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PECULIARITIES OF NEUROENDOCRINE AND METABOLIC EFFECTS OF SULFATE-CHLORIDE SODIUM-MAGNESIUM MINERAL WATERS "MYROSLAVA" AND "KHRYSTYNA" OF TRUSKAVETS' SPA IN HEALTHY FEMALE RATS

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Abstract

Background. Earlier we found that the newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa have similar neuroendocrine and metabolic effects on healthy old female rats significantly different from daily water. The aim of this study is to elucidate the effects of these mineral waters on the neuroendocrine status and metabolism of these animals. **Materials and Methods**. Experiment was performed on 50 healthy female Wistar rats. Animals of the first group remained intact, using tap water from drinking ad libitum. Rats of the control group for 6 days injected a tap water through the tube at a dose of 1,5 mL/100 g of body mass. The rats of the main groups received the water "Myroslava" and "Khrystyna". The day after the completion of the drinking course in all rats, at first, assessed the state of autonomous regulation by parameters of the HRV. The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine and testosterone (by the ELISA) as well as electrolytes: calcium, magnesium, phosphates, chloride, sodium and potassium; nitric metabolites: creatinine, urea, uric acid, medium molecular polypeptides, bilirubin; lipid peroxidation products and antioxidant enzymes, as well as cholesterol, amylase and glucose. Most of the listed parameters of metabolism were also determined in daily urine. In the adrenals the thickness of glomerular, fascicular, reticular and medullar zones was measured. **Results**. To identify exactly those parameters, the set of which all four groups of animals differ significantly from each other, the information field of the registered parameters was subjected to discriminant analysis. The program included in the model 8 endocrine and 16 metabolic parameters, information about which is condensed into three roots. The first root reflects directly the SOD and corticosterone and inversely the reticular

zone as well as plasma uric acid and glucose. The second root contains information about Nap/Kp ratio, natrihistia, amylasemia, magnesiumuria as well as inversely about kaliemia. The third root reflects directly the triiodothyronine, parathyroid activity, plasma Ca, natriuria and chloriduria as well as urine malondyaldehide. Inversely displays the root information about the testosterone, Ku/Nau ratio, glomerular zone, plasma katalase and Na as well as uricosuria and amylasuria. In the information space of the three discriminant roots, all four groups are quite clearly distinguished. Classification accuracy is 94% (three errors). **Conclusion**. The newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets resort have specific endocrine and metabolic effects on healthy old female rats with weekly use. This provides a basis for preclinical studies.
Keywords: sulfate-chloride sodium-magnesium drinking mineral waters, Truskavets' spa, neuroendocrine and metabolic parameters, female rats.

INRODUCTION

Earlier we found that the newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa have similar neuroendocrine and metabolic effects on healthy old female rats significantly different from daily water [9]. The aim of this study is to elucidate the effects of these mineral waters on the neuroendocrine status and metabolism of these animals.

MATERIALS AND METHODS

Experiment was performed on 50 healthy old female Wistar rats 220-300 g (M±SD=262±23 g) divided into 4 groups. Animals of the first group (10) remained intact, using tap water from drinking ad libitum. Rats of the second (control) group (10) for 6 days administered a single tap water through the tube at a dose of 1,5 mL/100 g of body mass. The rats of the main groups received the water "Myroslava" (15) and "Khrystyna" (15), prepared from the brine of the 27-*K* well of the Truskavetsian field by appropriate dilutions with fresh water. The chemical composition of the applied waters (as well as, for comparison, the "Sofiya" water of the Truskavets' spa), according to the Truskavetsian Hydrogeological Regime-operational station, is given in Table 1.

Table 1. Chemical composition of fresh and mineral waters

	Daily Water	Sofiya	Khrystyna	Myroslava
Electrolytes, mM/L				
SO_4^{2-}	1,2	13,1	**54,5**	**27,3**
Cl^-	3,4	142	**43**	**22**
Na^+	0,5	156	**127**	**64**
Mg^{2+}	0,5	4,3	**11,9**	**6,0**
Ca^{2+}	3,4	5,3	0,77	0,39
HCO_3^-	2,9	7,5	0,6	0,3
K^+	0,4	0,3	0,4	0,2
Trace elements, mg/L				
Br^-	8,3	6,7	2,68	1,34
F^-	0,95	0,52	1,16	0,58
H_2SiO_3	5	4,43	0,13	0,065
H_3BO_3	0,25	8,39	0,10	0,05
J^-	0,025	1,29	0,004	0,002
C organ	5,0	5,5	0,83	0,42

The day after the completion of the drinking course in all rats, at first, 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: mode (Mo), amplitude of the mode (AMo) and variational swing (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [2].

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) [7]; lipid peroxidation products: diene conjugates (spectrophotometry of the heptane phase of the lipids extract [6]) and malondyaldehide (in the test with thiobarbituric acid [1]), 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 [4,14]) and catalase plasma (at the rate of decomposition of hydrogen peroxide [13]), as well as cholesterol (by a direct method after the classic reaction by Zlatkis-Zack), amylase (Karavay's amyloclastic method with starch substrate) and glucose (glucose-oxidase method) [7].

Most of the listed parameters of metabolism were also determined in daily urine. The latter also determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene). By the size of the diuresis and the level of creatinine in plasma and urine, glomerular filtration and tubular reabsorption were calculated.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients $(Cap/Pp)^{0,5}$ and $(Pu/Cau)^{0,5}$, calcitonin by coefficients $(Cap•Pp)^{-0,5}$ and $(Cau•Pu)^{0,5}$ as well as mineralocorticoid by coefficients $(Nap/Kp)^{0,5}$ and $(Ku/Nau)^{0,5}$, based on their classical effects and recommendations by Popovych IL [18].

