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SIMILAR 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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Background. In order to expand the hydro-mineral base of Truskavets' spa by diluting brine (130 g/L), two new ***sulphate-chloride sodium-magnesium*** mineral waters "Myroslava" (5 g/L) and "Khrystyna" (10 g/L) were created. This report is the first in a series of experimental studies of their physiological activity in line with the concepts of neuroendocrine-immune complex and functional-metabolic continuum. **Materials and Methods**. Experiment was performed on 50 healthy female Wistar rats 220-300 g divided into 4 groups. Animals of the first group remained intact, using tap water from drinking ad libitum. Rats of the control group 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" and "Khrystyna". The day after the completion of the drinking course in all rats 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, nitric metabolites, lipid peroxidation products and antioxidant enzymes as well as cholesterol, glucose, amylase and middle mass molecules. Most of the listed parameters of metabolism as well as 17-ketosteroids were determined in daily urine. In the adrenal glands the thickness of glomerular, fascicular, reticular and medullar zones was measured. **Results**. To identify exactly those parameters, the set of which three 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 6 endocrine and 11 metabolic parameters, as well as glomerular filtration. **Conclusion**. 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.

Keywords: sulfate-chloride sodium-magnesium drinking mineral waters, Truskavets' spa, neuroendocrine and metabolic parameters, female rats.

INRODUCTION

In order to expand the hydro-mineral base of Truskavets' spa by diluting brine (130 g/L), two new ***sulphate-chloride sodium-magnesium*** mineral waters "Myroslava" (5 g/L) and "Khrystyna" (10 g/L) were created. This report is the first in a series of experimental studies of their physiological activity in line with the concepts of neuroendocrine-immune complex [14,18,21-23,26] and functional-metabolic continuum [8].

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 "Sofia" 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 [4,15]) 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 [23].

Urine lithogenicity index (Lith) was also calculated by the Tiselius' HS [24] 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".

RESULTS AND DISCUSSION

This article will look at the neuroendocrine and metabolic effects ***common*** to both mineral waters, so the rats they load are grouped together in the "Salt Waters" group. The specific effects of waters will be the subject of the next study, theses of which were published earlier [9]. To identify exactly those parameters, the set of which three groups of animals differ significantly from each other, the information field of the registered parameters was subjected to discriminant analysis [12]. The program forward stepwise included in the model 6 **endocrine** and 11 metabolic parameters, including 7 **electrolytes** of **plasma** and **urine** and 4 **non-electrolytes** of **plasma** and **urine**, as well as **glomerular filtration** (Tables 2 and 7). The rest of the registered parameters were outside the discriminant model (Tables 3-6).

Table 2. Discriminant Function Analysis Summary
Step 18, N of Variables currently in the model: 18; Grouping: 3 groups
Wilks' Lambda: 0,1058; approx. $F_{(37)}$=3,46; $p<10^{-5}$

