Zavidnyuk Yuriy V, Mysula Igor R, Klishch Ivan M, Zukow Walery, Popovych Igor L, Korda Mykhaylo M. General non-specific metabolic, neuroendocrine and immune reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018;8(3):513-524. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1237109
http://ojs.ukw.edu.pl/index.php/johs/article/view/5454
https://pbn.nauka.gov.pl/sedno-webapp/works/864250

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eissn 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 05.03.2018. Revised: 10.03.2018. Accepted: 27.03.2018.

GENERAL NON-SPECIFIC METABOLIC, NEUROENDOCRINE AND IMMUNE REACTIONS TO VARIOUS WATER-SALT LOADS IN FEMALE RATS

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Abstract

Background. This article begins with a series of articles on the effects on parameters of water-salt, nitrous and lipid metabolism, as well as the neuroendocrine-immune complex of mineral water, extracted from the bore located in the city Gertsa (Bukovyna, Ukraine). The chemical analysis prompted us to use waters Sophiya and Naftussya from spa Truskavets' as a reference as well as an artificial salt analogue of Gertsa water, which contains no organic matter or trace elements. **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 the parameters of metabolism and neuroendocrine-immune complex were registered. **Results.** Screening registered parameters found 42 among them who in rats subjected to 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. **Conclusion.** Takes place nonspecific (general) reaction neuroendocrine-immune complex and metabolism in water-salt load as such, regardless of the specific chemical composition of fluids applied.

Keywords: Water-salt loads, water-salt, nitrous and lipid metabolism, neuroendocrine-immune complex, female rats.

INTRODUCTION

The long-term studies of the Truskavetsian scientific school of balneology have proven that drinking mineral waters have a modulating effect on the functions of blood circulation,

digestion and urination systems, as well as on the chronic inflammatory process in them and metabolism through the mediation of the nervous, endocrine and immune systems [10,20], which function as a triple complex [7,9,17,18,21-24]. The priority is that the operating principles of mineral waters are not only salts and trace elements, but also organic matter and autochthonous microflora [3,10,19,20]. On the basis of these principles an algorithm for research of newly opened drinking mineral waters was created.

This article begins with a series of articles on the effects on parameters of water-salt, nitrous and lipid metabolism, as well as the neuroendocrine-immune complex of mineral water, extracted from the bore located in the city Gertsa (Bucovyna, Ukraine). The chemical analysis showed that this water for mineralization and the content of the main electrolytes is very close to the water Sophiya spa Truskavets'. However, it contains organic matter, as in Naftussya water of the same spa. This prompted us to use Sophiya and Naftussya waters as a reference as well as an artificial salt analogue of Gertsa water, which contains no organic matter or trace elements.

Since, firstly, the procedure of water-salt loading (removal from the cage, fixation in the hand of the experimenter, insertion into the esophagus of the metal probe) is for the rats to be averted, that is, it causes stress, and secondly, an additional introduction into the body of the fluid as such, regardless of its chemical composition, it also causes changes, at least, of water and salt metabolism, in the first stage we have analyzed changes in the registered parameters common to all applied mineral waters.

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 administered a 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 1.

Table 1. 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					
SiO_2	5	4,43	9,88	0	9,5
B	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	0,8	0,14	0	0,33

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), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (both in plasma and in erythrocytes) by flamming photometry; nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method), medium molecular polypeptides (by spectrophotometric method), bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method); lipid peroxidation products: diene conjugates (spectrophotometry of the heptane phase of the lipids extract) and malonic dyaldehide (in the test with thiobarbituric acid), antioxidant enzymes: superoxide dismutase erythrocytes (according to the degree of inhibition of reduction of nitroblue tetrazolium in the presence of N-methylphenazonium metasulphate and NADH) and catalase plasma (at the rate of decomposition of hydrogen peroxide), as well as amylase (Karavay's amyloclastic method with starch substrate) and glucose (glucose-oxidase method).

Most of the listed parameters of metabolism were also determined in daily urine. By the size of the diuresis and the level of creatinine in plasma and urine, glomerular filtration and tubular reabsorption were calculated. In addition, the osmolarity of the urine was measured by the cryostatic method.

