CANONICAL ANALYSIS OF NEUROENDOCRINE-METABOLIC AND NEUROENDOCRINE-IMMUNE RELATIONSHIPS AT FEMALE RATS

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Abstract

Background. It has been shown before that drinking mineral waters of different composition has a significant effect on the neuroendocrine, metabolic and immune parameters of healthy rats. We analyzed the canonical correlations between neuroendocrine parameters, on the one hand, and metabolic and immune parameters, on the other hand. Materials and Methods. Experiment was performed on 58 healthy female Wistar rats 240-290 g. HRV parameters, major adaptation hormones, metabolism and immunity parameters were recorded. Results. The method of canonical correlation analysis revealed causal relationships between neuroendocrine and metabolic and neuroendocrine and immune parameters of the organism. The degree of neuroendocrine determination of individual sets of metabolic parameters ranges from 60% to 92%, and immune status from 87% to 96,5%. Conclusion. Effects of mineral waters on metabolism and immunity are realized through nervous and hormonal mechanisms. Keywords. HRV, hormones, metabolites, immunity, relationships, female rats.

INTRODUCTION

It has been shown before that drinking mineral waters of different composition has a significant effect on the neuroendocrine, metabolic and immune parameters of healthy rats [24]. A priori, effects of mineral waters, by analogy with other natural and reshaped factors, on metabolism and immunity are realized through nervous and hormonal mechanisms [8,10,13-23]. To confirm this position, we analyzed the canonical correlations between neuroendocrine parameters, on the one hand, and metabolic and immune parameters, on the other hand.
MATERIALS AND METHODS

Experiment was performed on 58 healthy female Wistar rats 240-290 g. Ten animals remained intact, using tap water from drinking ad libitum, and others received mineral water and water-salt solution of various compositions [24].

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: 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 flameless photometry; nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolphochlorite), uric acid (uricase method), medium molecular polypeptides (by spectrophotometric method), bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method) [6]; lipid peroxidation products: diene conjugates (spectrophotometry of the heptane phase of the lipids extract) [5] and malonic dyaldehyde (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) [11] and catalase plasma (at the rate of decomposition of hydrogen peroxide) [9], as well as amylase (Karavay’s amyloclastic method with starch substrate) and glucose (glucose-oxidase method) [6].

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 analyzers “Tecan” (Oesterreich), “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets and a fiery spectrophotometer “CF-47”.

According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients (Cap/Pp)0.5 and (Cap•Pu/Pp•Cau)0.25, calcitonin by coefficients (1/Cap•Pp)0.5 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].

In the blood, the parameters of immunity were determined, as described in the manual [12]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant (T-helper) and theophyllin-susceptible (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline); the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep. 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 [7,15]. 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 DISCUSSION

At the first stage, a matrix of neuroendocrine-metabolic correlations was created (Table 1).

Table 1. Matrix of correlations between neuroendocrine and metabolic parameters of rats