Variables currently in the model	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Salt Waters (30)	Wilks' Λ	Partial Λ	F-remove (2,30)	p-level	Tolerancy
Calcium Plasma, mM/L	3,35 1 0	2,08 0,62 -1,24	2,71 0,81 -0,63	0,110	0,964	0,56	0,579	0,437
Potassium Plasma, mM/L	4,23 1 0	3,54 0,84 -0,98	3,38 0,80 -1,21	0,157	0,673	7,28	0,003	0,355
Sodium Excretion, µM/24h•100 g Body Mass	135 1 0	76 0,56 -0,70	219 1,63 +1,00	0,118	0,897	1,72	0,196	0,366
$(Cap/Pp)^{0.5}$ as Parathyroid Activity	2,56 1 0	1,58 0,62 -0,84	1,83 0,71 -0,63	0,150	0,706	6,25	0,005	0,127
Glomerular Filtration, µL/min•100 g Body Mass	86,0 1 0	85,2 0,99 -0,03	146,7 1,71 +1,97	0,115	0,922	1,27	0,296	0,613
Glomerular Zone of Adrenal Cortex, µM	193 1 0	207 1,07 +0,29	184 0,95 -0,21	0,120	0,881	2,02	0,151	0,484
Katalase Activity Plasma, µM/h•L	103 1 0	148 1,43 +1,58	125 1,21 +0,77	0,138	0,769	4,50	0,019	0,138
Mode HRV as Humoral channel, msec	124 1 0	105 0,85 -1,27	119 0,96 -0,34	0,133	0,795	3,87	0,032	0,415
Diene conjugates Plasma, E^{232}/mL	1,34 1 0	1,42 1,06 +0,20	1,50 1,12 +0,39	0,149	0,710	6,11	0,006	0,389
Sodium Plasma, mM/L	128,6 1 0	131,9 1,03 +0,65	127,7 0,99 -0,16	0,135	0,784	4,12	0,026	0,065
Cholesterol Plasma mM/L	1,57 1 0	1,70 1,08 +0,28	1,57 1,00 -0,01	0,114	0,927	1,19	0,319	0,591
Medullar Zone of Adrenals, µM	94 1 0	65 0,69 -0,93	94 1,00 -0,01	0,124	0,855	2,55	0,095	0,366
Triiodothyronine Plasma, nM/L	2,14 1 0	2,11 0,99 -0,05	2,35 1,10 +0,36	0,122	0,869	2,26	0,122	0,509
Phosphate Plasma, mM/L	0,72 1 0	1,01 1,41 +0,65	0,96 1,34 +0,53	0,129	0,823	3,24	0,053	0,104
Chloride Plasma, mM/L	94,3 1 0	95,4 1,01 +0,14	90,7 0,96 -0,51	0,120	0,882	2,00	0,153	0,061
Katalase Activity Urine, µM/h•L	123 1 0	149 1,22 +0,96	146 1,19 +0,86	0,124	0,853	2,59	0,092	0,132
Testosterone Plasma, nM/L	3,93 1 0	6,04 1,54 +1,97	4,75 1,21 +0,77	0,114	0,928	1,16	0,326	0,551
Magnesium Plasma, mM/L	0,88 1 0	0,99 1,13 +0,19	0,73 0,83 -0,24	0,113	0,933	1,09	0,351	0,412

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 variables currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Salt Waters (30)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
MxDMn HRV as Vagal tone, msec	53 1 0	37 0,70 -0,39	55 1,04 +0,05	0,100	0,948	0,79	0,463	0,179
Amplitude Mode HRV as Sympathetic tone, %	56 1 0	70 1,26 +0,84	56 1,00 -0,01	0,099	0,940	0,93	0,406	0,112
Corticosterone Plasma, nM/L	482 1 0	383 0,80 -0,78	413 0,86 -0,55	0,103	0,970	0,45	0,641	0,701
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	5,57 1 0	6,22 1,12 +1,18	6,26 1,12 +1,25	0,105	0,988	0,18	0,839	0,038
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,44 1 0	2,34 1,63 +1,09	1,39 0,97 -0,05	0,103	0,976	0,36	0,699	0,226
17-Ketosteroide Excretion, nM/24h•100g Body Mass	61 1 0	59 0,97 -0,04	75 1,22 +0,24	0,104	0,986	0,20	0,817	0,453
Adrenals Mass Index, mg/100 g Body Mass	25,2 1 0	26,8 1,06 +0,31	26,1 1,04 +0,18	0,105	0,990	0,15	0,863	0,842
Fascicular Zone of Adrenal Cortex, µM	391 1 0	398 1,02 +0,09	420 1,08 +0,34	0,104	0,983	0,25	0,778	0,483
Reticular Zone of Adrenal Cortex, µM	43 1 0	40 0,95 -0,29	43 1,01 +0,04	0,101	0,958	0,63	0,540	0,614
$(Cap•Pp)^{-0,5}$ as Calcitonin Activity	0,79 1 0	0,78 0,98 -0,05	0,78 0,92 -0,18	0,105	0,994	0,08	0,918	0,034
$(Cau•Pu)^{0,5}$ as Calcitonin Activity	3,63 1 0	3,63 1,00 0,00	3,50 0,97 -0,15	0,103	0,973	0,41	0,668	0,582
$(Pu/Cau)^{0,5}$ as Parathyroid Activity	1,76 1 0	1,80 1,02 +0,08	1,82 1,03 +0,13	0,102	0,966	0,51	0,605	0,527

