International Medical University, Odesa, Ukraine
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The monograph systematizes these writers and highlights the results of their own priority experimental and clinical-physiological studies of the impact of drinking mineral waters of Ukraine on neuroendocrine regulation, metabolism and immunity of healthy rats and patients in the process of rehabilitation of chronic pyelonephritis and cholecystitis in remission. In line with the concepts of functional-metabolic continuum and neuroendocrine-immune complex using the methods of factor, discriminant and canonical correlation analysis, it is demonstrated that mineral waters have both similar and specific physiologically favorable modulating effects on the parameters of the studied body systems.

For specialists in medical rehabilitation, endocrinologists, immunologists, biochemists, pathophysiologists.

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CONTENT

SUMMARY

The monograph reflects the results of priority experimental and clinical-physiological studies, carried out in line with the concepts of functional-metabolic continuum and neuroendocrine-immune complex, the impact of drinking mineral waters of Ukraine on neuro-endocrine regulation, metabolism and immunity of healthy rats and patients of the spa in the process of restorative treatment of chronic pyelonephritis and cholecystitis in the phase of remission.

In the first experiment on healthy female rats, factor analysis shows that information about 100 neuroendocrine, metabolic and immune parameters is recorded in 12 principal components and three common factors. The method of canonical correlation analysis revealed causal relationships between neuroendocrine and metabolic as well as neuroendocrine and immune parameters of the body. The measure of neuroendocrine determination of individual sets of metabolic parameters ranges from 60% to 92%, and immune status – 87%.

The method of discriminant analysis revealed 6 endocrine (adrenal mass, mineralocorticoid, calcitonin and parathyroid activity, as well as heart rhythm rate), 9 metabolic (creatinineemia and creatinineuria, concentration of potassium in plasma and urine, plasma levels of calcium, malone dialdehyde and medium-mass molecules, as well as glomerular filtration) and 6 immune (spleen mass and its reticulocyte content, content of epitheliocytes and macrophages in the thymus, content in the blood the rod-shaped neutrophils, as well as the microbial number of microphages) of indicators that together characterize the **nonspecific** (general) reaction of the body to the water-salt load as such, without reference to the specifics of the chemical composition of the applied liquids.

The search for **specific** manifestations of the integral reaction to various water-salt loads revealed 35 parameters, among them, 4 reflect endocrine regulation (the level in the plasma of testosterone and the thickness of their secreting reticular zone of the adrenal cortex, as well as parathyroid and calcitonin activity, assessed by their subordinate effects by the parameters of calcium and phosphate metabolism), 9 - glomerular filtration and mineral metabolism, 5 - nitrogenous and carbohydrate metabolism, 3 – lipoperoxidation, 4 - elements of splenocytogram, 8 - elements of immunocytogram and leukocytogram of blood and parameters of phagocytosis, as well as the mass of the thymus and the content of thymocytes in it, according to the totality of which intact female rats and drunk with tap water, medicinal waters of Naftussya, Sofiya, Hertsa and its artificial salt analogue are identified with an accuracy of 100%.

Through canonical correlation analysis of the relationship between the chemical composition of irritant liquids, on the one hand, and the metabolic and neuroendocrine-immune complex of animals, on the other hand, it was found that the content of organic nitrogen and carbon in liquids determines the level of a number of endocrine parameters by 39%, metabolic - by 53%, immune - by 77%. The measure of determination on the part of trace elements is 59%, 32% and 84%, while on the part of electrolytes 33%, 36% and 66%, respectively.

In the second experiment on healthy female rats, divided into 4 groups (intact, control tap water and two main) show that the sulphate-chloride sodium-magnesium mineral waters Myroslava (5 g/l) and Khrystyna (10 g/l), consumed for 6 days, equally effectively prevent the increase in the thickness of the glomerular zone of the adrenal cortex and the mineralocorticoid activity caused by it, as well as glycemia and amylaseuria, thymus mass and content in thymocytogram of endotheliocytes and macrophages in splenocytogram as well as phagocytic index of blood neutrophils. Significantly increased due to stress levels of testosterone and plasma catalase, thymocytogram plasmacytes and immunocytogram entropy under the influence of mineral waters are reduced to the upper zone of normal. On the other hand, both mineral waters equally prevent stress-induced reduction in the thickness of the reticular zone of the adrenal cortex, triiodothyronineemia, parathyroid gland activity, calciemia, excretion of sodium and chloride in the urine, concentrations in the urine of malone dialdehyde, as well as the relative content of monocytes in the blood and the activity and intensity of phagocytosis of Staphylococcus aureus by monocytes.

At the same time, differences in the integral effects of mineral waters have been identified. The Myroslava water deepens the stress-induced decrease in plasma corticosterone levels, activity of superoxide dismutase of red blood cells, levels of lymphoblasts in thymocytogram, plasma cells in splenocytogram and spleen mass, general leukocytes in the blood and levels in the leukocytogram of eosinophils, as well as the intensity of phagocytosis of bacteria by neutrophils and the reaction of transformation of T-lymphocytes into blasts under the influence of PhHA. Instead, Khrystyna water does not affect this constellation of parameters. Stress-insensitive parameters (amylasemia, red blood cell sodium, magnesiumuria, levels of lymphoblasts and reticulocytes in splenocytogram, T-cytolytic lymphocyte content in immunocytogram, and killing index of blood neutrophils) are increased under the influence of Khrystyna water, while Myroslava water is ineffective with respect to these parameters. Instead, Myroslava water, unlike Khrystyna water, initiates an increase in the entropy of leukocytogram and thymocytogram, the content of epitheliocytes, macrophages and reticulocytes in thymocytogram, as well as eosinophils in splenocytogram and natural killers in the blood.

In clinical-physiological observation of men with chronic pyelonephritis and cholecystitis in the remission phase, who received three variants of drinking balneotherapy (only bioactive water Naftussya; water Naftussya

in combination with mineral water Myroslava or Khrystyna), the same physiologically favorable changes were found:

a) parameters of the electroencephalogram: an increase within the norm of the initially normal levels of power spectral density (PSD) of the beta-rhythm in the loci Fp2 and T4, theta-rhythm in locus F7 and entropy of PSD in locus T5 in combination with a reduction within the norm of the initially normal levels of PSD and its entropy in the Fp2 locus, as well as a left-sided displacement of the originally symmetrical theta- and beta-rhythms;

b) normalization of moderately elevated plasma testosterone level;

c) metabolic parameters: increase of moderately elevated daily diuresis, movement of phosphaturia from the lower zone of normal to the upper zone, reduction of hypocreatinuria and hypercreatininemia, decrease within the norm of urine concentration of sodium;

d) immune parameters: an increase to the lower zone of the norm of reduced bactericidity of blood neutrophils against both Staphylococcus aureus and Escherichia coli and an increase in normal serum IgG level;

e) increase to the lower zone of the norm of reduced content in the feces of lactic acid Bifidobactera and Lactobacilli in combination with a decrease in the increased content of the strain Escherichia coli with weakened enzymatic activity, that is, reduction of dysbiosis;

f) reduction of moderately expressed bacteriuria;

e) normalization of reduced reactivity of the gallbladder to a standard cholekinetic.

Complex balneotherapy by interval use of sulfate-chloride sodium-magnesium mineral waters together with Naftussya water causes significant changes in the constellation of neuroendocrine, metabolic and immune parameters that are different from the effects of monotherapy by Naftussya water.

In particular, initially reduced neuro-endocrine (VLF and ULF bands of HRV, calcitonin, triiodothyronine, aldosterone), metabolic (concentration in urine of phosphates, calcium, magnesium and creatinine, phosphaturia, kaliemia, calciemia and cholecystokinetics activity) variables, as well as the completion of phagocytosis of Staphylococcus aureus increase, as a rule, to the normal zone. On the other hand, initially elevated urinary excretion and concentration of sodium as well as plasma levels of creatinine and urea decrease.

At the same time, there is an increase in the initially normal levels of vagal tone, parathyroid activity, plasma testosterone, magnesium and chloride excretion and plasma interleukins 1 and 6, as well as a decrease in the initially normal levels of uric acid concentration in urine and glucose and low-density lipoprotein cholesterol in plasma, as well as the intensity of phagocytosis of Staphylococcus aureus. The latter pattern forms initially reduced plasma levels of sodium, phosphates and chloride, urine chloride and potassium, as well as “active” T-lymphocytes, which continue to decline.

When analyzing EEG, two patterns of different-core neurotropic effects of mineral waters were identified. The first pattern reflects more or less pronounced activation of the delta-rhythm generating neurons, projected onto O2 and F7 loci, the alpha-rhythm generating neurons, projected onto P3, F4, T4 and C3 loci, the beta-rhythm generating neurons, projected onto F8, Fp2 and F4 loci, as well as right-sided symmetry shear of beta- and alpha-rhythms. Instead, the antipode pattern reflects the inhibition of the delta-rhythm generating neurons projected onto the T5, P3, C4, and F8 loci, the alpha-rhythm generating neurons projected onto the F8 locus and the theta-rhythm generating neurons projected onto Fp2, T4, F7, F8, O2 and T4 loci, as well as left-sided symmetry shift of theta-rhythm and decreased EEG entropy in T5, T4, O2, T6, P3 and F7 loci.

A screening of differences in mineral waters balneoeffects revealed 37 parameters grouped into 5 patterns. The first pattern combines 11 parameters, which, under the influence of Myroslava water, decrease, and under the influence of Khrystyna water increase. In particular, these are PSD of beta-rhythm in loci Fp1, F4, F8, C4, T6 and O2, as well as LF band HRV; diastolic but not systolic blood pressure; plasma aldosterone, potassium excretion, and blood monocyte relative levels. As for the other 7 parameters of the second pattern (PSD T5-β and F4-α; plasma sodium and chloride; blood “active” T-lymphocytes, as well as both variants of Popovych’s Strain Index of leukocytogram) Myroslava water acts similarly, while Khrystyna water is ineffective. At 8 parameters, both mineral waters have a stimulating effect, while Myroslava is inferior to Khrystyna. In particular, this is diuresis and excretion of creatinine, uric acid and magnesium; serum IgG level, completion of E. coli phagocytosis by blood neutrophils; content in feces of the usual strain E. coli, as well as cholecystokinetics index. It should be noted that the content in feces of E. soli strains with hemolytic and weakened enzymatic ability, as well as Klebsiela&Proteus, decreases equally. On 9 parameters of the fourth pattern water Myroslava water has a stimulating effect, while Khrystyna water inhibitory. In particular, these are the PSD of delta-rhythm in the loci Fp1, F4, C3 and C4, as well as the VLF band of the HRV and the Kerdoe’s vegetative index; plasma cortisol and serum IgM levels. Finally, sodium excretion and leukocyturia decrease under the influence of both mineral waters, but to a greater extent under the influence of Khrystyna water.

So, mineral waters Myroslava and Khrystyna have both similar and different effects on the body. It is important that the differences are manifested not only in the severity of changes in the registered parameters, but even in their directness. Since the chemical composition of both mineral waters is qualitatively identical, the difference in physiological effects is obviously due to their total mineralization (5 g/l and 10 g/l, respectively). In

addition, it should be borne in mind that patients consumed these waters in combination with biologically active Naftussya water. However, the specificity of physiological effects is confirmed in the experiment in rats.

Based on the data of previous research of Truskavetians Scientific School of Balneology, the authors put forward and the obtained data confirmed the hypothesis that the primary effect of mineral waters is the modulation of the activity of the structures of the autonomic and central nervous and endocrine systems, which, in turn, have a regulatory modulating effect on the immune system, microbiota, metabolism, cholekinetics, blood pressure and, apparently, other, not yet recorded parameters of the body.

The materials of the monograph are reflected in the following articles of the authors

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INTRODUCTION

According to the modern paradigm, three regulatory systems participate in the maintenance of homeostasis: nervous, endocrine and immune systems. It is the close and continuous functional relationship of the nervous, hormonal and immune systems, which is based on the existence of common and similar receptor structures, that determines the high adaptive ability of the body (Akmaev, 1996). The interactions of the nervous and endocrine systems in this process are well studied and became the basis for the allocation of an independent field of knowledge - neuroendocrinology (Akmaev, 1990). Interactions of neuroendocrine and immune systems are intensively studied and considered as the most intriguing area of modern research - immunoneuroendocrinology (Korneva et al., 1993; Neuroimmunomodulation, 2000; Markovich, 2004; Sternberg, 2006; Nance, Sanders, 2007; Thayer, Sternberg, 2010; Chavan et al., 2017; Chavan, Tracey, 2017; Pavlov et al., 2018; Chang et al., 2019; Gozhenko et al., 2019; Popovych et al., 2020).

Since the landmark discovery of Selye, (1936) it is known that the nonspecific pathogenetic basis of many chronic diseases is stress (general adaptation syndrome). This concept is developed by its numerous adepts (Horizontov et al., 1983; Meerson, 1986, Meerson, 1993; Kolyada et al., 1995; Reznikov, 1998; 2019; Stress of Life, 1998; Radchenko, 2004; Reznikov et al., 2004; Baraboy, Reznikov, 2013; Dhabhar, 2009; 2018; Popovych, 2011; Gozhenko et al., 2019; Popovych et al., 2020).

The physiological antipode of stressors are adaptogens that have a stress-limiting effect by inducing anti-stress general adaptation reactions. The level of resistance of the organism is determined by the quality of its general adaptation reaction (Garkavy et al., 1990, Garkavy et al., 1998; Garkavy et al., 2000). By Garkavy et al., (1990), adaptogens should be considered all stimuli and effects that, when exposed to the body, can cause a particular general adaptive reaction. However, most authors to adaptogens include only substances that can cause a state of "***nonspecific increased resistance***" of the body to the effects of adverse environmental factors of physical, chemical and biological nature (Brekhman, 1957; Brekhman, 1968; Brekhman, 1987; Dardymov, 1976; Saratikov, Krasnov, 1987; Kaplan et al., 1990; Yakovlev et al., 1990; Popovych, 2011). Another hypostasis of the adaptogenic action of the means is their regulatory effect, that is, the normalization of the deviated parameters of the body, regardless of their orientation (Saratikov, Krasnov, 1987).

Panossian et al., (2021) gave the following definition in a recent excellent review. ***Adaptogens comprise a category of herbal medicinal and nutritional products promoting adaptability, resilience, and survival of living organisms in stress.*** The authors summarized the growing knowledge about common adaptogenic plants used in various traditional medical systems and conventional medicine and to provided a modern rationale for their use in the treatment of stress-induced and aging-related disorders. Adaptogens have pharmacologically pleiotropic effects on the neuroendocrine-immune system, which explain their traditional use for the treatment of a wide range of conditions. They exhibit a biphasic dose-effect response: at low doses they function as mild stress-mimetics, which activate the adaptive stress-response signaling pathways to cope with severe stress. That is in line with their traditional use for preventing premature aging and to maintain good health and vitality. However, the potential of adaptogens remains poorly explored. Treatment of stress and aging-related diseases require novel approaches. Some combinations of adaptogenic plants provide unique effects due to their synergistic interactions in organisms not obtainable by any ingredient independently. Further progress in this field needs to focus on discovering new combinations of adaptogens based on traditional medical concepts. Robust and rigorous approaches including network pharmacology and systems pharmacology could help in analyzing potential synergistic effects and, more broadly, future uses of adaptogens. In conclusion, the evolution of the adaptogenic concept has led back to basics of traditional medical systems and a new level of understanding of holistic approach. It provides a rationale for their use in stress-induced and aging-related diseases.

One of the long-known and widely used adaptogenic agents are balneofactors. The theoretical basis of balneotherapy and balneorehabilitation is the Gozhenko's & Gozhenko's concept of sanogenesis (Gozhenko, Gozhenko, 2007). Their main task is to increase the resistance of the body, both general and immune, in order to prevent the encumbrance of aseptic inflammation with infectious, metaphylaxis of relapses in patients in the phase of remission, deepening and prolongation of the latter, suppression of the latent inflammatory process (Smiyan, 1967; Smiyan, 1973; Smiyan, 2008; Bogolyubov, Zubkova, 1995; Seredyuk et al., 1995; Seredyuk, 1998; Popovych et al., 2003; Kostyuk et al., 2006; Babov et al., 2009; Popovych, 2011).

Back in 1993, Korneva, (1993) put forward the hypothesis of the existence of "***the triune immune-neuro-endocrine complex that is involved in ensuring the permanence of the internal environment of the body***". Popovych, barely changing the term "**neuroendocrine-immune complex**" (Popovych, 2009), initiated large-scale studies of the immediate and long-term (course) effects on it of the famous bioactive water **Naftussya** ("***Queen of medicinal waters***"), which is a combination of non-antigenic (organic oil-like substances) and antigenic (autochton microflora) stimuli. The results were published in a number of monographs (Dranovskiy, Popovych, 2010; Popovych, 2011; Chebanenko et al., 2011; Chebanenko et al., 2012; Chebanenko et al., 2013; Chebanenko et al., 2015; Popovych et al., 2014; Kozyavkina et al., 2015; Sydoruk et al., 2018; Gozhenko et al., 2019; Popovych et al., 2020; Gozhenko et al., 2021) and numerous articles of Truskavetsian Scientific School of

Balneology, which aroused great interest among specialists and were recognized by the expert as the main trend of the last decade in Ukrainian balneology (Portnichenko, 2015).

Together with the components of the neuroendocrine-immune complex, the object of research was the traditional parameters of water-salt, lipid and nitrogenous metabolism, hemodynamics, cholekinetics, gastric and pancreatic secretion (Ivassivka et al., 2004; Popovych et al., 2005; Kostyuk et al., 2006; Gumega et al., 2011) and their connections with neuroendocrine and immune parameters in line with the Gozhenko's concept of functional-metabolic continuum (Gozhenko, 2016).

Against this background, studies in line with the concepts of the neuroendocrine-immune complex and the functional-metabolic continuum of other drinking mineral waters of Ukraine are very inferior in methodical level (Babov et al., 2009).

Therefore, the topic of research remains very relevant.

CHAPTER 1

INFLUENCE OF DRINKING MINERAL WATERS ON METABOLISM AND NEUROENDOCRINE-IMMUNE COMPLEX (literature review)

Mineral waters are a natural healing resource that has long been used by humans to correct health problems. In accordance with Directives 98/38/EC and 2009/54/EC, natural mineral waters are "waters originating from an aquifer or underground reservoir derived from one or more natural sources or wells which have specific hygienic features and specific properties" (Comission …1998; 2009).

According to the federal rules, section 21; paragraph 165 (drinks) - mineral waters (MW) - "waters containing at least 250 ppm of dissolved solids (TDS) that come from the source through one or more wells, springs, reservoirs and arise from a geologically and physically protected underground source". According to the dry residue content, the following groups of MWs are distinguished: <0,5 g/l - light mineralization; 0,5-5 g/l - water with a low mineral content; 5–15 g/l – middling mineralization; >15 g/l – significant mineralization (Quattrini et al., 2016).

In Ukraine, due to the long-term study and use of MW, classifications original in relation to European classifications have been developed. This direction of classifications is based not only on the assessment of the content of the main mineral, but also on the presence of a biologically active agent (organic carbon, iron, radon, silicon, boron, etc.).

In addition to classifications of MWs according to physical and chemical characteristics, classifications of mineral waters are now proposed on the basis of their biological action. The first direction is characterized by a classification that is carried out in accordance with the criteria proved by DSTU-878-93. Despite the fact that in mineral waters the content of trace elements is insignificant, their physiological effect is very clear and causes, in some cases, recommendations for the use of these waters (arsenic, iron, cobalt, copper, manganese, iodine, zinc, etc.) (Albertini et al., 2007; Rilander, 2008).

The leading institution of Ukraine for the study of balneofactors (drinking mineral waters, waters for external use, therapeutic mud, etc.) is considered the Ukrainian Scientific Research Institute of Medical Rehabilitation and Balneology of the Ministry of Health (Odesa). The results of the research by the staff of the Institute of drinking mineral waters are concentrated in their monograph "Features of the Biologic Effects of Mineral Waters of Different Mineralization" (Babov et al., 2009) and a number of articles (Alekseyenko, 2005; Nasibullin, Gushcha, 2005; Babov et al., 2006; Kolodenko et al., 2016; Gushcha et al., 2018; Gushcha et al., 2018a; Gushcha et al., 2018b; Gushcha et al., 2019; Gushcha et al., 2019a; Dragomiretska et al., 2020).

Next, **we quote the authors with the preservation of their style**.

Course use of MWs without specific components causes, first of all, a reaction from peripheral blood, which is manifested by an increase in the total number of leukocytes and changes in the ratio of cellular elements of blood. The number of neutrophils (nonspecific cellular protection) increases 1,6 times; the number of lymphocytes decreases by 10%. The number of monocytes (specific phagocytosis of cell residues) is significantly reduced. The humoral link of immunity in terms of peripheral blood also reacts to the reception of waters of this type. This reaction is determined in the tendency to increase the content of heterophilic antibodies, complements and circulating immune complexes. These changes indicate increased regulation of protein synthesis (?) and increased protective potential. As for the cellular component of immunity, no significant changes are observed: the number of phagocytes and their metabolic and absorption capacity remain within the control values.

The excretory system of the body also responds to the intake of MW without specific components: the volume of daily diuresis increases by 35% only by increasing the filtration rate (by 27%) of primary urine while maintaining the value of tubular reabsorption at the level of control. The excretion of creatinine and urea increases by 27% and 28%, respectively, that is, the excretion of the final metabolic products of nitrogenous compounds increases. Changes in ion-exchange function of the kidneys are determined by a decrease in the excretion of chlorides by 38%. At the same time, the reaction of daily urine slightly, but reliably shifts in an acidic direction.

Regarding the state of metabolic reactions, changes in the activity of reamination (AsAT and AlAT) are not observed. The level of total bilirubin does not differ from the level of intact animals, but there is an increase in direct bilirubin and a decrease in the level of indirect. The authors believe that this may be due to active re-amination and decreased red blood cell decay activity.

The study of the state of structural-functional parameters of the internal organs of rats during course intake of MW without specific components revealed neither structural changes nor changes in the functional activity of the investigated organs.

The next group of MW, the biological activity of which was investigated by Odessian scientists, were waters with a high content of organic carbon. It is stated that the use of MV of this type causes a redistribution of formed elements in peripheral blood. This is manifested by an increase in the content of leukocytes and neutrophils, that is, the protective capabilities of the blood increase. The contents of other formed elements

remain close to the control data. Taking MW with a high carbon content has an effect on the state of the immune response of peripheral blood. This is determined by an increase in the content of CIC and heterophilic antibodies, which the authors interpret as an increase in the potential of typical pathophysiological reactions of the body, and since the metabolic function of neutrophils increases at the same time, such reactions will be with increased efficiency.

The functional state of the kidneys when taking MW with a high content of organic carbon is significantly activated. In particular, the volume of daily diuresis increased compared to control by 113%. This is due to a significant increase in the rate of glomerular filtration by 73% and a significant decrease in tubular reabsorption by 0,75%. At the same time, creatinine excretion almost doubled - by 70%, urea excretion increased by 39%, and chloride excretion decreased by 23%. That is, this type of MW significantly stimulates both urinary and excretory function of the kidneys. The peculiarity of the influence of these MW is also in the fact that they reduce the excretion of chloride-ions, that is, they ensure the preservation of ion homeostasis.

The activity of enzymes of reamination is somewhat reduced (trend level) compared to control, the ratio of their activities remains. The authors believe that as a result, the detoxification function of the liver has weakened somewhat. Since at the same time decreases the total bilirubin of blood, mainly due to indirect bilirubin, it is believed that the weakening of the change is due to a more intensive removal of adverse metabolites with bile. In addition, the use of these MW is accompanied by a weakening of carbohydrate metabolism, as evidenced by a decrease in amylase activity.

Conducting histological examinations of the heart, liver, stomach, kidneys did not reveal changes in the structure that could be attributed to the manifestations of changes in the functional activity of these organs. Only in the liver, unlike control, observed the absence of dual-core cells, which is interpreted by the authors as a weakening of reparative processes in the liver.

The next group of MW is water that contains metasilicic acid. According to existing materials, they have a complex effect on the functional systems of the body. Changes in red blood indicators do not cause the use of MW of this group. At the same time, the erythrocytes sedimentation rate is growing significantly, which may be the result of changes in protein-synthesizing processes, since metasilicic-ion inhibits the intensity of energy formation reactions. White blood showed a decrease in the content of leukocytes due to a decrease in the number of neutrophils, acidophils and monocytes. The content of lymphocytes does not change with the use of these MW, ie the protective function of peripheral blood is somewhat weakened, but the recognition system of others operates normally. The change also occurs in the functional system of the immune response. First of all, the total number of neutrophils (nonspecific phagocytes) decreases slightly, but their absorbing function and the number of active phagocytes remain at the level of control. At the same time, the concentration of CIC increases and the content of heterophilic antibodies changes, that is, the humoral component of the immune response, which is not only a protective, but also a regulatory system, is at a fairly active level. In general, according to the authors, it is possible to believe that under the influence of MW enriched with meta-silicic acid, the protective system becomes more effective, since its usual activity is provided by fewer elements.

Assessing the effect of MW enriched with meta-silicic acid on the state of metabolic processes, the authors state that the inhibitory effect of silicon is determined in reducing the activity of AlAT and AsAT. At the same time, the ratio of the activity of these enzymes is maintained at the level of control, that is, the processes of conversion in hepatocytes proceed optimally, but somewhat slower. The weakening of the transamination causes an increase in the content of total bilirubin in the blood and a tendency to increase the content of indirect bilirubin.

Regarding the effect of MW with a high content of silicon on the state of the excretory system, the authors found an increase in daily diuresis by 96%, which is due, on the one hand, to an increase in glomerular filtration by 63%, and on the other - a decrease in tubular reabsorption by 1,36%. This is accompanied by an increase in the excretion of creatinine and urea (by 60% and 50%, respectively), that is, the release of the body from toxic metabolites increases. Excretion of chlorides and urine pH remained at the level of reference values.

Structural and functional adjustments in internal organs under the influence of MW enriched with meta-silicic acid are not acute or pathological in nature. There are only changes in functional activity. In the stomach - eosinophilia of the cytoplasm of part of the cells of the gastric glands and the appearance of dark granules in some of the epitheliocytes; in the liver - the expansion of part of the inter-balkan spaces, the granular structure of the cytoplasm of hepatocytes, the equally high blood filling of the vessels of the triad and the central vein. In the kidneys - the expansion of Bowman's spaces, swelling and bright color of the epitheliocytes of the tubular.

Course use of MW with a high content of ortho-boric acid, according to the Odessian authors, causes an increase in the blood content of leukocytes by 39%, neutrophils by 35,3% and a decrease in the content of monocytes by 40% with maintaining the content of lymphocytes and acidophiles at the level of control. That is, according to the authors, the use of MW containing ortho-boric acid somewhat enhances the nonspecific defenses of the blood system and inhibits the subtle specific phagocytosis (?). The immune response is manifested by an increase in the level of heterogeneous antibodies by 24%, complement by 12,5% and circulating immune complexes by 30%. In general, according to the authors, the humoral link of immunity is activated, which is more associated with the function of regulation, and not protection (?).

Regarding excretory system stated an increase in diuresis by 92% due to an increase in glomerular filtration by 54% (the amount of tubular reabsorption remains unchanged and does not differ from the control group). At the same time, increased excretion of urea by 46%, creatinine by 60%, chloride by 40%. The pH of daily urine is slightly (6%?), but significantly shifts to the alkaline side.

At the same time, MW with a high content of ortho-boric acid does not have a significant effect on protein metabolism in the liver. The activity of AlAT and AsAT and the Ritis's index remain at the level of control. At the same time, the content of total bilirubin and its fractions in the blood increases, which indicates some inhibition of the processes of bile formation and bile secretion, which, according to the authors, may be associated with the calming effect of boron on protein metabolism. The calming nature of the effect of boron ions on the course of metabolic reactions confirms the tendency to reduce the activity of amylase - the first enzyme of the multi-link chain of carbohydrate metabolism that supply the main energy substrate to the body.

Changes in the state of functional systems occur against the background of changes in the structural and functional organization of internal organs. From the stomach, this is manifested by signs of activation of the function of the epitheliocytes of the glands and the presence of granules of unrealized secretion. Protein inclusions are detected in the liver in individual hepatocytes, which indicates the activation of some metabolic processes. The kidneys are characterized by manifestations of increased excretion of metabolites, up to their appearance in the lumen of the tubulins.

Odessian authors summarize that the internal intake of MWs has an effect on all (?) the main functional systems of the body. This effect is unidirectional for MWs of different types and is obviously due to their macro-component composition. The presence of a specific bioactive agent makes some adjustments (features) in the course of life processes and thus ensures the specificity of the impact of each individual mineral water.

As you can see, studies of the physiological effect of drinking mineral waters of various composition are focused on the urinary and digestive systems; The metabolic aspect of most studies is limited to blood content and excretion with urine of electrolytes and nitrogenous metabolites, as well as bilirubinemia and transaminases and blood amylase activity. The immunotropic effect of mineral waters is assessed extremely superficially. The influence of mineral waters on the parameters of the autonomic nervous and endocrine systems as regulators of metabolism and immunity was not investigated at all.

Against this background, the Truskavetsian Scientific School of Balneology stands out, the research of which over the past decade is carried out in line with the concept of the neuroendocrine-immune complex, which has become a trend of Ukrainian Balneology (Portnichenko, 2015). The creativity of this school, founded by Yessypenko BYe in 1973, explains the fact that the lion's share among the objects of balneological research belongs to the bioactive water Naftussya of Truskavets' spa, which is informally considered the "***Queen of medicinal waters***". By the way, representatives of Truskavetsian Scientific School of Balneology make up the vast majority of the top 20 rating "Google Scholar" in the rubric "Rehabilitation Therapy" headed by its head Popovych IL (h=31; 2800 citations).

We give a systematic review of the literature.

1.1. Metabolism

Among the metabolic parameters, the greatest attention of researchers was paid to water-electrolyte exchange.

In fundamental experimental studies on rats Yessypenko (1981) was shown that daily watering with Naftussya at a dose of 10 ml/kg, which corresponds to the balneotherapy used in practice, leads during the first 6 days to a decrease in blood volume by 13.8% (due to circulating plasma - by 7.5% and erythrocyte mass - by 6.3%). Over the next 6 days, the volume deficit is already only 4.4%, on the 13-18th day the blood volume exceeds the initial volume by 6.1%, and by 19-21 - by as much as 27%, almost exclusively due to plasma. This is accompanied by a decrease in blood viscosity in the second half of the course by 9% in the absence of its change - in the first.

In experiments on dogs (Flyunt, 1991; Chebanenko et al., 1997) under similar conditions during the first 12 days, the volume of circulating plasma increased by 14.9%, during the second half of the course - by 19.2%, while in control experiments (tap water) an increase was not likely. Such changes were caused not so much by an increase in the total volume of water in the body, which amounted to only 4.1% and 11.4% in the first and second half of the course, respectively, but by a significant redistribution of water between sectors. Thus, the volume of extracellular water increased by 28.3% and 28.8%, respectively, while intracellular water decreased by 10.9% and 0.6%. Explanations of the mechanism of such redistribution should be found in the data on the activation of Naftussya transmembrane water transfer and Na^+ ions in incubated sections of hepatocytes and an increase in ATP-ase activity in the liver and skeletal muscles of rats drunk by it (Yessypenko, 1981).