Urine lithogenicity index (Lith) was also calculated by the Tiselius' HS [19] formula modifed by Flyunt VR et al [5]: $Lith = (Uric\ acid•Calcium/Magnesium•Creatinine)^{0,25}$.

The analyzes were carried out according to the instructions. The analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a flaming spectrophotometer "СФ-47".

After decapitation, the adrenal glands were removed and weighed, then the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope [3].

Digital material is statistically processed on a computer using the software package "Statistica 64".

Abstracts of the article are published in the conference proceedings [8].

RESULTS AND DISCUSSION

In order to identify those metabolic and neuro-endocrine parameters, the combination of influences on which both mineral waters differ from each other and from tap water, discriminant analysis [12] was used. The forward stepwise program included 24 parameters in the model (Tables 2 and 6), including 8 **endocrine** parameters, 5 **blood electrolyte**

parameters, 3 **urine electrolyte** parameters, 5 **blood enzyme and non-electrolyte** parameters, and 3 **urine** parameters. Other recorded parameters were outside the model (Tables 3-5).

Table 2. Discriminant Function Analysis Summary
Step 24, N of Variables currently in the model: 24; Grouping: 4 groups
Wilks' Lambda: 0,0253; approx. $F_{(73)}$=2,34; p=0,0002

Variables currently in the model	Groups (n)				Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Myr-osla-va (15)	Khry styna (15)	Wil ks' Λ	Par-tial Λ	F-re-move	p-level	Tole-rancy
Calcium Plasma, mM/L	3,35 1 0	2,08 0,62 -1,24	2,91 0,87 -0,43	2,51 0,75 -0,83	0,042	0,598	5,16	0,007	0,011
Superoxide Dismutase Erythrocytes, un/mL	58,0 1 0	58,2 1,00 +0,02	49,9 0,86 -0,75	57,7 0,99 -0,03	0,030	0,838	1,48	0,247	0,326
Sodium Excretion, µM/24h•100 g Body Mass	135 1 0	76 0,56 -0,70	167 1,24 +0,39	271 2,01 +1,62	0,029	0,872	1,12	0,360	0,256
Potassium Plasma, mM/L	4,23 1 0	3,54 0,84 -0,98	3,42 0,81 -1,15	3,33 0,79 -1,27	0,032	0,785	2,09	0,129	0,021
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	2,56 1 0	1,58 0,62 -0,84	1,91 0,75 -0,56	1,75 0,68 -0,70	0,027	0,942	0,47	0,705	0,408
Triiodothyronine Plasma, nM/L	2,14 1 0	2,11 0,99 -0,05	2,31 1,08 +0,30	2,38 1,11 +0,42	0,038	0,657	4,01	0,020	0,166
Glucose Plasma, mM/L	4,95 1 0	5,49 1,11 +0,49	5,55 1,12 +0,55	5,22 1,05 +0,25	0,048	0,529	6,83	0,002	0,264
Sodium Plasma, mM/L	128,6 1 0	131,9 1,03 +0,65	128,1 1,00 -0,09	127,3 0,99 -0,24	0,036	0,710	3,13	0,045	0,047
Katalase Activity Plasma, µM/h•L	103 1 0	148 1,43 +1,58	122 1,18 +0,67	128 1,24 +0,88	0,036	0,712	3,10	0,046	0,367
Chloride Excretion, µM/24h•100 g Body Mass	144 1 0	107 0,74 -0,38	195 1,35 +0,51	244 1,69 +1,02	0,041	0,619	4,72	0,010	0,007
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,44 1 0	2,34 1,63 +1,09	1,37 0,95 -0,08	1,42 0,99 -0,02	0,037	0,690	3,45	0,033	0,214
Corticosterone Plasma, nM/L	482 1 0	383 0,80 -0,78	365 0,76 -0,92	460 0,96 -0,17	0,033	0,768	2,31	0,103	0,580
Glomerular Zone of Adrenal Cortex, µM	193 1 0	207 1,07 +0,29	182 0,94 -0,25	185 0,96 -0,18	0,040	0,628	4,53	0,012	0,307
Amylase Activity Urine, g/h•L	202 1 0	217 1,07 +0,26	204 1,01 0,04	204 1,01 +0,02	0,029	0,879	1,05	0,389	0,351
Reticular Zone of Adrenal Cortex, µM	43 1 0	40 0,95 -0,29	44 1,04 +0,20	42 0,98 -0,12	0,036	0,702	3,26	0,040	0,306
Testosterone Plasma, nM/L	3,93 1 0	6,04 1,54 +1,97	4,97 1,27 +0,98	4,50 1,15 +0,53	0,031	0,827	1,61	0,215	0,546

Amylase Activity Plasma, g/h•L	152 1 0	154 1,02 +0,10	155 1,02 +0,14	163 1,07 +0,46	0,031	0,810	1,79	0,177	0,384
Magnesium Urine, mM/L	2,56 1 0	2,34 0,91 -0,12	2,49 0,97 -0,04	2,89 1,13 +0,18	0,031	0,804	1,87	0,162	0,175
(Nap/Kp)^{0,5} as Mineralocorticoid Activity	5,57 1 0	6,22 1,12 +1,18	6,20 1,11 +1,15	6,32 1,13 +1,36	0,034	0,747	2,59	0,077	0,012
Chloride Plasma, mM/L	94,3 1 0	95,4 1,01 +0,14	90,5 0,96 -0,54	90,9 0,96 -0,48	0,030	0,841	1,44	0,256	0,074
Sodium Erythrocytes, mM/L	22,0 1 0	22,6 1,03 +0,13	21,8 0,99 -0,04	24,2 1,10 +0,51	0,034	0,736	2,76	0,066	0,130
Uric Acid Plasma, µM/L	662 1 0	620 0,94 -0,12	944 1,43 +0,83	630 0,95 -0,09	0,034	0,744	2,63	0,074	0,230
Malondialdehyde Urine, µM/L	92 1 0	75 0,81 -0,40	88 0,95 -0,10	96 1,04 +0,09	0,031	0,809	1,81	0,173	0,248
Uric Acid Excretion, µM/24h•100 g Body Mass	5,72 1 0	6,02 1,05 +0,05	5,32 0,93 -0,08	5,35 0,93 -0,07	0,029	0,867	1,17	0,342	0,242

Note. In each column, the first line is the average value, the second is the fraction of the norm, and the third is the Z-score.