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 a fiery spectrophotometer "СФ-47".

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyrine by coefficients $(Cap/Pp)^{0,5}$ and $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonine by coefficients $(1/Cap•Pp)^{0,25}$ and $(Cau•Pu/Cap•Pp)^{0,25}$ as well as mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$ and $(Nap•Ku/Kp•Nau)^{0,25}$, based on their classical effects and recommendations by IL Popovych [10,20].

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 [11]: 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 [6], 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 [12]; 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 [10,20,25]. 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

In order to make a comparative assessment of changes, we used the recommendations of IL Popovych [10,20] express the values of the indices in Z-units calculated by the formula:

Z=(L/I – 1)/Cv, where

L - the individual value of the variable of the loaded rat;

I - the average value of the variable in the intact group;

Cv - coefficient of variation of the variable in the intact group

This approach allows us to estimate the values expressed in different units (μL/min, μM/L, %, nM/h•mL, msec, etc.), not only on the same scale, but also taking into account their variability, since the "physiological price" 1 % deviation from the norm of a stable variable is, to a certain extent, higher than that which normally fluctuates widely (for example, fluctuations in the concentration of electrolytes in the blood and urine).

Screening of registered parameters revealed significant deviations from intact control of a number of metabolic parameters of **blood** and daily **urine**, as well as **immune** and **neuroendocrine** parameters. At the same time, 26 variables increase (Fig. 1), while the other 16 decrease (Fig. 2) with respect to intact control.

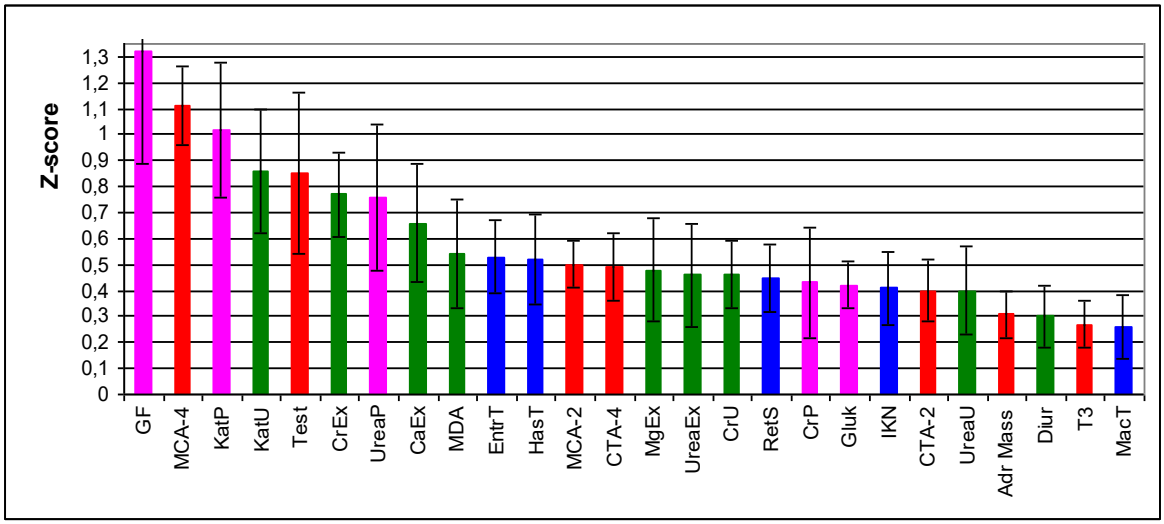

Fig. 1. Ranking of significant increasing changes (Z±SE) of the parameters of the **neuroendocrine-immune** complex and metabolic parameters of **blood** and **urine** caused by the water-salt load