In clinical observations, the results are not so unambiguous. Only 14 patients out of 22 volumes of circulating plasma at the end of the course increased by 15%, while the rest of the natural changes were not detected (Balanovs'kyi, 1993; Chebanenko et al., 1997).

Since the exchange of water is closely related to the exchange of electrolytes, primarily Na^+, K^+ and Cl^-, it is logical to consider their changes under the conditions of use Naftussya. Litvinenko and Gaske (1975) with 2% course loads of dogs with water Naftussya No.1, an increase in water diuresis by 10-32% due to glomerular

filtration was detected; daily diuresis increased by 34%, excretion of chloride and non-protein nitrogen increased. MW wells 21-N and 8-NO is even more pronounced. 1% 32-day load of dogs with water Naftussya No.10 of the Skhidnytsia deposit increased daily diuresis by 36%, while in control experiments with tap water it decreased slightly (Alekseyenko, 1975). According to the Pribylskaya (1975), the 2% to 14-day load of dogs of the Naftussya from Shklovsky field increased 3.5-hour diuresis by 243-311% compared to tap water. In another work shown that 3-week drinking of dogs with water wells 16-NO, 17-NO, 22-N of Truskavetsian deposit at a dose of 1% two times a day did not affect spontaneous diuresis, but increased water diuresis by 274-337%. At the same time, filtration increased by 46-90%, and reabsorption decreased, excretion of chloride increased to a lesser extent than diuresis, that is, its concentration in urine decreased by 42-64% (Kapskaya, 1980). Water from Kala-Alty (Azerbaijan), close to Naftussya, increased the rate of urination in dogs on the 5th day of the course by 150-161%, on the 15th - by 160-306%, on the 26th - by 122-234%, while the intake of fresh water gave an increase of only 41%, 160% and 100%, respectively. The concentration of urine chlorides in the first period of loads increased, and in the second - fell below the background, the concentration of calcium - did not change significantly, chloridemia - too (Balajaeva et al., 1975).

In experiments on rats, it is shown that with a 1% course load of waters such as Naftussya of Ray-Yelenivka, Zbruch, Zhabyntsi, Makiv, Sataniv and Hussyatyn deposits, daily diuresis, excretion of chloride and nitrogenous metabolites are progressively increasing (Alekseyenko, 1975; Naumenko, 1988; Lopan', 1988). Close to Naftussya water Mizun' did not affect the daily diuresis of rats due to the simultaneous increase in glomerular filtration and tubular reabsorption of water (Lopan', 1988).

The use by rats of Guta water, identified as an analogue of Naftussya, caused an increase in daily diuresis at the end of the 1st week of the course by 15%, the 2nd - by 48%, the 3rd - by 45%, mainly due to glomerular filtration. At the same time, the concentration of potassium in the urine remained unchanged, of sodium decreased significantly at the end of the 1st week to 62%, remaining at this level in the future (63% and 60%), while of calcium, magnesium and H^+ progressively increased. Taking into account the increase in daily diuresis, it was stated that there was an increase in the excretion of the listed ions, with the exception of sodium, the excretion of which at the end of the 1st week decreased by 32%, and further it was no different from control. The Na/K coefficient decreased from 1.08 to 0.57-0.75, and Ca/Mg - from 1.2 to 0.45-0.02. The content of serum urates during the first two weeks increased by 18 and 36%, in urine - by 34 and 38%, after another week the uricemia returned to normal, and uricosuria grew in the future - by 40%. Daily excretion of urates at the same time increased by 60-100% from the initial level (Ivassivka et al., 1990). In another study at 1.5% 3-week loads of water well 21-H was confirmed to increase daily diuresis to 10.2 ml versus 4.5 ml in control, reduction of Na/K-coefficient to 0.23 vs. 1.29 due to an increase in potassium excretion to 1.51 vs. 0.62 mM/day and a decrease in sodium to 0.34 vs. 0.68 mM/day (Levkut, 1994; Popovych et al., 1995).

In more detail, the effect of course loads of water Naftussya on the excretory function of the kidneys was studied in the experiments of Yessypenko (1978, 1981), Flyunt et al.,(1974, 1978, 1991), Lakhin et al.,(1990) and clinical observations of Yessypenko (1981), Balanovskyi (1993), Shymonko (1987), Flyunt (1999).

In experiments on rats, it is shown that after the first 1% load of Naftussya well 21-N daily diuresis increases by 180%, but already on the 3rd day of the course its level drops to the initial, and on the 5th - even below it, after which the second rise begins, as a result of which on the 21st day of the course the daily diuresis reaches the level of 240% of the original, and then decreases again quickly (Yessypenko, 1981). On average, the daily diuresis rate increases by 57%. Excretion of sodium during the first 8 days of the course decreased, reaching about 50% of the original, after which it began to grow, reaching the 13th day of the baseline, and at the end of the 3rd week exceeding it by 1.5 times. On average, during the first 12 days, sodium excretion decreased by 30%, and the next 12 increased by 25% compared to the original. Potassium excretion, on the contrary, increased throughout the course of drinking: for the first half - by 73%, for the second - by 110%, as a result of which the Na/K coefficient decreased in the first half of the course by 60%, in the second - by 41% (Yessypenko, 1978).

In experiments on dogs, a 1%-load of Naftussya well 21-N increased daily diuresis during the first 6 days by an average of 26%, the second - by 44%, the third - by 82%, the fourth - by 54%, in general for the course - by 52%, while drinking a similar volume of tap water by 13% reduced it (Lakhin et al., 1990; Flyunt, 1991). According to other data, tap water during the first half of the course increased daily diuresis by 5%, the second - by 13% against 52% and 61% in experiments with water Naftussya (Yessypenko, 1981). In an earlier study by Yessypenko (1978) the increase in daily diuresis averaged 31% per course. Along with the increase in basal diuresis, water diuresis accelerated within 2 hours after the load of Naftussya. So, on the first day of the course, water diuresis was 174 ml, on the second - 214 ml, on the third - 252 ml, on the fifth - 275 ml. In the future, the intensity of water diuresis decreased to the initial level on the 11th day of the course, after which it increased again. On average, for the first half of the course, 2-hour urination after the introduction of Naftussya was dominated by this after the introduction of tap water by 136%, for the second - by 112% (Yessypenko, 1981). In another experiment, the increase in water diuresis during the first half of the course in dogs drunk with Naftussya was 46%, the second - 42%, while in dogs drunk with tap water - only 7% and 10%, respectively (Lakhin et al., 1990; Flyunt, 1991). Daily excretion of sodium increased on average for the course by 63%,

potassium - by 64% and 130%, calcium - by 26% and 17%, respectively, for the first and second half of the course (Yessypenko, 1978). According to other data, for the first half of the course, sodium excretion increased by 41%, potassium - by 45%, calcium - by 26%, magnesium - by 330%, in the second half of the course excretion Na, K and Mg grew even more - respectively by 82%, 130% and 617%, and calcium and phosphates - to a lesser extent - by 17% and 9% (Yessypenko, 1978). In the experiment of Flyunt (1991) daily excretion of sodium increased on average for the course of 1% loads of Naftussya by 50%, while with similar loads of tap water - decreased by 17%; potassium excretion increased in both groups, but to a greater extent in the experimental group - by 77% against 54% in the control group.

In the study of the mechanisms of stimulating effect of course load with water Naftussya on the release of urine and excretion of electrolytes Yessypenko (1981) to its main links attributed a significant increase in plasma flow, glomerular filtration and a decrease in tubular reabsorption. The increase in renal plasma flow, in turn, is due to an increase in the volume of circulating plasma as part of the body's extracellular water space, which is significantly expanded by both increasing the total water content in the body (to a lesser extent) and mainly due to the redistribution of water between the intracellular and extracellular spaces in favor of the latter. At the same time, the transition of electrolytes, first of all, sodium, from tissues to the blood is intensified, which leads to an increase in their filtration charge, and this, along with a decrease in tubular reabsorption, leads to an increase in their excretion with urine.

Analysis of correlation between diuretic and partial functions of dog kidneys in the process of water loads led Flyunt (1991) to the conclusion that the growth of basal diuresis is mainly due to a decrease in tubular water reabsorption (r=-0.81), while the growth of renal plasma flow and glomerular filtration plays a smaller role (r=0.30 and 0.29, respectively). This also applies to water diuresis.

Significant changes in the excretion of electrolytes affect their concentration in the plasma. Thus, as a result of 1% course loads of dogs, the level of sodium increased by 20.6%, 2% - by 22.2%, 3% - by 22.8%. The level of potassium at the same time almost did not change (respectively 1.3%, -1.4% and 0%) (Chebanenko et al., 1997).

At the same time, in patients with urolithiasis, in the presence of significant increases in sodium excretion (by 21-35%), chloride (by 21-45%) and potassium (by 13-19%) there is only a tendency to increase their plasma content by 4.9-6.2% (sodium) and 2.6-10.7% (potassium) within the norm (Flyunt et al., 1999).

According to Alyeksyeyev et al.,(1994), at the end of course treatment, there is a likely increase in serum potassium concentration by 0.42±0.09 mM/L and its excretion with urine by 11±2 mM/day in the absence of significantly changes in the exchange of the remaining electrolytes.

Komissarov et al., (1988) indicate that in patients with IHD with concomitant chronic cholecystitis or hepatitis who consumed water Naftussya-Shklo (3.3 ml/kg 30 minutes before meals) in combination with hydrogen sulfide baths, mud applications to the liver, dosed walking and diet, serum sodium levels decreased from 142.4±1.1 mM/L to 138.3±0.5 mM/L in the absence of likely changes in potassium levels (4.09±0.14 mM/L and 3.86±0.11 mM/L).

Studies of the dependence of the diuretic effect of Naftussya water on its dose revealed the "phenomenon of scissors" (Yessypenko, 1981; Lakhin et al., 1990). Its essence lies in the fact that with an increase in the load dose from 1% to 2% and 3%, the effect of Naftussya decreases, while of tap water increases, which graphically resembles scissors. Thus, daily diuresis in dogs as a result of 1% loads of Naftussya on average increased by 52% per course, 2% - by 40%, 3% - only by 27%, while the 1% load of tap water reduced it by 13%, 2% - increased by 55%, 3% - by 172%. Thus the ratio of the effects of therapeutic and tap water for a dose of 1% is 1.75, 2% - 0.91, 3% - 0.47. Calculations carried out by Yessypenko (1981) for indicators of water diuresis gave the following figures: 2.27; 1.04 and 0.63, which in principle was confirmed in the experiments of Lakhin et al., (1990): 1.33; 1.23 and 0.69. A similar pattern is noted for other indicators of kidney function, which gave rise to Yessypenko (1981) to draw a general conclusion about the high diuretic efficiency of Naftussya at a dose of 1%, the absence of its specific effect at a dose of 2% and the inhibitory effect on water-filled funcion of the kidneys at a dose of 3%.

This provision was used by Yessypenko (1981) to explain extremely ambiguous data of clinical observations of changes in urination in urological patients in the Truskavets' spa. Such facts have been known for a long time. Even in the monograph "Resorts of the western regions of Ukraine" (1959) it was noted that in 20% of urological patients, the diuretic effect of Naftussya is poorly or completely absent. According to Bajkalov (1966), after taking Naftussya, considerable increase in diuresis is observed only in 20% of patients, moderate - in 50%, and in 30% of patients the diuretic effect has no place. Among patients with chronic pyelonephritis, a moderate increase in the intensity of diuresis was stated in 29%, more than 50% - in 34%, almost twice - in 20%, and in 16% diuresis decreased (Yessypenko, 1981). The proportion of cases of increased daily diuresis in different groups of patients with urolithiasis and chronic pyelonephritis was 46%, 74%, 76% (Flyunt et al., 1974), 67%, 71%, 54% (Yessypenko, 1981), 47%, 61%, 45% (Gabor et al., 1984), 60%, 80%, 58% (Markovetskyi, Gabor, 1984). In the rest of the patients, diuresis did not change significantly or even decreased.

Developing his concept of the dependence of the diuretic effect of Naftussya on its dose, which is based

on these experiments, Yessypenko (1981) showed, that in patients with chronic pyelonephritis who received a daily dose of 0.6-0.8%, daily diuresis at the end of treatment increased from 1417 ml by 13%, dose 0.9-1.0% gave an increase from 1568 ml by 20%, a dose of 1.1-1.4% - from 1539 ml by 4%; elsewhere the monograph says, that the dose of 0.6-0.7% increases diuresis by 7%, 0.8-1.0% - by 19%. In patients with urolithiasis, a dose of 0.7-0.8% caused an increase in diuresis from 1532 ml by 11%; 0.9-1.2% - with 1603 ml by 16%; 1.3-1.6% - with 1680 ml by 1%.

So, according to Yessypenko (1981), for both nosological forms, there are three dose ranges: ineffective, optimal and unfavorable (overdose).

However, subsequent researchers tried to prove that other doses are optimal, quite different (1.5-3 times). Thus, for the treatment of pyelonephritis as optimal recommended doses Naftussya 0.8-1.2% (Markovetskyi, Gabor, 1984), 1.0-1.2% (Shymonko, Skorobohatov, 1990), 1.2-1.5% (Khokhlov et al., 1988) and 1.5-1.7% (Khokhlov, Borzhyevskyi, 1990). For the treatment of urolithiasis, the optimal doses are: 1.4-1.5% (Gabor et al., 1984), 1.5% (Shymonko, Skorobohatov, 1990), 2.0% (Skorobohatov et al., 1988).

The above raises doubts about the existence of dependence of diuretic and/or therapeutic effect on the dose of Naftussya in the range of 0.7-2.0%. One of the additional arguments in favor of these doubts may be to compare the scope of the diuretic effect in urological patients (45-71%, the authors have already cited) and the effectiveness of treatment in the spa of Truskavets': 79-97% (Dudchenko, 1960; Smiyan, 1967, Yessypenko, 1981; Shymonko, 1987; Alyeksyeyev et al., 1994; 1995).

An even stronger argument in this regard is the results of clinical observations of Balanovskyi (1993). The author shows that the direction and magnitude of changes in daily diuresis in patients with urolithiasis are determined by its initial level. Thus, a natural increase in daily diuresis was observed, as a rule, in patients with its initial level below 1.44 liters; in persons with diuresis in the range of 1.5-2.3 l/day, the changes were immeasurable, and in cases of initial urination in the range of 2.4-2.8 l/day at the end of the drinking course, its obligate decrease was noted. On average, in 60% of patients, daily diuresis increased from 1376 ml by 526 ml (or 38%), and in 40% decreased from 2038 ml by 518 ml (or 25%). The same pattern was found in relation to the concentration of electrolytes in the urine and excretion of electrolytes with it: sodium, potassium, calcium, magnesium, chloride, phosphates, which gave grounds to Balanovskyi, Popovych and Karpynets'-Ruzhylo (1993) formulate the concept of ambivalent-equilibrator nature of the action of medicinal water Naftussya. The essence of the concept is that the effect of Naftussya water on the parameters of water-salt exchange is differently directed (ambivalent), while the direction of action is determined by the initial level of the parameter: at a low level, the action is stimulating, and at a high level - inhibitory, that is, the nature of the action is normalizing, leveling (equalizer). The concept was confirmed in the following researches (Nishcheta et al., 1995, Nishcheta et al., 1999).

Data on the effects of balneotherapy on **lipid** metabolism are mixed.

Komissarov et al., (1988) indicate that in patients with Ischemic Heart Disease with concomitant chronic cholecystitis or hepatitis who consumed Naftussya-Shklo water (3.3 ml/ kg 30 minutes before meals) in combination with hydrogen sulfide baths, mud applications on the liver area, dosed walking and diet, there was a decrease in the level of total cholesterol and β-lipoproteins. Unfortunately, the authors did not provide any digital data.

In patients with pathology of the digestive system, which was accompanied by hypercholesterolemia and an increase in serum prebeta- and beta-lipoproteins, basic balneotherapy in the Truskavets' spa caused a decrease in both indicators by 21%, to the upper limit of the norm (Bul'ba, 2000; Kit et al., 1994). In liquidators of the Chernobyl accident, the initially reduced indicators of total cholesterol and lipoproteins of very low and low density under the influence of standard treatment did not change. In patients with diabetes mellitus, there was a probable decrease in the latter indicator by 11%, while the serum content of cholesterol and triglycerides showed only a tendency to decrease, respectively, by 9% and 6% (Alyeksyeyev et al., 1994).

The methodological disadvantage of these studies is their fragmentation. Therefore, of particular interest are the works in which the influence of balneotherapy on the entire spectrum of lipids is investigated. According to Popovych et al., (1998), in women with chronic digestive pathology, the initially elevated serum content of triglycerides and prebeta-lipoproteins did not change or continued to grow. At the same time, the content of total cholesterol, being in the lower zone of the norm, showed a tendency to decrease by 5%, while its level in the composition of beta-lipoproteins decreased by 11-18%, and in the composition of alpha-lipoproteins increased by 5-6%. As a result, the coefficient of atherogenicity, initially increased, in one group of women decreased from 3.46±0.31 to 3.21±0.34, and in the other - from 3.06±0.32 to 2.83±0.37, not reaching, however, the optimal level (2.45±0.24).

Shown (Flyunt et al., 2002), that in liquidators with urological pathology, the level of general serum lipids, as well as total cholesterol, are within the normal range, however, the atherogenicity coefficient was probably lower than the control by 27% due to a 24% increase in the content of alpha-lipoproteins and a decrease of 22% in beta-lipoproteins. This is consistent with the data on the increase in this contingent content of estrogen and a decrease in testosterone, so that the estrogen/testosterone coefficient rises to 0.30-0.61 at a normal level 0.19 (Bazhan, 1998). At the end of balneotherapy, the content of general lipids and cholesterol did not differ from the

original, while the level of alpha-lipoproteins decreased to normal, which in the absence of significant growth in beta-lipoproteins indicates an improvement in the testosterone/estrogen ratio.

In the experiment on rats, Naftussya did not affect the level of total lipids, nor total cholesterol, or its fractions. Naftussya, passed through a membrane sieve that traps its microflora, causes an increase in total lipids by 39%, cholesterol by 28%, but almost equally in both fractions, so that the atherogenicity coefficient probably does not increase. At the same time, Naftussya, exposed to ultraviolet irradiation, increases the content of lipids and cholesterol similarly (by 30% and 29%), but the fraction of alpha-lipoproteins to a much greater extent (+42%) than beta-lipoproteins (+10%), so that the atherogenicity coefficient decreases by 22% (Ivassivka et al., 1999; Kovalchuk et al., 1997).

Beyda (1997) has been shown that drinking Naftussya water in during the load of rats ^{137}Cs reduces the serum content, compared with water control, cholesterol of lipoproteins of very low density by 49%, low density - by 30%, while the content of lipoproteins of high density even shows a tendency to increase by 5%.

1.2. Immune system

The first direct evidence of the immunotropic effect of Naftussya water contained in the monograph Yessypenko (1981). A 6-day course of rat drinking was shown to increase serum β-globulins content by 35%, γ-globulins by 28%, while the increase in α-globulins was only 3%, the total proteins was 22%. The even more noticeable stimulating effect of Naftussya on the synthesis of immunoglobulins and complement is manifested during the next 6 days of the course: the content of β-globulins increases by 51%, γ-globulins by 73%, while α-globulins – only by 6%, total proteins – by 7.5%, which is combined with a 16.5% decrease in albumins level. Consequently, there is a selective effect of Naftussya on those protein fractions, which contain complement and immunoglobulins. Unfortunately, neither the author nor other researchers paid due attention to this fact over the next decade. This can be explained by the dominance in these times of the concept of the predetermination of the therapeutic effect of Naftussya in patients with chronic pyelonephritis and urolithiasis by its diuretic and saluretic effects.

Another manifestation of the immunotropic action of Naftussya was discovered in 1989 by chance, as part of a study of its trophic effects on the digestive organs of rats. Having stated the expected increase after 4 weeks of use of Naftussya liver mass by 16%, intestines - by 10%, kidneys - by 12%, Popovych et collegues, suddenly found that to the greatest extent (44%) increases under these conditions, the mass of the spleen. This fact prompted the researchers to think about the immunotropic effect of Naftussya. In the next, already purposeful experiment Popovych et al., (1995), with the watering of rats with Naftussya water (15 ml/kg, 3 weeks), along with confirmation of the previously discovered fact of an increase in the mass of the spleen by 41%, was first detected an increase in the relative (by 15%) and, especially, absolute (42%) content of neutrophils, their phagocytic activity (by 60%), the phagocytic capacity of blood neutrophils (by 127%), their ability to absorb latex particles (by 100%). The mass of the thymus, the content of lymphocytes and monocytes in the blood under these conditions did not change, still showing a tendency to decrease, which is associated with a 45% increase in the mass of the adrenal glands.

Around the same period, the publication of Khokhlov (1990) appeared, which should be considered a priority in relation to the clinical study of the immunotropic effect of balneotherapy in the resort Truskavets'. Naturally, it concerned 20 patients with chronic calculous pyelonephritis. Based on the modest methodological possibilities available at this time, the author found that after a course of balneotherapy against the background of the absence of significant changes in the initially normal indicators of leukocytosis, absolute and relative lymphocytosis, the significantly reduced relative content of rosette-forming lymphocytes shows a tendency to increase from 60% to 63%. Initially, the normal content of IgG increased, while the severity of hyperimmunoglobulinemia A and M decreased. The author concluded that complex balneotherapy in the resort leads to "a tendency to normalize the number of T-lymphocytes and the approximation of immunoglobulins of all classes to the average values of healthy ones, which was the result of a decrease in antigenic stimulation". The latter, in turn, according to the author, is due to the elimination or subsidence of the inflammatory process in the kidneys. Found in some cases, the author interprets a further increase in immune indicators as an indication of "activation of nonspecific defenses of the body against the background of a withering infectious process".

In 1994, the publication of Rayniger et al., (1994), which noted that in patients with chronic pyelonephritis balneotherapy in Truskavets' resort causes an increase in the number of T-helpers and average levels of immunoglobulins. Unfortunately, the authors do not provide any digital data. At the same conference, the results of priority observations of Aksentiychuk et al., (1994) were published according to the dynamics of immunological indicators in liquidators of the Chernobyl accident with chronic pathology of the digestive and urinary organs. The authors did not register reliable changes in the content of rosette-forming lymphocytes in patients with their initially normal level, while in cases of T-lymphopenia, a stimulating effect was noted in most patients. The level of IgM and IgA, initially reduced, respectively, in 22.2% and 12% of patients, also showed a tendency to increase. Instead, changes in IgG concentrations occurred according to the "initial level law": the initially reduced level probably increased, while the initially increased (45%) decreased. In patients with an increased level of CIC, its decrease was noted, but under the normal initial level, dynamics were not detected.

In the monograph Alyeksyeyev et al., (1994) it was noted that among patients with chronic stone-free cholecystitis who arrived in the resort Truskavets', an increase in IgG levels at 41% was detected; IgA – at 67%; IgM – at 59.7%, while decrease in RBTL – at 27.7%. The authors argue that under the influence of balneotherapy "there was a tendency to reduce the level of immunoglobulins in the blood and normalize the number of T-lymphocytes" (p. 87), without giving, however, any digital data. Among patients with chronic hepatitis hyperimmunoglobulinemia G is stated with a yield at 60%, IgA - at 42.3%, IgM - at 64.6%, increase in CIC – at 27%, which was combined with a decrease in the level of T-lymphocytes at 80.1%, the indicator of neutrophil damage – at 49.0% of the surveyed. Against the background of T-lymphopenia, there was a decrease in T-suppressors at 31.5% and an increase in T-helpers at 45%. As a result of balneotherapy, the authors found a tendency to normalize immunological disorders in the systems of B- and T-lymphocytes. Thus, the severity of hyperimmunoglobulinemia M and G decreased, as did its occurrence. Instead, the dynamics of the IgA had a slight multi-core character. The content of T-lymphocytes increased, the occurrence of T-lymphopenia decreased from 80% to 60%, with a less pronounced restoration of normal ratios in the subpopulation composition of T-lymphocytes (p. 93).

In the next monograph Alyeksyeyev et al., (1995) the results of studies on the effect of balneotherapy on the immune status of liquidators with pathology of the digestive and urinary organs, conducted at the same methodological level, are presented. It is shown that in the process of treatment there was an increase in the reduced average level of T-lymphocytes to normal, while in cases of initially normal levels of T-lymphocytes, no possible changes were recorded. At the same time, the content of T-helpers probably increased, while the content of T-suppressors practically did not change. No IgA and IgM changes were detected, while IgG content changed according to "initial level law." The level of CIC showed a tendency to decrease in different groups of liquidators.

In the third monograph Alyeksyeyev et al., (1996) the assessment of immune status was carried out at a slightly higher level: along with the parameters of T- and B-links, separate indicators of phagocytosis and nonspecific protection were determined. In addition, the observed contingent was expanded at the expense of schoolchildren living in radiation-contaminated areas. It was found that the immune status of the latter responds to balneotherapy according to the "initial level law". Thus, the relative content of lymphocytes in schoolchildren with an initial range of 18-36% increased, while lymphocytosis (37-52%) decreased. The level of T-lymphocytes in the initial range of 36-53% increased, while in children with normal or elevated indicators (54-74%) it did not change unambiguously. The relative content of T-helpers increased, while T-suppressors showed a tendency to decrease. Reduced concentrations of immunoglobulins increased, while normal ones did not change. For the first time, it was found that the reduced activity of saliva lysozyme, the phagocytic index of blood neutrophils, their phagocytic number are increasing. Instead, in liquidators, the increase in the activity of saliva lysozyme was combined with the lack of dynamics of activity and intensity of phagocytosis.

A number of reports concerning the effect of balneotherapy in spa Truskavets' on the immune status of schoolchildren, residents of radiation-contaminated areas, as well as women with thyroid hyperplasia, published by Sarancha et al., (1998), Sarancha et al., (1999), Hrinchenko et al., (1998), Hrinchenko et al., (1999), Grinchenko et al., (2001) as well as Bul'ba (2001; 2002; 2004). The authors demonstrated that the nature and severity of the effects of the standard balneotherapeutic complex on both individual immune parameters and immunity links have their own characteristics due to the initial state of immune status.

In particular, in schoolchildren with moderate immunodisfunction by type of relative hypersuppression with the normal state of B-link and suppression of the phagocytic link, the integral index D of T-links increased by 46%, the integral index D of B-links - by 50%, while the index D of 0-lymphocytes decreased by 53%, that, taking into account a 23% increase in the index of the content of total lymphocytes, reduces the severity of the integral index D suppression of T- and B-links from -1.41 to -0.76 (by 46%). Index D inhibition of the phagocytic link under the influence of balneotherapy decreased by 77% (from -2.09 to -0.49).

In another group of schoolchildren, during the initial examination, the authors stated weak immunodisfunction according to the type of activation of the B-link while maintaining the helper/suppressor balance in combination with the suppression of the phagocytic link. Under the initial conditions, the effects of balneotherapy were ambiguous. Thus, the slight suppression of the T-link deepened, the index of 0-lymphocytes increased, the activation of the B-link reversed in its suppression, while the degree of suppression of the phagocytic link decreased significantly. It is here that it is appropriate to cite Seredyuk (1995; 1998) that patients with chronic hepatitis with hyperreactive condition of the B-immune system internal use of Morshyn's mineral water is contraindicated in view of the possibility of transformation of chronic persistent hepatitis into autoimmune.

Schoolchildren of the third group (Bulba & Sarancha, 2001) during admission stated moderate immunodisfunction according to the type of absolute hypersupression with inhibition of B- and phagocytic links. Under the influence of balneotherapy, there was a significant transformation of the helper/suppressor balance: an excessive increase in the relative content of T-helpers (from 75% to 110% of the norm) in combination with the opposite dynamics of T-suppressors (from 109% to 80%), so that immunoregulatory index transformed from a reduced (82% norm) to an increased (148% norm). Accordingly, there was also a reversal of the integral index D

of T-link, due, according to the authors, the transformation of 0-lymphocytes into T-helpers. The described changes were accompanied by the complete reduction of deficit of the B-link and the phagocytic link.

In line with the above, significant interest is aroused by the results of the study by Yaremenko et al., (1997) of immunotropic action of anaerobically preserved water Naftussya of Zbruch and Truskavets' deposits in monotherapy of patients with gastroenterological profile. According to the authors, clinical improvement is accompanied by a likely increase in the relative and absolute content of the entire $CD3^+$ lymphocyte population, as well as their "active" subpopulation, subpopulations of $CD4^+$- and $CD8^+$-cells, which in general led to the elimination of reversal of the helper/suppressor ratio and the restoration of the immunoregulatory index. With regard to the humoral part of the immune system, the authors noted a reduction in B-lymphocytosis and an increase in the level of antibodies of the IgG and IgA classes in serum and sIgA in saliva and bile. At the same time, the absolute content of undifferentiated 0-lymphocytes probably decreased and the pool of D-lymphocytes disappeared, which are not detected in healthy ones. A 50% reduction in medium-sized CIC content was also found, which the authors attribute to the activation of the macrophagic link of immunity.

In the same year, Sherstyuk (1997) was published the results of the study of the dynamics of immune indicators in patients with chronic inflammatory diseases of the biliary system by the treatment of them at the Sataniv resort with mineral water Naftussya of Zbruchansk' deposit. As can be judged from the digital material given by the author, in persons with initially reduced reactivity of T- and B-immunity systems, balneotherapy caused only a tendency to normalize most parameters. In particular, the index of relative content of T-lymphocytes increased from 0.66 to 0.85, absolute - from 0.81 to 0.90; RBTL index with PhHA – from 0.65 to 0.70; index of relative content of B-lymphocytes – from 0.88 to 0.95; concentrations of IgA – from 0.70 to 0.79, while hyperimmunoglobulinemia G decreased from 1.28 to 1.22; complement binding reaction – from 1.94 to 1.46 in the absence of dynamics of the initially normal indices of absolute content of B-cells and IgM. No parameter dynamics were detected in individuals with initially normal immunological reactivity, with the exception of IgA growth (from 1.05 to 1.19) and reduction of hyperimmunoglobulinemia M (from 1.29 to 1.02) and G (from 1.37 to 1.29) and CIC level. In patients with immunodisfunction (the combination of hyporeactivity of the T-system with hyperreactivity of the B-system), an immunoregulatory effect was manifested: the parameters of the first increased, the second - decreased. In particular, the index of T-lymphocytes from 0.75 to 0.84 and from 0.79 to 0.98; RBTL with PhHA – from 0.72 to 0.81; B-lymphocytes – from 1.33 to 1.10 and from 1.57 to 1.33; IgM – from 1.77 to 1.11; IgG – from 1.74 to 1.39; complement binding reaction – from 8.8 to 5.7 in the absence of dynamics of the normal level of IgA. The author, noting the generally favorable immunomodulatory effect of balneotherapy, is not at all inclined to overestimate its effectiveness and comes to the conclusion that it is necessary to include immunostimulating drugs in the sanatorium-therapeutic complex, which is difficult to disagree with.