Table 4. Discriminant Function Analysis Summary. Urine and erythrocytes electrolytic variables currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Salt Waters (30)	Wilks' Λ	Parti-al Λ	F to en-ter	p-level	Tole-rancy
Magnesium Urine, mM/L	2,56 1 0	2,34 0,91 -0,12	2,69 1,05 +0,07	0,103	0,976	0,36	0,699	0,226
Potassium Urine, mM/L	131 1 0	130 0,99 -0,02	122 0,93 -0,23	0,103	0,976	0,36	0,699	0,226
Calcium Urine, mM/L	2,10 1 0	2,17 1,03 +0,19	2,08 0,99 -0,03	0,104	0,986	0,20	0,817	0,453
Phosphate Urine, mM/L	6,39 1 0	6,20 0,97 -0,24	6,13 0,96 -0,33	0,105	0,990	0,15	0,863	0,842
Sodium Urine, mM/L	105 1 0	55 0,52 -0,76	126 1,20 +0,32	0,104	0,983	0,25	0,778	0,483
Chloride Urine, mM/L	115 1 0	70 0,61 -0,56	137 1,19 +0,28	0,101	0,958	0,63	0,540	0,614
Phosphates Excretion, µM/24h•100 g Body Mass	9,4 1 0	9,9 1,05 +0,08	11,5 1,22 +0,33	0,105	0,988	0,18	0,839	0,038
Potassium Excretion, µM/24h•100 g Body Mass	189 1 0	203 1,08 +0,12	197 1,05 +0,07	0,105	0,994	0,08	0,918	0,034
Magnesium Excretion, µM/24h•100 g Body Mass	3,30 1 0	3,55 1,07 +0,12	4,46 1,35 +0,56	0,099	0,940	0,93	0,406	0,112
Chloride Excretion, µM/24h•100 g Body Mass	144 1 0	107 0,74 -0,38	220 1,52 +0,76	0,100	0,943	0,88	0,424	0,022
Calcium Excretion, µM/24h•100 g Body Mass	2,90 1 0	3,22 1,11 +0,21	3,86 1,33 +0,63	0,103	0,970	0,45	0,641	0,701
Potassium Erythrocytes, mM/L	87,0 1 0	85,8 0,99 -0,18	87,5 1,01 +0,08	0,100	0,948	0,79	0,462	0,684
Sodium Erythrocytes, mM/L	22,0 1 0	22,6 1,03 +0,13	23,0 1,05 +0,23	0,104	0,986	0,20	0,817	0,453

Table 5. Discriminant Function Analysis Summary. Urine non-electrolytic variables currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Salt Waters (30)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Malondialdehyde Urine, µM/L	92 1 0	75 0,81 -0,40	92 1,00 0,00	0,103	0,973	0,41	0,668	0,582
Diene conjugates Urine, E^{232}/mL	1,86 1 0	1,68 0,91 -0,26	1,87 1,01 +0,03	0,102	0,966	0,51	0,605	0,527
Urea Excretion, µM/24h•100 g Body Mass	169 1 0	179 1,06 +0,08	262 1,55 +0,69	0,100	0,948	0,79	0,462	0,684
Urea Urine, mM/L	107 1 0	110 1,03 +0,07	131 1,22 +0,58	0,105	0,988	0,18	0,839	0,038
Uric Acid Urine, mM/L	3,68 1 0	4,29 1,17 +0,33	3,30 0,90 -0,20	0,103	0,976	0,36	0,699	0,226
Middle Mass Molecules Urine, units	182 1 0	174 0,95 -0,16	158 0,87 -0,46	0,104	0,986	0,20	0,817	0,453
Creatinine Excretion, µM/24h•100 g Body Mass	8,7 1 0	10,7 1,23 +0,46	12,5 1,43 +0,86	0,105	0,990	0,15	0,863	0,842
Creatinine Urine, mM/L	6,41 1 0	7,23 1,13 +0,45	7,16 1,12 +0,41	0,104	0,983	0,25	0,778	0,483
Amylase Activity Urine, g/h•L	202 1 0	217 1,07 +0,26	204 1,01 +0,03	0,105	0,994	0,08	0,918	0,034
Uric Acid Excretion, µM/24h•100 g Body Mass	5,72 1 0	6,02 1,05 +0,05	5,33 0,93 -0,07	0,100	0,948	0,79	0,462	0,684