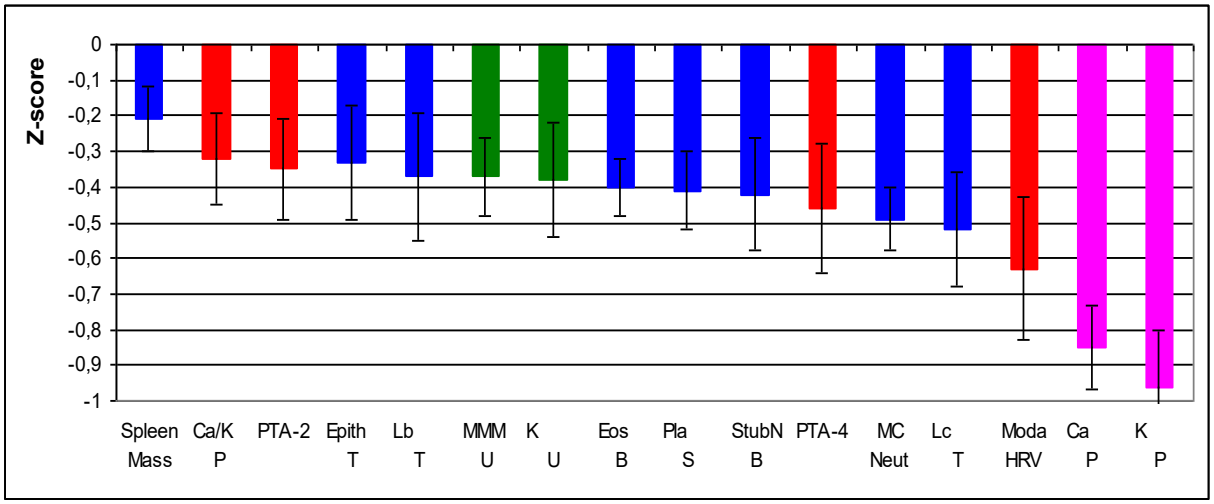

Fig. 2. Ranking of significant decreasing changes (Z±SE) of the parameters of the **neuroendocrine-immune** complex and metabolic parameters of **blood** and **urine** caused by the water-salt load

As we can see, is most heavily grown glomerular filtration (GF) and mineralocorticoid activity, which is evaluated by the exchange of sodium and potassium [MCA_4=(NaP•KU/KP•NaU)^{0,25}; MCA_2=(NaP/KP)^{0,5}], the activity of catalase plasma (KatP) and urine (KatU), as well as the plasma testosterone (Test), urea (UreaP) and malonic dialdehyde (MDA) levels. Further, in the ranking, follow: urine excretion of calcium (CaEx) and associated with it and calciumemia calcitonin activity [CTA_4=(CaU•PU)/(CaP•PP)^{0,25}; CTA_2=(CaP•PP)$^{-0,5}$], as well as excretion of creatinine (CrEx), magnesium (MgEx) and urea (UreaEx), concentration of creatinine in urine (CrU) and plasma (CrP), urea concentration in urine (UreaU) and plasma glucose (Gluk).

Among the immune parameters, the content in the thymocytogram of endotheliocytes (EndT) and the Hassalle body (HasT) increases, while in the splenocytogram - reticulocytes (RetS), as well as the index of killing by neutrophils Staph. aureus (IKN). In addition, increased diuresis (Diu), adrenals mass (AdrMass) and triiodothyronine (T_3) levels were found.

Instead, decreases the weight of the spleen, the relative content in the thymocytogram of the epitheliocytes (EpithT), lymphoblasts (LbT) and lymphocytes (LcT), in the blood of eosinophils (EosB) and of the rodenuclear (stub) neutrophils (StubN B), in splenocytogram - plasmocytes (PlaS), as well as microbial number of neutrophils of blood (MC Neut). In urine, the concentration of medium mass molecules (MMM U) and potassium (KU) decreases. The maximum level of potassium (KP) and calcium (CaP) in plasma is reduced.

The listed changes in metabolism of electrolytes reflect the increase of mineralocorticoid and calcitonin activity in conjunction with the decrease of paratyrin [PTA_2=(CaP/PP)^{0,5}; PTA_4=(CaP•PU)/(CaU•PP)^{0,25}] activity. In this case, the Cap/Kp ratio, which is considered as a sympathetic-vagal balance marker, decreases, however, Moda decreases, that is, an increase in the heart rate.