The article by Raksha-Slusareva (1997) refers to the possibility of using preserved Zbruchans'ka Naftussya as a new immunocorrector for eco-crisis regions, in particular Donbas. The declared conclusion of the author is based on the results of the study of the impact of 3-week use of this water on the immunity indicators of "conditionally healthy" medical workers, but with symptoms of chronic fatigue and immunodisfunction. From the presented material it follows that the index of absolute content of $CD3^+$ cells increase from 0.72 to 0.91; $CD4^+$- from 0.60 to 0.86; $CD8^+$- from 0.77 to 0.97; $CD22^+$- from 0.82 to 0.94; IgG – from 0.56 to 0.83; IgM – from 0.58 to 0.84 in the absence of IgA dynamics (1.03 and 0.94) and phagocytic neutrophil activity (0.73 and 0.79). Instead, the index of increased indicators decreases: CIC – from 1.46 to 0.89; NBT-test – from 1.43 to 1.17. The described favorable changes are combined with the normalization of plasma cytotoxicity (according to the paramecial test), pathological cytomorphological indicators of leukocytes, hemogram.

Yaremenko et al., (1997a) for the first time, the immunotropic effect of Naftussya water of the Zbruch deposit in vitro was demonstrated in the test of "active" E-rosette formation. According to their data, in the presence of Naftussya water in the environment of incubation of human lymphocytes in breeding 1:6 – 1:3, the number of "active" T-lymphocytes increases by an average of 90%. Preserved in anaerobic conditions Naftussya well 1-NO Truskavets' deposit increases the level of "active" T-lymphocytes by 24-37%, and well 17 by 31-81%.

Experiments conducted according to the same method by Zavyalova et al., (2001) with native Naftussya wells 1-NO, 21-N, 8-NO and 22-N Truskavets' deposit through 1, 3 and 5 hours after selection and aerobic storage, showed its ability not only to activate, but also to inhibit the "active" rosette formation, which is more fully consistent with both clinical and immunological observations, and with the existing concept of the simultaneous presence of activators and inhibitors of a number of enzymes and processes in Naftussya, the relationship between which is subordinated to different influences (Yaremenko et al., 1989; Ivassivka, 1997; Ivassivka et al., 1999).

Flyunt et al., (2002) it was found that a deep suppression of the functional state of microphages, assessed as insufficiency of the IIIa stage, which occurred when given in patients with an active inflammatory process, as a result of balneotherapy was reduced by 74%, rising to the boundary between Ia and Ib st. At the same time, the most significant changes were observed in relation to a spontaneous NBT-test, significantly increased initially, which, together with less pronounced changes in the activated by Zymosan NBT- test, indicates a tendency to restore the functional reserve of oxygen-dependent mechanisms of bactericidity. Nevertheless, the degree of

completion of phagocytosis remained unchanged, which is due, perhaps, to its deep violations, instead, the intensity (to a lesser extent) and activity (to a greater extent) of phagocytosis increased, which is based on the activation expression of receptor to the C_{3b}-component of the complement and the Fc-fragment of IgG on the surface of neutrophils. In patients admitted for rehabilitation in the latent phase, the functional level of microphages was on the border between Ia and Ib st insufficiency, and no significant impact on this link has been registered (an increase of 11%). At the same time, the most favorable to impact of balneofactors was the intensity of phagocytosis, which even exceeded the average level of donors. At the same level was on admission the function of microphages in liquidators with pyelonephritis calculosa in phase remission of inflammation or its absence. Similar (+13%) was the enhancing effect of balneotherapy. At the same time, under these conditions, the completeness of phagocytosis was most restored. The average activity of macrophages in all groups during admission exceeded the average level of donors, which should be regarded as a compensatory reaction to reduced microphage activity. In favor of this is evidenced by both the inverse relationship between these indicators, and a decrease in the degree of activation during the cupping of inflammation. Complement was insensitive to balneotherapy, and only the activity of lysozyme - a marker of oxygen-independent bactericidity - probably increased, most affectingly in patients with latent pyelonephritis calculosa. In general, a block of 6 parameters of nonspecific protection and macrophagocytic link did not respond clearly enough to balneofactors: the degree of deviation from the norm decreased from the IIb st. by 16% in patients with an active process, from the Ia st. by 24% - with latent process, from Ib st. by 10% - in remission. Integral index D of the phagocytic link, being on the border between the IIb and the IIIa st. of insufficiency in patients with active process decreased by 60%, moving to the middle of Ib st. In patients with latent process an improvement of 19% occurred within the Ib st., while in cases of remission of inflammation or its absence of significant shifts around the boundary between Ib and Ia st. didn't happen (+11%).

Flyunt et al., (2002, 2003) it was shown that, unlike the phagocytic link, there were no significant differences between the integral state of the T-cell link of immunity, judging by the D index, in liquidators with active and latent pyelonephritis. In both groups on admission was noted the insufficiency of the IIa st. At the same time, in the active phase there was a more significant decrease in the content of T-lymphocytes, while in the latent phase – their functional activity. Therefore, it is quite logical that the effect of balneotherapy in the last group was somewhat more pronounced: 45% vs 38%; in the first case the insufficiency was reduced to the level of Ia st, while in the second - to the level of Ib st. In liquidators of the first group with a minimum deficiency of T-links, minor favorable changes occurred within the Ia st., the most favorable to impact of balneofactors was RBTL, that is, a functional parameter. In general, judging by the dynamics of the content of 0-lymphocytes, it seems that balneotherapy activates the expression of CD8 receptors in patients with an active or latent inflammatory process. The B-cell link of immunity, in contrast to the phagocytic and T-cell links, is indicated activated, most of all in patients with an active process (up to the level of IIb st.), less pronounced - in persons with latent inflammation (IIa st.), minimally (within Ib st.) - in its remission or absence of inflamation. It turned out that balneotherapy reduces the severity of B-link activation to almost the same level (Ib st.) in liquidators of all three groups. At the same time, the relative effect in the most burdened nosologically group was 51%, in the intermediate group - 38%, while in the most valiant group integral change was not registered. Among some parameters, the most dynamic changes in cases of active process are recorded in relation to the content of B-lymphocytes and IgG, which are fully normalized, as well as the titer of incomplete heterophilic antibodies. The latter parameter, along with the level of small-molecular CIC, was the most subordinate to balneotherapy in patients with latent process, while among the liquidators of the first group, significant dynamics was registered in relation to only the most pathogenic CIC. The parameters that characterize the condition of NK- and K-lymphocytes were deviated from the norm to the least extent, in liquidators with active or latent inflammation - to the level of insufficiency of Ib st., and in cases of its remission or absence - within the Ia st. Balneotherapy caused both quantitative and qualitative favorable changes in the killer link of immunity. In both groups, the integrated index improved by 31% and 34%, respectively, marking the reduction of insufficiency from Ib to Ia st., and in the first group, a shift of 32% occurred within the initial Ia st. failure.

So, the balneotherapeutic complex of Truskavets' spa, which is based on bioactive water Naftussya, has a beneficial modulation effect on the immune system - the main component of the body's protective systems.

1.3. Neuro-hormonal regulation

Kurkudym (1963), based on the obtained data on the positive inotropic and chronotropic effects of Naftussya water on the isolated heart of a frog, gave it sympathomimetic properties caused by organic substances. Popovych (1989) in the conditions of a whole organism showed that Naftussya, injected into the stomach of intact dogs, inhibits basal acid formation, instead, against the background of the previous blockade of alpha-adrenoreceptors by phentolamine activates acidogenesis. In another experiment on dogs, author found that phentolamine several times increases the release of insulin caused by Naftussya (Popovych, 2000). Clinical-physiological observations revealed a significant increase in the cholecystokinetic effect of Naftussya taken against the background of α-adrenoceptor blockade (Chebanenko et al., 1997). All these facts also indicate the adrenomimetic properties of Naftusya water. However, Zahorodniuk (1989), having failed to prevent the

positive ino- and chronotropic effects of Naftussya on the isolated heart of the frog neither alpha-nor beta-blockers, rejected this hypothesis, at the same time explaining their effect of carboxylic acids.

However, a number of facts indicate Naftussya's ability to activate neuro-hormonal regulatory mechanisms. Yes, half a century ago Markov et al., (1971) was reported that gastroenterological patients have increased daily excretion with urine of catecholamines, 17-ketosteroids, 17-ketogenic steroids, and 5-oxyindolacetate after three meals a day. The activation of the adrenal cortex after course use of Naftussya was indirectly evidenced by data on the decrease in the Na/K coefficient of urine in dogs (Flyunt, 1991), rats (Levkut, 1994) and people (Ivassivka et al., 1999), as well as an increase in the mass of adrenal glands (Levkut, 1994).

Of particular interest are the data on the effect of balneofactors on the vegetative support of cardiac activity. For the first time, Perchenko et al., (1999) by cardiointervalography method showed that even a single use of 200 ml of Naftussya has a tangible effect on cholinergic-adrenergic heart regulation in humans. At the same time, 49% of people had different variants of sympathotonic reactions, 24% - vagotonic reactions, and the remaining 27% did not change.

A similar variety of vegetative reactions was obtained as a result of a course of balneotherapy in children (Velychko, 1998). In the first variant, the initial vegetative homeostasis was characterized as vagotonia. In 73% of cases, standard balneotherapy caused an increase in sympathetic tone by 31%, to the lower limit of normotony, a decrease in vagal tone by 12% in the absence of significant changes in the humoral channel of regulation. As a result, the vegetative balance index (VBI) increased by 49%, and the Baevskiy's Stress Index (BSI) - by 45%, so that vegetative homeostasis shifted towards the weakening of the vagotonia. In the remaining 27% of children with initial vagotonia under similar conditions, the sympathetic tone increased by 121%, and the vagal tone decreased by 75%, which gave an increase in VBI by 8.5 times, and BSI - by 8.9 times, so that the vagotonia was transformed into sympathicotonia. At the third option, normothonia took place at the beginning, at the end of the course, the tone of the vagus decreased by 18.5%, which, with a tendency to increase the sympathetic tone, gave an increase in VBI by 29%, BSI - by 19%, but within normality. Finally, in several cases of initial sympathotony, standard balneotherapy further burdened it due to a further increase in sympathetic tone by 40%, while with a 23% weakening of humoral stimulating effects. As a result, BSI grew by only 14%. In general, as we can see, standard balneotherapy has caused a sympathotonic effect.

In this context, we should mention the results of observations of Alyeksyeyev et al., (1995), although they are based on an insufficiently high methodological level, since the authors judged the state of vegetative homeostasis according to the Kerdoe and Weyne indices. It is shown that among the children of the "Chernobyl zone" was dominated the sympathotonia (51.7%), while normothonia took place only in 15.6%. After the course of spa rehabilitation, the share of normotony increased to 45% due to the fall of cases of sympathotonia to 24.6% at the previous level of vagotonia.

Vis'tak (2009; 2010; 2011; 2012) studied the effects of Naftussya on vegetative homeostasis and their prediction in women. 122 women of reproductive age with chronic gynecological and endocrine pathology who used during the individual ovarian-menstrual cycle of Naftussya were examined. There are three variants of the vegetative effect: vagotonic (at 25%), neutral (at 37%) and sympathotonic (at 38% of women). In observations of 30 women aged 32-59 years with thyroid hyperplasia in combination with chronic stoneless cholecystitis in the remission phase, the polyvariant nature of the vegetative effect of Naftussya is confirmed: vagotonic at 33%, neutral at 30% and sympathonic in 37% of women.

In the first observation, vagotonic effect is characterized by a decrease in increased sympathetic tone in combination with an increase in slightly reduced vagal tone in the absence of significant changes in the normal humoral channel of vegetative regulation (Mo). Instead, the sympathotonic effect is manifested by an increase in slightly reduced sympathetic tone and a decrease in slightly increased vagal tone, as well as sympathotonic shifts of normal Mo. As a result of balneotherapy, the initially deviated parameters of vegetative homeostasis move to the middle zone of the norm in both vagotonic and sympathotonic effects. According to neutral effect, the initially normal regulatory parameters do not change significantly.

In order to identify predictors of a vegetative effect, the matrix of all registered initial parameters was objected to discriminant analysis. The program is included in the model 23 predictors, which for the convenience of further consideration are grouped into three pleyads: neuro-hormonal, gynecological and immune. The first pleyad of predictors is headed by a sympathetic tone, immediately there are other indicators of vegetative homeostasis: humoral channel, vagal tone and BSI, as well as the Kerdoe's vegetative index with its components and vegetative reactivity, assessed by the ratio of BSI standing and lying down. Among a number of hormonal predictors were only progesterone and triiodothyronine. The gynecological pleyad of predictors is headed by the size of the fibromyoma, this also includes the volume of the right (dominant) ovary, the expressiveness and echogency of its cystosis and similar characteristics of mastopathy (at the same time, left-sided mastopathy was much more informative for the prognosis then the right-sided one), as well as the duration of the ovarian-menstrual cycle. The immune pleyad of predictors is headed by a subpopulation of "active" T-lymphocytes, the company consists of the immune-regulatory index and its component - subpopulation of T-helpers, population of natural killers, IgM, as well as skin alkaline resistance, which is considered a marker of adaptive and protective mechanisms.

The predictive information contained in the predictors is based on two radicals. At the same time, the first radical contains 69.2% of predictive capabilities and significantly correlates with sympathetic (r=-0.48) and vagal (r= 0.37) tone and duration of OMC (r=-0.17), and the second - the remaining 30.8% and correlates with heart rate standing (r=-0.34), expressiveness of right ovarium cystosis (r=0.28) and its volume (r=0.21), immunoregulatory index (r=-0.23), levels of "active" T-lymphocytes (r=-0.19) and $CD3^+CD4^+$-lymphocytes (r=-0.17). The calculation of classifying discriminant functions makes it possible to retrospectively predict vagotonic effect with an accuracy of 90.3%, neutral - 91.1%, sympathjtonic - 87.0% with a total correctness of the forecast of 89.3%.

With individual analysis, it was found that at 39% of women, the vegetative reactivity index (VR) decreases, at 13% - remains unchanged, at 48% increases. In cases of a decrease in the VR index, the share of hypersympathicotonic VR decreases from 35.4±7.0% to 14.6±5.1%, normal - from 62.5±7.1% to 50.0±7.3%, while asympathicotonic VR increases from 2.1±2.1% to 35.4±7.0%. An increase in the VR index is associated with an increase in the proportion of hypersympathicotonic VR from 10.3±4.0% to 39.7±6.5%, and normal - from 41.4±6.5% to 51.7±6.6% in combination with a decrease in cases of asympathicotonic VR from 48.3±6.6% to 8.6±3.7%. In the group with no changes, the proportions of different types of VR remains stable: normal - 56.3±12.8%, hypersympathicotonic - 25.0±11.2%, asympathicotonic - 18.7±10.0%.

At the same time, the decrease in VR is accompanied by a decrease in the vagal tone from the upper zone of the norm to its middle and a sympathotonic shift in the optimal state of the humoral channel to the lower zone of the norm by maintaining optimal sympathetic tone. Instead, the increase in VR is associated with a decrease in sympathetic and an increase in the vagal tone within the norm with a similar sympathotonic shift in the humoral channel of vegetative regulation. The stability of VR corresponds to the stability of vegetative homeostasis indicators.

In the observations of 30 women of the other contingent, the multivariate nature of the vegetative effect of Naftussya is confirmed: vagotonic in 33%, neutral in 30% and sympathotonic in 37% of women. Vagotonic effect, verified by a decrease in BSI from 128±15 to 75±9 units, accompanied by an increase in HRV-markers of vagal tone (MxDMn, TI, SDNN, RMSSD, pNN_{50}, HF) in combination with a decrease in HRV- markers of sympathetic tone (AMo and LFnu), as well as the LF/HF. Instead, sympathotonic effect, verified by an increase in BSI from 72±9 to 125±17 units, is accompanied by opposite changes in the HRV-markers of vegetative homeostasis. According to neutral effect (BSI 116±11 and 111±12 units before and after the course, respectively), the latter do not change significantly, however, a significant increase in the spectral power of the VLF component of the HRV from 658±84 ms^2 to 539±184 ms^2 was detected, which may indicate an increase in blood levels of glucocorticoids, catecholamines or renin (Kotelnikov et al., 2002). Significant links were found between changes in HRV parameters and plasma levels of triiodothyronine (LF/HF: 0.57; LFnu: 0,57; HF: -0.53), cholesterol of α-lipoproteins (LF/HF: 0.52), chloride (AMo: 0.63), sodium (HF: -0.41), magnesium (LFr: -0.41), activity of Ca-ATPase of red blood cells (HF: -0.41), potassium of red blood cell content (VLF: -0.47). The canonical correlation between changes in HRV parameters and metabolism was very strong (R=0.96).

Kozyavkina (2008, 2009, 2009a, 2012, 2015) in an experiment on 60 healthy female and 50 male rats studied the vegetative effects of course use Naftussya and their endocrine and immune accompaniments.

It is shown that in females, bioactive water Naftussya has at 44% vagotonic, and at 26% - sympathotonic effect, without significantly affecting the autonomous status at 30% of animals. It was found that the vagotonic effect is achieved due to both a significant increase in the vagal tone and a vagotonic shift of the humoral channel, as well as a significant decrease in sympathetic tone. Instead, the sympathotonic effect is associated with a significant increase in sympathetic tone, a sympathotonic shift in the humoral channel and a significant decrease in vagal tone. With a neutral vegetative effect, a moderate decrease in MxDMn is compensated by a slight increase in AMo and a decrease in Mo. In this case, changes in the parameters of autonomic regulation are reciproque in nature, as evidenced by a significant inverse correlation between MxDMn and AMo (r=-0.72) and between Mo and AMo (r=-0.82), as well as a direct correlation between MxDMn and Mo (r=0.84).

Such correlation is a reflection of long-known facts that the strengthening of sympathetic effector influences on the β_1-adrenoreceptors of post-synaptic membranes is accompanied by a reciprocal weakening of the vagal effects on post-synaptic membranes through β_2- and, it is possible, α_2-adrenoreceptors of presynaptic membranes of parasympathetic terminals, which reduces their release of acetylcholine. Conversely, increased vagal effector influences on postsynaptic M-cholinoreceptors are associated with reciprocal attenuation of sympathetic effects via M-cholinoreceptors of presynaptic membranes of adrenergic nerve endings by inhibiting their release of norepinephrine. (Henning et al., 1991; McGrattan et al., 1987; Tkachenko et al., 1998).

When analyzing the accompanying changes in the morpho-functional parameters of the adrenal cortex, first of all, a significant decrease in the level in the plasma of corticosterone, associated with an increase in the thickness of its fascicular zone in rats subordinate to the sympathotonic effect of Naftussya, attracts attention. Opposite changes in these morpho-functional parameters, but only in the form of a trend, occur under the vagotonic effect of Naftussya.

Despite the lack of reciprocity at a neutral effect, a total of 60 animals found a significant inverse correlation between corticosteroneemia and the thickness of the fascicular zone of the adrenal cortex (r=-0.64). This gives

the impression that under the influence of Naftussya the release of glucocorticoid hormone from the cells of this zone is inhibited, significantly expressed by its sympathotonic effect, moderately - by neutral and only in the form of a trend - by vagotonic effect. On the other hand, the thickness of the glomerular zone by vagotonic effect decreases significantly, by neutral effect only in the form of a tendency, and by sympathotonic effect, it tends to increase. Therefore, by analogy, it can be assumed that the vagotonic effect of Naftussya is accompanied by an increase in the release of aldosterone into the blood, which is reduced to its neutral effect and reversed into inhibition – by a sympathotonic effect. The thickness of the reticular zone of the adrenal cortex does not change, as well as the mass of the adrenal glands, yet it is possible to note opposite trends in the alternative vegetative effects of Naftussya.

Screening of correlation relationships between vegetative regulation parameters on the one hand and morpho-functional parameters of the adrenal cortex on the other hand, found significant coefficients of correlation of AMo with the thickness of the glomerular (r= 0.40) and fascicular (r = 0.37) zones, Mo - with them, but of the opposite nature (r= -0.25 and -0.33, respectively). Noteworthy are the boundary connections of MxDMn with the glomerular zone (r=-0.23) and AMo with corticosterone (r=-0.22). The adrenal mass is weakly related only to AMo (r=-0.18), as is the thickness of the reticular zone (r=0.14).

In relation to thyroid hormones, significant changes were detected only by the neutral vegetotropic effect of Naftussya, at the same time, the level of prohormone thyroxine increases, and the true thyroid hormone triiodothyronine decreases (there is a significant inverse relationship between their levels: r=-0.68). Hence the assumption that Naftussya activates the release of T_4, but inhibits its transformation into T_3, but only under conditions of stable vegetative status. With the parameters of vegetative regulation, thyroid hormones are not significantly correlated, only the relationship between T_4 and Mo (r = 0.20) can be noted. The procedure of canonical correlation analysis revealed a significant relationship between vegetative and endocrine statuses: R=0.60.

Analysis of concomitant changes in the parameters of the immune status is appropriate to start with the central organ of immunity – thymus. According to average values, it was found that the pattern of autonomous dynamics corresponds more or less only to the pattern of relative content of epitheliocytes in the thymocytogram: the tendency to decrease by the vagotonic effect, the absence of changes - by neutral and the tendency to increase - by the sympathotonic effect of Naftussya. Screening revealed significant links of sympathetic tone to the relative content in the thymus not only of epitheliocytes (r=0.27), but also of lymphocytes (r=-0.31) and thymocytogram entropy (r=0.29) as well as the borderline relationship with the thymus mass (r=0.23). The humoral channel of regulation correlates with these parameters in the opposite way: r= -0.29; 0.27 and -0.25, respectively, and vagal tone – only with T-lymphocytes (r =0.25) and borderline with entropy (r=-0.23). Canonical correlation analysis shows only a moderate relationship between vegetative status and morpho-functional state of the thymus: R=0.44.

It seems that the thymotropic effects of Naftussya found in one or more groups of animals (a decrease in the mass of the thymus, the relative content of lymphoblasts and endotheliocytes in it and an increase in the content of macrophages and Hassal's bodies) are not at all associated with its vegetotropic effects, but caused by other factors.

Among the accompanying changes in the morpho-functional parameters of the spleen, first of all, the pattern of the relative content of macrophages in it draws attention: significant decrease by the vagotonic effect, the absence of changes by neutral and significant increase by the sympathotonic effect of Naftussya. The susceptibility of the content of splenic macrophages to vegetative influences is evidenced by its significant direct association with the sympathetic correlate AMo (r=0.68) and inverse - with vagal MxDMn (r=-0.41) as well as Mo (r=-0.64).

In the opposite way and only moderately related to the parameters of vegetative regulation, the content in the spleen of lymphoblasts - the corresponding correlation coefficients are: -0.27 (AMo); 0.24 (MxDMn) and 0.30 (Mo). A borderlinely significant inverse correlation of sympathetic tone with lymphocytosis and plasmocytosis of the spleen (r=-0.25 in both cases) was also identified, as well as a noteworthy one with reticulocytosis (r=0,20). As a result, the canonical correlation between vegetative status and the morpho-functional state of the spleen is very strong: R=0.75.

Moving to the analysis of leukocytogram of peripheral blood, we note that a significant direct correlation between eosinophilia and Mo (r=0.51) and MxDMn (r=0.48) was found. Among the parameters of the phagocytic function of neutrophils-microphages and monocytes-macrophages of peripheral blood, a weak correlation with the parameters of vegetative regulation is found only for the phagocytic number of neutrophils (r=0.33 with a vagal tone and r=0.30 with a humoral channel) and a phagocytic monocyte index (r=-0.29 with a sympathetic tone). This is manifested in a minimal degree of inhibition of the intensity of phagocytosis of **microphages** in the vagotonic effect of Naftussya and opposite changes in the activity of **macrophage** phagocytosis in its alternative effects, while the activity of microphage phagocytosis decreased equally, and the microphage killing index and the intensity of macrophage phagocytosis increased equally in all animal groups. Despite the weak binary correlation between autonomous regulation parameters – on the one hand, and

leukocytogram and phagocytosis – on the other hand, the canonical correlation between these sets was very strong: R=0.89.

The binary correlation between vegetative regulation and peripheral blood immunocytogram was quite weak. Attention should be paid only to the relationship of the vagal tone with the relative content of T-helpers (r=-0.21), B-lymphocytes (r=-0.19) and natural killers (r=0.15). While there is a significant correlation (r=0.31) with it of the entropy of the immunocytogram. The canonical correlation between vegetative status and immunocytogram was found to be moderate: R=0.50.

In conclusion Kozyavkina OV analyzed the canonical correlation between autonomic and immune-endocrine sets. It was found that immune-endocrine radical is represented directly by macrophages of the spleen (r= 0.67), glomerular (r= 0.43) and fascicular (r= 0.41) adrenal cortex zones, blood eosinophilia (r=0.41), thymus mass (r=0.28), thymocytogram entropy (r=0.25) and its epitheliocytes (r=0.24) while inversely by plasmocytosis of the spleen (r=-0.35), macrophage phagocytosis activity (r=-0.32), blood T-helpers (r=-0.32), adrenal mass (r=-0 31), spleen (r=-0.27) and thymus (r=-0.25) lymphocytosis, as well as corticosteroneemia (r=-0.23). The canonical correlation between radicals is also very strong: R=0.92. This suggests that sympathetic (mostly nervous, to a lesser extent humoral) influences determine changes in the listed endocrine and immune parameters on 85%.

In the experiment on male rats, Kozyavkina OV shows that on 57.5% of animals Naftussya have a sympathotonic effect, while on 42.5% - vagotonic.

It was found that with the vagotonic effect the decrease in the BSI is due to a decrease in sympathetic tone by 26% in combination with a 39% increase in the vagal tone and a vagotonic shift in the state of the humoral channel. Instead, the sympathotonic effect is characterized by a 48% increase in sympathetic tone associated with a 65% decrease in vagal tone and a sympathotonic shift of 20% in the state of the humoral channel. The Mode value, in turn, closely correlates with MxDMn (r= 0.88) and inversely with AMo (r= -0.76). The impression of reciprocal changes in the sympathetic and vagal links of vegetative regulation is confirmed by correlation analysis: the correlation coefficient between AMo and MxDMn is -0.90.

The identified significant changes in vegetative regulation are accompanied by certain changes in a number of morpho-functional parameters of the adrenal glands.

In particular, the mass of the adrenal glands and excretion of 17-ketosteroids by the vagotonic effect of Naftussya increase, while by sympathotonic effect, they tend to decrease. The vagotonic effect is also accompanied by thickening of the fascicular and reticular zones of the adrenal cortex and an increase in the level in the plasma of corticosterone, while by sympathotonic effect, the first two parameters show only a tendency to increase, and corticosteronemia increases to a lesser extent.

On the other hand, the thickness of the glomerular zone of the adrenal cortex does not change under the vagotonic effect, while by sympathotonic it decreases significantly. At the same time, the thickness of the medullar zone of the adrenal glands does not change with any vegetatropic effect of Naftussya.

No changes have been detected in relation to plasma levels of testosterone and thyroxine. Instead, the level of triiodothyronine by sympathotonic effect increases significantly, showing only a tendency to increase by vagototonic effect.

Taking into account the well-known facts that parathyroide hormone increases plasma level of calcium and reduces the level of phosphates, and calcitonin reduces the levels of both electrolytes, Popovych (2007) proposed indices of parathyroide and calcitonin activity calculated by plasma levels of these electrolytes.

Applying this approach, Kozyavkina OV state that the sympathotonic effect of Naftussya is accompanied by an increase of 14% of calcitonin activity and a decrease of 9% of parathyroid, as evidenced by a decrease in plasma levels of calcium and phosphates. Instead, by vagotonic effect the levels of these electrolytes are shifted in opposite directions, which can be interpreted as an increase of 9% of parathyroide and a decrease of 11% of calcitonin activity.

Analysis of the peculiarities of the state of parameters of sodium and potassium exchange by alternative vegetotropic effects of Naftussya found that the vagotonic effect is accompanied by an increase in the level of potassium in the plasma and its excretion with urine in the absence of changes in the potassium content in red blood cells. Instead, the sodium content increases in red blood cells, while neither its concentration in the plasma nor the excretion with urine change. The sympathotonic effect is associated with a decrease in sodium excretion with urine, combined with a tendency to reduce its plasma concentration at normal content in red blood cells. All three parameters of potassium exchange do not differ from the control ones.

In order to assess the strength of the relationship between the parameters of vegetative regulation - on the one hand, and endocrine and metabolic parameters - on the other hand, the procedure of canonical correlation analysis was carried out. The program identifies two canonical roots. The vegetative canonical root receives a negative factor load from AMo (r=-0.97) and positive - from MxDMn (r=0.88) and Mo (r=0.65). The endocrine-metabolic root is represented by the mass of the adrenal glands (r=0.54), the thickness of the glomerular (r=0.53) and fascicular (r=0.33) zones of their cortex, excretion with urine of 17-ketosteroids (r=0.33), as well as plasma levels of potassium (r=0,59), calcium (r=0.41) and sodium (r=0.31). The canonical correlation between radicals was significant: R=0.66.

The mass of the thymus increases equally for both types of vegetotropic effects of Naftussya. Instead, the content of lymphocytes in the thymocytogram exhibits opposite tendencies: to increase by vagotonic while to decrease by sympathotonic effect. Opposite changes were also found for Hassal's bodies, the content of which decreases significantly due to the vagotonic effect and tends to increase due to the sympathotonic effect. The levels of the other two elements of the thymocytogram change unidirectionally, but to varying degrees. In particular, the increase in the content of macrophages in the thymus is more noticeable by sympathotonic effect, and the fall in the content of endotheliocytes is deeper by vagotonic effect of Naftussya. The remaining elements of the thymocytogram – lymphoblasts, epitheliocytes and reticulocytes – are not object to significant effects of Naftussya.

The canonical correlation between HRV and thymocytogram parameters was significant in strength: R=0.67. The vegetative canonical radical is represented to the greatest extent by the humoral channel (r=0.86), smaller in module and opposite in nature, factor loads on the radical give vagal (r=0.74) and sympathetic (r=0.66) tones. The thymic canonical radical receives negative loads from the relative mass of the thymus (r=-0.53) and the levels of macrophages in it (r=-0.57), the Hassal's bodies (r=-0.48) and endotheliocytes (r=-0.32), while lymphoblasts (r=0.47) and lymphocytes (r=0.33) give positive factor loads on the radical.

Mass, especially relative, of the spleen exhibits opposite tendencies to change by alternative vegetatotropic effects of Naftussya: to increase by vagotonic while to decrease by sympathotonic. The vagotonic effect is associated with an increase in the content of plasma cells, fibroblasts and eosinophils in combination with a decrease in the content of reticulocytes in the absence of significant changes in the lymphocytes, lymphocytes, neutrophils and macrophages. Instead, by sympathotonic effect, the content of neutrophils decreases significantly, and macrophages increase even more significantly, while the levels of other elements of the splenocytogram do not differ significantly from the control ones.

A very strong canonical correlation between vegetative parameters and splenocytogram parameters has been identified: R=0.94. In this case, the vegetative radical is represented in an inversely sympathetic tone (r=-0.99) and in a direct way – by a vagal tone (r=0.97) and a humoral channel (r=0.80). Splenic canonical radical receives negative factor loads from macrophages (r=-0.90) and reticulocytes (r=-0.23) while positive from the mass of the spleen (r=0.48) and the content in it of neutrophils (r=0,36), lymphocytes (r=0.31) and eosinophils (r=0.30).

Concomitant changes in the immune parameters of peripheral blood will begin to analyze with leukocytogram. It was found that the vagotonic effect of Naftussya is accompanied by a slight but significant increase in the blood content of leukocytes. At the same time, among the form elements of leukocytogram, significant changes in relative content were detected only in relation to eosinophils (elevation) and basophils (decrease). By sympathotonic effect the total content of leukocytes does not differ from the control, however, the relative content of lymphocytes decreases significantly and the relative content of monocytes increases.