| Variables          | Symp tone | Glom Mass | Fasc Mass | Retic Mass | T3 | Mode | Vag tone | Corticosterone |
|--------------------|-----------|-----------|-----------|------------|----|------|----------|---------------|
| Na Urine           | -0.39     | -0.15     | -0.03     | -0.24      | -0.16 | -0.23 | 0.24     | 0.16          |
| Cl Urine           | -0.22     | -0.18     | 0.05      | -0.20      | -0.20 | -0.08 | 0.06     | 0.01          |
| K Urine            | 0.29      | 0.08      | -0.02     | 0.06       | -0.09 | 0.10  | -0.28    | -0.30         |
| Mg Urine           | 0.24      | 0.03      | -0.46     | -0.41      | 0.06  | 0.68  | -0.20    | -0.18         |
| Ca Urine           | -0.11     | -0.00     | -0.25     | -0.07      | 0.17  | -0.38 | 0.11     | 0.04          |
| Pi Urine           | -0.02     | 0.14      | -0.15     | -0.14      | 0.17  | -0.23 | 0.09     | 0.05          |
| Urea Urine         | -0.18     | 0.07      | -0.11     | -0.13      | 0.03  | -0.11 | 0.18     | 0.23          |
| Creatinine Urine   | 0.30      | -0.13     | -0.23     | -0.11      | -0.03 | 0.28  | -0.25    | -0.15         |
| Uric acid Urine    | -0.07     | 0.07      | -0.50     | 0.04       | -0.21 | -0.54 | 0.14     | 0.25          |
| Amylase Urine      | 0.28      | 0.30      | 0.16      | 0.10       | -0.16 | -0.03 | -0.29    | -0.19         |
| MMM Urine          | -0.07     | -0.12     | -0.20     | -0.16      | 0.21  | -0.26 | 0.09     | -0.03         |
| Katalase Urine     | -0.20     | -0.33     | -0.28     | -0.21      | 0.15  | -0.33 | 0.19     | 0.05          |
| MDA Urine          | 0.01      | -0.00     | -0.03     | -0.30      | -0.19 | -0.15 | -0.04    | 0.00          |
| DC Urine           | -0.33     | 0.04      | 0.03      | -0.17      | -0.25 | -0.25 | 0.07     | 0.33          |
| Osmolality Urine   | -0.29     | -0.14     | -0.05     | -0.24      | -0.18 | -0.18 | 0.14     | 0.12          |
| Na Excretion       | -0.21     | -0.26     | -0.08     | -0.27      | 0.01  | -0.24 | 0.23     | 0.23          |
| Cl Excretion       | -0.21     | -0.28     | 0.04      | -0.27      | 0.10  | -0.11 | 0.10     | 0.07          |
| K Excretion        | 0.15      | 0.22      | 0.00      | 0.01       | 0.01  | -0.22 | -0.02    | -0.14         |
| Mg Excretion       | 0.15      | 0.03      | -0.45     | -0.45      | 0.02  | 0.69  | -0.13    | -0.16         |
| Ca Excretion       | -0.07     | -0.18     | -0.23     | -0.12      | 0.23  | -0.29 | 0.07     | 0.02          |
| Pi Excretion       | -0.07     | -0.17     | -0.12     | -0.09      | 0.11  | -0.15 | -0.10    | 0.03          |
| Creatinine Excretion| 0.09     | -0.09     | 0.18      | -0.08      | 0.07  | -0.20 | 0.09     | -0.10         |
| Urea Excretion     | -0.14     | -0.12     | -0.14     | -0.11      | -0.14 | 0.12  | 0.13     | 0.03          |
| Uric acid Excretion| -0.08     | -0.19     | -0.44     | 0.06       | -0.12 | -0.50 | 0.18     | 0.20          |
| Diurese            | -0.08     | 0.21      | 0.02      | -0.01      | 0.07  | -0.03 | 0.08     | 0.02          |
| Canalicicular Reabsorption| 0.25 | 0.22 | 0.27 | 0.01 | -0.04 | 0.33 | -0.14 | -0.02 |
| Glomerular Filtration| -0.03 | 0.11 | 0.27 | -0.04 | -0.07 | 0.20 | 0.09 | 0.12 |
| Creatinine Plasma  | -0.02     | -0.19     | -0.15     | -0.13      | 0.05  | -0.12 | 0.09     | -0.17         |
| Na Erythrocytes    | -0.31     | -0.19     | -0.09     | -0.14      | -0.06 | -0.37 | 0.22     | 0.26          |
| K Erythrocytes     | 0.15      | 0.03      | -0.16     | 0.07       | 0.10  | 0.14  | -0.20    | -0.13         |
| Na Plasma           | 0.32      | -0.06     | -0.03     | 0.02       | 0.01  | -0.05 | -0.28    | -0.16         |
| K Plasma           | -0.01     | -0.21     | -0.16     | -0.08      | 0.03  | -0.34 | 0.06     | -0.07         |
| Mg Plasma          | -0.24     | 0.10      | -0.16     | -0.14      | -0.10 | -0.14 | 0.16     | 0.22          |
| Ca Plasma          | -0.01     | 0.16      | 0.31      | -0.06      | 0.06  | 0.36  | 0.03     | 0.05          |
| Pi Plasma          | 0.20      | 0.08      | -0.47     | 0.29       | -0.04 | 0.65  | -0.16    | -0.19         |
| Cl Plasma          | 0.19      | 0.06      | -0.04     | 0.03       | -0.02 | -0.08 | -0.16    | -0.08         |
| Glucose Plasma | -17 | .10 | -.03 | .18 | -.08 | -01 | .13 | .11 | -04 | -01 |
| Cholesterol Plasma | -.08 | .06 | .11 | -.25 | .02 | -.16 | .13 | .11 | -.01 | -.11 |
| Bilirubine Plasma | -.04 | -.01 | -.28 | -.30 | -.34 | .05 | -.07 | .03 | -.23 |
| Urea Plasma | .20 | -.11 | -.09 | .13 | -.09 | .02 | -.25 | -.31 | -.38 | .11 |
| Uric acid Plasma | -.23 | .02 | -.22 | -.06 | -.15 | -.27 | .24 | -.37 | -.10 | -.09 |
| MMM | -.15 | -.02 | -.00 | .01 | -.12 | .11 | -.02 | .25 | -.11 |
| Amylase Plasma | .23 | .21 | .08 | .20 | .04 | .23 | -.29 | -.23 | -.12 | .10 |
| SOD Erythrocytes | .06 | -.24 | .22 | .23 | -.14 | .00 | .00 | -.10 | .18 | .01 |
| Katalase Plasma | -.24 | -.08 | -.29 | -.23 | .11 | -.31 | .19 | .07 | .16 | .06 |
| MDA Plasma | -.29 | -.02 | -.38 | .03 | .04 | -.33 | .15 | .12 | .11 | .06 |
| DC Plasma | -.65 | .19 | -.13 | .08 | -.10 | -.15 | .48 | .52 | -.13 | -.02 |

