-,0255	-,0257
Testosterone, nM/L	-80,28	-78,66	-81,76	-79,83	-81,48	-79,97
Eosinophils Blood, %	-45,813	-45,928	-47,798	-45,722	-46,448	-46,676
Macrophages Spleen, %	175,7	172,5	173,5	174,2	179,6	175,2
Entropy of Splenocytogram $•10^3$	51,610	51,144	52,030	51,379	51,558	51,423
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyrine Activ	114,2	108,0	112,2	115,3	115,8	113,1
Killing Index of Neutrophils, %	24,28	23,89	24,60	24,48	24,29	24,32
Lymphocytes Thymus, %	-53,70	-52,97	-56,01	-53,32	-53,61	-53,24
MxDMn HRV as Vagal tone, msec	2,975	3,030	3,292	2,975	2,839	2,977
Eosinophils Spleen, %	-386,4	-386,6	-396,6	-384,7	-383,1	-386,2
Plasmocytes Thymus, %	-146,4	-145,0	-149,7	-146,6	-143,5	-145,1
Reticular Zone of Adrenal Cortex, µM	6,129	6,207	6,500	6,072	5,830	5,989
Lymphoblastes Thymus, %	-61,32	-61,49	-63,42	-61,44	-58,14	-61,57
Constants	-33823	-33223	-34480	-33430	-33719	-33342

Table 12. Classification Matrix. Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,172	W p=,172	G p=,190	SAG p=,138	N p=,155	S p=,172
Intact	90,0	**9**	0	0	0	0	**1**
Daily Water	100	0	**10**	0	0	0	0
Gertsa Water	100	0	0	**11**	0	0	0
Salt Analogue Gertsa	100	0	0	0	**8**	0	0
Naftussya Water	100	0	0	0	0	**9**	0
Sofiya Water	100	0	0	0	0	0	**10**
Total	98,3	9	10	11	8	9	11

CONCLUSION

The method of discriminant analysis revealed 29 parameters of the neuroendocrine-immune complex (10 of them reflect the neuroendocrine regulation, 4 thymus mass and thymocytogram elements, 5 elements of splenocytogram, 10 reflect elements of

immunocytogram and leukocytogram of blood and parameters of phagocytosis), according to which the reaction on various water-salt loads are identified with an accuracy of 98,3%. The peculiarities of the reactions of the parameters of the neuroendocrine-immune complex are due to the content of water in sulfate, bicarbonate and magnesium, as well as organic carbon and nitrogen.

In the next article, an analysis of canonical correlation between the described effects of water-salt loads and their chemical composition will be carried out.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The carrying out of experiments was approved by the Ethics Committee of the Horbachevskyi Ternopil' State Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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