Regarding concomitant changes in the parameters of phagocytosis of monocytes and neutrophils of peripheral blood, it is found that vagotonic effect of Naftussya is accompanied by a slight decrease in the activity of phagocytosis (phagocytic index) of monocytes combined with a more noticeable increase in its intensity (microbial number), while under the sympathonic effect, the phagocytic index of macrophages decreases to a slightly greater extent, and the increase in the microbial number is significantly less pronounced compared to such a vagotonic effect.

By the vagotonic effect, both the activity and intensity of neutrophil/microphage phagocytosis are significantly reduced, while by the sympathotonic effect, the first parameter decreases only in the form of a trend, and the second - to a lesser extent. Instead, the completeness of microphage phagocytosis remains stable for both types of vegetotropic effects.

The absolute content of lymphocytes in peripheral blood does not change significantly with any vegetotropic effects of Naftussya. Among the elements of the immunocytogram, the most noticeable changes by the vagotonic effect were found in relation to 0-lymphocytes, the relative content of which increases. At the same time, accordingly, the relative content of T-killers, natural killers and B-lymphocytes decreases, along with a tendency to reduce the content of T-helpers and plasma cells. The mitogenic ability of T-lymphocytes is also reduced. Consequently, the vagotonic effect is accompanied by a decrease or tendency to decrease content in blood and activity of all lymphocyte populations that express surface differentiation receptors, in combination with increased lymphocyte content, which these receptors do not express or have lost. The sympathotonic effect is also accompanied by an increase in the content of 0- lymphocytes, but significant to a lesser extent than the vagotonic effect. Accordingly, the population of T-lymphocytes and NK-lymphocytes show only a tendency to decrease, and the level of B-lymphocytes does not differ from the control.

The canonical correlation between vegetative parameters and the immune parameters of the blood was strong: R=0.79. At the same time, the vegetative canonical radical receives a negative factor load from the sympathetic tone (r=-0.72) and positive from the vagal tone (r=0.72) and the humoral channel (r=0.74). On the other hand, the immune radical of peripheral blood is represented inversely by B lymphocytes (r=-0.57), basophils (r=-0.37), segmental (r=-0.33) and rod-shaped (r=-0.26) neutrophils and plasma cells (r=-0.26); while positive factor loads on the immune radical are: phagocytic index of monocytes (r=0.36), leukocytosis (r=0.31),

levels of eosinophils (r=0.27) and total lymphocytes (r=0.22) as well as completion of neutrophil phagocytosis (r=0.21).

Kozyavkina NV (2008, 2009, 2009a, 2012, 2015) in experiments on rats of both sexes purposefully studied the effects of weekly use of Naftussya water on plasma levels of thyroid hormones and their metabolic and neuroendocrine-immune support.

The author identified a wide range of thyrotropic effects, which were accompanied by certain changes in metabolic parameters. The level of triacylglycerides does not change significantly in any group, showing only a tendency to increase, most expressive than the inhibitory thyrotropic effect. Instead, changes in plasma's total cholesterol (ChS) levels clearly correlate with changes in the total thyroid index (TTI), calculated by formula:

$TTI=(4 \bullet T_3/2{,}29+T_4/55{,}6)/5$, where

4 is ratio of activities of T_3 and T_4 (Braverman and Vagenakis, 1982);

2,29 and 55,6 are averages of T_3 and T_4 respectively in intact female rats.

Thus, the decrease in TTI is associated with an increase in ChS by 19±7%, a moderate increase with a decrease in ChS by 18±3%, an even more significant increase in TTI corresponds to a deeper drop in the level of ChS - by 28±3%. Finally, the absence of changes in TTI is accompanied by the absence of changes and ChS. The accompanying changes in the content of ChS in the composition of lipoproteins (LP) of different densities are not so unambiguous. If the ChS of the nonα-LP changes according to a pattern similar to such a general ChS, then the ChS of α-LP by the inhibitory thyrotropic effect shows only a tendency to increase (by 7±7%), and decreases equally with both neutral (11±4%), and moderately stimulating (by 9±4%) effects, and only the maximum increase in TTI corresponds to the deepest drop in ChS of α-LP (by 18±4%). Therefore, the Klimov's cholesterol coefficient of atherogenicity is significantly reduced only by stimulating thyrotropic effect, moreover to approximately the same extent, instead, it shows a tendency to increase both by inhibitory and neutral effects.

In order to assess the state of exchange of major cations – sodium and potassium, Kozyavkina NV determined their content in plasma and red blood cells, as well as excretion with daily urine. It appears that the content in plasma both sodium and potassium almost the same in rats of all groups and did not differ from the control. Instead, the content of both cations in erythrocytes, as a marker of their content in the intracellular space, varies significantly and reciprocally by different thyrotropic effects. In particular, the inhibitory effect is accompanied by a decrease in potassium level in combination with an increase in sodium level. The neutral thyrotropic effect corresponds to the absence of significant changes in both sodium and potassium. Instead, the moderately stimulating effect is characterized by opposite changes in the levels of these cations.

Regarding the indicators of neuro-endocrine regulation, it was found that the inhibitory thyrotropic effect of Naftussya is accompanied by a significant increase in sympathetic tone and a decrease in the vagal tone in combination with a sympathotonic deviation of the humoral channel of vegetative regulation. At the same time, the level in the plasma of corticosterone increases significantly, while testosterone levels tend to decrease, and excretion with urine of androgen metabolites 17-KS decreases significantly. The absence of changes in the total thyroid index (neutral tyrotropic effect) is associated with the absence of significant deviations from the norm of indicators of neuro-hormonal regulation. Instead, the stimulating thyrotropic effects are again accompanied by a sympathotonic shift in vegetative homeostasis, somewhat more noticeable by significant than by moderately stimulating effects on thyroid status. However, the indicators of steroid hormones do not significantly deviate from the norm, except for a decrease in testosterone by moderately stimulating effect.

The relative mass of the adrenals by inhibition of thyroid function also significantly decreases, including due to an increase in body weight; by an unchanging thyroid function, this parameter also does not change, but a moderate increase in function is accompanied by adrenal hypertrophy, but the latter comes to naught in cases of a significantly stimulating thyrotropic effect.

The decrease in the mass of the adrenal glands is obviously due to the thinning of their glomerular zone and, to a lesser extent, reticular zone, while hypertrophy occurs due to thickening of the fascicular, reticular and medullary zones. At the same time, in the absence of changes in the mass of the adrenal glands, there is a combination of thickening of the fascicular and reticular zones with thinning of the medullary and glomerular.

Mineralocorticoid activity of the adrenal cortex, carried out in rats, as is known, not only aldosterone as product of glomerular zone cells, but also corticosterone secreted by corticocytes of the fascicular zone, does not change in any of the groups.

Along with the endocrine function of thyrocytes, the function of thyroid C-cells is also suppressed, as evidenced by a significant decrease in the calcitonin activity index, the reflection of which is hypercalcemia. In cases of neutral thyrotropic effect, calcitonin activity remains unchanged, as in a moderately stimulating effect, and only a significantly stimulating thyrotropic effect is accompanied by a significant increase in calcitonin activity, which is manifested by hypocalcemia.

Parathyroid activity varies reciprocally to calcitonin, which is confirmed by the high (r=-0.92) coefficient of inverse correlation between them.

With regard to immune support, it was found that the inhibitory thyrotropic effect is accompanied, first of all, by a decrease in the blood content of common leukocytes. The activity of macrophage phagocytosis under the inhibitory thyrotropic effect is significantly suppressed, Instead, its intensity is even more elevated, so that

the bactericidal capacity of macrophages (BCCM) of blood is significantly higher than in control. The neutral thyrotropic effect is also accompanied by reciprocal changes in the phagocytic index and microbial count of macrophages, but less pronounced and more proportionate, so that BCCM shows only a tendency to increase. This applies to both stimulatory thyrotropic effects. A weak inverse correlation with TTI is found only for the microbial number of monocytes (r=-0.23). A similar but direct correlation with TTI (r=0.25) occurs for the bactericidal capacity of neutrophils (BCCN) of blood. BCCN is significantly reduced by the inhibitory thyrotropic effect (due to the suppression of activity, intensity and completeness of phagocytosis of microphages) while practically does not differ from the control by neutral effect. However, the increase in STI is not accompanied by a significant increase in BCCN.

Regarding the indicators of the T-link of immunity, it was found that the inhibititory thyrotropic effect is accompanied by a significant decrease in the levels of both T-helpers and T-killers. By neutral effect, this decrease comes to naught, and this condition persists at both stimulating effects, with the exception of a repeated decrease in T-killers by significantly stimulating thyrotropic effect. The reaction of blast transformation of T-lymphocyte to phytohemagglutinin remains close to the control by all thyrotropic effects of Naftussya, except its inhibition in cases of neutral effect.

The level of 0-lymphocytes approximately equally increases in all research groups. The level of natural killers by the inhibitory thyrotropic effect remains normal, while in other cases significantly decreases. Weak inverse correlation of NK with STI (r=-0.26) was revealed.

Among the indicators of B-link immunity, significant concomitant changes were found only by the inhibitory thyrotropic effect: a decrease in the concentration of IgM in combination with an increase in the level of circulating immune complexes. Entropy of immunocytogram, consisting of 0.524±0.004 in intact rats, found to be reduced in all groups, but not significant (0.515±0.007; 0.519±0.005; 0.516±0.003 and 0.515±0.006).

In relation to the elements of splenocytogram by the inhibitory thyrotropic effect, a decrease in the content of lymphoblasts and, to a lesser extent, lymphocytes in combination with an increase in the content of macrophages and fibroblasts was found. By neutral effect, the marked changes are leveled or reduced (in relation to macrophages), at the same time, the content of plasmocytes increases while of reticulocytes decrease. Both stimulating effects are accompanied by a repeated increase in the content of macrophages, as well as a significant decrease in the content of neutrophils, which, by the inhibitory effect, manifested only as a trend. A weak correlation with TTI was found only in relation to lymphoblasts (r=0.24). The entropy of splenocytogram (normally: 0.591±0.007) was increased both by inhibitory (up to 0.610±0.006) and by significantly stimulating (up to 0.602±0.003) effects, unchanged in other cases (0.589±0.011 and 0.589±0.007).

The mass of the thymus, unlike the spleen, increases significantly, while most of all by the neutral thyrotropic effect of Naftussya, remaining unchanged only by the inhibitory effect. The correlation with TTI is significant (r=0.32). Content in thymus of lymphoblastes is most noticeably reduced by a significantly stimulating effect, in the form of a tendency - by moderately stimulating, does not differ from the control by neutral and tends to increase by inhibitory thyrotropic effect. Both inhibitory and stimulating thyrotropic effects are accompanied by a significant increase in the content of macrophages while a decrease in the content of endotheliocytes. By neutral effect, the expressiveness of the first accompaniment decreases, while the second increases.

A similar design Kozyavkina NV (2013) used in the experiment on female rats.

Regarding the cholesterol accompaniment of thyrotropic effects of Naftussya, it was found that a significantly inhibitory thyrotropic effect is accompanied by an increase in the concentration in the plasma of total cholesterol by 30%, while the composition of nonα-(pre-β- and β-) lipoproteins (LP) to a greater extent, than in the composition of α-LP (by 51% and 8%, respectively), so that the Klimov's cholesterol coefficient of atherogenicity increases by 42% relative to intact control. By a moderately inhibitive thyrotropic effect, the total cholesterol content increases by only 10%, almost entirely due to pro-atherogenic fractions (+18%), while the anti-atherogenic fraction does not differ from the control, which gives an increase in the atherogenicity coefficient by 17%. In the absence of significant changes in the total thyroid index, the atherogenicity coefficient is moderately reduced (by 17%) due to a decrease in cholesterol content in the pre-β and β-LP to a greater extent (by 26%) than in the α-LP (by 8%).

At the same time, the stimulating thyrotropic effect is accompanied by a significant anti-atherogenic effect due to a decrease in cholesterol content in the composition of atherogenic fractions by 40% in combination with a tendency to increase its content in the composition of the α-LP by 6%, which reduces the atherogenicity factor by 43%.

Regarding another aspect of lipid status – lipoperoxidation – it was found that by a significantly inhibitory thyrotropic effect, the plasma content of primary (diene conjugates, DC) and secondary (malone dialdehyde, MDA) lipid peroxidation products increases significantly along with the activation of the antioxidant enzyme catalase, but not superoxide dismutase (SOD).

A smaller degree of inhibition of thyroid function is accompanied by lower levels of MDA and only a tendency to increase the level of DC with similar activity of antioxidant enzymes. At the same time, in the absence of changes in the thyroid status regarding control, the maximum levels of MDA and catalase activity in

combination with normal levels of DC and SOD are stated. The stimulating thyrotropic effect of Naftussya is accompanied by a pattern of lipoperoxidation parameters, very similar to that of a significantly inhibitory thyrotropic effect.

The plasma content of nitrogenous metabolites by a significantly inhibittory thyrotropic effect of Naftussya does not change, while a significant increase in glucose levels has been detected. Instead, a moderate decrease in the total thyroid index is accompanied by an increase in plasma levels of urea and creatinine in combination with a decrease in the content of medium-mass molecules (MMM), while the levels of urates, bilirubin and glucose do not differ from the control ones. The neutral thyrotropic effect is associated with the highest possible levels of urea and creatinine in quasi-null deviations from the norm of the remaining 4 parameters. The increase in the total thyroid index is accompanied by less pronounced changes in the same direction of the mentioned nitrogenous metabolites while maintaining the stability of the levels of urates, MMM and bilirubin. At the same time, the maximum increase in the level of glycemia is stated.

As for electrolytes, it was found that a significant decrease in the total thyroid index is combined with a slight but significant decrease in the levels of the main electrolytes of plasma - sodium and chloride, combined with tendention to a decrease in potassium and to increase phosphates by normal levels of magnesium and calcium. By a moderately inhibitory thyrotropic effect, these trends in potassium and phosphates are transformed into a pattern, while changes in sodium and chloride are reduced. At the same time, there is a tendency to reduce the levels of calcium and magnesium. Quasi-zero deviation of the total thyroid index are associated with a similar quasi-normal state of sodium, chloride, potassium and magnesium levels. At the same time, a significant decrease in calciemia was found in combination with an increase in phosphatemia. The stimulating thyrotropic effect is accompanied by a significant decrease in potassium, chloride and calcium levels, combined with a tendency to increase the level of phosphates.

In relation to endocrine accompaniment, it was found that significantly inhibitory thyrotropic effect of Naftussya is accompanied by a moderate increase in mineralocorticoid activity (MCA) in combination with a moderate decrease in calcitonin (CTA) and a tendency to decrease parathyroid (PTA) activity. The weakening of the degree of suppression of thyroid function is associated with further increase in MCA and deepening of PTA suppression, while CTA suppression is reduced. The quasi-normal state of the total thyroid index against the background of the use of Naftussya is combined with maximum suppression of PTA, repeated decrease in CTA and reduction of the increased MCA. By the stimulating thyrotropic effect, different-directional changes in the MCA and KTA with a consistently suppressed PTA are noted.

Regarding concomitant changes in the parameters of autonomous regulation, it was found that a significant suppression of thyroid function is accompanied by a significant vagotonic shift in the humoral channel of regulation, combined with a tendency to increase the vagal tone, in the absence of significant changes in sympathetic tone. However, by a moderately inhibitory thyrotropic effect of Naftussya, the parameters of vegetative regulation do not differ significantly from the control ones. In the absence of changes in the total thyroid index, a significant decrease in vagal tone was detected with a completely normal sympathetic tone. Activation of thyroid function is accompanied by opposite changes (in the form of trends) of tonic autonomous regulatory influences towards vagotonia.

Regarding the accompanying changes in the morpho-functional parameters of the adrenal glands, it was found that by a significantly inhibitory thyrotropic effect, Naftussya decreases by 9% their weight (but not the mass index, since body weight also decreases by 6%). At the same time, the thickness of the glomerular zone of the cortex decreases, which, taking into account the previously noted increase in the MCA, can be interpreted as a manifestation of its release by endocrinocytes into the blood of aldosterone, especially since the absence of changes in the thickness of the fascicular zone of the cortex is combined with the absence of changes in the level in the plasma of corticosterone secreted by it.

Discordant changes in the thickness of the glomerular zone of the cortex and MCA are also observed in the neutral and stimulating thyrotropic effects of Naftussya. Conversely, the more thickened under these conditions the fascicular zone of the cortex, the lower the level of corticosteroneemia (r=-0.65), which, apparently reflects the deposition of glucocorticoid in endocrinocytes. However, for mineralocorticoids, this pattern is violated by a moderate inhibitory thyrotropic effect, when the maximum elevated MCA is combined with a slight thickening of the glomerular zone of the adrenal cortex, so that in general there is no morpho-functional correlation (r=0,06). The absence of changes in the thickness of the reticular zone of the cortex, apparently, is evidenced by the absence of significant changes in the androgens it secreted.

Analysis of concomitant changes in immunity parameters is logical to start with blood, the composition of immunocytes which reflects the nature and intensity of their migration (traffic) between the thymus, bone marrow and spleen.

It was found that by a significantly inhibitory thyrotropic effect of Naftussya, the total content of leukocytes in the blood practically does not change, as well as the relative content in the leukocytogram of lymphocytes, basophils and segmental neutrophils (PMNN). At the same time, the content of eosinophils and rod-shaped neutrophils (RSN) increases slightly, while monocytes significantly decreases. The weakening of the degree of inhibition of the thyroid function is accompanied by a weakening of monocytopenia. At the same time,

the relative content of lymphocytes and basophils increases, eosinophilia comes to naught, and the level of RSN decreases. The absence of changes in the total thyroid index is associated with the emergence of the most pronounced lymphocytosis, monocytopenia and PMN-penia, as well as with a decrease in levels of RSN and eosinophils and the disappearance of basophils. Against the background of stimulation of the thyroid function, the severity of lymphocytosis and monocytopenia decreases, PMN-penia does not change, and RSN-penia decreases somewhat, the level of eosinophils normalizes, and basophils reach a maximum.

As for populations of lymphocytes, under conditions of significant inhibition of thyroid function, no changes were detected. A similar situation is recorded in the diametrically opposite state of thyroid function – its stimulation. Instead, the moderately inhibitory thyrotropic effect of Naftussya is accompanied by a significant increase in the relative content in the immunocytogram of B-lymphocytes, natural killers and a tendency to increase the content of subpopulation of T-killers; at the same time, of course, the share of 0-lymphocytes decreases. The neutral thyrotropic effect of Naftussya is associated with a maximum increase in the content of NK lymphocytes in combination with a maximum decrease in the content of B lymphocytes and a tendency to reduce the content of T-killers.

Analysis of concomitant changes in the parameters of phagocytosis of the Staphylococcus aureus culture revealed the following. Significantly inhibitory thyrotropic effect is accompanied by maximum inhibition of activity and intensity of phagocytosis of microphages in combination with the maximum increase in the measure of the completeness of phagocytosis. Instead, the parameters of the phagocytic function of macrophages under these conditions increase as much as possible. The weakening of inhibition of the thyroid function is also associated with less pronounced changes in the parameters of phagocytosis, and in the absence of significant changes in the thyroid status, all parameters of microphages and macrophages do not differ significantly from the control ones. However, stimulation of thyroid function again leads to inhibition of activity and intensity of absorption of microbes and increase their killing by microphages, but not by macrophages.

The mass of the thymus by a significantly inhibitory thyrotropic effect of Naftussya significantly decreases. At the same time, the proportion of lymphoblasts and epitheliocytes in the thymocytogram decreases, while the share of plasma cells and Hassal's corpuscles increases. The weakening of the degree of inhibition of thyroid function is associated with the ascent of the hypoplasia of the thymus in general and epitheliocytopenia in particular; in this case, the degree of lymphoblastopenia decreases. The increased content of Hassal's bodies is stored at the previous level, at the same time, plasmocytosis of the thymocytogram comes to naught, instead the level of macrophages increases. The neutral thyrotropic effect of Naftussya is accompanied by the same hypoplasia of the thymus as significantly inhibitory, in combination with a similarly elevated content of Hassal's bodies and a reduced content of epitheliocytes. At the same time, the content of endotheliocytes decreases, and the levels of the remaining elements of the thymocytogram do not differ significantly from the control ones. Stimulation of thyroid function is accompanied by a further increase in the content of Hassal's bodies in the thymocytogram in combination with a decrease in the content of lymphoblasts and an increase in macrophages to a degree similar to that of moderate suppression of thyroid function.

The mass of the spleen is also reduced by the significantly inhibitory thyrotropic effect of Naftussya. However, splenocytogram does not differ significantly from the control, with the exception of the tendency to increase the share of lymphoblasts. Instead, the moderately inhibitory thyrotropic effect is accompanied by a decrease in the content of plasma cells and fibroblasts in combination with an increase in the content of eosinophils against the background of the normal mass of the spleen. In the absence of significant changes in the thyroid index, a decrease in the content of neutrophils in the splenocytogram and an increase in reticulocytes were detected. Stimulation of thyroid function is accompanied by a decrease in the degree of reticulocytosis and neutropenia in combination with the development of fibroblastosis and plasmocytopenia.

1.4. Gastroentero-pancreatic endocrine system (GEPES)

There is a concept on the implementation of the physiological and therapeutic effect of mineral waters due to their modulating effect on GEPES.

Schmidt-Kessen (1978) pioneered this plan. In a thorough clinical-physiological experiment on healthy volunteers, he showed that taking 300 ml of Karlsbad mineral water (almost isotonic, chloride-sulfate sodium-calcium-magnesium) within a few minutes after the start of drinking caused an increase in serum gastrin, which peaked - 31 pg/ml against 11 pg/ml on an empty stomach - after 10-15 minutes, and by the 30th minute the level of gastrinemia dropped to the basal. Ordinary drinking water also caused an increase in plasma gastrin, but to a lesser extent - only up to 22 pg/ml, while with periodic blood sampling during 30 minutes of monitoring in the same person, the level of gastrin did not naturally change, remaining within 9-14 pg/ml. Mineral water not only increased the level of gastrin on an empty stomach, but also significantly - by 7.4 times - increased the gastrinincretory reaction to food, which followed 15 minutes after the end of drinking water. Drinking water under these conditions increased the peak of gastrinemia by 5.5 times, while for the food itself, the level of gastrin increased by only 4.6 times. The duration of the postprandial gastrinincretory reaction, which was 45 minutes, was not affected by drinking or mineral water.

In the same person at the same time studied insulinincretory reaction to water intake and subsequent consumption of food. It is established that drinking water causes a gradual increase in insulinemia levels by an

average of 22 μU/ml 15 minutes after the start of drinking, followed by a steep decline to the basal at the 25th minute. In contrast, mineral water contributes to the rise in insulin levels by 30 μU/ml after 5 minutes, keeping it for the next 15 minutes, after which this level fell steeply, even below the basal, in the 25th minute. Interestingly, the insulinincretory reaction to the food itself was even somewhat inferior to that of mineral water. Under the condition of preliminary intake of the latter, the postprandial insulinincretory reaction increased significantly: this applies both to the value of the increase in insulinemia (by 70 μU/ml) and its duration (25 minutes versus 18 minutes in control).

The cited work of the German balneologist gave a powerful impetus for similar studies of other mineral waters.

In the research of the Pyatigorsk Research Institute of Balneology on healthy rats (Polushina et al., 1990; 1993; 1994) it was found that a single introduction into the stomach of Yessentuki water No. 17 stimulates the release of the hormones GEPES - gastrin, glucagone, VIP, insulin, serotonin, as well as ACTH and aldosterone, while inhibits the release of cortisol and, to a small extent**,** triiodothyronine and thyroxine.

The content of somatostatin - another hormone of GEPES, given the paracrine nature of its action, was determined not in the blood, but in the tissues. It turned out that under the influence of Yessentuki water No. 17, the somatostatinhistia of the small intestine and pancreas decreased, and the stomach increased (Kuznetsov et al., 1984).

The data presented relate to the immediate effects of mineral waters. Of much greater practical interest are the results of the study of the effect on GEPES course use of drinking medicinal waters, since they are used in this way.

According to one data (Saakyan et al., 1983), the course use of water Yessentuki No. 17 or Slavyanovskaya does not affect the basal level of insulin in rats (healthy and with experimental ulceration) and in patients with duodenal ulcer (DU) in the phase of remission (complete or incomplete). According to other data of the same group of authors, the basal concentration of insulin in patients with DU (adults and children) in the remission phase and in rats with acetate ulcer for Okabe as a result of the course of drinking treatment increases (Ossipov et al., 1981; Tarverdyan et al., 1983), while in patients with DU in the phase of fading exacerbation - decreases (Saakyan et al., 1983). In patients with diabetes mellitus treated with mineral water, basal insulinemia decreased (Kuznetsov, 1980) or increased (Rachmanova et al., 1984).

It is shown that in healthy rats and patients with diabetes mellitus or DU in the phase of complete remission or fading exacerbation course drinking is accompanied by a decrease in insulin incretion stimulated by glucose dissolved in mineral waters Yessentuki No. 17 or Slavyanovskaya (Kuznetsov, 1978; 1980; Saakyan et al., 1983). In patients with DU in the phase of incomplete remission, reactivity did not change, while in rats with acetate ulcer by Okabe - increased (Saaakyan et al., 1983). Therefore, both in normal and in certain pathological conditions, the course of consumption of mineral waters, as a rule, does not affect the basal level of insulin in the blood. At the same time, the reactivity of the insular link of GEPES changes, as a rule, in the direction of weakening.

Another hormone of GEPES - gastrin - is more subordinate to the effects of course drinking of mineral waters. According to Ossipov et al., (1981) and Tarverdyan et al., (1983) as a result of balneotherapy in Zheleznovodsk children with DU reduced basal level of gastrin increases; in adults with a similar pathology at the beginning of treatment, basal hypergastrinemia occurred, which at the end of treatment became even more pronounced. Vygodner (1987) showed that after the use of Moscowskaya mineral water, reduced and normal levels of plasma gastrin increased at the beginning of treatment at only 44% of patients, while after the course of drinking normal and reduced levels increased, while increased levels decreased. According to Dzvonkovskyi (1986), course treatment of patients with chronic gastroduodenitis with the use of Morshyns'ka chloride-sulfate potassium-magnesium-sodium water, regardless of drinking regime, probably did not affect either the basal or postprandial levels of gastrinemia.

The level of glucagon in adults with DU, initially increased, as a result of treatment in Zheleznovodsk increased even more, while in children hyperglucagonemia decreased by 33%, not reaching the norm (Ossipov et al., 1981; Tarverdyan et al., 1983). Polushina et al., (1990; 1993; 1994) showed that at rats course drinking with Yessentuki water No. 17 increases the basal plasma gastrin by 80%, glucagon by 102%, insulin by 43%, cortisol by 3%. ACTH by 33%, serotonin by 74%, while reduces the level of aldosterone by 19%, triiodothyronine by 33%, thyroxine by 41%. Control rats drunk with tap water had similar shifts in relation to insulin (+31%), triiodothyronine (-17%) and thyroxine (-29%), so we can talk about the specificity of the effect of course drinking mineral water on the level of gastrin, glucagon, cortisol and aldosterone. Unlike insulin, the reactivity of gastrin and serotonin to the introduction of mineral water at the end of the course of drinking increases.

The morphological substrate for the development of hypergastrinemia by the course administration of calcium salts is hyperplasia of G-cells of the antral mucosa, as evidenced by the data of experiments in rats with a 5-day administration of 0.11 M of calcium chloride at a dose of 2 ml/200 g (Katić et al., 1981) or 8-week - 1-2 ml of antacid containing calcium bicarbonate (Kaduk, Häuser, 1980).

The influence of Naftussya water on GEPES in humans is investigated by Popovych et al., (1987-2000). The authors show that the bonding and the degree of reaction of gastrin are determined by its initial serum level.

In particular, in all patients with normal basal gastrinemia (57÷93 pg/ml) and in 16% of patients with minor hypergastrinemia (115÷127 pg/ml) 15 minutes after Naftussya, gastrin levels increase by 50±14 pg/ml. While in 84% of patients with hypergastrinemia, it decreases by an average of 35±20 pg/ml. A close inverse correlation (r=-0.76) was found between basal gastrinemia and the magnitude of the gastrinincretory reaction to Naftussya. The nature of the glucagonincretory reaction is also determined by the initial level of the hormone: in cases of hypoglucagonemia (210÷350 pg/ml), the glucagon level increased by an average of 59±16 pg/ml after 15 minutes, at the same time, the normal levels of the hormone (380÷450 pg/ml) decreased by an average of 73±28 pg/ml. The correlation coefficient between basal glucagonemia and glucagonincretory response to Naftussya is -0.90. The serum insulin in 2/3 of patients in response to Naftussya increased from 1÷3 μU/ml to 8÷10 μU/ml, on average by 7.0±0.5 μU/ml, and in the rest no significant changes were detected or there was a slight decrease in insulinemia. There is a close relationship between reactions to Naftussya of insulin and gastrin (r=0.84) and insulin and glucagon (r=0.99). There is no relationship between gastrin and glucagon reactions (r=0.03).

In the same study, it is shown that the course of balneotherapy with drinking Naftussya significantly affects both the basal level of hormones and their immediate reactions to Naftussya. In particular, at the end of drinking treatment in all observed patients, the basal level of gastrinemia was detected within the normal range, at the same time, the reaction to Naftussya was significantly reduced, both gastrinenhancing (from 60% to 4%), and gastriningibitory (up to -17%); at the same time, the inverse relationship between the basal level of the hormone and its reaction to Naftussya disappeared (r=-0.20).

The basal level of glucagonemia under the influence of balneotherapy also normalized, while the reactivity of the glucagone to Naftussya, unlike gastrin, increased: from -17% to -30%, judging by the change in basal level. At the same time, a close inverse relationship between the basal level and the degree of its decrease remained (r=-0.91).

Basal insulin levels at the end of treatment showed only a tendency to increase. In 43% of people, in response to Naftussya, insulin levels increased from 3÷15 μU/ml to 8÷19 μU/ml, on average by 4.7±0.8 μU/ml, while in 57% of patients remained unchanged or decreased by 1÷2 μU/ml, that is, the reactivity of the insulin decreased slightly. No correlation was found between basal insulinemia and insulinincretory reaction to Naftussya (r=-0.17).

The described functional changes in GEPES were accompanied by an increase in the antral mucosa of the total density of G- and Ec-endocrinocytes from 7÷9 cells/mm^2 to 38÷63 cells/mm^2.

Experiments on rats and dogs give grounds to attribute functional and morphological changes in GEPES under the influence of complex treatment to the account of Naftussya. Yes, it is shown that at rats the course use of Naftussya causes increase the density of argyrophilic endocrinocytes by 19% (up to 205±5 celis/mm^2 versus 172±3.5 cells/mm^2 in control). This is accompanied by a 25% increase in basal gastrinemia (up to 76±6 pg/ml versus 61±1 pg/ml) and a nearly three-fold increase in gastrin in the antrum.

The experiment on dogs tracked changes in the gastrin and insulin on the 4th, 7th and 15th day of the course of drinking them Naftussya (3 ml/kg for 4 hours and 4 hours after meals), as well as 7 days after the completion of the course. Control dogs received an artificial salt analogue of Naftussya. It was found that already on the 4th day of the course, the basal level of gastrinemia increases by 70% against 9% in control; on the 7th day, the increase reaches 77% and 16%, respectively. On the last, 15th day of the course in control dogs, basal gastrinemia exceeded this at the beginning by only 5%, while in dogs of the main group by 58%. The level of gastrin was increased (by 30%) and on the 7th day of the recovery period. Regarding basal insulinemia, no natural changes have been detected.