Note. For a sample of 58 animals, the critical level of the modulus of the correlation coefficient at p<0,05 ($t>2,00$) is 0.26, at p<0,01 ($t>2,66$) is 0,34, at p<0,001 ($t>3,66$) is 0,45.

On the basis of the created matrix the canonical correlation analysis, ie the analysis of correlation between neuroendocrine and metabolic sets is carried out. The last set is divided into three subsets for convenience: urinary concentration, excretory and blood concentration. The program identified 6 pairs of canonical radicals. Neuroendocrine root was taken as causal, and metabolic - as consequential.

The factor structure of the first neuroendocrine radical is formed, in descending order of load, triiodothyronine, the thickness of the fascicular and reticular zones of the adrenal cortex as well as testosterone (Table 2).

### Table 2. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and concentration or activity in the urine of metabolites

| Neuroendocrine factors | Root 1 | Root 2 |
|------------------------|--------|--------|
| Triiodothyronine       | .973   | .015   |
| Fascicular ZAC         | .712   | .172   |
| Reticular ZAC          | -.326  | .245   |
| Testosterone           | .268   | .169   |
| Sympathetic tone       | .286   | .639   |
| Catecholamines (1/Mode)| -.285  | .585   |
| Adrenals Mass Index    | .029   | .562   |
| Glomerular ZAC         | -.035  | .274   |
| Corticosterone         | -.045  | .166   |
| Vagal tone             | -.283  | -.585  |

| Metabolic parameters   | Root 1 | Root 2 |
|------------------------|--------|--------|
| Uric acid Urine Concen| -.578  | -.220  |
| Ca Urine Concentration | -.422  | .225   |
| Katalase activity Urine| -.328  | -.049  |
| Middle Mass Molecules U| -.280  | .186   |
| Pi Urine Concentration | -.273  | .285   |
| Mg Urine Concentration | .717   | .057   |
| Creatinine Urine Concen| .317   | .155   |
| Malonic dyaldehyde Urine| .137  | -.252  |
| K Urine Concentration  | .112   | .082   |
| Diene conjugates Urine | .066   | -.505  |
| Na Urine Concentration | -.209  | -.323  |
| Cl Urine Concentration | -.044  | -.269  |
| Osmolality Urine       | -.165  | -.264  |
| Urea Urine Concentration| -.148  | -.048  |
| Amylase activity Urine | -.019  | .469   |

359
The concentration or activity in the urine of metabolites is represented in the canonical radical by uric acid, calcium, catalase, medium mass molecules and phosphates inversely, therefore, their level is negatively affected by the listed hormonal constellation. In contrast, the positive effects of urine concentrations of magnesium, creatinine, malonic dialdehyde and potassium. As a result, we state the determination of endocrine factors levels in the urine of these metabolites by 92% (Fig. 1 above).