Table 6. Discriminant Function Analysis Summary. Blood non-electrolytic variables as well as kidney function variables currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Salt Waters (30)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Superoxide Dismutase Erythrocytes, un/mL	58,01 0	58,21,00 +0,02	53,80,93 -0,39	0,106	0,998	0,03	0,972	0,602
Malondyaldehide Plasma, µM/L	631 0	791,25 +0,74	681,08 +0,24	0,105	0,992	0,11	0,896	0,205
Creatinine Plasma, µM/L	72,51 0	921,26 +0,79	761,05 +0,14	0,104	0,983	0,25	0,778	0,483
Bilirubin Plasma, µM/L	4,631 0	4,651,00 +0,01	4,340,94 -0,11	0,101	0,958	0,63	0,540	0,614
Urea Plasma, mM/L	7,421 0	9,461,27 +1,19	8,321,12 +0,53	0,105	0,994	0,08	0,918	0,034
Middle Mass Molecules Plasma, units	1541 0	1751,14 +0,41	1290,84 -0,48	0,099	0,940	0,93	0,406	0,112
Glucose Plasma, mM/L	4,951 0	5,491,11 +0,49	5,391,09 +0,40	0,100	0,943	0,88	0,424	0,022
Amylase Activity Plasma, g/h•L	1521 0	1541,02 +0,10	1591,05 +0,30	0,103	0,970	0,45	0,641	0,701
Uric Acid Plasma, µM/L	6621 0	6200,94 -0,12	7871,19 +0,37	0,105	0,988	0,18	0,839	0,038
Diuresis, mL/24h•100 g Body Mass	1,441 0	1,481,03 +0,05	1,831,27 +0,43	0,103	0,976	0,36	0,699	0,226
Canalicular Reabsorbtion, %	98,71 0	98,61,00 -0,05	98,91,00 +0,23	0,104	0,986	0,20	0,817	0,453
$(Ca•UA/Mg•Cr)^{0,25}$ as Lithogenicity Urine Index	0,901 0	0,901,00 0,00	0,820,91 -0,31	0,100	0,948	0,79	0,462	0,684

Table 7. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Calcium Plasma	5,92	0,005	0,799	5,92	0,005
Potassium Plasma	4,33	0,019	0,672	5,05	0,001
Sodium Excretion	5,34	0,008	0,543	5,35	10^{-4}
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	4,43	0,018	0,452	5,36	10^{-4}
Glomerular Filtration	3,21	0,050	0,393	5,11	10^{-5}
Glomerular Zone of Adrenal Cortex	1,93	0,157	0,360	4,66	10^{-5}
Katalase Plasma	1,94	0,157	0,329	4,35	10^{-5}
Mode HRV as Humoral channel	2,45	0,099	0,293	4,24	10^{-5}
Diene conjugates Plasma	2,68	0,081	0,258	4,20	10^{-5}
Sodium Plasma	1,86	0,169	0,235	4,04	10^{-5}
Cholesterol Plasma	2,13	0,133	0,210	3,97	10^{-5}
Medullar Zone of Adrenals	2,27	0,118	0,187	3,94	10^{-5}
Triiodothyronine Plasma	1,37	0,268	0,173	3,78	10^{-5}
Phosphate Plasma	2,12	0,136	0,154	3,76	10^{-5}
Chloride Plasma	1,74	0,192	0,139	3,69	10^{-5}
Katalase Urine	2,16	0,131	0,123	3,71	10^{-5}
Testosterone Plasma	1,28	0,294	0,113	3,59	10^{-5}
Magnesium Plasma	1,09	0,351	0,106	3,46	10^{-5}

The dividing information contained in 18 variables is condensed in 2 canonical discriminant roots (Table 8). The major root contains 68,7% of discriminative opportunities (r^*=0,866; Wilks' Λ=0,1058; $\chi^2_{(36)}$=86; $p<10^{-5}$) and the minor root 31,3% (r^*=0,760; Wilks' Λ=0,4227; $\chi^2_{(17)}$=33; p=0,011).

Table 8 shows standardized (normalized) and non-standardized (raw) coefficients for discriminant variables. The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots (Fig. 1).