Table 3. Discriminant Function Analysis Summary. Neuro-endocrine and kidney variables currently not in the model

Variables	**Groups (n)**				**Parameters of Wilks' Statistics**				
	Intact rats (10)	**Daily Water (10)**	**Myr-osla-va (15)**	**Khry styna (15)**	Wilks' Λ	Parti-al Λ	F to en-ter	p-level	Tole-rancy
Amplitude Mode HRV as Sympathetic tone, %	56 1 0	70 1,26 +0,84	54 0,96 -0,13	58 1,03 +0,11	0,023	0,897	0,84	0,485	0,412
MxDMn HRV as Vagal tone, msec	53 1 0	37 0,70 -0,39	62 1,18 +0,22	47 0,89 -0,14	0,022	0,883	0,97	0,423	0,260
Mode HRV as Humoral channel, msec	124 1 0	105 0,85 -1,27	122 0,98 -0,13	115 0,93 -0,57	0,024	0,936	0,50	0,686	0,324
Adrenals Mass Index, mg/100 g Body Mass	25,2 1 0	26,8 1,06 +0,31	27,4 1,09 +0,42	24,9 0,99 -0,06	0,025	0,987	0,10	0,959	0,505
Fascicular Zone of Adrenal Cortex, µM	391 1 0	398 1,02 +0,09	411 1,05 +0,23	430 1,10 +0,46	0,024	0,943	0,45	0,722	0,344
Medullar Zone of Adrenals, µM	94 1 0	65 0,69 -0,93	94 1,01 +0,02	93 0,99 -0,03	0,024	0,935	0,51	0,680	0,320
17-Ketosteroide Excretion, nM/24h•100g Body Mass	61 1 0	59 0,97 -0,04	73 1,19 +0,22	76 1,24 +0,27	0,024	0,945	0,43	0,737	0,241
(Cau•Pu)^{0,5} as Calcitonin Activity	3,63 1 0	3,63 1,00 0,00	3,36 0,93 -0,32	3,65 1,01 +0,03	0,024	0,941	0,46	0,712	0,419

(Cap•Pp)$^{-0,5}$ as Calcitonin Activity	0,79 1 0	0,78 0,98 -0,05	0,72 0,91 -0,20	0,74 0,93 -0,16	0,024	0,960	0,28	0,840	0,490
(Pu/Cau)^{0,5} as Parathyroid Activity	1,76 1 0	1,80 1,02 +0,08	1,82 1,03 +0,14	1,81 1,03 +0,11	0,024	0,940	0,40	0,737	0,241
Glomerular Filtration, µL/min•100 g Body Mass	86,0 1 0	85,2 0,99 -0,03	158 1,84 +2,35	134 1,56 +1,56	0,022	0,882	0,98	0,421	0,340
Canalicular Reabsorbtion, %	98,7 1 0	98,6 1,00 -0,05	99,1 1,00 +0,50	98,6 1,00 -0,06	0,025	0,999	0,00	0,999	0,481
Diuresis, mL/24h•100 g Body Mass	1,44 1 0	1,48 1,03 +0,05	1,77 1,23 +0,37	1,89 1,31 +0,50	0,024	0,941	0,46	0,712	0,419
(Ca•UA/Mg•Cr)^{0,25} as Lithogenicity Urine Index	0,90 1 0	0,90 1,00 0,00	0,85 0,95 -0,19	0,79 0,88 -0,43	0,024	0,961	0,28	0,838	0,497

Table 4. Discriminant Function Analysis Summary. Electrolytic variables currently not in the model

Variables	**Groups (n)**				**Parameters of Wilks' Statistics**				
	Intact rats (10)	**Daily Water (10)**	**Myr-osla-va (15)**	**Khry styna (15)**	Wilks' Λ	Parti-al Λ	F to en-ter	p-level	Tole-rancy
Potassium Urine, mM/L	131 1 0	130 0,99 -0,02	128 0,98 -0,06	115 0,88 -0,41	0,025	0,983	0,13	0,942	0,314
Potassium Excretion, µM/24h•100 g Body Mass	189 1 0	203 1,08 +0,12	207 1,10 +0,15	187 0,99 -0,02	0,025	0,985	0,11	0,953	0,269
Calcium Urine, mM/L	2,10 1 0	2,17 1,03 +0,19	2,04 0,97 -0,16	2,13 1,02 +0,10	0,024	0,961	0,30	0,827	0,435
Calcium Excretion, µM/24h•100 g Body Mass	2,90 1 0	3,22 1,11 +0,21	3,67 1,26 +0,50	4,07 1,40 +0,76	0,024	0,961	0,30	0,827	0,435
Phosphate Urine, mM/L	6,39 1 0	6,20 0,97 -0,24	5,85 0,91 -0,69	6,43 1,01 +0,05	0,024	0,941	0,46	0,712	0,419
Phosphates Excretion, µM/24h•100 g Body Mass	9,4 1 0	9,9 1,05 +0,08	10,9 1,16 +0,23	12,1 1,29 +0,44	0,024	0,945	0,43	0,736	0,295
Sodium Urine, mM/L	105 1 0	55 0,52 -0,76	102 0,97 -0,05	153 1,45 +0,72	0,024	0,941	0,46	0,712	0,419
Chloride Urine, mM/L	115 1 0	70 0,61 -0,56	125 1,09 +0,13	150 1,31 +0,44	0,022	0,882	0,98	0,421	0,340
Magnesium Excretion, µM/24h•100 g Body Mass	3,30 1 0	3,55 1,07 +0,12	4,17 1,26 +0,42	4,77 1,45 +0,71	0,024	0,951	0,38	0,769	0,242
Magnesium Plasma, mM/L	0,88 1 0	0,99 1,13 +0,19	0,64 0,73 -0,39	0,83 0,95 -0,08	0,024	0,945	0,43	0,736	0,295
Phosphate Plasma, mM/L	0,72 1 0	1,01 1,41 +0,65	0,98 1,36 +0,57	0,94 1,31 +0,49	0,023	0,891	0,89	0,459	0,076