Fig. 1. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and urinary concentrations of metabolites (Y-axis)

The second neuroendocrine radical is represented directly by sympathetic tone, circulating catecholamines (marked by the inverse value of Mode HRV), adrenals mass, thickness of the glomerular zone of their cortex and corticosteronemia, while inverse by vagal tone. The metabolic canonical radical receives negative factor loads, primarily from the concentration of diene conjugates, as well as the osmolality of urine and its forming concentrations of sodium, chloride and urea. Instead, amylase activity gives a positive load.
As a result, the determination of neuroendocrine factors in the levels of urine of these metabolites is 69% (Fig. 1 below).

Canonical analysis of neuroendocrine-excretory connections revealed the following (Table 3).

Table 3. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and metabolites excretion parameters

| Neuroendocrine factors          | Root 1 | Root 2 |
|---------------------------------|--------|--------|
| Triiodothyronine                | .980   | .041   |
| Fascicular ZAC                  | .712   | -.157  |
| Reticular ZAC                   | .317   | .342   |
| Testosterone                    | .182   | .072   |
| Catecholamines (1/Mode)         | .235   | .045   |
| Vagal tone                      | -.225  | .189   |
| Sympathetic tone                | .201   | .457   |
| Glomerular ZAC                  | .103   | .331   |
| Adrenals Mass Index             | -.045  | .154   |
| Corticosterone                  | -.126  | -.362  |

| Metabolic parameters            | Root 1 | Root 2 |
|---------------------------------|--------|--------|
| Mg Excretion                    | .713   | .070   |
| Glomerular Filtration           | .268   | .025   |
| Creatinine Excretion            | .214   | -.026  |
| Uric acid Excretion             | -.532  | .196   |
| Ca Excretion                    | -.348  | -.106  |
| Pi Excretion                    | -.170  | -.119  |
| Urea Excretion                  | -.158  | -.116  |
| Cl Excretion                    | -.074  | -.468  |
| Osmolality Urine                | -.155  | -.440  |
| Na Excretion                    | -.226  | -.366  |
| Diurese                         | -.028  | -.156  |
| K Excretion                     | .020   | -.136  |
| Canalicular Reabsorption        | .397   | .382   |

The factor structure of the first neuroendocrine radical receives positive loads from triiodothyronineemia, thickness of the fascicular and reticular zones of the adrenal cortex, testosteroneemia and catecholaminemia, while negative from the vagal tone. Glomerular filtration and excretion of magnesium and creatinine are directly represented in the effective canonical radical. Instead, negative loads give the levels of excretion of uric acid, calcium, phosphates and urea. As a result, the determination of neuroendocrine factors of these parameters of excretory function of the kidneys is 88% (Fig. 2 above).

The second neuroendocrine radical is represented directly by sympathetic tone, thickness of the glomerular zone of the adrenal cortex and their mass, while inverse by corticosteronemia. The metabolic canonical radical receives significant negative factor loads from urinary excretion of chloride and sodium, as well as related urine osmolality, and minor from diuresis and potassium excretion, which reflects the negative impact on these parameters of sympathetic tone and mineralocorticoids. In contrast, tubular water reabsorption is negatively related to plasma corticosterone levels. As a result, we state the determination by neuroendocrine factors of this set of parameters of excretory function of the kidneys by 60% (Fig. 2 below).
The analysis of the canonical correlation of regulatory factors with metabolic parameters of blood revealed that the factor structure of the first radical is exclusively endocrine and usually receives significant positive loads, in descending order, from triiodothyronemia, fascicular, reticular and glomerular zones thickness as well as testosteroneemia (Table 4).
Table 4. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and blood concentration of metabolites