In another experiment, dogs were drinking Naftussya at a dose of 15 ml/kg once for 20 days. On the 5th, 10th, 15th and 20th days of the course, gastrinemia was registered in basal conditions and 30 minutes after the intragastric introduction of Naftussya. It was found that already on the 5th day of the course, the basal level of gastrins reached 246% of the background, then it fell to 213% on the 10th day and 167% on the 15th day, after which it again increased to 291% on the 20th day. At the same time, the postprandial (reactive) level of gastrinemia did not differ significantly from the background on either the 5th or 10th day of the course, so that the severity of the gastrinincretory reaction decreased to 124±9% and 124±5% vs 215±54% at the beginning. On the 15th day of the course, reactivity was restored (199±46%) due to a decrease in basal and an increase in reactive gastrin levels. At the end of the course of drinking, the reversion of the gastrinincretory reaction was stated: the maximum basal level (70.7±3.5 pg/ml) after the introduction of Naftussya decreased to 16.3±3.2 pg/ml.

Based on the obtained facts, Popovych et al., (1990) suggest that the active factors of Naftussya (hypotension, ions, organic substances) realize their gastrinincretory effect due to irritation of the interoreceptors (osmo- and chemoreceptors) of the antral-duodenal mucosa, the impulses from which enter the afferent neurons of the intramural (meta-sympathetic) nervous system, which are Dogel's cells of type II; cholinergic axons of the latter through N-cholinoreceptors excite effector neurons – Dogel's cells of type I, both cholinergic and adrenergic. Axons of the first neurons, in turn, terminate in activating M-cholinoreceptors of gastrin-containing G-cells and insulin-containing β-cells and inhibitory M-cholinoreceptors D-, H-, A- and possibly α-cells containing somatostatin, VIP, respectively. enteroglucagon and glucagon respectively. Axons of other neurons,

on the contrary, excite the α- and β-adrenoreceptors of the listed GEPES cells. It is known that the release of gastrin is realized through M-cholino-, α- and β-adrenoreceptors, the release of somatostatin, glucagon, other polypeptides of the secretin family - through adrenoreceptors of both types, instead, the release of insulin during the excitation of β-adrenoreceptors is activated, while the α-adrenoreceptors is inhibited. On the other hand, endocrinocytes of different types interact with each other through their products (incretes) in a paracrine or endocrine way, in particular somatostatin, glucagon, VIP inhibit the release of gastrin, that is, they act as gastrons; somatostatin inhibits, while gastrin, glucagon, VIP, GIP stimulate the release of insulin, that is, act as incretins. It is with this interaction that the variety of reactions of GEPES to Naftussya can be explained.

In "**Conclusion**", readers will probably be interested to compare these views 32 years ago with modern ones.

CHAPTER 2

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

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 (Popovych et al., 2014; Kozyavkina et al., 2015), which function as a triple complex (Korneva, 1993; Kolyada et al., 1995; Khaitov, 2005; Sternberg, 2006; Uchakin et al., 2007; Tracey, 2009; Popovych, 2008; 2009; Thayer, Sternberg, 2010). The priority is that the operating principles of mineral waters are not only salts and trace elements, but also organic matter and autochthonous microflora (Bilas, Popovych, 2009; Popovych, Ivassivka, 2009). On the basis of these principles an algorithm for research of newly opened drinking mineral waters was created.

This chapter begins with a series of investigations 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 Hertsa (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 Hertsa 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.

Experimental designe. 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 Hertsa 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 2.1.

Table 2.1. The chemical composition of the applied mineral waters

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

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).

Methods. 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 (Baevskiy et al., 1984).

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 (Gavrilov, Mishkorudnaya, 1983)) and malonic dyaldehide (in the test with thiobarbituric acid (Andreyeva et al., 1988)), 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 (Makarenko, 1988)) and catalase plasma (at the rate of decomposition of hydrogen peroxide (Korolyuk et al., 1988)), 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 (Goryachkovskiy, 1998). The analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a flamming spectrophotometer "СФ-47".

According to the parameters of electrolyte exchange, hormonal activity was evaluated: Parathytoid 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 (2000).

In rats a sample of peripheral blood (by incision of the tip of the tail) was taken for analysis of Leukocytogram (LCG), ie the relative content of lymphocytes (L), monocytes (M), eosinophils (Eo), basophils (Bas), rod-shaped (RN) and polymorphonuclear (PMN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the formula derived by IL Popovych on the basis of the classical CE Shannon (1948) formula:

$$hLCG = - (L•\log_2 L+M•\log_2 M+Eo•\log_2 Eo+Bas•\log_2 Bas+RN•\log_2 RN+PMN•\log_{2PM} N)/\log_2 6.$$

The parameters of immunity were determined according to the tests of the 1st and 2nd levels of the WHO, as described in the manual (Perederiy et al., 1995): the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by Jondal et al., (1972), their theophylline-resistant (T-helper) and theophyllin-susceptible (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline by Limatibul et al., (1978); the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by Bianco, (1970). Natural killers were identified as large granules contain lymphocytes. The content of zero-lymphocytes (0L) was calculated by the balance method. For these components, as well as plasma cells (Pla), the Entropy of the Immunocytogram (hICG) was calculated:

$$hICG = - (Th•\log_2 Th+Tc•\log_2 Tc+B•\log_2 B+Pla•\log_2 Pla+NK•\log_2 NK+0L•\log_2 0L)/\log_2 6.$$

The blast transformation reaction of T-lymphocytes to phytohemagglutinin was performed separately (Perederiy et al., 1995).

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) (Douglas, Quie, 1981; Bilas, Popovych, 2009).

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 (Belousova, Fedotova, 1968; Bilas, Popovych, 2009). For them, as well as leukocytogram, CE Shannon's entropy was calculated.

In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope.

Digital material is statistically processed on a computer using the software package "Statistica 5.5" and "Statistica 64".

In order to make a comparative assessment of changes, we expressed the values of the indices in Z-score 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. 2.1), while the other 16 decrease (Fig. 2.2) with respect to intact control.

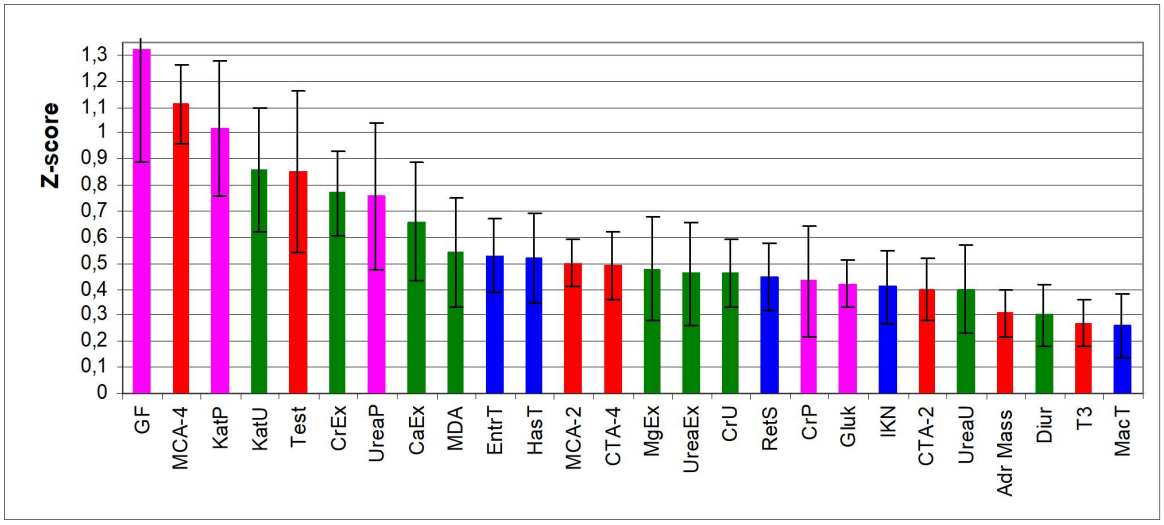


Fig. 2.1. Ranking of significant increasing changes (Z±SE) of the parameters of the **neuroendocrine**-**immune** complex and metabolic parameters of **blood** and **urine** caused by the water-salt load

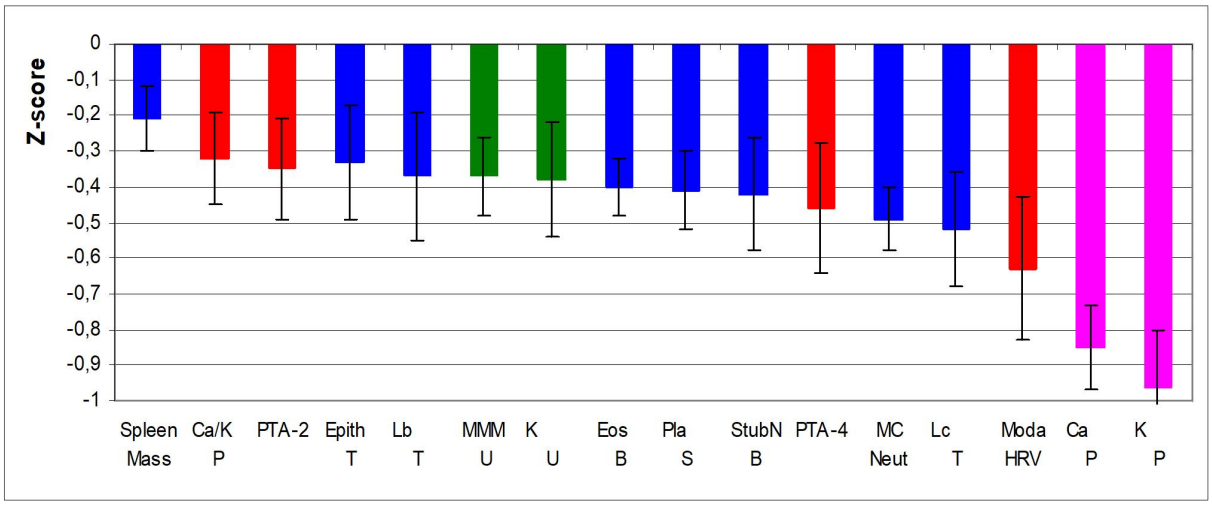


Fig. 2.2. Ranking of significant decreasing changes (Z±SE) of the parameters of the **neuroendocrine**-**immune** complex and metabolic parameters of **blood** and **urine** caused by the water-salt load

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 Hassall 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 parathyroid ($PTA_2=(CaP/PP)^{0,5}$; $PTA_4=(CaP•PU)/(CaU•PP)^{0,25}$) 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 (Polovynko et al., 2013; 2016; Polovynko, Zukow, 2016; Zajats et al., 2017; 2017a; Gozhenko et al., 2019; Popovych et al., 2018; 2020).

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 (Klecka, 1986). To include in the model (Table 2.2), the program has selected only 21 variables, while the other 21 were outside the discriminant model (Table 2.3).

Table 2.2. 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 Paratyroide 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 Dialdehid 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, µM/h•L	,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 Calcitonin 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 2.3. 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 Calcitonin 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
Glucose 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 Parathyroid 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 2.4 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 parathyroid 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 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 2.4. 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 Parathyroide 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 Calcitonin 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).

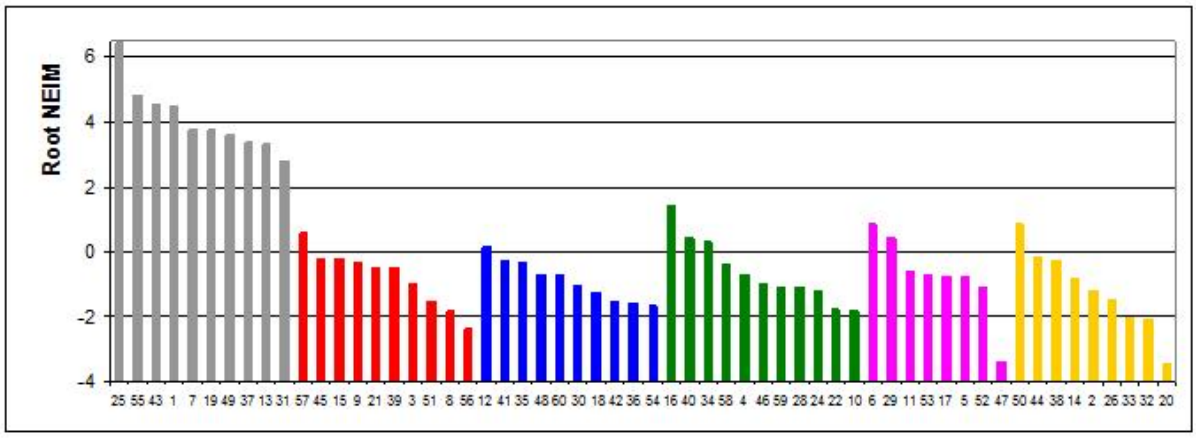


Fig. 2.3. Individual values of discriminant root of parameters of metabolism and neuroendocrine-immune complex of **intact** rats and loaded with **daily** water, mineral waters **Sofiya** and **Hertsa**, artificial **salt analogue** of Hertsa water, as well as bioactive water **Naftussya**. Below are 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. 2.4).

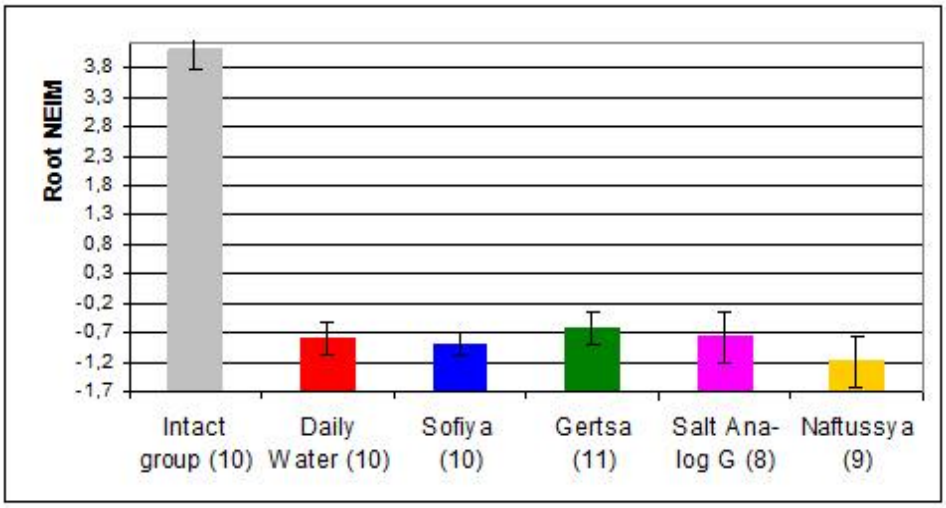


Fig. 2.4. Average values of discriminant root of parameters of metabolism and neuroendocrine-immune complex of **intact** rats and loaded with **daily** water, mineral waters **Sofiya** and **Hertsa**, artificial **salt analogue** of Hertsa water, as well as bioactive water **Naftussya**.

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 discriminant 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 2.5).

Table 2.5. 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 Parathyroide 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 Calcitonin 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 Mineralocorticoid 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 chapter.

CHAPTER 3
FEATURES OF METABOLIC REACTIONS TO VARIOUS WATER-SALT LOADS IN FEMALE RATS

In the previous chapter, we reported that screening registered parameters of water-salt, nitrous and lipid metabolism as well as the neuroendocrine-immune complex found 42 among them who in rats subjected to various water-salt loads, significantly different from that of intact rats, but on average the same group of animals that received liquids with different mineralization and chemical composition. The purpose of this chapter is to find out the features of the reactions of the parameters of metabolism.

In the first stage of the analysis, all registered parameters were divided into 6 patterns. The pattern is a characteristic sequence of localization of rats in a plane whose Y axis represents the mean of the Z-scores. At the second stage of the analysis, the quasi-mirror patterns were paired.

The first pattern (Table 3.1 and Fig. 3.1) combines nine parameters, the average Z-scores which is maximal for rats that received artificial saline analogue of mineral water Hertsa and dominates this natural mineral water. Next, the therapeutic waters Sofia and Naftussya, as well as daily water, whose effects on these parameters are approximately equally moderate.

Significantly, that most likely to increase are glomerular filtration and excretion of creatinine and urea, as well as diuresis. Back in 2004, we showed that one-time loading with 0,5% solution NaCl (5 mL/kg of body mass) as compared with daily water load is followed immediately by reliably increased excretion of creatinine and nitrous metabolites at healthy volunteers (Gozhenko et al., 2004). A simple recount shows that in the Hertsa native water the concentration of NaCl is 1,28% and in Sofiya water is 0,86%. Nevertheless, their influence on these parameters of the kidneys is much weaker, due, apparently, to the presence in their composition of sulphate and/or of trace elements and organic substances. Contrary to the expectation inspired by extensive literature (Yessypenko, 1981; Yaremenko et al., 1989; Chebanenko et al., 1997; 2014; Ivassivka, 1997; Ivassivka et al., 1999; 2004), the influence of Naftussya water on diuresis and urine excretion of metabolites was weaker.

The second pattern combines seven parameters, the mean Z-score of which, by contrast, is minimal in similar circumstances, while under the influence of other loads, they decrease to a lesser extent. First of all, it is the concentration of potassium in urine, as well as phosphates and medium molecules. Configurations of patterns are close to mirror, which became the basis for their visualization on a common plane.

Table 3.1. The first pair of patterns of reactions of metabolic parameters to water-salt loadings

Variables	Salt Anal G (8)	MW Hertsa (11)	MW Sofiya (10)	Naftussya (9)	Daily Water (10)	Intact rats (10)
Glomerulary Filtration, µL/min•100 g Body Mass	194 2,26 +1,75	142 1,65 +0,91	112 1,30 +0,41	109 1,27 +0,38	86,5 1,01 +0,01	85,9 1 0
Creatinine Excretion, µM/24h•100 g Body Mass	16,03 1,84 +1,68	10,53 1,21 +0,42	12,30 1,41 +0,82	12,27 1,41 +0,82	10,12 1,16 +0,32	8,72 1 0
Katalase Urine, nM/h•mL	163 1,33 +1,47	141 1,15 +0,65	136 1,11 +0,48	145 1,18 +0,80	151 1,23 +1,04	123 1 0
Urea Excretion, µM/24h•100 g Body Mass	315 1,86 +1,08	283 1,68 +0,85	192 1,14 +0,17	201 1,19 +0,24	164 0,97 -0,04	169 1 0
Diurese, mL/24h•100 g Body Mass	2,37 1,65 +1,05	1,66 1,15 +0,25	1,53 1,06 +0,10	1,65 1,14 +0,23	1,44 1,00 0,00	1,44 1 0
Diene conjugates Plasma, E^{232}/mL	1,63 1,21 +0,72	1,65 1,23 +0,76	1,30 0,96 -0,13	1,31 0,97 -0,10	1,45 1,08 +0,25	1,35 1 0
Glukose Plasma, mM/L	5,76 1,16 +0,74	5,15 1,04 +0,19	5,31 1,07 +0,33	5,32 1,08 +0,34	5,61 1,13 +0,60	4,95 1 0
Phosphates Excretion, µM/24h•100 g Body Mass	13,7 0,15 +0,69	10,8 0,12 +0,23	9,2 0,10 -0,03	10,4 0,11 +0,16	9,3 0,10 -0,01	9,4 1 0
Urea Urine,	129 1,20	141 1,32	122 1,14	118 1,10	104 0,97	107 1

mM/L	+0,52	+0,83	+0,36	+0,27	-0,08	0
Pattern I (9)	**+1,08 ±0,15**	**+0,57 ±0,10**	**+0,28 ±0,10**	**+0,35 ±0,10**	**+0,23 ±0,13**	**0**
Potassium Urine, mM/L	95 0,73 -0,91	122 0,93 -0,23	128 0,98 -0,08	104 0,79 -0,70	125 0,96 -0,14	131 1 0
Middle Mass Molecules Urine, units	147 0,80 -0,68	165 0,91 -0,32	159 0,87 -0,44	159 0,87 -0,44	181 0,99 -0,02	182 1 0
Phosphates Urine, mM/L	5,89 0,09 -0,64	6,26 0,10 -0,16	6,13 0,10 -0,33	6,38 0,10 -0,01	6,35 0,10 -0,05	6,39 1 0
Potassium Plasma, mM/L	3,82 0,90 -0,58	3,35 0,79 -1,25	3,12 0,74 -1,58	3,86 0,91 -0,53	3,71 0,88 -0,73	4,23 1 0
Superoxide Dismutase Plasma, units/mL	53,3 0,92 -0,44	51,4 0,89 -0,62	57,8 1,00 -0,02	56,8 0,98 -0,12	56,3 0,97 -0,16	58,0 1 0
Bilirubine Plasma, µM/L	3,94 0,85 -0,27	4,77 1,03 +0,06	4,20 0,91 -0,17	4,70 1,02 +0,03	5,04 1,09 +0,16	4,63 1 0
Pattern II (7)	**-0,54 ±0,09**	**-0,40 ±0,16**	**-0,34 ±0,22**	**-0,30 ±0,10**	**-0,10 ±0,12**	**0**

Notes. In each graph, the first line is the actual mean values, the second line is the portion of the average value of the intact group (L/I), the third row is Z=(L/I-1)/Cv. Patterns display average values of Z and their standard errors.

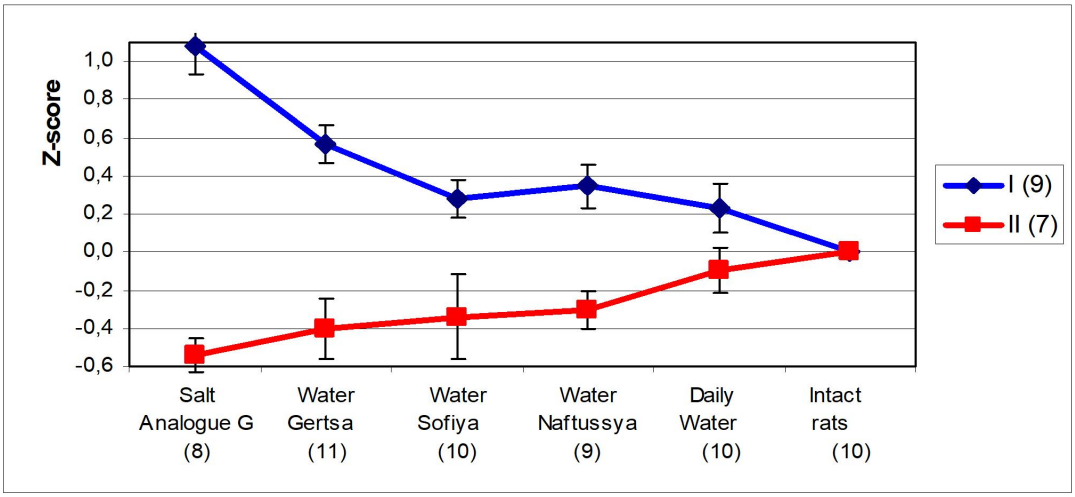


Fig. 3.1. The first pair of patterns of reactions of metabolic parameters to water-salt loads

The third pattern (Table 3.2 and Figure 3.2) combines 12 parameters, the mean of Z-score which is reduced to a greater extent under the influence of bioactive water of Naftussya and less noticeable in the rats receiving daily water from the tap, while the tendency towards increasing is in animals, which received the mineral waters Sofiya and Hertsa and significantly increased under the influence of the salt analogue of the latter.

Mostly, the excretion and concentration in urine of sodium and chloride decreases, which leads to a decrease in the osmolarity of the urine. In addition, the content in the urine of diene conjugates, in the plasma of uric acid and calcium, in erythrocytes of sodium and potassium is reduced, as well as tubular reabsorption of water.

On the opposite end of the pattern, on the contrary, the opposite changes in these parameters are reflected, with the exception of a significant increase in urinary concentration of chloride and potassium and caused by these ions the urinary osmolality, as well as the concentration in calcium plasma.

Table 3.2. The second pair of patterns of reactions of metabolic parameters to water-salt loadings

Variables	Salt Anal G (8)	MW Hertsa (11)	MW Sofiya (10)	Intact rats (10)	Daily Water (10)	Naftussya (9)
Osmolarity Urine, mOsm/L	581 1,04 +0,16	623 1,11 +0,46	598 1,07 +0,28	559 1 0	464 0,83 -0,69	424 0,76 -0,98
Sodium Excretion, µM/24h•100 g Body Mass	282 2,09 +1,75	225 1,67 +1,08	175 1,30 +0,48	135 1 0	89 0,66 -0,54	66 0,49 -0,81
Chloride Excretion, µM/24h•100 g Body Mass	244 1,69 +1,01	203 1,41 +0,60	195 1,35 +0,52	144 1 0	102 0,71 -0,43	66 0,46 -0,80
Sodium Urine, mM/L	135 1,28 +0,45	128 1,22 +0,34	117 1,11 +0,18	105 1 0	64 0,61 -0,62	53 0,50 -0,78
Chloride Urine, mM/L	129 1,12 +0,17	132 1,15 +0,21	133 1,15 +0,22	115 1 0	69 0,61 -0,56	47 0,41 -0,85
Calcium Plasma, mM/L	3,36 1,00 +0,01	2,32 0,69 -1,01	2,57 0,77 -0,76	3,35 1 0	1,88 0,56 -1,44	2,44 0,73 -0,89
Diene conjugates Urine, E^{232}/mL	2,14 1,15 +0,43	1,66 0,89 -0,30	1,90 1,03 +0,07	1,86 1 0	1,70 0,92 -0,23	1,45 0,78 -0,61
Uric Acid Plasma, µM/L	781 1,18 +0,35	935 1,41 +0,80	550 0,83 -0,33	662 1 0	716 1,08 +0,16	504 0,76 -0,46
Potassium Erythrocytes, mM/L	90,1 1,04 +0,46	85,8 0,99 -0,18	88,5 1,02 +0,21	87,0 1 0	86,9 1,00 -0,02	83,9 0,96 -0,45
Sodium Erythrocytes, mM/L	25,5 1,16 +0,78	22,7 1,03 +0,16	21,8 0,99 -0,04	22,0 1 0	23,7 1,08 +0,38	20,6 0,93 -0,33
Canalicular Reabsorbtion, %	98,8 1,00 +0,11	99,0 1,00 +0,33	98,9 1,00 +0,24	98,7 1 0	98,7 1,00 -0,02	98,5 1,00 -0,27
Potassium Excretion, µM/24h•100 g Body Mass	197 1,04 +0,07	173 0,92 -0,13	189 1,00 +0,01	189 1 0	191 1,01 +0,02	179 0,95 -0,08
Pattern III (12)	**+0,48 ±0,14**	**+0,20 ±0,16**	**+0,09 ±0,10**	**0**	**-0,33 ±0,14**	**-0,61 ±0,09**
Calcium Urine, mM/L	1,93 0,92 -0,44	2,32 1,11 +0,59	2,07 0,99 -0,07	2,10 1 0	2,15 1,02 +0,13	3,05 1,46 +2,55
Calcium Excretion, µM/24h•100 g Body Mass	4,50 1,55 +1,05	4,25 1,47 +0,88	3,09 1,07 +0,13	2,90 1 0	3,08 1,06 +0,12	4,76 1,64 +1,22
Urea Plasma, mM/L	7,44 1,00 +0,01	7,85 1,06 +0,25	9,29 1,25 +1,09	7,42 1 0	8,92 1,20 +0,88	10,03 1,35 +1,52
Katalase Plasma, nM/h•mL	135 1,30 +1,12	122 1,18 +0,66	120 1,16 +0,60	103 1 0	145 1,40 +1,49	142 1,37 +1,38
Creatinine Plasma, µM/L	73 1,01 -0,04	68 0,94 -0,18	90 1,24 +0,72	73 1 0	86 1,18 +0,55	98 1,35 +1,04
Cholesterol Plasma,	1,46 0,93	1,53 0,97	1,62 1,03	1,57 1	1,60 1,02	1,67 1,06

mM/L	-0,23	-0,09	+0,11	0	+0,06	+0,21
Amylase Urine, mg/h•mL	181 0,90 -0,39	215 1,06 +0,23	210 1,04 +0,14	202 1 0	212 1,05 +0,18	210 1,04 +0,15
Pattern IV (7)	**+0,15 ±0,25**	**+0,33 ±0,15**	**+0,39 ±0,16**	**0**	**+0,49 ±0,20**	**+1,15 ±0,31**

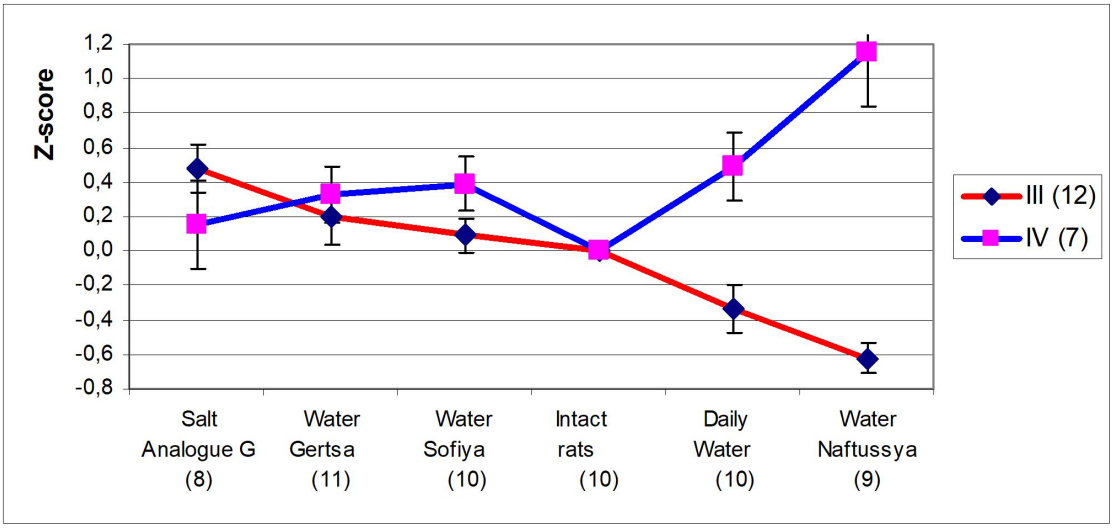


Fig. 3.2. The second pair of patterns of reactions of metabolic parameters to water-salt loads

The fifth pattern (Table 3.3 and Figure 3.3) combines 6 parameters, the mean value of Z which increases significantly under the influence of Sofiya water. Urinary excretion and concentration of magnesium, as well as the concentration of creatinine in urine and plasma amylase activity, are the most prevalent, while phosphateemia and urine concentration of malonic dialdehyde are slightly increased. Under the influence of Naftussya water, the average Z value tends to increase, whereas neither Hertsa native water nor its salt analogue causes significant changes in these parameters, while daily water causes a weak tendency to decrease them.

The sixth pattern, mirrored to the fifth, reflects the maximum drop in plasma chloride and middle mass molecules levels, urinary concentration and excretion of uric acid, as well as the tendency towards a decrease in the plasma level of malonic dialdehyde, sodium and magnesium. Instead, under the influence of water from the crane, these parameters increase to varying degrees, without reacting substantially to Hertsa native water or to its salt analogue.