Described deviations from the norm of endocrine, immune and metabolic parameters, we consider as a reaction to averted stress, as it was detected earlier under conditions of immobilization stress [13-16,26].

In order to identify exactly those variables whose constellation is characteristic for all rats subjected to water-salt loading, regardless of its quality, the available informational field was subjected to discriminant analysis by the method of forward stepwise [8]. To include in the

model (Table 2), the program has selected only 21 variables, while the other 21 were outside the discriminant model (Table 3).

Table 2. Summary of discriminant analysis of parameters of the neuroendocrine-immune complex and metabolism. Variables currently in the model
Step 21, N of variables in model: 21; Grouping: 2 groups
Wilks' Lambda: 0,217; approx. $F_{(21,4)}$=6,2; $p<10^{-6}$

Variables currently in the model	Parameters of Wilks' Statistics					Rats		Z-score
	Wilks Λ	Partial Λ	F-remove	p-level	Tolerancy	Intact Group (10)	Loaded (48)	
Calcium Plasma, mM/L	,332	,653	19,1	10^{-4}	,165	3,35 0,32	2,48 0,12	-0,85 0,12
Microbian Count of Neutrophils, Bacteras/Phagocyte	,225	,962	1,43	,239	,299	8,6 0,6	7,7 0,2	-0,49 0,09
$(CaP/PP)^{0,5}$ as Paratyrine Activity-2	,251	,864	5,65	,023	,214	2,02 0,19	1,80 0,09	-0,35 0,14
Creatinine Excretion, µM/24h•100 g Body Mass	,222	,977	,83	,367	,154	8,7 1,4	12,1 0,7	+0,77 0,16
Malonic Dyaldehid Plasma, µM/L	,240	,904	3,84	,058	,675	63 7	75 4	+0,54 0,21
Potassium Plasma, mM/L	,239	,908	3,65	,064	,060	4,23 0,22	3,55 0,11	-0,96 0,16
Reticulocytes of Spleen, %	,236	,916	3,30	,077	,577	14,3 0,6	15,2 0,2	+0,45 0,13
Adrenals Mass, mg/100 g Body Mass	,272	,795	9,27	,004	,666	25,2 1,6	27,3 0,6	+0,31 0,09
Stub Neutrophils of Blood, %	,218	,993	,27	,608	,700	3,60 0,34	3,15 0,17	-0,42 0,16
Potassium Urine, mM/L	,244	,886	4,64	,038	,545	131 12	116 6	-0,38 0,16
Epitheliocytes of Thymus, %	,269	,806	8,64	,006	,336	8,80 0,63	9,45 0,31	+0,33 0,16
Katalase Plasma, nM/h•mL	,268	,809	8,48	,006	,364	103 9	132 7	+1,02 0,26
Glomerulary Filtration, µL/min•100 g Body Mass	,251	,863	5,69	,022	,135	86 10	127 13	+1,32 0,43
Creatinine Plasma, µM/L	,251	,863	5,72	,022	,206	73 8	83 5	+0,43 0,21
Spleen Mass Index, mg/100 g Body Mass	,262	,828	7,48	,010	,434	312 32	291 9	-0,21 0,09
Moda HRV, msec	,241	,900	3,99	,053	,625	124 5	115 3	-0,63 0,20
$(CaU•PU)/(CaP•PP)^{0,25}$ as Calcitonine Activity-4	,236	,919	3,18	,083	,151	3,14 0,41	3,76 0,17	+0,49 0,13
$(NaP•KU/KP•NaU)^{0,25}$ as Mineralocorticoid Activity-4	,242	,894	4,28	,046	,440	2,73 0,25	3,04 0,14	+1,11 0,05
Middle Mass Molecules Urine, units	,232	,931	2,65	,112	,416	182 17	163 6	-0,37 0,11
Macrophages of Thymus, %	,227	,953	1,77	,192	,584	2,70 0,42	3,04 0,16	+0,26 0,12
$(NaP/KP)^{0,5}$ as Mineralocorticoid Activity-2	,224	,969	1,16	,289	,061	5,57 0,17	6,14 0,10	+0,50 0,09

Note. The lower lines of each graph are standard errors (SE).