| Neuroendocrine factors   | Root 1 | Root 2 |
|--------------------------|--------|--------|
| Triiodothyronine         | .875   | -.037  |
| Fascicular ZAC           | .677   | -.122  |
| Reticular ZAC            | .436   | .298   |
| Glomerular ZAC           | .295   | .241   |
| Testosterone             | .231   | .184   |
| Vagal tone               | -.210  | .630   |
| Sympathetic tone         | -.399  | -.603  |
| Catecholamines (1/Mode)  | .287   | -.395  |
| Adrenals Mass Index      | -.195  | -.250  |
| Corticosterone           | -.054  | -.063  |
| **Metabolic parameters** |        |        |
| Bilirubine               | -.516  | -.346  |
| Malonic dialdehyde       | -.447  | .352   |
| Katalase activity        | -.439  | .036   |
| Na Erythrocytes          | -.367  | .254   |
| K                         | -.346  | -.246  |
| Middle Mass Molecules    | -.218  | -.090  |
| Creatinine               | -.187  | .054   |
| Cholesterol              | -.116  | -.162  |
| Pi                       | .704   | -.067  |
| Amylase Activity         | .234   | .016   |
| K Erythrocytes           | .210   | .026   |
| Superoxide dismutase     | .185   | -.072  |
| Na                       | .090   | -.054  |
| Cl                       | .030   | .008   |
| Diene conjugates         | -.246  | .730   |
| Mg                       | -.102  | .490   |
| Uric acid                | -.206  | .358   |
| Glucose                  | -.010  | .220   |
| Urea                     | -.009  | -.108  |

This endocrine network has a **negative** effect on plasma levels of bilirubin, malonic dialdehyde, potassium, medium weight molecules, creatinine and cholesterol, catalase activity, as well as sodium levels in erythrocytes. In contrast, these endocrine factors have a **positive** effect on plasma phosphate, sodium and chloride levels, plasma amylase activity and erythrocyte superoxide dismutase, as well as their potassium content. The degree of endocrine-metabolic determination is 81% (Fig. 3 above).
The second neuroendocrine radical directly represents the vagal tone, while the inverse - sympathetic tone, catecholamines, adrenal mass and corticosteronemia. Positive factor loads on the corresponding metabolic radical from plasma levels of diene conjugates, magnesium, uric acid and glucose reflect their direct dependence on vagal tone and inverse - on sympathetic tone and catecholamines. Significant negative load on the radical from bilirubinemia reflects its positive relationship with the mass of the adrenal glands. In contrast, plasma urea levels are directly dependent on corticosteronemia, catecholaminemia, and sympathetic tone (see Table 3.4). As a result, the determination of neuroendocrine factors of this set of blood plasma metabolites is 72% (Fig. 3 below).

Following the accepted algorithm, a matrix of notable correlations between neuroendocrine indicators, on the one hand, and immunity indicators, on the other, was first created (Table 5).
Table 5. Matrix of correlations between neuroendocrine and immune parameters

| r | HL CG | HI CG | HT CG | FN N | FN M | Fi M | FN I | Fl N | Pla S | Ret S | Fib S | Mac S | Thy mus | Lc T | Ret T | Epi T | Mo noc | Ly mph | NK | Th | Te | B |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tr | -0.89 | ,22 | ,47 | -0.60 | ,27 | -0.40 | -0.24 | ,36 | ,87 | ,23 | ,90 | -0.21 | |
| AMo | ,28 | -0.28 | -0.31 | ,31 | -0.31 | ,69 | -0.30 | ,29 | ,29 | ,30 | |
| CA | ,28 | -0.23 | -0.34 | ,34 | -0.22 | ,65 | -0.26 | ,31 | ,30 | ,27 | |
| DX | -0.25 | ,28 | ,27 | -0.24 | ,30 | -0.43 | 0.24 | -0.22 | -0.33 | ,24 | -0.29 | ,22 | |
| Med | ,28 | ,53 | ,52 | -0.29 | -0.38 | ,23 | -0.37 | -0.35 | ,28 | |
| CTA | -0.25 | ,24 | ,28 | ,41 | ,35 | -0.29 | -0.36 | -0.21 | -0.23 | |
| PTA | -0.25 | ,24 | ,28 | ,41 | ,35 | -0.29 | -0.36 | -0.21 | -0.23 | |
| MC | ,23 | ,34 | ,30 | -0.22 | ,24 | ,28 | ,28 | ,20 | ,20 | |
| Glo | -0.21 | -0.25 | -0.32 | ,46 | ,24 | |
| Cort | -0.27 | ,31 | ,37 | ,25 | 0.19 | ,23 | ,63 | ,56 | ,23 | |
| Test | -0.23 | ,28 | ,32 | ,32 | 0.19 | ,23 | ,63 | ,56 | ,23 | |
| Ret | -0.26 | |

For further consideration, two pairs of significantly related pairs of canonical radicals were selected (Table 6).