Potassium Erythrocytes, mM/L	87,0 1 0	85,8 0,99 -0,18	85,9 0,99 -0,16	89,3 1,03 +0,33	0,024	0,963	0,2 8	0,839	0,497

Table 5. Discriminant Function Analysis Summary. Nonelectrolytic variables currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Myr-osla-va (15)	Khry styna (15)	Wilks' Λ	Parti-al Λ	F to en-ter	p-level	Tole-rancy
Cholesterol Plasma mM/L	1,57 1 0	1,70 1,08 +0,28	1,49 0,95 -0,16	1,64 1,05 +0,16	0,025	0,992	0,0 6	0,981	0,477
Bilirubin Plasma, µM/L	4,63 1 0	4,65 1,00 +0,01	4,34 0,94 -0,11	4,35 0,94 -0,11	0,025	0,999	0,0 0	0,999	0,481
Creatinine Plasma, µM/L	72,5 1 0	92 1,26 +0,79	64 0,88 -0,37	89 1,23 +0,69	0,024	0,949	0,4 0	0,756	0,310
Creatinine Urine, mM/L	6,41 1 0	7,23 1,13 +0,45	7,25 1,13 +0,46	7,07 1,10 +0,36	0,024	0,963	0,2 8	0,839	0,497
Creatinine Excretion, µM/24h•100 g Body Mass	8,7 1 0	10,7 1,23 +0,46	12,4 1,43 +0,85	12,5 1,43 +0,87	0,022	0,883	0,9 7	0,423	0,260
Urea Plasma, mM/L	7,42 1 0	9,46 1,27 +1,19	7,65 1,03 +0,13	9,05 1,22 +0,95	0,024	0,939	0,4 8	0,702	0,313
Urea Urine, mM/L	107 1 0	110 1,03 +0,07	124 1,16 +0,40	139 1,30 +0,77	0,024	0,945	0,4 3	0,736	0,295
Urea Excretion, µM/24h•100 g Body Mass	169 1 0	179 1,06 +0,08	234 1,39 +0,48	292 1,73 +0,91	0,023	0,919	0,6 5	0,591	0,243
Uric Acid Urine, mM/L	3,68 1 0	4,29 1,17 +0,33	3,42 0,93 -0,14	3,18 0,86 -0,27	0,024	0,951	0,3 8	0,769	0,242
Middle Mass Molecules Plasma, units	154 1 0	175 1,14 +0,41	133 0,87 -0,40	126 0,82 -0,55	0,023	0,928	0,5 7	0,641	0,467
Middle Mass Molecules Urine, units	182 1 0	174 0,95 -0,16	154 0,85 -0,53	161 0,89 -0,40	0,024	0,935	0,5 1	0,681	0,438
Diene conjugates Plasma, E^{232}/mL	1,34 1 0	1,42 1,06 +0,20	1,56 1,16 +0,55	1,44 1,07 +0,23	0,024	0,938	0,4 8	0700	0,453
Diene conjugates Urine, E^{232}/mL	1,86 1 0	1,68 0,91 -0,26	1,79 0,97 -0,10	1,96 1,06 +0,16	0,025	0,994	0,0 4	0,988	0,393
Malondyaldehide Plasma, µM/L	63 1 0	79 1,25 +0,74	74 1,16 +0,47	63 1,00 -0,01	0,025	0,978	0,1 7	0,917	0,487
Katalase Activity Urine, µM/h•L	123 1 0	149 1,22 +0,96	145 1,18 +0,81	148 1,21 +0,92	0,024	0,930	0,5 5	0,654	0,128

Table 6. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Calcium Plasma	4,49	0,008	0,773	4,49	0,008
Superoxide Dismutase Plasma	4,18	0,011	0,605	4,29	0,001
Sodium Excretion	2,90	0,045	0,505	3,86	10^{-3}
Potassium Plasma	4,48	0,008	0,385	4,13	10^{-4}
(Cap/Pp)^{0,5} as Parathyroid Activity	3,39	0,026	0,310	4,10	10^{-5}
Triiodothyronine	2,53	0,071	0,261	3,93	10^{-5}
Glucose Plasma	2,47	0,076	0,221	3,81	10^{-5}
Sodium Plasma	1,74	0,174	0,194	3,60	10^{-5}
Katalase Plasma	1,63	0,198	0,172	3,42	10^{-5}
Chloride Excretion	1,60	0,190	0,150	3,30	10^{-5}
(Ku/Nau)^{0,5} as Mineralocorticoid Activity	2,24	0,100	0,128	3,26	10^{-5}
Corticosterone	1,25	0,306	0,116	3,11	10^{-5}
Glomerular Zone of Adrenals	1,29	0,295	0,104	2,99	10^{-5}
Amylase Urine	1,73	0,180	0,090	2,94	10^{-5}
Reticular Zone of Adrenals	1,52	0,227	0,079	2,88	10^{-5}
Testosterone	1,33	0,284	0,070	2,81	10^{-5}
Amylase Plasma	1,29	0,297	0,062	2,74	10^{-4}
Magnesium Urine	1,27	0,303	0,055	2,67	10^{-4}
(Nap/Kp)^{0,5} as Mineralocorticoid Activity	1,42	0,257	0,047	2,64	10^{-4}
Chloride Plasma	1,11	0,363	0,042	2,56	10^{-4}
Sodium Erythrocytes	1,01	0,403	0,038	2,49	10^{-4}
Uric Acid Plasma	1,04	0,393	0,034	2,42	10^{-3}
Malondialdehyde Urine	1,21	0,327	0,029	2,38	10^{-3}
Uric Acid Excretion	1,17	0,342	0,025	2,34	10^{-3}