Table 3. Summary of discriminant analysis of parameters of the neuroendocrine-immune complex and metabolism. Variables currently not in the model

Variables currently not in the model (Df for all F-tests:1,35)	Parameters of Wilks' Statistics					Rats		Z-score
	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy	Intact Group (10)	Loaded (48)	
Testosterone, nM/L	,216	,999	,03	,865	,597	3,93 0,34	4,84 0,33	+0,85 0,31
Killing Index of Neutrophils, %	,217	1,00	,00	,969	,757	50,7 2,0	53,3 0,9	+0,41 0,14
Hassal corpuscles of Thymus, %	,213	,983	,60	,445	,260	1,70 0,17	1,98 0,06	+0,52 0,17
Entropy of Thymocytogram	,216	,997	,10	,748	,395	0,439 0,009	0,454 0,004	+0,53 0,14
Magnesium Excretion, μM/24h•100 g Body Mass	,211	,973	,96	,334	,251	3,30 0,66	4,30 0,43	+0,48 0,20
Urea Urine, mM/L	,216	,999	,03	,870	,402	107 13	124 7	+0,40 0,17
Katalase Urine, nM/h•mL	,215	,993	,23	,631	,201	123 9	146 7	+0,86 0,24
Creatinine Urine, mM/L	,215	,994	,22	,641	,280	6,41 0,58	7,26 0,24	+0,46 0,13
$1/(CaP•PP)^{0,5}$ as Calcitonine Activity-2	,216	,996	,13	,719	,079	0,65 0,09	0,76 0,03	+0,40 0,12
$(Ca/K)^{0,5}$ as Sympatho-Vagal Balance marker	,215	,993	,24	,625	,022	0,89 0,06	0,84 0,02	-0,32 0,13
Triiodothyronine, nM/L	,217	1,00	,00	,975	,032	2,14 0,18	2,29 0,05	+0,27 0,09
Diurese, mL/24h•100 g Body Mass	,216	,996	,16	,694	,070	1,44 0,28	1,70 0,11	+0,30 0,12
Lymphoblastes of Thymus, %	,217	1,00	,00	,949	,534	7,40 0,27	7,09 0,15	-0,37 0,18
Plasmocytes of Spleen, %	,216	1,00	,01	,919	,245	2,50 0,50	1,85 0,18	-0,41 0,11
Lymphocytes of Thymus, %	,214	,988	,42	,522	,275	70,3 0,8	69,1 0,4	-0,52 0,16
Eosinophiles of Blood, %	,212	,978	,80	,378	,717	4,60 0,95	3,42 0,22	-0,40 0,08
Calcium Excretion, μM/24h•100 g Body Mass	,212	,978	,79	,380	,090	2,90 0,48	3,90 0,36	+0,66 0,23
Urea Excretion, μM/24h•100 g Body Mass	,215	,995	,18	,670	,146	169 43	231 27	+0,46 0,20
Glukose Plasma, mM/L	,215	,995	,18	,677	,509	4,95 0,35	5,41 0,10	+0,42 0,09
Urea Plasma, mM/L	,216	,998	,08	,777	,130	7,42 0,54	8,71 0,48	+0,76 0,28
$(CaP•PU)/(CaU•PP)^{0,25}$ as Parathyrine Activity-4	,216	,998	,08	,773	,098	3,30 0,15	3,08 0,09	-0,46 0,18

Next, the 21-dimensional space of **discriminant variables** transforms into one-dimensional space of a **canonical discriminant function** (canonical root), which is a linear combination of discriminant variables. The discriminating (differentiating) ability of the root characterizes the canonical correlation coefficient (r*) as a measure of connection, the degree of dependence between groups (intact and subjected to water-salt load rats) and a discriminant function. It is 0,885 (Wilks' Λ=0,217; $\chi^2_{(21)}$=70; $p<10^{-6}$).