Table 8. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Calcium Plasma	-0,209	-0,292	-0,254	-0,355
Potassium Plasma	0,967	0,616	1,277	0,813
Sodium Excretion	-0,494	-0,413	-0,0028	-0,0024
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	1,685	0,567	2,468	0,830
Glomerular Filtration	-0,121	-0,449	-0,0014	-0,0053
Glomerular Zone of Adrenal Cortex	0,474	0,364	0,013	0,010
Katalase Plasma	-1,484	0,187	-0,032	0,004
Mode HRV as Humoral channel	0,812	-0,0004	0,0412	-0,00002
Diene conjugates Plasma	-0,996	-0,035	-2,176	-0,076
Sodium Plasma	-1,856	1,139	-0,346	0,212
Cholesterol Plasma	-0,276	0,341	-0,650	0,803
Medullar Zone of Adrenals	0,439	-0,662	0,013	-0,020
Triiodothyronine Plasma	-0,112	-0,656	-0,276	-1,618
Phosphate Plasma	1,506	0,105	2,784	0,194
Chloride Plasma	1,368	-0,946	0,214	-0,148
Katalase Urine	1,221	0,039	0,031	0,001
Testosterone Plasma	-0,292	0,340	-0,142	0,166
Magnesium Plasma	0,228	0,465	0,452	0,921
	Constants		10,41	-16,16

	Eigenvalues	2,994	1,366
Cumulative Proportions		0,687	1

In the Table 9 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 the cluster of control rats in the extreme left zone of the first root axis (Fig. 1) reflects their maximally elevated levels of testosterone, circulating catechol amines, sympathetic tone and mineralocorticoid activity, on the one hand, and maximally reduced levels of parathyroid activity, corticosteronemia, vagal tone and thickness of medullar zone of adrenals.

Since control rats received the same water as intact, but through a metal tube with pre-fixation in the experimenter's hand, the detected changes in neuroendocrine status, apparently, is a manifestation of chronic aversive stress [16-20, 23,25,27].

Metabolic manifestations of chronic stress, apparently, are increased plasma levels of urea, creatinine and malondialdehyde as well as catalase activity in plasma and urine [27].

The tested mineral waters minimize or eliminate the neuroendocrine and metabolic manifestations of chronic stress, ie have a stress-limiting effect.

Table 9. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots		Daily Water	Salt Waters	Intact rats
Root 1 (68,7%)	**R1**	**R2**	**-1,73**	-0,50	+3,21
Testosterone Plasma	**-0,169**	0,148	**+1,97**	+0,77	0
1/Mode as Circulating Catechol amines	**-0,150**	0,169	**+1,27**	+0,34	0
Amplitude Mode as Sympathetic tone			**+0,84**	-0,01	0
(Ku/Nau)^{0,5} as Mineralocorticoid Activity			**+1,09**	-0,05	0
Katalase Plasma Activity	**-0,162**	0,086	**+1,58**	+0,77	0
Katalase Urine Activity	**-0,137**	-0,038	**+0,96**	+0,86	0
Malondyaldehide Plasma			**+0,74**	+0,24	0
Urea Plasma			**+1,19**	+0,53	0
Creatinine Plasma			**+0,79**	+0,14	0
Phosphate Plasma	**-0,122**	-0,043	**+0,65**	+0,53	0
Calcium Plasma	**0,268**	-0,164	**-1,24**	-0,63	0
(Cap/Pp)^{0,5} as Parathyroid Activity	**0,289**	-0,003	**-0,84**	-0,63	0
Medullar Zone of Adrenals	**0,099**	-0,241	**-0,93**	-0,01	0
Corticosterone Plasma			**-0,78**	-0,55	0
MxDMn HRV as Vagal tone			**-0,39**	+0,05	0
Root 2 (31,3%)	**R1**	**R2**	+1,94	**-0,86**	+0,65
Triiodothyronine Plasma	-0,044	**-0,229**	-0,05	**+0,36**	0
Fascicular Zone of Adrenal Cortex			+0,09	**+0,34**	0
Glomerular Filtration	-0,074	**-0,291**	-0,03	**+1,97**	0
Diuresis			+0,05	**+0,43**	0
Sodium Excretion	-0,010	**-0,297**	-0,70	**+1,00**	0
Chloride Excretion			-0,38	**+0,76**	0
Calcium Excretion			+0,21	**+0,63**	0
Magnesium Excretion			+0,12	**+0,56**	0
Creatinine Excretion			+0,46	**+0,86**	0
Urea Excretion			+0,08	**+0,69**	0
Urea Urine			+0,07	**+0,58**	
Phosphates Excretion			+0,08	**+0,33**	0
Sodium Urine			-0,76	**+0,32**	0
Chloride Urine			-0,56	**+0,28**	0
Diene conjugates Plasma	-0,059	**-0,090**	+0,20	**+0,39**	0
Uric Acid Plasma			-0,12	**+0,37**	0
Glomerular Zone of Adrenal Cortex	-0,019	**0,220**	+0,29	**-0,21**	0
Potassium Plasma	0,231	**0,178**	-0,98	**-1,21**	0
Chloride Plasma	0,040	**0,278**	+0,14	**-0,51**	0
Sodium Plasma	-0,060	**0,251**	+0,65	**-0,16**	0
Magnesium Plasma	-0,000	**0,167**	+0,19	**-0,24**	0
Middle Mass Molecules Plasmas			+0,41	**-0,48**	0
Middle Mass Molecules Urine			-0,16	**-0,46**	0
Uric Acid Urine			+0,33	**-0,20**	0
(Ca•UA/Mg•Cr)^{0,25} as Urolithogenicity			0,00	**-0,31**	0
Superoxide Dismutase Erythrocytes			+0,02	**-0,39**	0
Cholesterol Plasma	-0,033	**0,108**	+0,28	**-0,01**	0