The dividing information contained in 24 variables is condensed in 3 canonical discriminant roots (Table 7). At the same time, the first root contains 41,2% of discriminative opportunities (r^*=0,868; Wilks' Λ=0,025; $\chi^2_{(72)}$=129; $p<10^{-4}$), the second - 37,3% (r^*=0,857; Wilks' Λ=0,102; $\chi^2_{(46)}$=80; p=0,001), the third - 21,5% (r^*=0,784; Wilks' Λ=0,386; $\chi^2_{(22)}$=33; p=0,057).

The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients (Table 7) to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots.

Table 7. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Calcium Plasma	-2,644	6,571	0,014	-3,231	8,030	0,0165
Superoxide Dismutase Erythrocytes	0,266	-0,706	-0,351	0,0298	-0,0792	-0,0394
Sodium Excretion	-0,492	-0,546	0,399	-0,0029	-0,0032	0,0023
Potassium Plasma	3,450	1,103	0,991	4,5149	1,4429	1,2963
(Cap/Pp)^{0,5} as Parathyroid Activity	0,074	0,430	0,052	0,1082	0,6264	0,0756
Triiodothyronine	-0,675	-1,370	0,755	-1,6521	-3,3524	1,8481
Glucose Plasma	-1,488	0,379	0,156	-1,7945	0,4577	0,1888
Sodium Plasma	-0,706	-2,640	-1,007	-0,1302	-0,4869	-0,1858
Katalase Plasma	-0,699	0,579	-0,527	-0,015	0,012	-0,011
Chloride Excretion	-0,490	-0,540	0,400	-0,0030	-0,0036	0,0025
(Ku/Nau)^{0,5} as Mineralocorticoid Activity	1,184	-0,696	-0,261	1,2402	-0,7288	-0,2737
Corticosterone	0,310	-0,354	0,618	0,0018	-0,0021	0,0036
Glomerular Zone of Adrenals	1,090	-0,617	-0,242	0,0303	-0,0171	-0,0067
Amylase Urine	-0,645	-0,187	-0,082	-0,0161	-0,0047	-0,0020
Reticular Zone of Adrenals	-0,774	0,821	-0,207	-0,0725	0,0768	-0,0194
Testosterone	0,161	-0,001	-0,696	0,0778	-0,0004	-0,3366
Amylase Plasma	-0,545	0,057	0,659	-0,0157	0,0016	0,0190
Magnesium Urine	0,627	1,061	-0,027	0,3727	0,6301	-0,0158
(Nap/Kp)^{0,5} as Mineralocorticoid Activity	0,180	5,387	0,903	0,2587	7,7366	1,2966
Chloride Plasma	-0,009	1,612	0,602	-0,0014	0,2495	0,0932
Sodium Erythrocytes	0,586	-1,399	0,738	0,1222	-0,2915	0,1538
Uric Acid Plasma	-0,592	1,075	-0,027	-0,0013	0,0024	-0,0001
Malondialdehyde Urine	0,767	-0,543	0,426	0,0235	-0,0166	0,0130
Uric Acid Excretion	0,390	-0,759	0,135	0,1125	-0,2191	0,0391
			Constants	-3,048	23,12	-5,443
			Eigenvalues	3,051	2,766	1,593
	Cumulative Proportions			0,412	0,785	1

In the Table 8 together with discriminant variables are also variables that carry identifying/ separating information, but were outside the model due to its duplication/redundancy. For ease of comparison, the values of the variables are transformed into Z-scores.

The localization of rats loaded with "**Myroslava"** water in the extreme left zone of the first root axis (Fig. 1 above) reflects their maximally reduced levels of erythrocyte superoxide dismutase activity and plasma corticosterone in combination with maximally elevated levels of uric acid and glucose, also normal but maximal for sampling the thickness of the reticular zone of the adrenal cortex, while in the other two experimental groups, these parameters do not differ from the norm or less/greater, respectively.