Table 4 presents raw (actual) and standardized (normalized) coefficients for discriminant variables. The raw coefficient gives information on the **absolute** contribution of

this variable to the value of the discriminative function, whereas standardized coefficients represent the **relative** contribution of a variable independent of the unit of measurement. They make it possible to identify those variables that make the largest contribution to the discriminatory function value.

The same is the **full structural coefficients**, that is, the coefficients of correlation between the discriminant root and variables. The structural coefficient shows how closely variable and discriminant functions are related, that is, what is the fate of information about the discriminant function (root) contained in this variable.

As you can see, the root directly reflects the information on metabolic parameters: the plasma level of calcium and potassium, urine level of potassium and medium molecules; regulatory: the moda of HRV as a marker of the humoral channel of its regulation, and paratyrin activity, measured by plasma concentration and urine excretion of calcium and phosphates; as well as immune: the microbial number of neutrophils/microphages of blood and relative content in the leukocytogram of eosinophils, as well as the mass of the spleen, more precisely its mass-index. Instead, with a another constellation of metabolic parameters: electrolyte markers of mineralocorticoid and calcitonin activity, plasma catalase activity and malonic dialdehyde levels in it, the "creatinineuria, creatinineemia and glomerular filtration" triad, as well as the mass of the adrenal glands, the content of reticulocytes in the spleen and epitheliocytes and macrophages in the thymus, is discrimint root tied in a reverse manner.

Table 4. Summary of step-by-step analysis and standardized, structural and raw coefficients and constant for discriminant variables

Variables currently in the model	Parameters of Wilks' Statistics					Coefficients		
	F to enter	p-level	Λ	F-value	p-level	Standardized	Structural	Raw
Calcium Plasma	8,42	,005	,869	8,4	,005	1,637	**,204**	1,902
Potassium Plasma	3,24	,078	,469	9,6	10^{-6}	1,400	**,183**	1,861
Microbian Count of Neutrophils	10,4	,002	,731	10,1	10^{-3}	,405	**,143**	,307
Moda HRV	1,62	,211	,267	7,0	10^{-6}	,451	**,093**	,022
Middle Mass Molecules Urine	2,08	,157	,233	6,6	10^{-6}	,458	**,090**	,0106
Stub Neutrophils of Blood	2,33	,133	,398	8,1	10^{-6}	,116	**,080**	,101
Potassium Urine	2,49	,121	,378	7,7	10^{-6}	,517	**,075**	,013
$(CaP/PP)^{0,5}$ as Paratyrine Activity-2	13,3	,001	,586	12,7	10^{-5}	-,900	**,072**	-1,499
Spleen Mass Index	2,66	,110	,278	7,3	10^{-6}	,711	**,065**	,0106
$(NaP/KP)^{0,5}$ as Mineralocorticoid Activity-2	1,16	,289	,217	6,2	10^{-6}	,806	**-,170**	1,205
Creatinine Excretion	4,65	,036	,539	11,3	10^{-6}	-,433	**-,141**	-,091
Katalase Plasma	2,41	,128	,339	7,3	10^{-6}	-,818	**-,124**	-,0175
$(CaU•PU)/(CaP•PP)^{0,25}$ as Calcitonine Activity	1,76	,192	,256	6,8	10^{-6}	,829	**-,104**	,689
Adrenals Mass Index	2,69	,107	,417	8,5	10^{-6}	-,627	**-,100**	-,146
Reticulocytes of Spleen	3,24	,078	,440	9,1	10^{-6}	-,431	**-,098**	-,246
Glomerulary Filtration	3,36	,074	,315	7,4	10^{-6}	-1,135	**-,097**	-,013
Malonic Dyaldehid Plasma	4,20	,046	,499	10,5	10^{-6}	-,427	**-,079**	-,014
$(NaP•KU/KP•NaU)^{0,25}$as Mineralocortic Act-4	1,58	,217	,246	6,6	10^{-6}	,555	**-,066**	,583
Epitheliocytes of Thymus	2,72	,106	,357	7,5	10^{-6}	-,857	**-,062**	-,406
Macrophages of Thymus	1,61	,212	,224	6,4	10^{-6}	,320	**-,062**	,286
Creatinine Plasma	2,83	,100	,295	7,3	10^{-6}	-,922	**-,062**	-27,33
							Constant	-13,65

The sum of products of raw coefficients on the value of discriminant variables together with the constant gives the value of discriminant function (root) for each animal and allow its visualization (Fig. 3).