The stress-limiting effect of mineral waters is illustrated by the shift of the localization of their cluster towards the cluster of intact animals. However, the distinction with control animals is not entirely clear. Additional delimitation occurs along the axis of the second root. The lowest location of mineral-loaded rat points reflects the maximum sampling level of triiodothyronine and the thickness of the fascicular layer of the adrenal cortex combined with

maxima of glomerular filtration, diuresis and excretion of electrolytes and nitrogenous metabolites, as well as plasma levels of diene conjugates and uric acid. On the other hand, these rats are characterized by the minimum thickness of the glomerular layer of the adrenal cortex and the minimum plasma levels of electrolytes regulated by its hormones, as well as the activity of superoxide dismutase of erythrocytes and molecules of medium mass. The level of the latter is minimal also in urine, as well as uric acid and lithogenicity of urine.

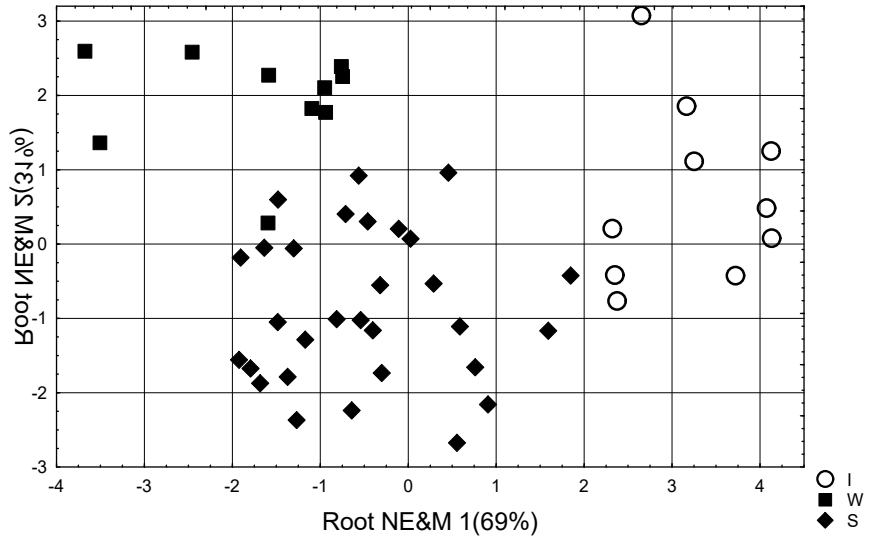

Fig. 1. Individual values of the first and second roots of the neuroendocrine and metabolic parameters in intact rats (I) and loaded with Daily water (W) and Salt waters (S)