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

	Correlations Variables-Roots			Myro-slava	Khry-sty-na	Daily Water	Intact rats
Root 1 (41,2%)	**R1**	**R2**	**R3**	**-2,06**	-0,12	0,58	**2,70**
Superoxide Dismutase Erythr	**0,203**	-0,149	-0,012	**-0,75**	-0,03	+0,02	**0**
Corticosterone	**0,092**	-0,035	0,129	**-0,92**	-0,17	-0,78	**0**
Phosphates Urine				**-0,69**	+0,05	-0,24	**0**
Middle Mass Molecules Urine				**-0,53**	-0,40	-0,16	**0**
Magnesium Plasma				**-0,39**	-0,08	+0,19	**0**
Uric Acid Plasma	**-0,140**	0,129	0,010	**+0,83**	-0,09	-0,12	**0**
Glucose Plasma	**-0,138**	-0,003	-0,125	**+0,55**	+0,25	+0,49	**0**
Reticular Zone of Adrenals	**-0,040**	0,062	0,044	**+0,20**	-0,12	-0,29	**0**
Diene conjugates Plasma				**+0,55**	+0,23	+0,20	**0**
Glomerular Filtration				**+2,35**	+1,56	-0,03	**0**
Canalicular Reabsorption				**+0,50**	-0,06	-0,05	**0**
Root 2 (37,3%)	**R1**	**R2**	**R3**	1,44	**-1,91**	-1,04	1,75
(Nap/Kp)^{0,5} as MCA	-0,175	**-0,164**	-0,036	+1,15	**+1,36**	+1,18	0
Fascicular Zone of Adrenals				+0,23	**+0,46**	+0,09	0
Sodium Erythrocytes	0,008	**-0,120**	0,074	-0,04	**+0,51**	-0,13	0
Amylase Plasma	-0,023	**-0,063**	0,054	+0,14	**+0,46**	+0,10	0
Magnesium Urine	0,002	**-0,040**	0,086	-0,04	**+0,18**	-0,12	0
Urea Excretion				+0,48	**+0,91**	+0,08	0
Magnesium Excretion				+0,42	**+0,71**	+0,12	0
Calcium Excretion				+0,50	**+0,76**	+0,21	0
Diurese				+0,37	**+0,50**	+0,05	0
Phosphates Excretion				+0,23	**+0,44**	+0,08	0
Potassium Plasma	0,211	**0,159**	0,005	-1,15	**-1,27**	-0,98	0
Lithogenicity Urine				-0,19	**-0,43**	0,00	0
Root 3 (21,5%)	**R1**	**R2**	**R3**	-0,01	1,15	**-2,25**	0,55
Sodium Excretion	-0,067	-0,118	**0,291**	+0,39	+1,62	**-0,70**	0
Sodium Urine				-0,05	+0,72	**-0,76**	0
Chloride Excretion	-0,063	0,089	**0,204**	+0,51	+1,02	**-0,38**	0
Chloride Urine				+0,13	+0,44	**-0,56**	0
Calcium Plasma	0,065	0,257	**0,247**	-0,43	-0,83	**-1,21**	0
Malondialdehyde Urine	0,007	-0,006	**0,175**	-0,10	+0,09	**-0,40**	0
(Cap/Pp)^{0,5} as PTA	0,164	0,222	**0,164**	-0,56	-0,70	**-0,84**	0
MxDMn HRV as Vagal tone				+0,22	-0,14	**-0,39**	0
Medullar Zone of Adrenals				+0,02	-0,03	**-0,93**	0
Triiodothyronine	-0,104	-0,055	**0,162**	+0,30	+0,42	**-0,05**	0
Testosterone	-0,073	-0,065	**-0,245**	+0,98	+0,53	**+1,97**	0
1/Mo as Circul Catecholamines				+0,13	+0,57	**+1,27**	0
AMo as Sympathetic tone				-0,13	+0,11	**+0,84**	0
(Ku/Nau)^{0,5} as MCA	0,055	-0,086	**-0,293**	-0,08	-0,02	**+1,09**	0
Glomerular Zone of Adrenals	0,086	-0,035	**-0,164**	-0,25	-0,18	**+0,29**	0
Katalase Plasma	-0,059	-0,124	**-0,154**	+0,67	+0,88	**+1,58**	0
Urea Plasma				+0,13	+0,95	**+1,19**	0
Middle Mass Molecules Plasma				-0,40	-0,55	**+0,41**	0
Malondyaldehide Plasma				+0,47	-0,01	**+0,74**	0
Sodium Plasma	0,047	-0,013	**-0,242**	-0,09	-0,24	**+0,65**	0
Creatinine Plasma				-0,37	+0,69	**+0,79**	0

Chloride Plasma	0,145	0,005	**-0,171**	-0,54	-0,48	**+0,14**	0
Amylase Urine	0,002	-0,025	**-0,093**	+0,04	+0,02	**+0,26**	0
Uric Acid Excretion	0,031	-0,003	**-0,051**	-0,08	-0,07	**+0,05**	0

"Khrystyna"-treated rats were characterized by maximally elevated plasma markers of mineralocorticoid activity, plasma amylase activity, erythrocyte natrihistia, urinary magnesium concentration, and daily diuresis in combination with maximal for sampling hypokalemia.