Table 3.3. The third pair of patterns of reactions of metabolic parameters to water-salt loadings

Variables	Daily Water (10)	MW Hertsa (11)	Intact rats (10)	Salt Anal G (8)	Naftu ssya (9)	**MW Sofiya (10)**
Magnesium Excretion, µM/24h•100 g Body Mass	2,65 0,80 -0,31	2,51 0,76 -0,38	3,30 1 0	5,85 1,77 +1,23	5,07 1,54 +0,85	5,98 1,81 +1,29
Magnesium Urine, mM/L	1,90 0,74 -0,37	1,73 0,68 -0,47	2,56 1 0	2,54 0,99 -0,01	3,27 1,26 +0,38	4,09 1,60 +0,86
Creatinine Urine, mM/L	7,15 1,12 +0,40	6,83 1,07 +0,23	6,41 1 0	7,01 1,09 +0,32	7,16 1,12 +0,41	8,12 1,27 +0,93
Amylase Plasma, mg/h•mL	145 0,96 -0,27	163 1,07 +0,45	152 1 0	134 0,88 -0,73	152 1,00 -0,01	171 1,13 +0,78
Phosphate Plasma, mM/L	0,87 0,84 -0,27	0,72 0,69 -0,52	1,04 1 0	0,92 0,88 -0,20	0,88 0,85 -0,26	1,22 1,18 +0,30
Malonic Dialdehyde Urine, µM/L	77 0,83 -0,36	91 0,99 -0,03	92 1 0	81 0,88 -0,25	87 0,95 -0,11	102 1,10 +0,22

Pattern V (6)	-0,20 ±0,12	-0,12 ±0,16	0	+0,06 ±0,27	+0,21 ±0,17	+0,78 ±0,17
Chloride Plasma, mM/L	95,0 1,01 +0,20	91,5 0,98 -0,38	93,8 1 0	92,9 0,99 -0,14	93,2 0,99 -0,10	89,7 0,96 -0,67
Middle Mass Molecules Plasma, units	193 1,25 +0,76	119 0,78 -0,67	154 1 0	148 0,96 -0,11	134 0,87 -0,38	126 0,82 -0,55
Uric Acid Urine, mM/L	4,70 1,28 +0,55	4,23 1,15 +0,30	3,68 1 0	2,91 0,79 -0,42	3,18 0,86 -0,27	2,56 0,69 -0,61
Uric Acid Excretion, µM/24h•100 g Body Mass	6,7 1,16 +0,18	6,0 1,04 +0,04	5,7 1 0	6,5 1,14 +0,15	4,9 0,86 -0,15	3,8 0,66 -0,36
Malonic Dialdehyde Plasma, µM/L	92 1,45 +1,30	81 1,28 +0,83	63 1 0	62 0,97 -0,08	80 1,26 +0,76	57 0,91 -0,27
Sodium Plasma, mM/L	131,8 1,03 +0,40	128,6 1,00 +0,01	128,6 1 0	127,8 0,99 -0,09	129,9 1,01 +0,16	127,0 0,99 -0,19
Magnesium Plasma, mM/L	1,05 1,19 +0,28	0,70 0,80 -0,29	0,88 1 0	0,79 0,89 -0,16	0,90 1,02 +0,03	0,80 0,90 -0,14
Pattern VI (7)	+0,52 ±0,15	-0,02 ±0,19	0	-0,12 ±0,07	+0,01 ±0,14	-0,40 ±0,08

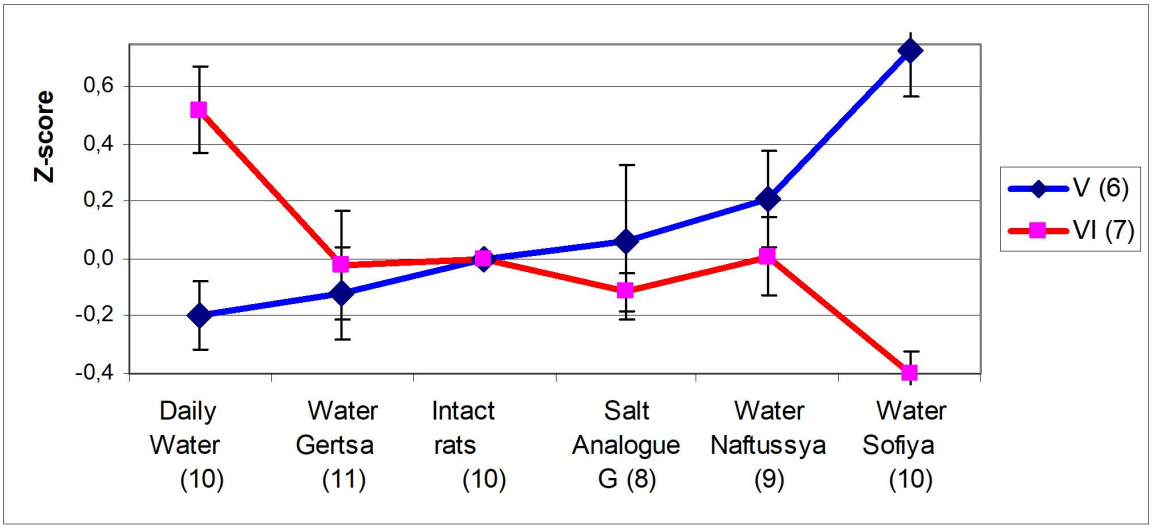


Fig. 3.3. The third pair of patterns of reactions of metabolic parameters to water-salt loads

At the next stage of the analysis, three pairs of mirror patterns were doubled and modulated by the reaction of the parameters to the dominant stimulus (Fig. 3.4).

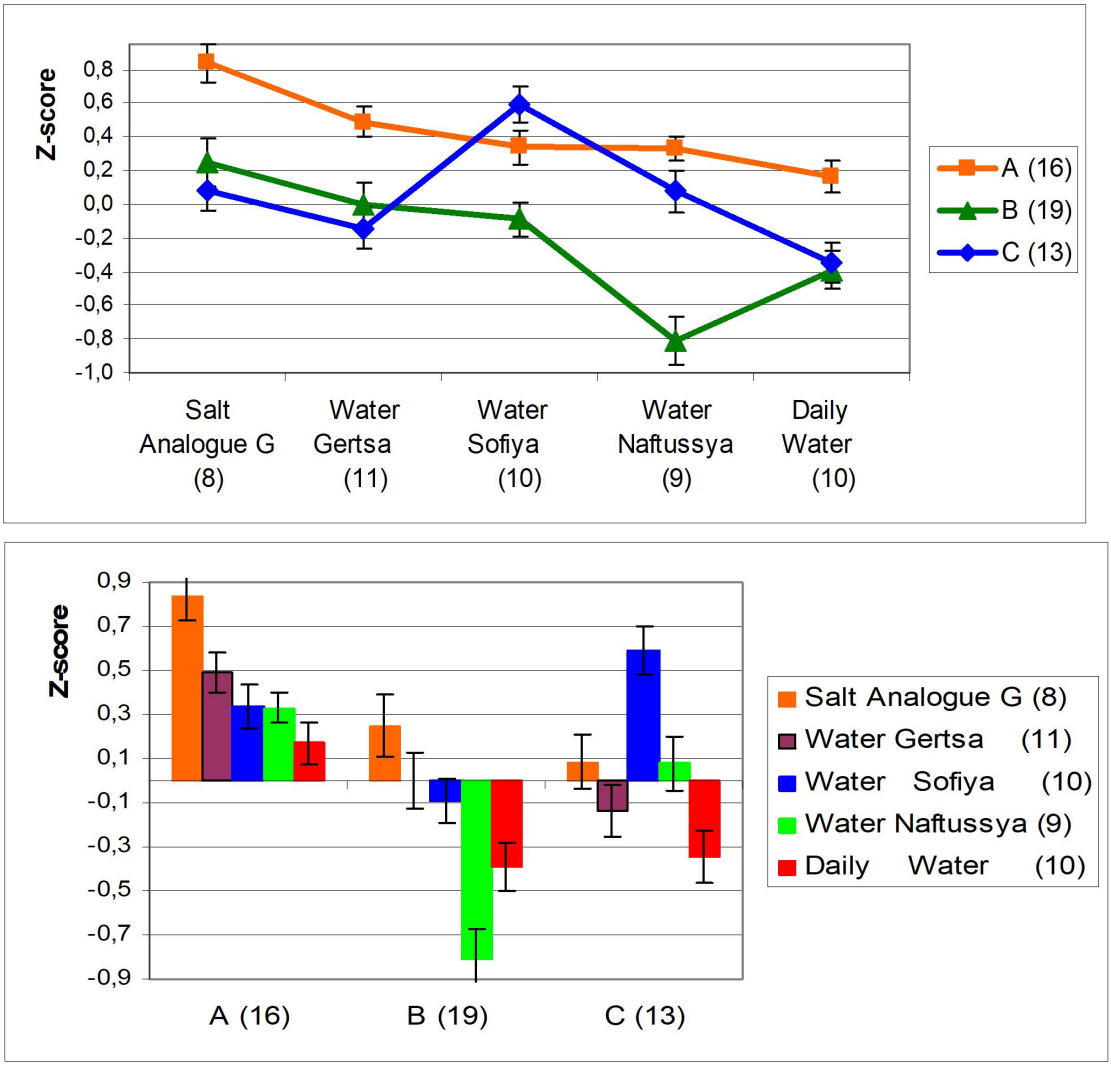


Fig. 3.4. Doubles and modulated superpatterns of reactions of metabolic parameters to water-salt loads

It can be seen that 16 parameters combined in superpattern A, the maximum deviates from the level of intact rats under the influence of the salt analogue of Hertsa water, a smaller, but tangible effect is made by the Hertsa native water, even fewer effective waters Sofiya and Naftussya, instead of ordinary water is almost ineffective in relation to these metabolic parameters. It seems that the deviation from control of these parameters of metabolism is most influenced by the cations of Na^+ and Cl^- entering the body, while the simultaneous receipt of trace elements and organic carbon, and possibly of sulphate anion, weakens the effect of NaCl, whereas organic nitrogen enhances it.

The other 19 parameters combined into superpattern B, deviates to a maximum extent from the reference level after the use of Naftussya water, fresh water is less effective, whereas quasi-isotonic liquids are practically inactive for these parameters. Apparently, their deviation is caused by stress, which accompanies the process of water loading, as well as hypotonicity of water. Organic substances deepen the deviation, whereas the quasi-isotonicity of the waters reduces them.

The remaining 13 parameters of superpattern C in animals that use daily water, deviates from intact control to the same extent as in the previous superpattern, which, apparently, is also due to the stressful effects of the load course. Both Naftussya and Hertsa water and its salt analogue prevent the stress deviations of these parameters. Instead, by consumption of Sofiya water stresses deviations of these parameters is reversed, which is apparently due to the presence of its composition sulphate anion and organic nitrogen, whose content in other liquids is negligible.

Another approach to detecting the features of metabolic reactions to different water-salt loads is a discriminant analysis. The program included 33 variables in the model (among them 8 refer to **plasma/erythrocytes electrolytes**, 7 to **electrolytes of urine**, to other metabolic parameters of **plasma** 5 and **urine** 8, separately allocated 4 **integral** parameters). Instead, other variables were out of the model (Tables 3.4 and 3.5).

Table 3.4. Discriminant Function Analysis Summary
Step 32, N of **Variables currently** in the model: 32; Grouping: 6 groups
Wilks' Lambda: 0,0004; approx. $F_{(165)}$=2,40; $p<10^{-6}$

Variables currently in the model	**Parameters of Wilks' Statistics**				
	Wilks' $\Lambda \cdot 10^{-3}$	Partial Λ	F-remove	p-level	Tolerancy
Calcium Plasma	,56	,756	1,29	,308	,101
Magnesium Excretion	1,18	,359	7,13	10^{-3}	,063
Sodium Excretion	,59	,721	1,55	,219	,026
Potassium Plasma	,70	,608	2,58	,059	,187
Glucose Plasma	,68	,621	2,44	,070	,248
Calcium Urine	,69	,613	2,53	,063	,043
Creatinine Urine	,94	,450	4,89	,004	,016
Phosphate Plasma	,92	,462	4,66	,006	,063
Urea Excretions	,60	,704	1,68	,184	,031
Middle Mass Molecules Urine	,64	,666	2,01	,122	,317
Glomerulary Filtration	,82	,518	3,72	,015	,065
Malonic Dialdehyde Plasma	,78	,544	3,36	,023	,173
Malonic Dialdehyde Urine	1,16	,366	6,92	,001	,073
Diene conjugates Urine	1,23	,345	7,58	10^{-3}	,094
Phosphate Urine	,70	,607	2,59	,058	,024
Potassium Erythrocytes	,75	,565	3,08	,032	,204
Canalicular Reabsorbtion	,86	,492	4,12	,010	,004
Creatinine Plasma	,95	,448	4,94	,004	,003
Chloride Excretion	,74	,570	3,01	,035	,018
Magnesium Plasma	,80	,527	3,58	,018	,062
Osmolarity Urine	,67	,633	2,32	,081	,046
Urea Plasma	1,19	,355	7,26	10^{-3}	,010
Chloride Plasma	,66	,646	2,19	,096	,006
Cholesterol Plasma	,84	,507	3,89	,013	,170
Amylase Urine	,84	,503	3,95	,012	,093
Uric Acid Urine	,55	,763	1,24	,327	,049
Katalase Urine	,77	,550	3,27	,026	,118
Sodium Plasma	,67	,636	2,29	,085	,008
Sodium Erythrocytes	,56	,760	1,26	,318	,146
Phosphates Excretion	,65	,652	2,14	,103	,004
Diurese	,59	,717	1,58	,212	,005
Calcium Excretion	,60	,710	1,64	,196	,018

Variables currently not in the model (df for all F-tests: 5,19)	**Parameters of Wilks' Statistics**				
	Wilks' $\Lambda \cdot 10^{-3}$	Partial Λ	F to enter	p-level	Tolerancy
Creatinine Excretion	,37	,868	,58	,716	,011
Sodium Urine	,41	,969	,12	,986	,038
Chloride Urine	,39	,913	,36	,868	,019
Potassium Excretion	,39	,922	,32	,894	,037
Urea Excretion	,39	,913	,36	,868	,053
Potassium Urine	,41	,977	,09	,993	,073
Magnesium Urine	,40	,949	,20	,957	,055
Bilirubine Plasma	,35	,830	,78	,579	,254
Amylase Plasma	,39	,912	,37	,864	,232
Urea Urine	,37	,885	,49	,778	,103
Uric Acid Plasma	,40	,949	,20	,957	,282
Superoxide Dismutase Plasma	,35	,824	,81	,554	,173
Middle Mass Molecules Plasma	,40	,950	,20	,958	,251
Katalase Plasma	,39	,913	,36	,868	,068
Diene conjugates Plasma	,36	,840	,72	,614	,085

Table 3.5. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Calcium Plasma	5,5	,0004	,656	5,45	10^{-3}
Magnesium Excretion	3,4	,009	,491	4,36	10^{-4}
Sodium Excretion	3,2	,014	,372	3,98	10^{-5}
Potassium Plasma	3,0	,018	,284	3,78	10^{-5}
Glukose Plasma	2,4	,048	,226	3,54	10^{-6}
Calcium Urine	2,5	,042	,178	3,41	10^{-6}
Creatinine Urine	2,2	,066	,143	3,28	10^{-6}
Phosphate Plasma	2,7	,033	,111	3,27	10^{-6}
Urea Excretion	2,4	,053	,087	3,23	10^{-6}
Middle Mass Molecules Urine	1,8	,134	,072	3,12	10^{-6}
Glomerulary Filtration	1,8	,141	,059	3,02	10^{-6}
Malonic Dialdehyde Plasma	2,1	,080	,047	3,00	10^{-6}
Malonic Dialdehyde Urine	1,3	,271	,040	2,89	10^{-6}
Diene conjugates Urine	1,9	,116	,032	2,86	10^{-6}
Phosphate Urine	1,5	,213	,027	2,79	10^{-6}
Potassium Erythrocytes	1,8	,146	,022	2,76	10^{-6}
Canalicular Reabsorbtion	1,6	,179	,018	2,73	10^{-6}
Creatinine Plasma	1,5	,211	,015	2,68	10^{-6}
Chloride Excretion	1,9	,126	,012	2,69	10^{-6}
Magnesium Plasma	1,7	,168	,009	2,67	10^{-6}
Osmolarity Urine	1,8	,137	,007	2,68	10^{-6}
Urea Plasma	1,3	,299	,006	2,63	10^{-6}
Chloride Plasma	1,3	,301	,005	2,59	10^{-6}
Cholesterol Plasma	1,2	,324	,004	2,54	10^{-6}
Amylase Urine	2,0	,114	,003	2,58	10^{-6}
Uric Acid Urine	1,5	,230	,002	2,57	10^{-6}
Katalase Urine	1,3	,295	,002	2,54	10^{-6}
Sodium Plasma	1,4	,256	,001	2,52	10^{-6}
Sodium Erythrocytes	1,3	,310	,001	2,50	10^{-6}
Phosphates Excretion	1,3	,288	,001	2,48	10^{-6}
Diurese	1,4	,257	,001	2,47	10^{-6}
Calcium Excretion	1,1	,384	,001	2,44	10^{-5}

The dividing information contained in 33 variables is condensed in 5 canonical discriminant roots (Table 3.6). At the same time, the first root contains 37,5% of discriminative opportunities, the second is 27,5%, the third is 17,3%, the fourth is 11,6%, and the fifth only 6,1%, therefore, will continue to be ignored.

Table 3.6. Chi-Square Tests with Successive Roots Removed

Roots Removed	Eigen-value	Canonical R	Wilks' Lambda	Chi-Sqr.	Degree freedom	p-level
0	8,04	,943	,0004	291	165	**10^{-6}**
1	5,90	,925	,0038	209	128	**10^{-5}**
2	3,71	,887	,0264	136	93	**,002**
3	2,48	,844	,1243	78	60	,058
4	1,31	,753	,4326	31	29	*,346*

Table 3.7 shows standardized (normalized) coefficients for discriminant variables, while Table 3.8 shows non-standardized (raw) coefficients and constants for discriminant variables.

Table 3.7. Standardized Coefficients for Canonical Variables

Variables currently in the model	Root 1	Root 2	Root 3	Root 4	Root 5
Calcium Plasma	,514	-,017	,948	1,432	,108
Magnesium Excretion	-1,612	-2,624	-1,514	-,307	-,162
Sodium Excretion	,081	,215	2,618	2,728	,178
Potassium Plasma	,781	-,855	-,509	,697	,820
Glucose Plasma	-,697	1,021	,486	-,073	-,154
Calcium Urine	-,113	-,500	3,236	,676	-,572
Creatinine Urine	-3,706	-,692	-4,852	,438	2,428
Phosphate Plasma	,907	2,799	1,107	,438	-,009
Urea Excretion	1,894	2,329	1,016	-,614	,974
Middle Mass Molecules Urine	-,413	,099	,012	-1,119	-,050
Glomerulary Filtration	-1,062	-,914	1,307	2,341	,859
Malonic Dialdehyde Plasma	,669	-1,431	,731	,016	,326
Malonic Dialdehyde Urine	2,825	-,328	-1,158	,557	-,645
Diene conjugates Urine	-2,501	1,029	,240	-,549	,596
Phosphate Urine	1,784	1,438	-3,773	-,816	,805
Potassium Erythrocytes	-,874	,656	,983	,141	,732
Canalicular Reabsorbtion	10,26	-1,391	4,265	-4,353	-6,035
Creatinine Plasma	13,14	-1,750	-,027	-3,437	-5,909
Chloride Excretion	-4,212	-1,244	-1,384	-2,562	1,232
Magnesium Plasma	-2,589	-,774	1,081	,138	,682
Osmolarity Urine	2,038	1,368	,016	,360	-2,143
Urea Plasma	-7,435	,817	3,484	2,112	2,790
Chloride Plasma	4,807	5,889	-2,173	-2,590	-1,846
Cholesterol Plasma	1,403	-,098	-,990	-,542	-,519
Amylase Urine	-1,483	-,173	2,019	,149	-,476
Uric Acid Urine	-1,849	-1,080	-,289	,683	-,785
Katalase Urine	-1,724	-,379	,564	,785	,778
Sodium Plasma	-4,214	-4,762	2,782	2,160	1,724
Sodium Erythrocytes	,917	-,224	,163	-,755	-,864
Phosphates Excretion	1,848	-8,133	6,753	,069	-2,558
Diurese	-,736	7,108	-4,596	-,522	-,677
Calcium Excretion	,490	,939	-4,294	-,364	,784
Discriminant Properties (%)	**37,5**	**27,5**	**17,3**	**11,6**	**6,1**

Table 3.8. Raw Coefficients and Constants for Canonical Variables

Variables currently in the model	Root 1	Root 2	Root 3	Root 4	Root 5
Calcium Plasma	,663	-,022	1,222	1,846	,1398
Magnesium Excretion	-,636	-1,035	-,597	-,121	-,064
Sodium Excretion	,0005	,0013	,0160	,0166	,0011
Potassium Plasma	1,071	-1,171	-,697	,956	1,124
Glucose Plasma	-,901	1,319	,628	-,095	-,199
Calcium Urine	-,137	-,6025	3,897	,814	-,689
Creatinine Urine	-2,204	-,411	-2,885	,260	1,444
Phosphate Plasma	1,791	5,525	2,185	,865	-,018
Urea Excretion	,586	,721	,314	-,190	,301
Middle Mass Molecules Urine	-,009	,002	,003	-,026	-,001
Glomerulary Filtration	-,0131	-,0113	,0161	,0289	,0106
Malonic Dialdehyde Plasma	,0236	-,0506	,0258	,0006	,0115
Malonic Dialdehyde Urine	,0909	-,0105	-,0372	,0179	-,0208
Diene conjugates Urine	-5,553	2,286	,532	-1,219	1,323
Phosphate Urine	,193	,155	-,407	-,088	,087
Potassium Erythrocytes	-,139	,104	,157	,022	,116
Canalicular Reabsorbtion	13,99	-1,896	5,814	-5,934	-8,227
Creatinine Plasma	,393	-,052	-,008	-,103	-,177

Chloride Excretion	-,0332	-,0098	-,0109	-,0202	,0097
Magnesium Plasma	-4,770	-1,426	1,992	,253	1,256
Osmolarity Urine	,0117	,0078	,0001	,0021	-,0123
Urea Plasma	-2,400	,264	1,125	,682	,901
Chloride Plasma	,760	,932	-,344	-,410	-,292
Cholesterol Plasma	3,263	-,229	-2,302	-1,260	-1,206
Amylase Urine	-,0390	-,0046	,053	,0039	-,0125
Uric Acid Urine	-1,019	-,595	-,159	,376	-,433
Katalase Urine	-39,88	-,009	,013	,018	,018
Sodium Plasma	-,788	-,890	,520	,404	,322
Sodium Erythrocytes	,191	-,047	,034	-,157	-,180
Phosphates Excretion	,036	-,157	,1308	,0013	-,0495
Diurese	-,988	9,544	-6,171	-,701	-,909
Calcium Excretion	,210	,402	-1,837	-,156	,335
Constants	**-1342**	**198,7**	**-605,7**	**570,7**	**786,6**

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.

On the plane of the first two roots, there is a clear distinction between clusters of intact rats and those subjected to loading by the salt analogue of water and Naftussya water. The less clearly separated cluster of animals loaded with native Hertsa water, instead, the other two clusters overlapped (Figures 3.5 and 3.6).

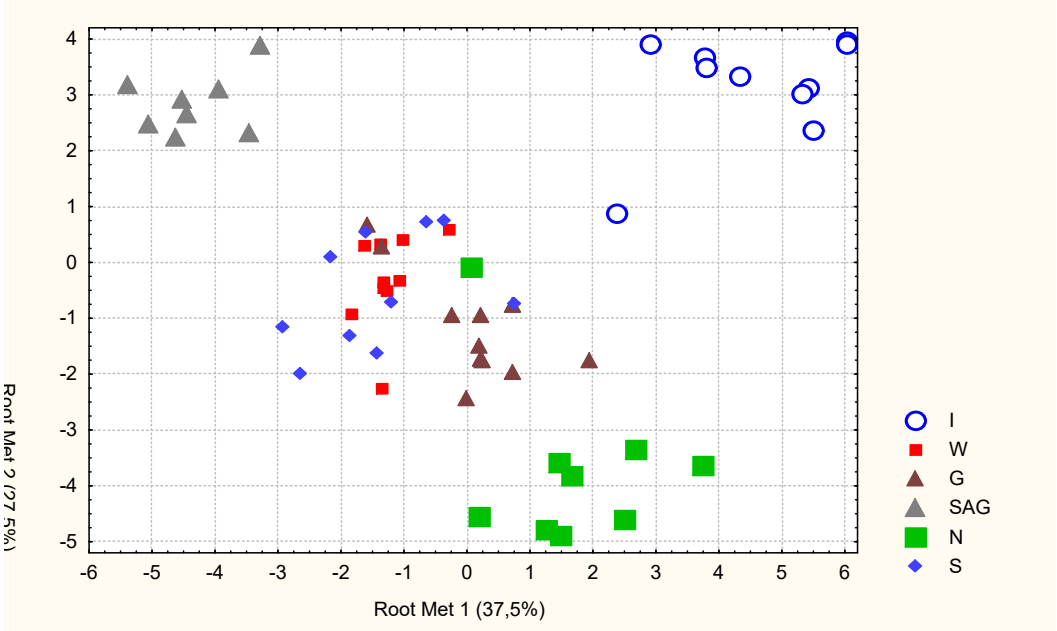


Fig. 3.5. Individual values of the first and second roots of the parameters of metabolism in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

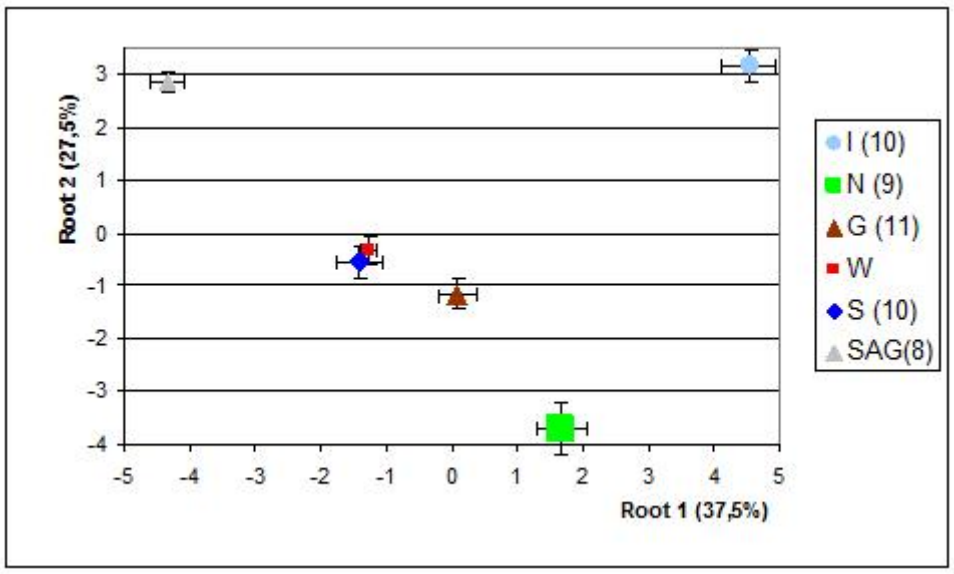


Fig. 3.6. Means of the first and second roots of the parameters of metabolism in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

However, the clusters of rats loaded with Sofiya water and tap water show themselves to be delimited on the plane of the first and third roots (Figures 3.7 and 3.8). In this case, takes place the interpenetration of the three members of clusters W and G.

However, on the plane of the first and fourth roots, and these two clusters are clearly delineated (Figures 3.9 and 3.10).

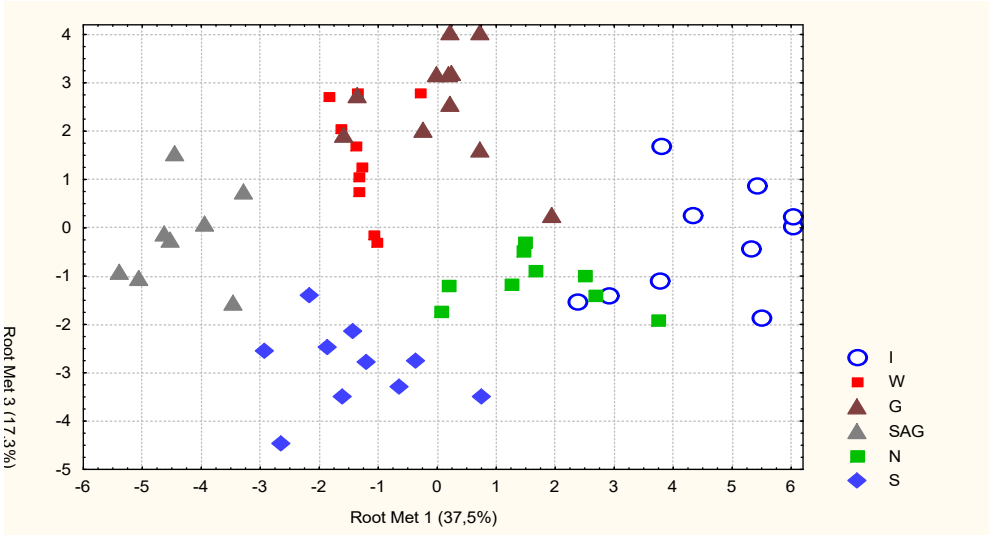


Fig. 3.7. Individual values of the first and third roots of the parameters of metabolism in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

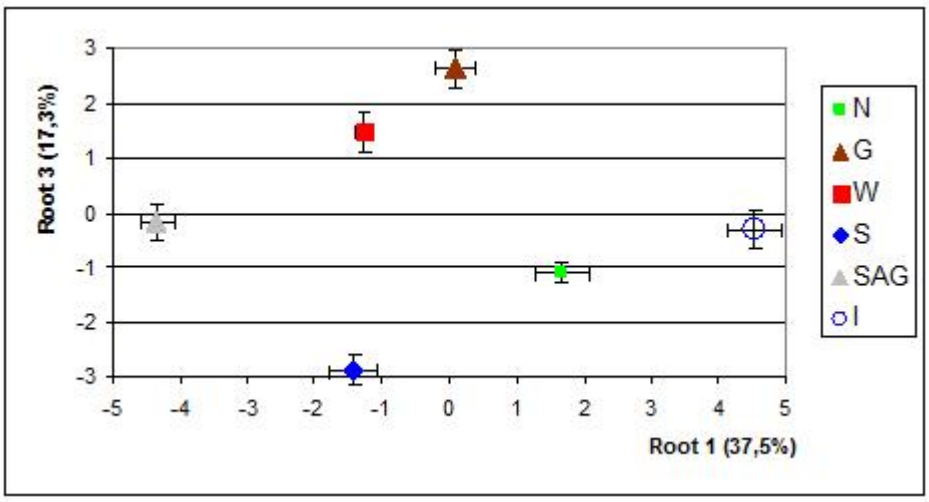


Fig. 3.8. Means of the first and third roots of the parameters of metabolism in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

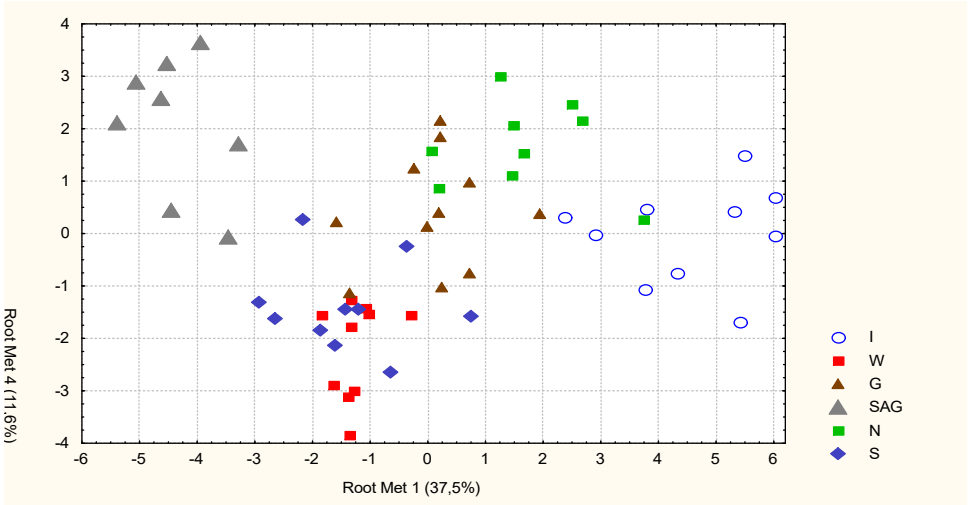


Fig. 3.9. Individual values of the first and fourth roots of the parameters of metabolism in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

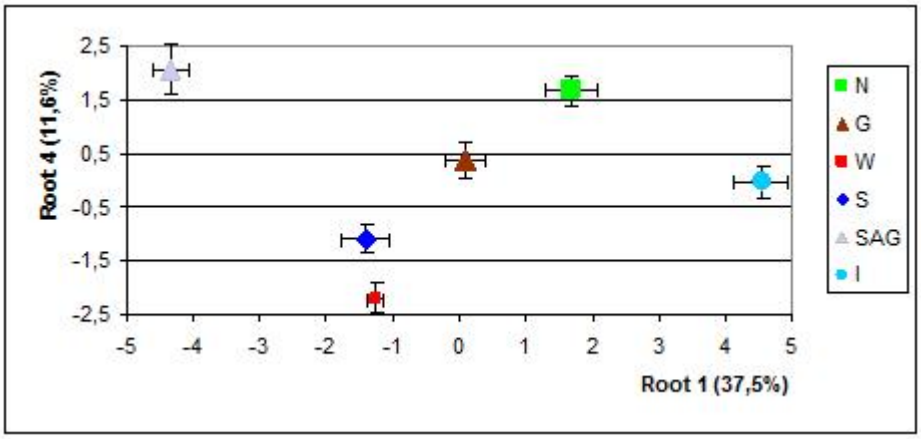


Fig. 3.10. Means of the first and fourth roots of the parameters of metabolism in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

On the whole, in the information space of the four discriminating roots, all six clusters are clearly delineated, that is, they differ from each other by constellation of 33 parameters of metabolism. This distinction is documented by calculating the squared Mahalanobis distances between them (Table 3.9).