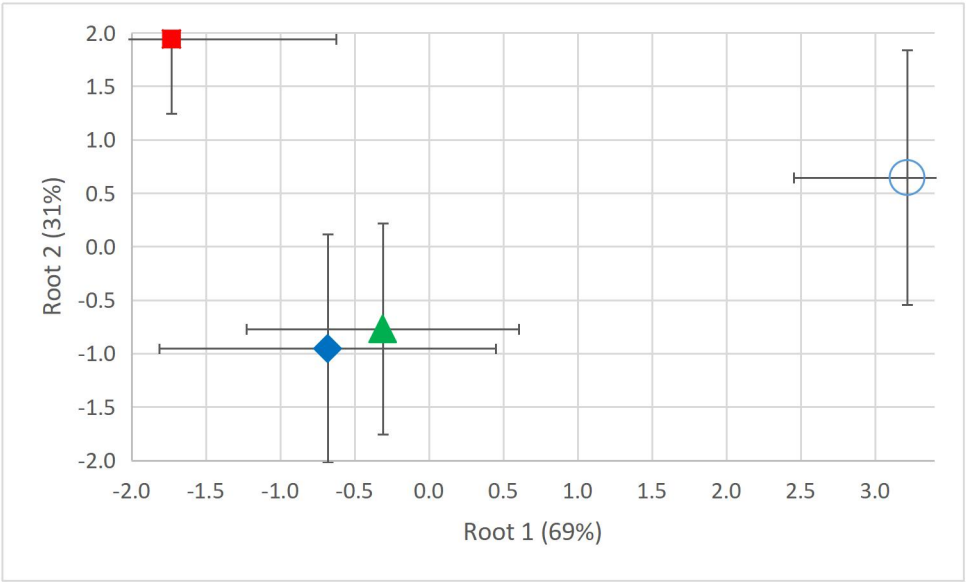

Fig. 2. Average values (Mean±SD) of the first and second roots of the neuroendocrine and metabolic parameters in intact rats (O) and loaded with Daily water and Salt waters Myroslava or Khrystyna

Figure 2 illustrates the lack of differences between the two mineral waters in the set of discriminant variables.

On the whole, in the information space of the discriminating roots, all groups are clearly delineated, that is, they differ from each other by constellation of 18 metabolic and

neuroendocrine parameters. This distinction is documented by calculating the squared Mahalanobis distances between them (Table 10).

Table 10. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=18,3) and p-levels (under diagonal)

Groups	I (10)	DW (10)	SW (30)
Intact rats (I)	**0,0**	26,1	16,1
Daily Water (DW)	**4,64 ,0001**	**0,0**	9,4
Salt Waters (SW)	**4,27 ,0002**	**2,50 ,0129**	**0,0**

The application of the classifying functions (Table 11) enables the retrospective identification of intact rats unmistakable, and the other two groups - with a single error (Table 12).

Table 11. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact rats	**Daily Water**	**Salt Waters**
Calcium Plasma	1,778	2,573	3,254
Potassium Plasma	19,33	14,07	13,36
Sodium Excretion	-0,014	-0,0032	0,0002
(Cap/Pp)^{0,5} as Parathyroid Activity	-7,293	-18,43	-17,71
Glomerular Filtration	-0,237	-0,236	-0,223
Glomerular Zone of Adrenal Cortex	0,237	0,185	0,172
Katalase Activity Plasma	-0,066	0,098	0,047
Mode HRV as Humoral channel	0,914	0,711	0,761
Diene conjugates Plasma	7,478	18,14	15,67
Sodium Plasma	24,44	26,42	25,40
Cholesterol Plasma	26,42	30,67	27,62
Medullar Zone of Adrenals	-0,240	-0,333	-0,259
Triiodothyronine Plasma	11,88	11,15	15,34
Phosphate Plasma	-41,86	-55,38	-52,48
Chloride Plasma	-16,73	-17,98	-17,30
Katalase Activity Urine	0,393	0,241	0,277
Testosterone Plasma	2,848	3,767	3,127
Magnesium Plasma	38,08	37,04	35,02
Constants	-941,7	-1012	-949,9

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

Groups	Percent correct	I p=,20	DW p=,20	SW p=,60
Intact rats (I)	100	**10**	0	0
Daily Water (DW)	90,0	0	**9**	1
Salt Waters (SW)	96,7	1	0	**29**
Total	96,0	11	9	30

CONCLUSION

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.

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).

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