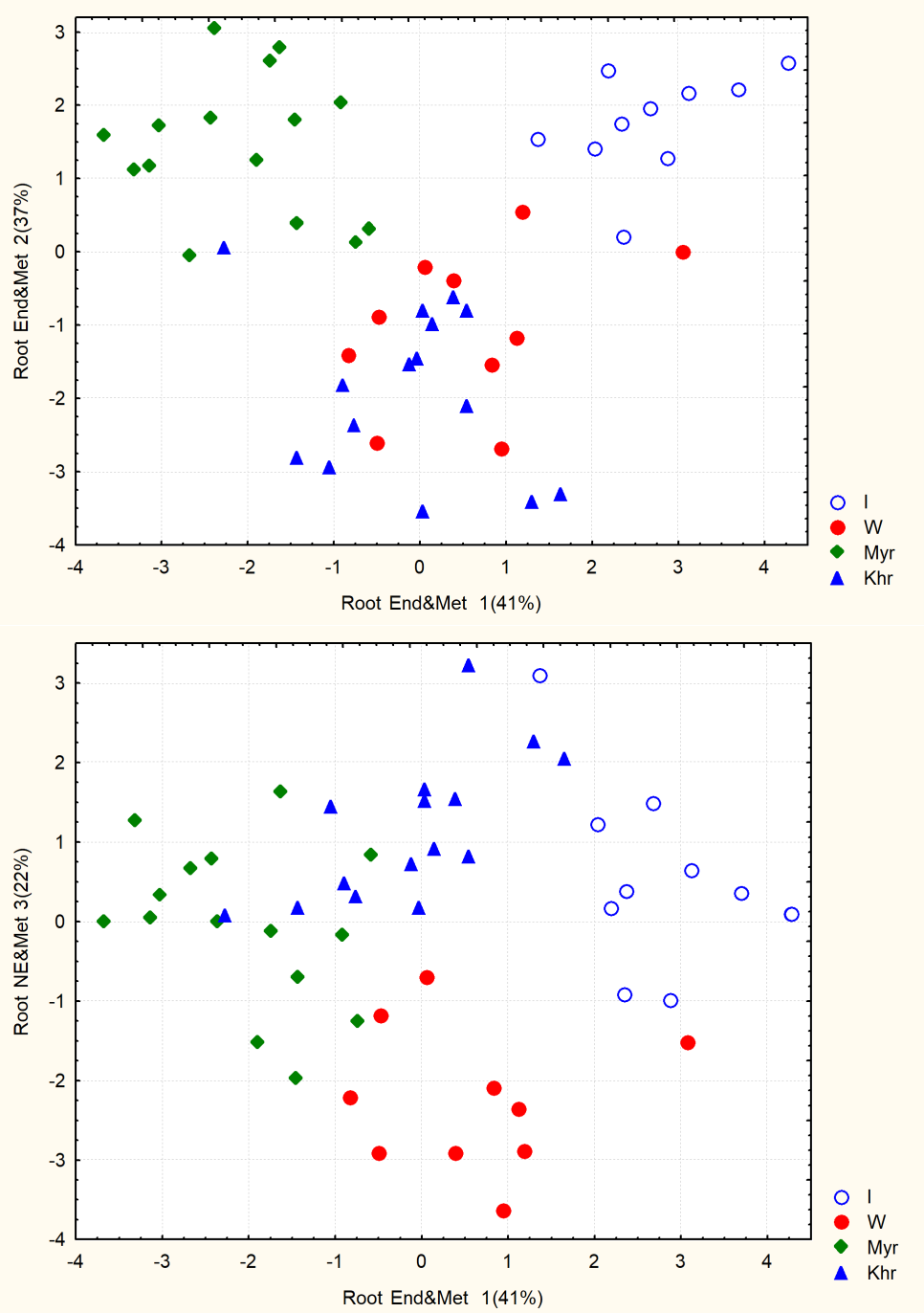

Fig. 1. Individual values of the first and second (above) and the first and third (below) roots of the endocrine and metabolic parameters in intact rats (o) and loaded with Daily water (W) and mineral waters "Myroslava" (Myr) and "Khrystyna" (Khr)

However, their demarcation with rats watered by **daily** water along the axis of the second root is not entirely clear. Instead, along the axis of the third root (Fig. 1 below) the

distinction is quite clear, but by a different constellation of variables. The localization of control rats in the lower zone of the third root axis reflects the maximum decrease in urine concentration and daily excretion of sodium and chloride, urine malonic dialdehyde concentration and plasma calcium, parathyroid activity, and the minimum sampling level of triiodothyronine. On the other hand, control rats are characterized by maximally elevated levels of urinary markers of mineralocorticoid activity, testosterone, sodium and plasma catalase and maximum sampling of chloridemia, amylasuria and uricosuria, as well as the thickness of the glomerular zone of the adrenal cortex. Obviously, the deviation of these parameters from the norm is due to aversion stress [15-18,20].

Despite some mutual penetrations, in the information field of the three discriminant roots, all four clusters are quite clearly delineated, as documented by the Mahalanobis distances between them (Table 9).

Table 9. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=33) and p-levels (under diagonal)

Groups	I (10)	DW (10)	Myr (15)	Khr (15)
Intact rats (I)	**0,0**	21,8	25,1	23,6
Daily Water (DW)	**2,05 ,045**	**0,0**	19,8	13,9
Water "Myroslava" (Myr)	**2,86 ,007**	**2,26 ,028**	**0,0**	17,7
Water "Khrystyna" (Khr)	**2,69 ,010**	**1,58 ,137**	**2,59 ,013**	**0,0**

The application of the classifying functions (Table 10) enables the retrospective identification of the intact rats without mistake, and the latter with a single error (Table 11).

Table 10. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact rats	Daily Water	Myro-slava	Khrys-tyna
Calcium Plasma	-290,0	-305,7	-277,1	-310,3
Superoxide Dismutase Plasma	-0,185	0,084	-0,280	-0,003
Sodium Excretion	0,147	0,156	0,161	0,168
Potassium Plasma	363,9	346,7	341,2	346,7
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	34,21	32,03	33,46	31,66
Triiodothyronine	99,38	107,1	107,2	117,4
Glucose Plasma	0,842	2,830	9,141	4,343
Sodium Plasma	20,80	22,95	21,67	22,83
Katalase Plasma	-0,050	-0,021	0,024	-0,060
Chloride Excretion	0,150	0,166	0,160	0,170
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	45,18	45,36	39,65	44,18
Corticosterone	0,174	0,166	0,164	0,179
Glomerular Zone of Adrenals	0,479	0,482	0,344	0,453
Amylase Urine	0,103	0,156	0,182	0,164
Reticular Zone of Adrenal Cortex	-1,325	-1,332	-0,993	-1,413
Testosterone	-4,555	-3,777	-4,736	-4,974
Amylase Plasma	1,046	1,021	1,109	1,095
Magnesium Urine	-15,25	-17,76	-17,21	-18,62
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	118,8	93,07	114,5	90,60
Chloride Plasma	-17,56	-18,51	-17,68	-18,41
Sodium Erythrocytes	12,42	12,55	11,85	13,24
Uric Acid Plasma	-0,024	-0,027	-0,018	-0,029
Malondialdehyde Urine	0,415	0,375	0,300	0,417
Uric Acid Excretion	-0,575	-0,310	-1,065	-0,068