Table 3.9. Mahalanobis D (over diagonal), F-values (df=33) and p-levels (under diagonal)

Groups	I (10)	W (10)	G (11)	SAG (8)	N (9)	S (10)
Intact rats (I)	**0,0**	61,9	55,7	93,2	66,6	66,0
Daily Water (W)	**3,25 ,004**	**0,0**	21,4	46,2	46,7	29,3
Water Hertsa (G)	**3,07 ,005**	**1,18 ,355**	**0,0**	55,9	34,1	40,5
Salt Analogue G	**4,28 ,001**	**2,12 ,040**	**2,68 ,012**	**0,0**	89,5	46,7
Water Naftussya (N)	**3,29 ,003**	**2,30 ,026**	**1,77 ,091**	**3,90 ,001**	**0,0**	40,8
Water Sofiya (S)	**3,46 ,002**	**1,53 ,158**	**2,24 ,031**	**2,14 ,038**	**2,01 ,051**	**0,0**

Now let's return to a more detailed analysis of the Figures.The polar localization along the axis of the first root of clusters of intact animals and loaded with salt analogues of Hertsa water reflects the maximum differences between them for the 8 parameters that correlate with this root **directly** or **inversely** (Table 3.10). At the same time, differences in these parameters between the other clusters are less clear (Figures 3.5 and 3.6). The localization of the cluster of rats drinking Naftussya water along the axis of the second root in its lower zone reflects the minimum values of parameters that correlate with this root **directly** and the maximum values of the parameters that correlate with it **inversely**. The localization of the cluster of rats fed with Sofiya water along the third root axis in its lower zone reflects the maximum values of parameters that correlate with this root **inversely** and the minimum values of parameters that correlate with it **directly** (Figures 3.7 and 3.8). Finally, along the axis of the fourth root, the cluster of the rats receiving water from the tap is the lowest, reflecting the minimum level of calcium in them and the maximum level of magnesium, as well as the activity of amylase urine.

Table 3.10. Factor Structure Matrix and Means of Roots and Variables

	Root 1	Root 2	Root 3	Root 4	Salt A H	Sofi-ya	Daily Water	Her-tsa	Naf-tussya	In-tact
Root 1 (37,5%)					**-4,34**	-1,41	-1,26	0,09	1,67	**4,54**
Glucose Plasma	**-,111**	-,002	,002	,005	**5,76**	5,31	5,61	5,15	5,32	**4,95**
Glomerul Filtratio	**-,110**	,033	,032	,193	**194**	112	86,5	142	109	**85,9**
Diurese	**-,098**	,048	,002	,189	**2,37**	1,53	1,44	1,66	1,65	**1,44**
Katalase Urine	**-,088**	-,015	,032	,053	**163**	136	151	141	145	**123**
Phospates Ex	**-,060**	,030	,029	,147	**137**	92	93	108	104	**91**
K Plasma	**,098**	,098	,024	,112	3,82	3,12	3,71	3,35	3,86	**4,23**
MMM Urine	**,067**	,022	,054	-,118	**147**	159	181	165	159	**182**
Phospates Urine	**,055**	-,028	,023	-,037	**58,9**	61,3	63,5	62,6	63,8	**63,9**
Root 2 (27,5%)					2,85	-0,54	-0,31	-1,16	**-3,69**	3,17
Diene Conj Urine	-,078	**,163**	-,066	,007	2,14	1,90	1,70	1,66	**1,45**	1,86
Chloride Ex	-,090	**,102**	,010	,057	244	195	102	203	**66**	144
K Erythrocytes	-,067	**,095**	-,044	-,021	90,1	88,5	86,9	85,8	**83,9**	87,0
Na Ex	-,088	**,087**	,039	,106	282	175	89	225	**66**	135
Osmolarity Urine	-,031	**,087**	,023	-,002	581	598	464	623	**411**	559
Na Erythrocytes	-,080	**,078**	,066	,006	25,5	21,8	23,7	22,7	**20,6**	22,0
Canalic Reabsorp	-,027	**,022**	,028	-,031	98,8	98,9	98,7	99,0	**98,5**	98,7
Ca Urine	,058	**-,150**	-,009	,112	1,93	2,07	2,15	2,32	**3,05**	2,10
Urea Plasma	-,005	**-,112**	-,076	-,044	7,44	9,29	8,92	7,85	**10,03**	7,42
Creatininemia	-,005	**-,088**	-,106	-,037	73	90	86	68	**98**	73
Ca Ex	-,032	**-,062**	,033	,179	4,50	3,09	3,08	4,25	**4,76**	2,90
Cholesterolemia	,023	**-,041**	-,037	-,034	1,46	1,62	1,60	1,53	**1,67**	1,57
Root 3 (17,3%)					-0,18	**-2,88**	1,47	2,62	-1,11	-0,32

Mg Ex	-,087	-,004	**-,268**	,129	5,85	**5,98**	2,65	2,51	5,07	3,30
Phosphatemia	,005	,039	**-,155**	-,072	0,92	**1,22**	0,87	0,72	0,88	1,04
Creatinine Urine	-,061	-,055	**-,107**	-,078	7,01	**8,12**	7,15	6,83	7,16	6,41
MDA Urine	,027	-,007	**-,080**	-,013	81	**102**	77	91	87	92
Uric Acid Urine	,030	-,009	**,198**	-,104	2,91	**2,56**	4,70	4,23	3,18	3,68
MDA Plasma	,005	-,101	**,172**	-,064	62	**57**	91	81	80	63
Uric Acid Ex	-,018	,050	**,134**	,020	6,5	**3,8**	6,7	6,0	4,9	5,7
Na Plasma	,012	-,039	**,080**	-,060	127,8	**127,0**	131,8	128,6	129,9	128,6
Cl Plasma	,023	,024	**,062**	-,004	92,9	**89,7**	95,0	91,5	93,2	93,8
Root 4 (11,6%)					2,07	-1,40	**-2,19**	0,40	1,68	-0,02
Ca Plasma	,044	,219	-,132	**,250**	3,36	2,57	**1,88**	2,32	2,44	3,35
Amylase Urine	,040	-,084	,031	**-,101**	181	210	212	215	210	202
Mg Plasma	,011	-,003	-,002	**-,065**	0,79	0,80	**1,05**	0,70	0,90	0,88

The classifying functions (Table 3.11) enables the retrospective identification of the five clusters to be unmistakable, and the latter with a single error (Table 3.12).

Table 3.11. Coefficients and Constants for Classification Functions

Variables currently in the model	I	W	H	SAG	N	S
Calcium Plasma, mM/L	3219	3214	3221	3218	3220	3210
Magnesium Excretion, µM/24h•100 g Body Mass	-1103,5	-1097,1	-1097,9	-1097,9	-1094,4	-1094,1
Sodium Excretion, µM/24h•100 g Body Mass	10,1	10,1	10,2	10,1	10,1	10,0
Potassium Plasma, mM/L	927,8	923,9	924,7	921,0	936,0	924,9
Glukose Plasma, mM/L	-892,6	-890,9	-892,2	-885,2	-899,9	-893,3
Calcium Urine, mM/L	4466,2	4473,4	4482,3	4469,6	4468,4	4459,0
Creatinine Urine, mM/L	-9227,6	-9217,2	-9226,7	-9207,3	-9214,4	-9207,7
Phosphate Plasma, mM/L	218,2	190,6	193,1	202,6	174,8	180,3
Urea Excretion, µM/24h•100 g Body Mass	476,8	472,2	471,4	471,1	469,8	469,7
Middle Mass Molecules Urine, units	4,648	4,750	4,672	4,677	4,614	4,732
Glomerulary Filtration, µL/min•100 g Body Mass	-42,8	-42,7	-42,7	-42,6	-42,7	-42,8
Malonic Dialdehyde Plasma, µM/L	68,3	68,4	68,4	68,1	68,5	68,2
Malonic Dialdehyde Urine, µM/L	105,3	104,7	104,8	104,5	105,1	104,9
Diene conjugates Urine, E^{232}/mL	-8622,4	-8592,8	-8608,7	-8575,9	-8623,5	-8599,2
Phosphate Urine, mM/L	29,6	27,5	26,7	27,6	28,2	28,9
Potassium Erythrocytes, mM/L	-299,7	-298,8	-299,2	-298,4	-300,0	-299,8
Canalicular Reabsorbtion, %	51871	51809	51845	51733	51822	51799
Creatinine Plasma, µM/L	1150	1148	1149	1147	1149	1149
Chloride Excretion, µM/24h•100 g Body Mass	-47,1	-46,9	-47,0	-46,9	-47,0	-46,9
Magnesium Plasma, mM/L	-4619,4	-4582,0	-4588,0	-4575,3	-4595,9	-4592,7
Osmolarity Urine, mOsm/L	16,4	16,3	16,3	16,3	16,3	16,3
Urea Plasma, mM/L	-5191,3	-5176,5	-5179,6	-5168,1	-5185,1	-5182,9
Chloride Plasma, mM/L	149,9	142,2	141,8	141,9	140,7	143,7
Cholesterol Plasma, mM/L	5832,9	5811,7	5813,9	5800,6	5823,7	5823,4
Amylase Urine, mg/h•mL	-11,7	-11,4	-11,3	-11,3	-11,6	-11,5
Uric Acid Urine, mM/L	-1139,1	-1132,8	-1131,7	-1129,3	-1131,8	-1130,5
Katalase Urine, nM/h•mL	-56,48	-56,21	-56,20	-56,07	-56,26	-56,29
Sodium Plasma, mM/L	-29,8	-21,7	-21,3	-21,5	-20,9	-24,1
Sodium Erythrocytes, mM/L	707,3	706,5	707,0	705,3	706,6	706,7
Phosphates Excretion, µM/24h•100 g Body Mass	183,5	184,0	184,5	183,2	184,3	183,6
Diurese, mL/24h•100 g Body Mass	-1146,2	-1184,5	-1200,2	-1143,2	-1206,0	-1157,9
Calcium Excretion, µM/24h•100 g Body Mass	-2647,8	-2652,9	-2656,5	-2650,2	-2649,7	-2646,0
Constants •10^3	-2560,2	-2554,4	-2557,9	-2547,0	-2555,6	-2553,1

Table 3.12. Classification Matrix.
Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,172	W p=,172	H p=,190	SAG p=,138	N p=,155	S p=,172
I	100	**10**	0	0	0	0	0
W	100	0	**10**	0	0	0	0
G	90,9	0	0	**10**	0	**1**	0
SAG	100	0	0	0	**8**	0	0
N	100	0	0	0	0	**9**	0
S	100	0	0	0	0	0	**10**
Total	98,3	10	10	10	8	10	10

Consequently, through the formation of patterns, as well as discriminant analysis, we were able to identify those metabolic parameters whose changes are specific in response to the loading of water-salt solutions of different composition. The specificity of the reactions is due to the content in solutions NaCl, SO_4^{2-}, as well as organic carbon and nitrogen.

CHAPTER 4
FEATURES OF NEURO-ENDOCRINE AND IMMUNE REACTIONS TO VARIOUS WATER-SALT LOADS IN FEMALE RATS

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

Given a significant number of registered parameters, a discriminant analysis was immediately applied. The program forward stepwise (8) included in the model 29 variables (Table 4.1). Among them, 10 reflects **neuroendocrine regulation**, 4 **thymus mass and timocytogram** elements, 5 elements of **splenocytogram**, 10 reflects elements of **immunocytogram and leukocytogram** of blood and parameters of phagocytosis. Instead, other variables were out of the model.

Table 4.1. Summary of Stepwise Analysis
Step 29, N of vars in model: 29; Grouping: 6 grps
Wilks' Lambda: 0,0013; approx. $F_{(145)}$=2,4; $p<10^{-6}$

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Microbian Count of Neutrophils, Bacterias/Phagocyte	5,4	10^{-3}	,659	5,4	10^{-3}
Monocytes Blood, %	4,1	,004	,471	4,7	10^{-4}
Moda HRV, msec	3,0	,018	,362	4,1	10^{-5}
Lymphocytes Spleen, %	3,0	,020	,278	3,9	10^{-6}
Entropy of Leukocytogram	2,6	,038	,219	3,6	10^{-6}
Stub Neutrophils Blood, %	3,1	,017	,165	3,6	10^{-6}
B-Lymphocytes Blood, %	2,6	,037	,128	3,5	10^{-6}
Basophils Blood, %	2,3	,057	,102	3,4	10^{-6}
Adrenals Mass Index, %	2,1	,080	,082	3,3	10^{-6}
$(Cau•Pu/Pp•Cap)^{0,25}$ as Calcitonin Activity	2,3	,059	,065	3,3	10^{-6}
Triiodothyronin, nM/L	2,1	,080	,051	3,2	10^{-6}
Neutrophils Spleen, %	2,2	,071	,041	3,2	10^{-6}
0-Lymphocytes Blood, %	1,7	,150	,033	3,1	10^{-6}
Leukocytes Blood, 10^9/L	2,0	,105	,027	3,1	10^{-6}
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	1,9	,119	,021	3,1	10^{-6}
Corticosterone, nM/L	1,5	,229	,018	3,0	10^{-6}
Thymus Mass Index, %	1,3	,269	,015	2,9	10^{-6}
Testosterone, nM/L	1,3	,293	,013	2,8	10^{-6}
Eosinophils Blood, %	1,5	,216	,010	2,8	10^{-6}
Macrophages Spleen, %	1,1	,357	,009	2,7	10^{-6}
Entropy of Splenocytogram	1,7	,158	,007	2,7	10^{-6}
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathytoid Activity	1,2	,330	,006	2,6	10^{-6}
Killing Index of Neutrophils, %	1,7	,155	,005	2,6	10^{-6}
Lymphocytes Thymus, %	1,2	,335	,004	2,6	10^{-6}
Variative Swing HRV as Vagal Tone, msec	1,3	,299	,003	2,6	10^{-6}
Eosinophils Spleen, %	1,6	,196	,002	2,6	10^{-6}
Plasmocytes Thymus, %	1,1	,369	,002	2,5	10^{-6}
Reticular Zone of Adrenal Cortex, µM	1,0	,418	,002	2,5	10^{-6}
Lymphoblastes Thymus, %	1,3	,304	,001	2,4	10^{-6}

Let's dwell on the individual components of the neuroendocrine-immune complex. As we see in Table 4.2, almost all of the registered parameters of neuroendocrine regulation were detected with regard to the specificity

of the balneoreaction, namely: a vagal tone, a humoral channel of regulation, levels in the plasma of triiodothyronine, testosterone and corticosterone, calcitonin, Parathytoid and mineralocorticoid activities, assessed by subordinates of their influence on the parameters of exchange of electrolytes, as well as the mass of the adrenal glands and the thickness of the reticular zone of their cortex. And only the thickness of the glomerular and fascicular zone of cortex of the adrenal glands, as well as the sympathetic tone, were beyond the discriminant model.

Table 4.2. Discriminant Function Analysis Summary for Neuroendocrine variables

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

Variables currently not in the model	**Parameters of Wilks' Statistics**					**Type of Water-Salt Load (n)**					
	WilksΛ $\bullet 10^{-3}$	Partial Λ	F to enter	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal G (8)	Naftussya (9)
AMo HRV as Sympathetic tone, %	5,5	,973	,16	,955	,369	56 1,00 0,00	69 1,23 +0,75	58 1,04 +0,14	60 1,07 +0,23	48 0,86 -0,47	69 1,25 +0,79
Glomerulary Zone of Adrenals, µM	5,4	,959	,25	,905	,581	193 1,00 0,00	187 0,97 -0,14	170 0,88 -0,52	198 1,03 +0,11	186 0,96 -0,17	192 0,99 -0,04
Fasciculary Zone of Adrenals, µM	5,5	,977	,14	,964	,342	391 1,00 0,00	386 0,99 -0,06	470 1,20 +0,92	371 0,95 -0,22	431 1,10 +0,46	394 1,01 +0,0

											4

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

Regarding the central organ of immunity, the thymus, its mass, more precisely the mass index, and the relative content in the thymocytogram of lymphoid elements: lymphocytes, lymphoblasts and plasmocytes (Table 4.3), was revealed as a recognizable, while the reticulo-endothelial elements of thymocytogram and its entropy appeared beyond the discriminant model.

Table 4.3. Discriminant Function Analysis Summary for Thymic variables

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

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

Among the elements of the splenocytogram, the discriminant model included lymphocytes, eosinophils, neutrophils/microphages and monocytes/macrophages, as well as its entropy, while the mass of the spleen and the contents of the splenocytogram of the two lymphoid (lymphoblasts and plasmocytes) and phagocyting (fibroblasts and reticulocytes) elements outside the model (Table 4.4).

Among the registered immune parameters of the blood, the overall content of leukocytes and the relative content in the leukocytogram of its minor elements: basophils, eosinophils, rodenuclear neutrophils and monocytes, as well as its entropy, were revealed. The second subgroup is composed of indicators of intensity and completeness of phagocytosis of neutrophils, and the third is the content in the immunocytogram of blood B- and 0-lymphocytes (Table 4.5). Outside the discriminant model, the major elements of the leukocytogram: segmental

neutrophils and general lymphocytes were, as well as most elements of the immunocytogram: natural killers and T-killers and T-helper lymphocytes, as well as its entropy. The same applies to the activity of phagocytosis of neutrophils/microphages and monocytes/macrophages and the intensity of phagocytosis of the latter.

Table 4.4. Discriminant Function Analysis Summary for Splenic variables

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

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

Table 4.5. Discriminant Function Analysis Summary for immune variables of Blood

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Partial Λ	F-remove	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal G (8)	Naftussya (9)
Leukocytes Blood, 10^{9}/L	1,7	,761	1,5	,224	,374	12,7 1,00 0,00	12,7 1,01 +0,01	10,7 0,84 -0,33	12,9 1,02 +0,03	8,70 0,69 -0,67	11,4 0,90 -0,22
Basophils	1,9	,652	2,6	,054	,286	0,30	0,20	0,60	0,45	0,13	0,11

Blood, %						1,00 0,00	0,67 -0,21	2,00 +0,62	1,52 +0,32	0,42 -0,36	0,37 -0,39
Eosinophils Blood, %	1,7	,755	1,6	,211	,477	4,60 1,00 0,00	3,60 0,78 -0,33	3,30 0,72 -0,43	3,64 0,79 -0,32	3,50 0,76 -0,37	3,00 0,65 -0,54
Stub Neutrophils Blood, %	1,9	,654	2,5	,056	,097	3,60 1,00 0,00	3,00 0,83 -0,56	3,90 1,08 +0,28	3,18 0,88 -0,39	2,50 0,69 -1,02	3,00 0,83 -0,56
Monocytes Blood, %	3,3	,388	7,6	10^{-3}	,034	4,80 1,00 0,00	3,40 0,71 -0,47	7,00 1,46 +0,73	3,27 0,68 -0,51	5,38 1,12 +0,19	4,78 1,00 -0,01
Entropy of Leukocytogram $\cdot 10^3$	2,5	,499	4,8	,003	,035	310 1,00 0,00	292 0,94 -0,42	338 1,09 +0,63	310 1,00 -0,01	299 0,96 -0,25	307 0,99 -0,08
Microbian Count of Neutrophils, Bacteras/Phagocyte	3,1	,415	6,8	10^{-3}	,021	8,60 1,00 0,00	8,30 0,97 -0,16	6,40 0,74 -1,16	8,45 0,98 -0,08	7,25 0,84 -0,71	7,78 0,90 -0,43
Killing Index of Neutrophils, %	1,7	,731	1,8	,158	,404	50,7 1,00 0,00	50,2 0,99 -0,08	55,2 1,09 +0,70	54,8 1,08 +0,64	52,6 1,04 +0,30	53,6 1,06 +0,45
B-Lymphocytes Blood, %	2,3	,552	3,9	,010	,108	16,0 1,00 0,00	16,7 1,04 +0,24	17,9 1,12 +0,65	15,1 0,94 -0,31	14,75 0,92 -0,42	14,2 0,89 -0,60
0-Lymphocytes Blood, %	2,5	,500	4,8	,003	,085	20,9 1,00 0,00	21,0 1,00 +0,02	16,6 0,79 -0,87	24,0 1,15 +0,63	22,2 1,06 +0,26	24,6 1,18 +0,75

Variables currently not in the model	**Parameters of Wilks' Statistics**					**Type of Water-Salt Load (n)**					
	Wilks Λ $\cdot 10^{-3}$	Par-tial Λ	F to en-ter	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Wate r (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal G (8)	Naftu ssya (9)
Phagocytose Index of Neutrophils, %	5,4	,95 9	,25	,90 5	,581	69,5 1,00 0,00	70,9 1,02 +0,32	66,9 0,96 -0,60	71,2 1,02 +0,39	69,1 0,99 -0,09	69,0 0,99 -0,12
Phagocytose Index of Monocytes, %	5,2	,92 5	,49	,74 4	,404	2,90 1,00 0,00	2,95 1,02 +0,07	2,70 0,93 -0,29	2,55 0,88 -0,51	3,50 1,21 +0,8 6	2,83 0,98 -0,10
Microbian Count of Monocytes, Bacteras/Phagocyte	5,5	,97 3	,16	,95 5	,369	4,98 1,00 0,00	4,02 0,81 -0,51	5,84 1,17 +0,46	3,63 0,73 -0,72	5,23 1,05 +0,1 3	4,38 0,88 -0,32
Segmented Neutrophils Blood, %	5,6	,99 3	,04	,99 6	,354	26,0 1,00 0,00	26,4 1,02 +0,06	26,1 1,00 +0,01	32,7 +1,26 +0,99	25,0 0,96 -0,15	27,1 1,04 +0,1 6
Panlymphocytes Blood, %	5,5	,97 7	,14	,96 4	,342	60,7 1,00 0,00	63,4 1,04 +0,29	59,1 0,97 -0,17	56,5 0,93 -0,44	63,5 1,05 +0,3 0	62,0 1,02 +0,1 4
Natural Killer Lymphocytes Blood, %	5,3	,94 4	,36	,83 6	,047	15,6 1,00 0,00	14,6 0,94 -0,35	17,7 1,13 +0,74	14,8 0,94 -0,31	16,7 1,07 +0,3 8	15,9 1,02 +0,0 9
T-cytolytic Lymphocytes Blood, %	5,2	,92 4	,49	,74 3	,425	16,0 1,00 0,00	16,6 1,04 +0,25	16,6 1,04 +0,25	15,7 0,98 -0,11	15,9 0,99 -0,05	14,6 0,91 -0,60
T-helper	5,5	,97	,17	,95	,407	31,5	31,1	31,2	30,5	30,5	30,8

Lymphocytes Blood, %		3		3		1,00 0,00	0,99 -0,13	0,99 -0,10	0,97 -0,34	0,97 -0,32	0,98 -0,23
Entropy of Immunocytogram •10^3	5,6	,99 6	,03	,99 9	,068	472 1,00 0,00	468 0,99 -0,60	468 0,99 -0,64	469 0,99 -0,45	474 1,00 +0,2 5	467 0,99 -0,74

The dividing information contained in 29 variables is condensed in 5 canonical discriminant roots (Tables 4.6 and 4.7). The first root contains 49,8% of discriminative opportunities, the second 21,1%, the third 10,7%, the fourth 9,6%, the fifth 8,8%.

Table 4.6. Chi-Square Tests with Successive Roots Removed

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

Table 4.7 presents standardized and raw coefficients and constants for discriminant variables.

Table 4.7. Standardized and Raw Coefficients and Constants for Canonical Variables

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

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

On the plane of the first two roots, which contain 71% of the discriminant information, there is a clear separation along the axis of the major root of the cluster of rats loaded by Hertsa water from other clusters (Fig. 4.1 and 4.2).

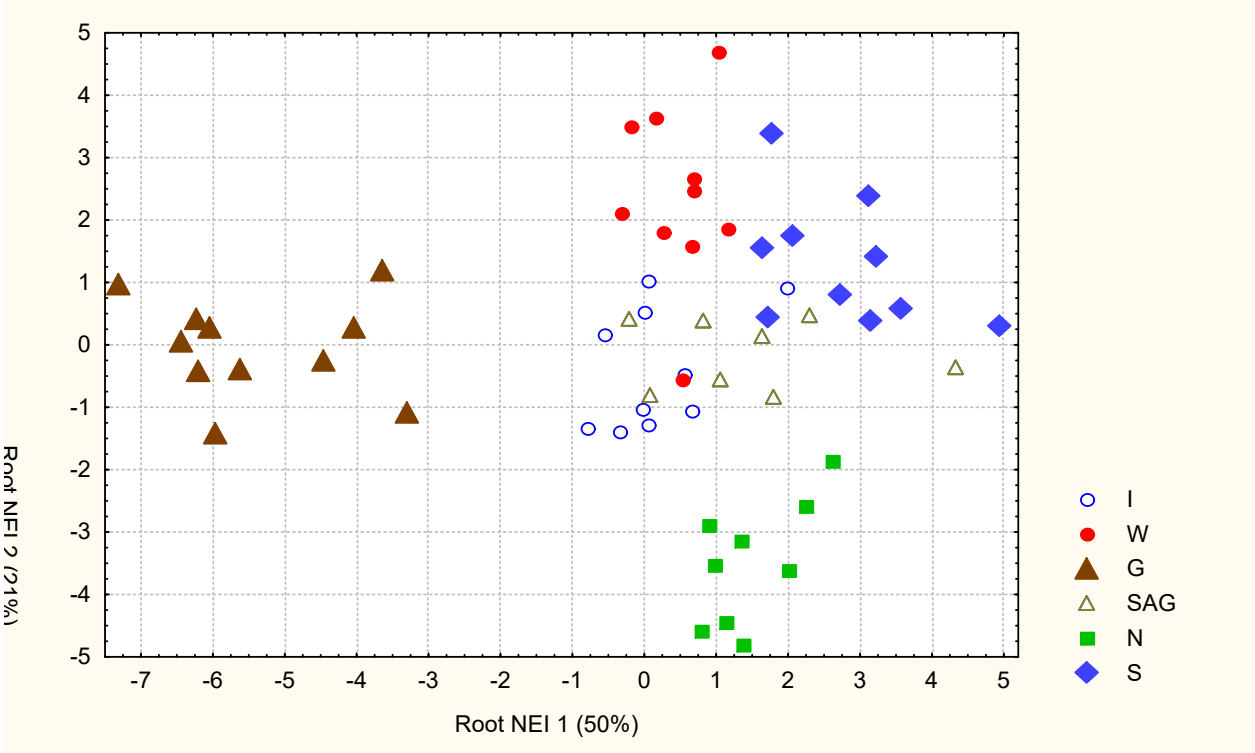


Fig. 4.1. Individual values of the first and second roots of the parameters of neuro-endocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

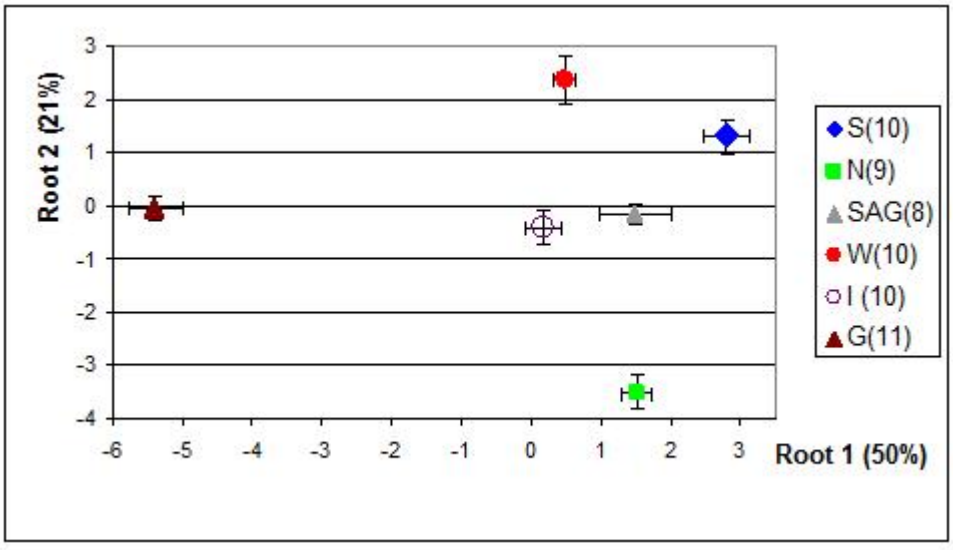


Fig. 4.2. Means of the first and second roots of the parameters of neuroendocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

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

of negative correlated with the root parameters (microbial number of neutrophils, content of leukocytes and 0-lymphocytes in blood and calcitonin activity) (Table 4.8).