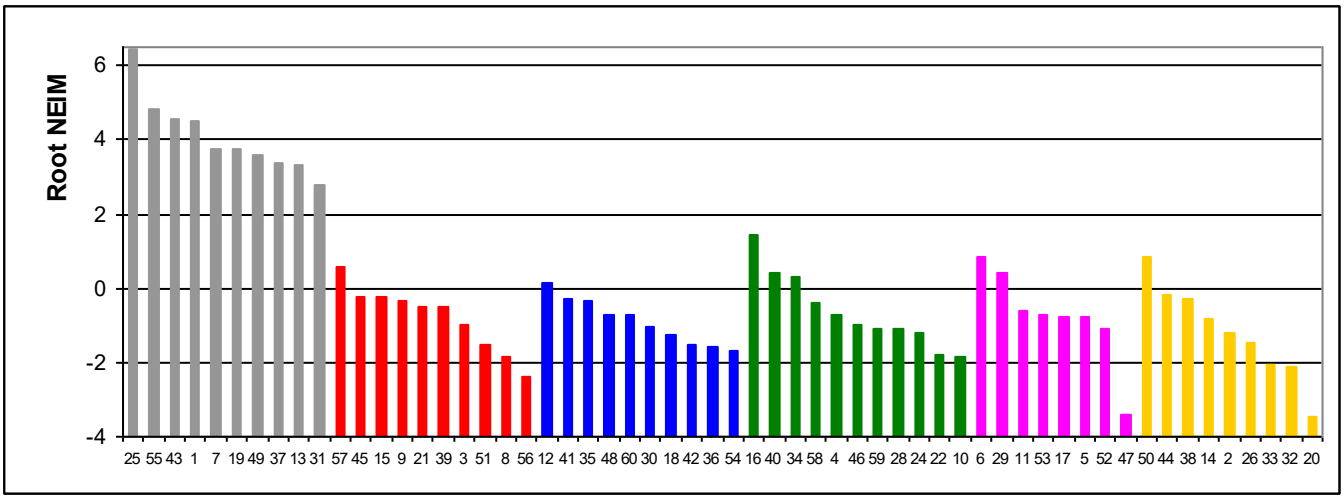

Fig. 3. Individual values of discriminant root of parameters of metabolism and neuroendocrine-immune complex of **intact** rats and loaded with **daily** water, mineral waters **Sofiya** and **Gertsa**, artificial **salt analogue** of Gertsa water, as well as bioactive water **Naftussya**. Below are the numbers of animals.

Even at first glance it is possible to state a clear difference between the status of intact rats and those subject to water-salt loading. Significantly lower individual columns of loaded rats show lower relative to intact rats values of these variables, which correlate with the canonical discriminant root **directly**, and the larger values of **inversely** correlated variables. The visual impression is documented by calculating the square of Mahalanobis distance between the values of discriminant roots: $D^2_M=25,4$ ($F=5,9$; $p<10^{-5}$).

Instead, between separate groups of rats, despite the different chemical composition of the received liquids, significant differences in the set of discriminant variables were not identified **by definition** (Fig. 4).