Constants	-2368	-2409	-2355	-2444

Table 11. Classification Matrix
Rows: Observed classifications; Columns: Predicted classifications

Groups	Per-cent correct	I p=,20	DW p=,20	Myr p=,30	Khr p=,30
Intact rats (I)	100	**10**	0	0	0
Daily Water (DW)	90,0	1	**9**	0	0
Water “Myroslava” (Myr)	93,3	0	1	**14**	0
Water “Khrystyna” (Khr)	93,3	0	0	1	**14**
Total	94,0	11	10	15	14

Thus, we confirmed the previously obtained data on a wide range of parameters of electrolyte metabolism [10,11], and also showed that the studied mineral waters have both the same and different effects on neuroendocrine and metabolic parameters of healthy old female rats. A detailed discussion of this situation will be conducted together with an analysis of the concomitant effects on the immune system.

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’ National 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).

REFERENCES

1. Andreyeva LI, Kozhemyakin LA, Kishkun AA. Modification of the method for determining the lipid peroxide in the test with thiobarbituric acid [in Russian]. Laboratornoye Delo. 1988; 11: 41-43.
2. Baevskiy RM, Kirillov OI, Kletskin SZ. Mathematical Analysis of Changes in Heart Rate by Stress [in Russian]. Moskva. Nauka; 1984: 221.
3. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009; 7(1): 68-102.
4. Dubinina YY, Yefimova LF, Sofronova LN, Geronimus AL. Comparative analysis of the activity of superoxide dismutase and catalase of erythrocytes and whole blood from newborn children with chronic hypoxia [in Russian]. Laboratornoye Delo. 1988; 8: 16-19.
5. Flyunt VR, Flyunt I-SS, Fil’ VM, Kovbasnyuk MM, Hryvnak RF, Popel SL, Zukow W. Relationships between caused by drinking of bioactive water Naftussya changes in urine lithogenicity and neuro-humoral-immune factors in humans with their abnormalities. Journal of Education, Health and Sport. 2017; 7(3): 11-30.
6. Gavrilov VB, Mishkorudnaya MI. Spectrophotometric determination of plasma levels of lipid hydroperoxides [in Russian]. Laboratornoye Delo. 1983; 3: 33-36.

7. Goryachkovskiy AM. Clinical Biochemistry [in Russian]. Odesa: Astroprint; 1998: 608.
8. Hrytsak MV, Barylyak LG, Usyns'kyi RS, Mysula IR. Endocrine and metabolic effects of sulfate chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets' spa in healthy female rats. In: Proceedings of the XII All-Ukrainian scientific-practical conference "Topical issues of pathology under the influence of extraordinary factors on the body". Galician Readings II (Ternopil', October 29-30, 2020). Ternopil'; 2020: 125-127.
9. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow W. Similar neuroendocrine and metabolic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets' spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(6): 320-334.
10. Hrytsan II, Gozhenko AI, Badiuk NS, Zukow W. Variants of the state of electrolyte exchange in female rates. In: Rehabilitation Medicine and Health-Resort Institutions Development. Proceedings of the 19th International Applied Research Conference (Kyïv, 11-12 December 2019). Edited by O. Gozhenko, W. Zukow. Toruń, Kyiv. 2019: 25-26.
11. Hrytsan II, Gozhenko AI, Badiuk NS, Zukow W. Variants of the state of electrolyte exchange in female rates. Journal of Education, Health and Sport. 2019; 9(10): 262-279.
12. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva: Finansy i Statistika; 1989: 78-138.
13. Korolyuk MA, Ivanova MI, Mayorova IG, Tokarev VYe. The method for determining the activity of catalase [in Russian]. Laboratornoye Delo. 1988; 1: 16-19.
14. Makarenko YeV. A comprehensive definition of the activity of superoxide dismutase and glutathione reductase in red blood cells in patients with chronic liver disease [in Russian]. Laboratornoye Delo. 1988; 11: 48-50.
15. Polovynko IS. Integrated quantitative estimation of neuro-endocrine manifestations of chronic stress in female rats. Experimental and Clinical Physiology and Biochemistry. 2017; 3(79): 5-10.
16. Polovynko IS, Zajats LM, Popovych AI, Popovych IL. Integral quantitative evaluation of neuroendocrine and immune reactions to chronic stress in male rats [in Ukrainian]. In: Pathophysiology and Pharmacy: ways of integration: Abstracts of the VII National Congress of Pathophysiologists of Ukraine with International Participation (October 5-7, 2016). Kharkiv. Nat Pharmac Univer; 2016: 182.
17. Polovynko IS, Zajats LM, Zukow W, Yanchij RI, Popovych IL. Quantitative evaluation of integrated neuroendocrine and immune responses to chronic stress in rats male. Journal of Education, Health and Sport. 2016; 6(8): 154-166.
18. Popovych IL, Gozhenko AI, Zukow W, Polovynko IS. Variety of Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment. Scholars' Press. Riga; 2020: 172.
19. Tiselius HS. A biochemical basis for grouping of patients with urolithiasis. Europ Urol. 1978; 4: 241-249.
20. Zajats LM, Polovynko IS, Zukow W. Features neuro-endocrine support diversity of immune responses to chronic stress in male rats. Journal of Education, Health and Sport. 2017; 7(3): 97-105.