Table 6. Factor structure of two pairs of canonical roots, which represent neuroendocrine and immune parameters

| Neuro-endocrine factors | Root 1 | Root 2 |
|------------------------|--------|--------|
| Triiodothyronine       | 0.935  | 0.181  |
| Fascicular Zone Adrenal Cortex | 0.596 | 0.348 |
| Mineralocorticoid activity | 0.408 | -0.357 |
| Reticular Zone Adrenal Cortex | 0.335 | -0.082 |
| Parathyroid activity | 0.290 | 0.009 |
| Catecholamines (1/Mode) | 0.289 | -0.287 |
| Testosterone           | 0.173  | -0.144 |
| Medullar Zone Adrenal | -0.435 | 0.503 |
| Calcitonin activity | -0.319 | 0.074 |
| Vagal tone (MxDMn) | -0.285 | 0.011 |
| Glomerular Zone Adrenal Cortex | 0.138 | -0.488 |
| Sympathetic tone (AMo) | 0.344 | -0.484 |
| Corticosterone | -0.056 | 0.379 |

| Immunity               | Root 1 | Root 2 |
|------------------------|--------|--------|
| NK Lymphocytes Blood   | 0.928  | 0.134  |
| Monocytes Blood        | 0.909  | 0.128  |
| Microbial Count Monocytes | 0.496 | 0.248 |
| Epithelioocytes Thymus | 0.435  | -0.214 |
| EntropyThymocyctogram | 0.256  | -0.287 |
| Reticuloocytes Thymus  | 0.213  | 0.078  |
| Phagocytic Index Monocytes | 0.185 | 0.201 |
| Reticuloocytes Spleen  | 0.139  | 0.172  |
| Microbial Count Neutrophils | -0.908 | -0.144 |
|                          | R   | R²  |
|--------------------------|-----|-----|
| Phagocytic Index Neutrophils | -0.610 | -0.275 |
| Plasmocytes Spleen       | -0.510 | 0.223 |
| Lymphoblasts Spleen      | -0.334 | 0.188 |
| Lymphocytes Thymus       | -0.310 | 0.261 |
| Pan-Lymphocytes Blood    | -0.257 | -0.043 |
| T-helper Lymphocytes Blood | -0.129 | -0.079 |
| Macrophages Spleen       | 0.218 | -0.568 |
| Thymus Mass Index        | 0.277 | -0.425 |
| Entropy Immunocytogram   | -0.028 | -0.423 |
| T-cytolytic Lymphocytes Blood | 0.097 | -0.347 |
| Entropy Leukocytogram   | 0.181 | -0.344 |
| B-Lymphocytes Blood      | -0.126 | -0.232 |
| Fibroblastes Spleen      | 0.094 | 0.155 |

R=0.982; R²=0.965; \( \chi^2_{(345)}=533; p<10^{-6} \); Λ Prime=10^{-6}
It was found that the neuro-endocrine root of the first pair receives the maximum positive factor load from triiodothyronine, less pronounced from markers of glucocorticoid, mineralocorticoid and androgenic functions of the adrenal cortex, circulating catecholamines, and parathyroid activity, instead negative from adrenaline-secreting adrenal medullary zone, vagal tone and calcitonin activity. And the immune root is represented by the parameters of the blood, thymus, as well as plasma cells and lymphoblastes of the spleen, which are subject to the types of upregulation/downregulation. The degree of neuroendocrine immunomodulation is very significant – 96.5%.

The neuro-endocrine root of the second pair is represented by sympathetic tone, glomerular zone of the adrenal cortex and, conversely, corticosterone. Sympathetic tone carries out upregulation of splenic macrophages. Corticosterone has a suppressive effect on T-killers and B-lymphocytes and reduces the entropy of the leukocytogram and thymocytogram. Mineralocorticoids are responsible for increasing the mass of the thymus and reducing the content of fibroblasts in the spleen. The degree of immunomodulation by this neuroendocrine constellation is less pronounced - 87%.

**CONCLUSION**

Effects of mineral waters on metabolism and immunity are realized through nervous and hormonal mechanisms.

**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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