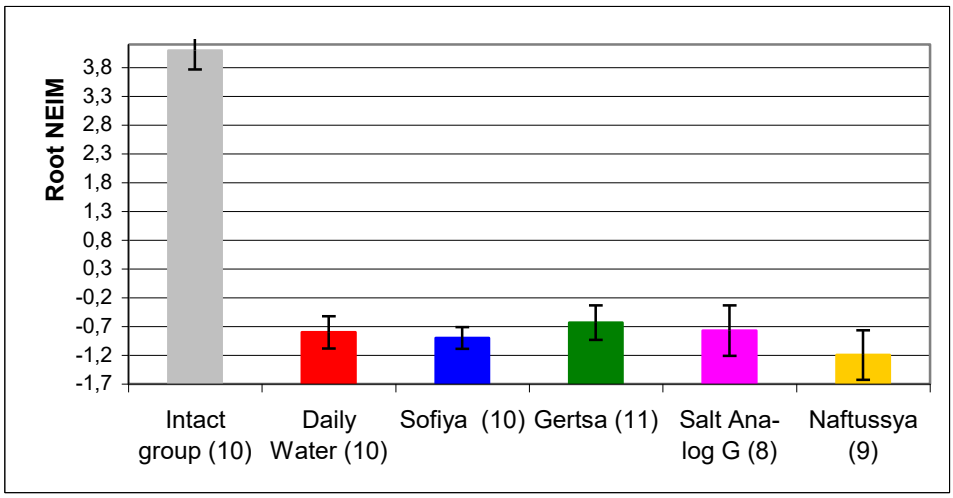

Fig. 4. Average values of discriminant root of parameters of metabolism and neuroendocrine-immune complex of **intact** rats and loaded with **daily** water, mineral waters **Sofiya** and **Gertsa**, artificial **salt analogue** of Gertsa water, as well as bioactive water **Naftussya**.

In other words, the selected parameters characterize the **non-specific (general)** reaction of the neuroendocrine-immune complex and the metabolism to the water-salt load as such (per se), regardless of the specificity of the chemical composition of the fluids used.

The same discriminan parameters can be used to identify (classify) the belonging of one or another rat to an intact group or subject to water-salt loading. This purpose of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 5).

Table 5. Coefficients and constants for classifying functions

Variables currently in the model	Intact rats n=10	Loaded rats n=48
Calcium Plasma, mM/L	78,62	69,21
Microbian Count of Neutrophils, Bacteras/Phagocyte	1,77	,25
$(CaP/PP)^{0,5}$ as Paratyrine Activity-2	-56,78	-49,37
Creatinine Excretion, µM/24h•100 g Body Mass	-13,29	-12,84
Malonic Dyaldehid Plasma, µM/L	,10	,17
Potassium Plasma, mM/L	319,8	310,6
Reticulocytes of Spleen, %	12,32	13,54
Adrenals Mass, mg/100 g Body Mass	-,519	,203
Stub Neutrophils of Blood, %	1,63	1,13
Potassium Urine, mM/L	,32	,25
Epitheliocytes of Thymus, %	-1,83	,18
Katalase Plasma, nM/h•mL	-,332	-,245
Glomerulary Filtration, µL/min•100 g Body Mass	,16	,23
Creatinine Plasma, µM/L	-118,2	17,05
Spleen Mass Index, mg/100 g Body Mass	,3665	,3143
Moda HRV, msec	1,03	,92
$(CaU•PU)/(CaP•PP)^{0,25}$ as Calcitonine Activity-4	57,04	53,63
$(NaP•KU/KP•NaU)^{0,25}$ as Minerelocorticoid Activity-4	29,49	26,60
Middle Mass Molecules Urine, units	,329	,277
Macrophages of Thymus, %	13,93	12,52
$(NaP/KP)^{0,5}$ as Minerelocorticoid Activity-2	366,4	360,4
Constants	**-2109**	**-2032**

These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. The coefficients of the classifying functions are not standardized, therefore they are not interpreted. An object belongs to a group with the maximum value of a function calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant. In this case, we can retrospectively recognize both intact rats and those subject to water-salt loading **unmistakably**.

Instead, other registered metabolic and neuroendocrine-immune complex components **do not respond equally** to the **procedure** of water-salt loadings.

In particular, there is no urine excretion of major electrolytes (sodium, chloride, potassium and phosphates), nor the content of sodium, chloride, phosphate and magnesium in plasma, nor level sodium and potassium in erythrocytes, do not differ in intact and loaded rats. But this applies only to the **average** (!) values, which hide the specificity of water-salt loads, which will be considered in the next article.

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 conduct of experiments was approved by the Ethics Committee of the Horbachevskyi Ternopil' 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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