Table 4.8. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Variables for Groups

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

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

Given the specificity of the chemical composition of the waters of Hertsa and Sofiya, it seems that the maximum effect on the listed endocrine and immune parameters of Hertsa water is carried out by organic carbon (Fig. 4.3) and trace elements (H_3BO_3, Br^-, J^-), the content of which is maximum among the liquids used.

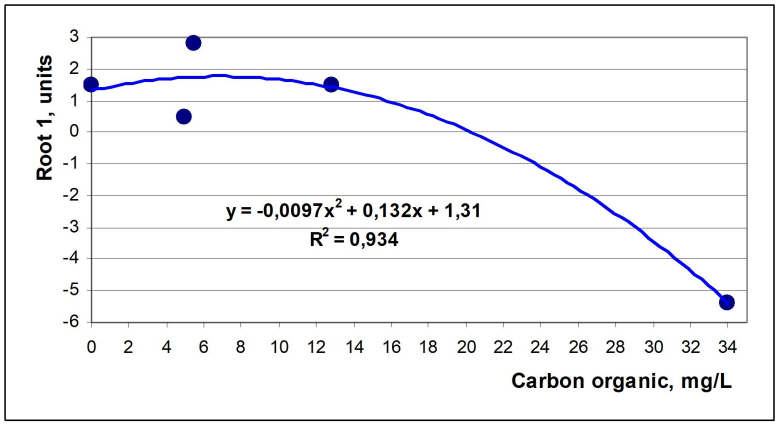


Fig. 4.3. Relationship between the concentration of organic carbon in water and its influence on the parameters condensed in the first canonical discriminant root

Water Sofiya realizes its opposite maximum effect on these parameters by sulfate (Fig. 4.4) and organic nitrogen, the content of which is also maximal.

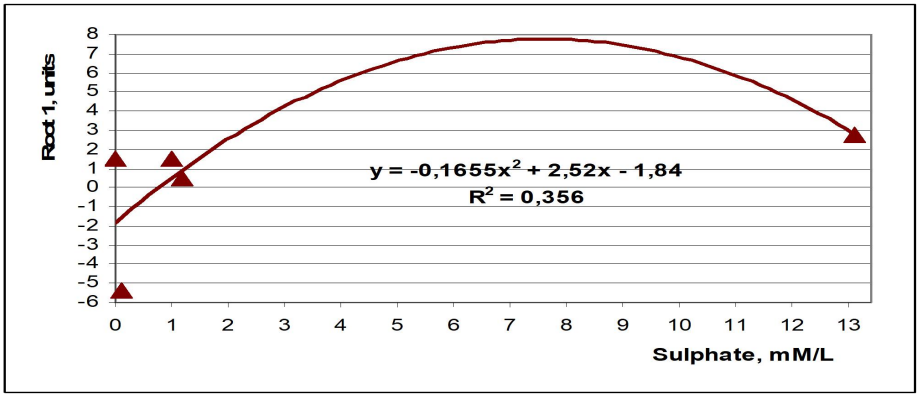


Fig. 4.4. Relationship between concentration in water of sulfate and its influence on parameters condensed in the first canonical discriminant root

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

In contrast, along the axis of the second root, a cluster of rats loaded with Naftussya water (centroid: -3,50) is clearly distinguished (Figs. 4.1 and 4.2). Such a localization of the cluster reflects the minimum values for parameters correlating with the second root positively (the level of corticosterone, the thickness of the reticular cortex of the adrenal glands, the content of the lymphoblasts in the thymus and neutrophils in the spleen, and the entropy of the splenocytogram) in conjunction with the maximum values of the negative correlates with the root parameters (vagal tonus, mineralocorticoid activity, plasma testosterone level, B-lymphocytes content in the blood and total lymphocytes in the spleen) (Table 4.8).

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

When comparing the parameters of the chemical composition, it was found that cluster ranking by average values of the second root coincides well with the ranking of liquids by content of bicarbonate in them (Fig. 4.5).

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

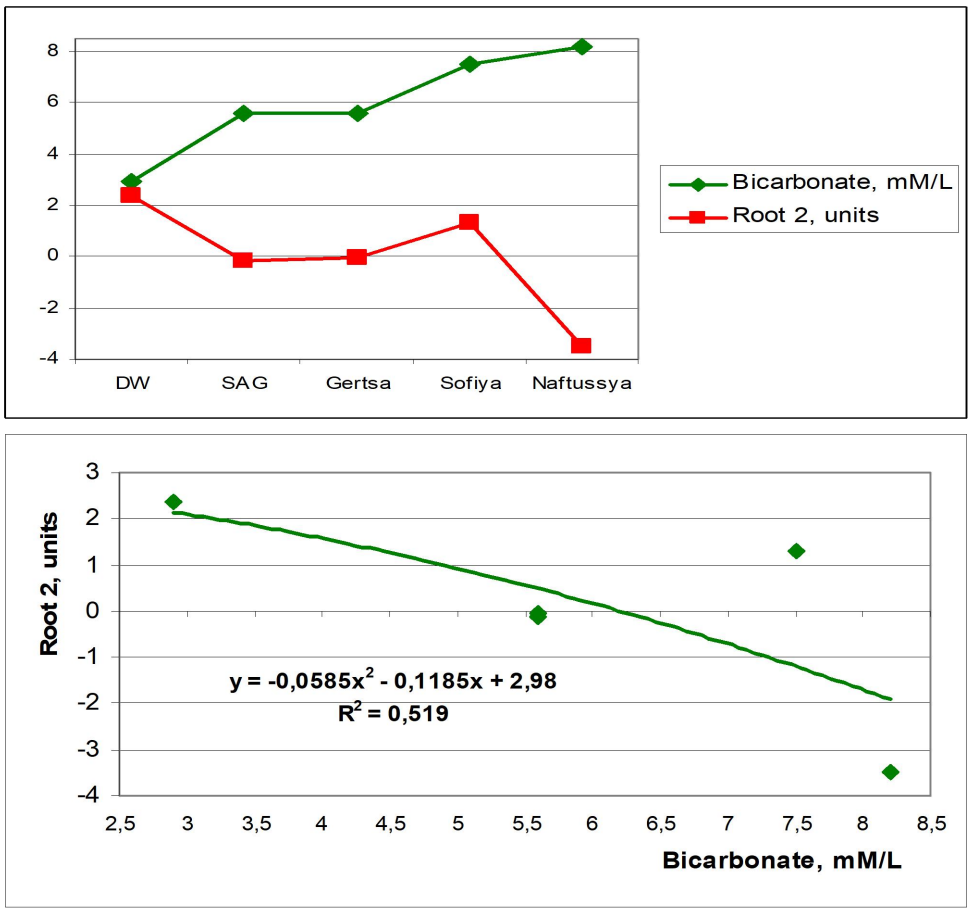


Fig. 4.5. Relationship between the concentration in water of bicarbonate and its influence on the parameters condensed in the second canonical discriminant root

Along the axis of the third root (Figures 4.6 and 4.7), the cluster of rats loaded with water Sofiya (centroid: +1,80) and daily water from the tap (centroid: -2,16) occupy the polar positions.

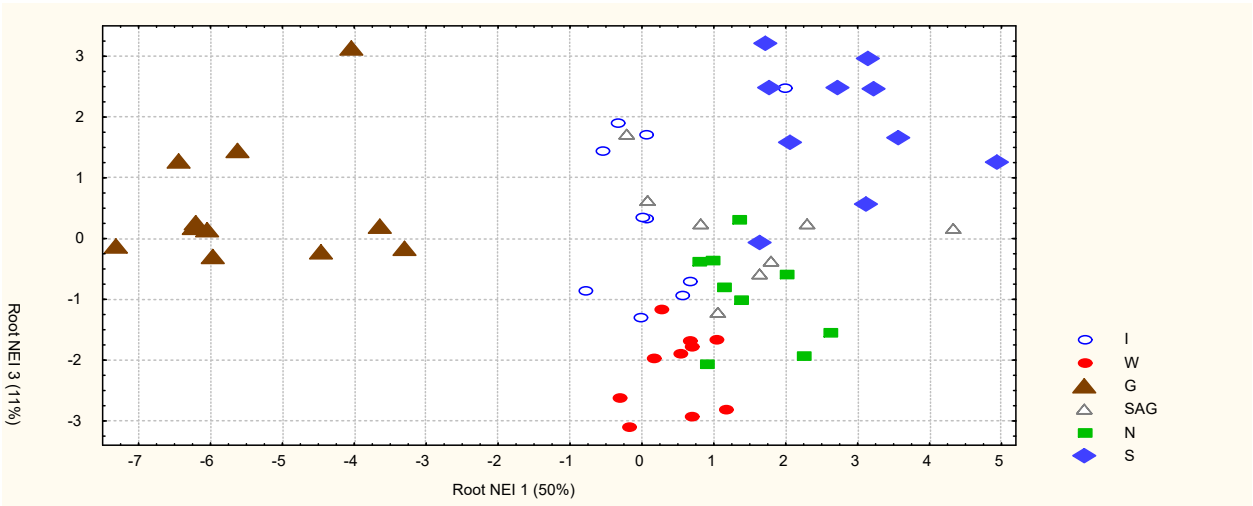


Fig. 4.6. Individual values of the first and third roots of the parameters of neuro-endocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

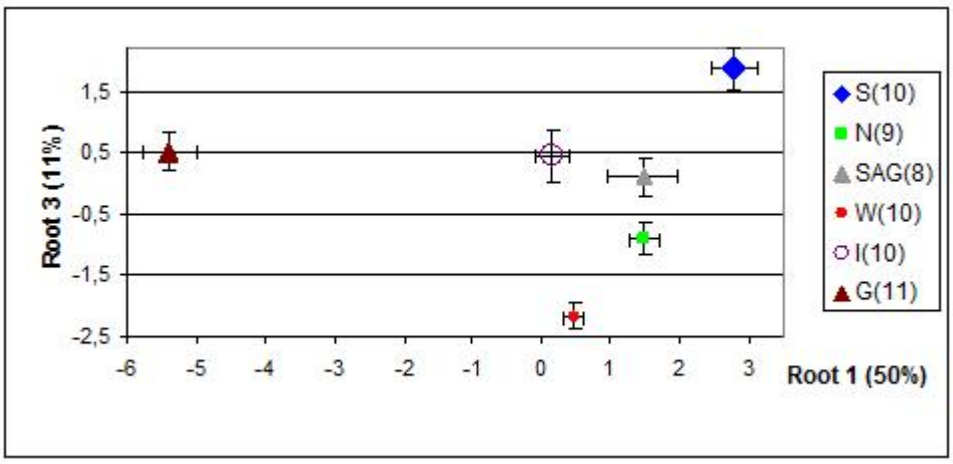
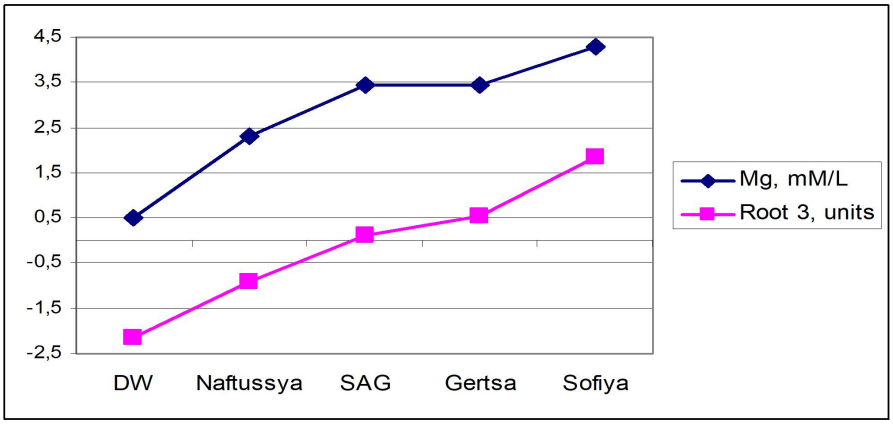


Fig. 4.7. Means of the first and third roots of the parameters of neuroendocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

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

When comparing the magnitudes of centroids with the chemical composition parameters, their almost linear relationship with the concentration of magnesium (Fig. 4.8) was found, suggesting a hypothesis about its main role in the effects of applied water loads on the listed immune parameters.



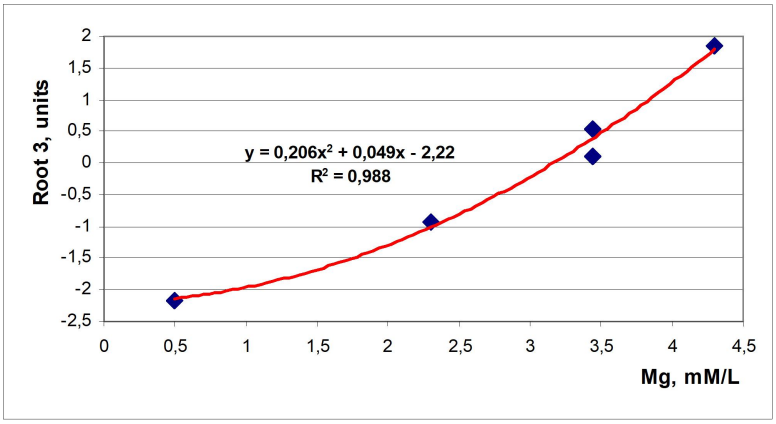


Fig. 4.8. Relationship between the concentration of magnesium in water and its effect on immune parameters condensed in the third canonical discriminant root

The top positions along the axis of the fourth root are placed in clusters of intact rats and loaded with the salt analogue of Hertsa water, while other clusters are located in the lower zone of the axis (Fig. 4.9).

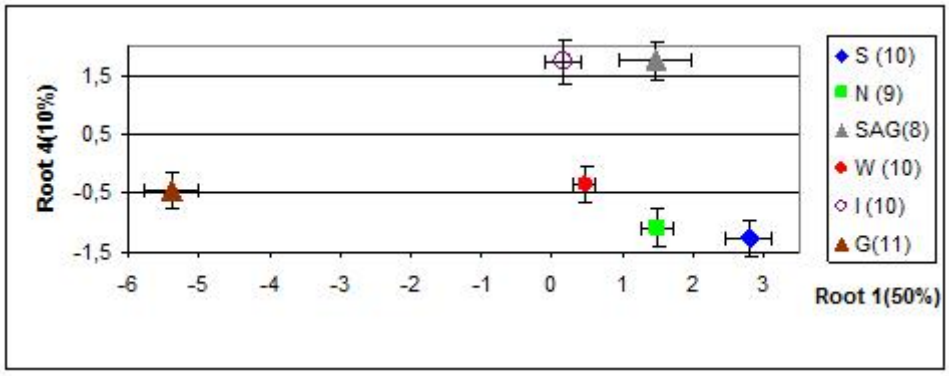


Fig. 4.9. Means of the first and fourth roots of the parameters of neuroendocrine-immune complex in intact rats (I) and loaded with Daily water (W), waters Naftussya (N), Sofiya (S), Hertsa (G) and its artificial salt analogue (SAG)

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

In general, in the information space of discriminatory roots, all six clusters are clearly delimited with one another, that is, they differ from each other by the constellation of 29 parameters of the neuroendocrine-immune complex, which is documented by calculating the squared Mahalanobis distances between them (Table 4.10).

Table 4.10. Squared Mahalanobis Distances, F-values (df=29) and p-levels between groups

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

The application of the classifying functions (Table 4.11) enables the retrospective identification of the five clusters unmistakably, and the intact cluster with a single error (Table 4.12).

Table 4.11. Coefficients and Constants for Classification Functions

Variables currently in the model	I p=,172	W p=,172	G p=,190	SAG p=,138	N p=,155	S p=,172
Microbian Count, Bac/Phag	887	880	907	878	875	8689
Monocytes Blood, %	-144	-148	-157	-144	-143	-140
Moda HRV as Humoral channel, msec	-9,467	-9,786	-10,541	-9,415	-8,947	-9,380

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

Table 4.12. Classification Matrix. Rows: Observed classifications; Columns: Predicted classifications

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

CHAPTER 5
PECULIARITIES OF INTEGRATED REACTIONS OF THE BODY ON WATER-SALT LOADS OF DIFFERENT CHEMICAL COMPOSITION

After finding out the peculiarities of reactions to water-salt loads of different chemical composition of metabolic parameters and neuroendocrine-immune complex, it is time to conduct a discriminant analysis of the entire information field. The forward stepwise program included 35 variables in the model (Table 5.1).

Table 5.1. Summary of step-by-step discriminant analysis of reactions of metabolic parameters and neuroendocrine-immune complex to water-salt loads

Step 35, N of vars in model: 35; Grouping: 6 grps; Wilks' Λ: 0,00007; appr. $F_{(176)}$=3,2; $<10^{-6}$

Variables currently in the model	F to enter	p-level	Lam-bda	F-value	p-level
Calcium Plasma, mM/L	5,5	10^{-3}	,656	5,45	10^{-3}
Microbian Count of Neutrophils, Bacter/Phagocyte	6,2	10^{-4}	,409	5,76	10^{-5}
Phosphate Plasma, mM/L	3,8	,006	,296	5,11	10^{-6}
Sodium Excretion, µM/24h•100 g Body Mass	3,3	,013	,222	4,69	10^{-6}
Entropy of Leukocytogram •10^3	2,9	,023	,171	4,38	10^{-6}
B-Lymphocytes Blood, %	3,0	,020	,130	4,22	10^{-6}
Amylase Urine, mg/h•mL	2,7	,031	,100	4,08	10^{-6}
Calcium Urine, mM/L	2,5	,042	,078	3,96	10^{-6}
Neutrophils Spleen, %	2,2	,073	,062	3,82	10^{-6}
Potassium Plasma, mM/L	2,1	,090	,050	3,69	10^{-6}
Thymus Mass Index, mg/100 g Body Mass	2,2	,077	,040	3,61	10^{-6}
Eosinophils Blood, %	1,9	,108	,032	3,52	10^{-6}
Testosterone, nM/L	2,4	,056	,025	3,51	10^{-6}
Lymphocytes Thymus, %	1,7	,159	,021	3,42	10^{-6}
(Cap•Pu/Pp•Cau)^{0,25} as Parathyroid Activity	2,5	,046	,015	3,45	10^{-6}
Glomerulary Filtration, µL/min•100 g Body Mass	1,7	,157	,013	3,38	10^{-6}
Malonic Dialdehyde Plasma, µM/L	1,8	,130	,010	3,34	10^{-6}
Malonic Dialdehyde Urine, µM/L	1,9	,120	,008	3,32	10^{-6}
Magnesium Excreti-on, µM/24h•100 g Body Mass	1,8	,148	,006	3,29	10^{-6}
Entropy of Splenocytogram •10^3	1,5	,209	,005	3,24	10^{-6}
Basophils Blood, %	1,5	,214	,004	3,19	10^{-6}
Stub Neutrophils Blood, %	1,3	,279	,003	3,13	10^{-6}
Leukocytes Blood, 10^9/L	2,3	,070	,002	3,18	10^{-6}
Macrophages Spleen, %	1,4	,265	,002	3,13	10^{-6}
Lymphocytes Spleen, %	1,5	,233	,002	3,10	10^{-6}
Phosphates Urine, mM/L	1,4	,257	,001	3,06	10^{-6}
Diene conjugates Urine, E^{232}/mL	1,7	,181	,001	3,06	10^{-6}
Potassium Erythrocytes, mM/L	1,4	,251	,001	3,04	10^{-6}
Reticular Zone of Adrenal Cortex, µM	1,7	,182	,001	3,05	10^{-6}
Creatinine Excretion, µM/24h•100 g Body Mass	2,3	,080	,000	3,13	10^{-6}
Uric Acid Excretion, µM/24h•100 g Body Mass	1,7	,169	,000	3,16	10^{-6}
Middle Mass Molecules Urine, units	2,0	,113	,000	3,23	10^{-6}
(Cau•Pu/Pp•Cap)^{0,25} as Calcitonin Activity	1,5	,221	,000	3,23	10^{-6}
0-Lymphocytes Blood, %	1,1	,398	,000	3,17	10^{-6}
Glucose Plasma, mM/L	1,5	,247	,000	3,18	10^{-6}

Among them 9 reflect **glomerular filtration** and **mineral** exchange, 5 - **nitrogen and carbohydrate** metabolism, 3 - **lipoperoxidation**, 4 - **endocrine regulation**, 2 - **thymus mass and element of thymocytogram**, 4 - **elements of splenocytogram**, 8 - **elements of immunocytogram, leukocytogram and phagocytosis**. Instead, other variables were outside the model.

Among the parameters of electrolyte metabolism (Table 5.2) in the discriminant model were included (hereinafter - in descending order of criterion Λ): calciumemia, phosphatemia, natriuria, calcium concentration in urine, kaliemia, glomerular filtration, magnesiumuria, urinary phosphate concentration, as well as erythrocyte kalihistia.

Table 5.2. Summary of discriminant analysis of reactions of electrolyte exchange parameters to water-salt loads

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Partial Λ	F to enter	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal H (8)	Naftussya (9)
Calcium Plasma, mM/L	14	,488	3,8	,016	,091	3,35 1 0	1,88 0,56 -1,44	2,57 0,77 -0,76	2,32 0,69 -1,01	3,36 1,00 +0,01	2,44 0,73 -0,89
Phosphate Plasma, mM/L	12	,596	2,4	,075	,032	1,04 1 0	0,87 0,84 -0,27	1,22 1,18 +0,30	0,72 0,69 -0,52	0,92 0,88 -0,20	0,88 0,85 -0,26
Sodium Excretion, µM/24h•100 g Body Mass	14	,497	3,6	,019	,194	135 1 0	89 0,66 -0,54	175 1,30 +0,48	225 1,67 +1,08	282 2,09 +1,75	66 0,49 -0,81
Calcium Urine, mM/L	11	,656	1,9	,146	,093	2,10 1 0	2,15 1,02 +0,13	2,07 0,99 -0,07	2,32 1,11 +0,59	1,93 0,92 -0,44	3,05 1,46 +2,55
Potassium Plasma, mM/L	13	,530	3,2	,031	,293	4,23 1 0	3,71 0,88 -0,73	3,12 0,74 -1,58	3,35 0,79 -1,25	3,82 0,90 -0,58	3,86 0,91 -0,53
Glomerulary Filtration, µL/min•100 g	10	,673	1,7	,174	,291	85,9 1 0	86,5 1,01 +0,01	112 1,30 +0,41	142 1,65 +0,91	194 2,26 +1,75	109 1,27 +0,38
Magnesium Excreti-on, µM/24h•100 g Body Mass	27	,264	10	10^{-3}	,025	3,30 1 0	2,65 0,80 -0,31	5,98 1,81 +1,29	2,51 0,76 -0,38	5,85 1,77 +1,23	5,07 1,54 +0,85
Phosphates Urine, mM/L	10	,679	1,7	,185	,123	6,39 1 0	6,35 0,99 -0,05	6,13 0,96 -0,33	6,26 0,98 -0,16	5,89 0,92 -0,64	6,38 1,00 -0,01
Potassium Erythrocytes, mM/L	26	,268	9,8	10^{-3}	,102	87,0 1 0	86,9 1,00 -0,02	88,5 1,02 +0,21	85,8 0,99 -0,18	90,1 1,04 +0,46	83,9 0,96 -0,45

Among non-electrolytes (Table 5.3), urinary amylase activity occupies a prominent place in the range, followed by levels of malonic dialdehyde in plasma and urine, diene conjugates in urine, creatinineuria, uricosuria, concentration of medium weight molecules in urine and glucose in plasma.

Only 4 endocrine parameters were recognizable in terms of the specifics of integrated balneoreactions (Table 5.4), namely: plasma testosterone levels and the thickness of the adrenal reticular zone secreting them, as well as parathyroid and calcitonin activity, assessed by regulated parameters of calcium and phosphate exchange.

Table 5.3. The result of discriminant analysis of the reactions of non-electrolyte parameters to water-salt loads

Variables currently in the model	Parameters of Wilks' Statistics					Type of Water-Salt Load (n)					
	Wilks Λ •10^{-3}	Partial Λ	F to enter	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal H (8)	Naftussya (9)
Amylase Urine, mg/h•mL	10	,698	1,6	,222	,180	202 1 0	212 1,05 +0,18	210 1,04 +0,14	215 1,06 +0,23	181 0,90 -0,39	210 1,04 +0,15
Malonic Dialdehyde Plasma, µM/L	15	,461	4,2	,010	,177	63 1 0	92 1,45 +1,30	57 0,91 -0,27	81 1,28 +0,83	62 0,97 -0,08	80 1,26 +0,76
Malonic Dialdehyde Urine,	17	,406	5,3	,004	,088	92 1 0	77 0,83 -0,36	102 1,10 +0,22	91 0,99 -0,03	81 0,88 -0,25	87 0,95 -0,11

µM/L											
Diene conjugates Urine, E^{232}/mL	10	,682	1,7	,190	,209	1,86 1 0	1,70 0,92 -0,23	1,90 1,03 +0,07	1,66 0,89 -0,30	2,14 1,15 +0,43	1,45 0,78 -0,61
Creatinine Excretion, µM/24h•100 g	22	,324	7,5	10^{-3}	,044	8,72 1 0	10,12 1,16 +0,32	12,30 1,41 +0,82	10,53 1,21 +0,42	16,03 1,84 +1,68	12,27 1,41 +0,82
Uric Acid Excretion, µM/24h•100 g Body Mass	11	,626	2,1	,106	,040	5,7 1 0	6,7 1,16 +0,18	3,8 0,66 -0,36	6,0 1,04 +0,04	6,5 1,14 +0,15	4,9 0,86 -0,15
Middle Mass Molecules Urine, units	10	,691	1,6	,208	,265	182 1 0	181 0,99 -0,02	159 0,87 -0,44	165 0,91 -0,32	147 0,80 -0,68	159 0,87 -0,44
Glucose Plasma, mM/L	10	,709	1,5	,247	,223	4,95 1 0	5,61 1,13 +0,60	5,31 1,07 +0,33	5,15 1,04 +0,19	5,76 1,16 +0,74	5,32 1,08 +0,34

Table 5.4. Summary of discriminant analysis of reactions of endocrine parameters to water-salt loads

Variables currently in the model	**Parameters of Wilks' Statistics**					**Type of Water-Salt Load (n)**					
	Wilks Λ •10^{-3}	Partial Λ	F to enter	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal H (8)	Naftussya (9)
Testosterone, nM/L	11	,660	1,9	,153	,320	3,93 1 0	5,98 1,52 +1,92	5,53 1,41 +1,49	3,98 1,01 +0,05	4,76 1,21 +0,78	4,11 1,05 +0,17
(Cap•Pu/Pp•Cau)^{0,25} as Parathyroid Activity	10	,725	1,4	,284	,038	3,51 1 0	2,70 0,77 -0,80	2,69 0,77 -0,81	3,33 0,95 -0,18	4,11 1,17 +0,59	2,86 0,81 -0,65
Reticular Zone of Adrenal Cortex, µM	13	,553	2,9	,043	,206	43 1 0	42 0,98 -0,10	42 0,98 -0,10	42 0,98 -0,09	46 1,08 +0,44	45 1,05 +0,28
(Cau•Pu/Pp•Cap)^{0,25} as Calcitonin Activity	10	,682	1,7	,192	,088	2,35 1 0	3,08 1,31 +0,71	2,13 0,91 -0,22	3,24 1,38 +0,87	2,38 1,01 +0,07	3,61 1,53 +1,22

With regard to the central organ of immunity - the thymus - its mass index and relative content in the thymocytogram of T lymphocytes were recognizable, and among the elements of the splenocytogram in the discriminant model included neutrophils/microphages and monocytes/macrophages, T and B lymphocytes as well as entropy of splenocytogram (Table 5.5).

Table 5.5. The result of discriminant analysis of the reactions of the parameters of the spleen and thymus to water-salt loads

Variables currently in the model	**Parameters of Wilks' Statistics**					**Type of Water-Salt Load (n)**					
	Wilks Λ •10^{-3}	Partial Λ	F to enter	p-level	Tolerancy	Intact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal H (8)	Naftussya (9)
Neutrophils Spleen, %	14	,505	3,5	,021	,166	13,0 1 0	11,8 0,91 -0,85	12,7 0,98 -0,21	13,2 1,01 +0,13	12,7 0,98 -0,18	14,1 1,09 +0,78
Thymus Mass Index, mg/100 g Body Mass	09	,798	,9	,494	,178	28 1 0	30 1,06 +0,14	25 0,88 -0,31	29 1,03 +0,07	28 0,98 -0,04	30 1,06 +0,14

Lymphocytes Thymus, %	24	,288	8,9	10^{-3}	,112	70,3 1 0	69,0 0,98 -0,54	68,7 0,98 -0,69	68,6 0,98 -0,73	69,9 0,99 -0,15	69,4 0,99 -0,36
Entropy of Splenocytogram $\bullet 10^3$	09	,747	1,2	,34 2	,086	613 1 0	605 0,99 -0,30	615 1,00 +0,09	606 0,99 -0,29	610 1,00 -0,09	627 1,02 +0,5 7
Macrophages Spleen, %	12	,604	2,4	,08 2	,141	7,90 1 0	8,80 1,11 +0,56	8,30 1,05 +0,25	8,55 1,08 +0,40	7,25 0,92 -0,41	9,00 1,14 +0,6 9
Lymphocytes Spleen, %	11	,616	2,2	,09 4	,063	48,7 1 0	49,0 1,01 +0,11	47,7 0,98 -0,37	49,4 1,01 +0,24	48,5 1,00 -0,07	46,0 0,94 -0,99

Among the registered immune parameters of blood, the intensity of phagocytosis of neutrophils as well as the entropy of the leukocytogram and the relative content of its minor elements in the leukocytogram: eosinophils, basophils and rod-shaped neutrophils were recognizable. The second subgroup consists of indicators of the immunocytogram of blood: B- and 0-lymphocytes and the total content of leukocytes in the blood (Table 5.6).

Table 5.6. The result of discriminant analysis of the reactions of the immune parameters of the blood to water-salt loads

Variables currently in the model	**Parameters of Wilks' Statistics**					**Type of Water-Salt Load (n)**					
	Wilks Λ $\bullet 10^{-3}$	Par-tial Λ	F to en-ter	p-le-vel	Tole-ran-cy	In-tact (10)	Daily Water (10)	MW Sofiya (10)	MW Hertsa (11)	Salt Anal H(8)	Naftu ssya (9)
Microbian Count of Neutrophils, Bacteras/Phagocyte	09	,787	1,0	,460	,057	8,60 1 0	8,30 0,97 -0,16	6,40 0,74 -1,16	8,45 0,98 -0,08	7,25 0,84 -0,71	7,78 0,90 -0,43
Entropy of Leukocytogram $\bullet 10^3$	23	,304	8,3	10^{-3}	,026	310 1 0	292 0,94 -0,42	338 1,09 +0,63	310 1,00 -0,01	299 0,96 -0,25	307 0,99 -0,08
B-Lymphocytes Blood, %	14	,514	3,4	,024	,077	16,0 1 0	16,7 1,04 +0,24	17,9 1,12 +0,65	15,1 0,94 -0,31	14,75 0,92 -0,42	14,2 0,89 -0,60
Eosinophils Blood, %	17	,412	5,1	,004	,190	4,60 1 0	3,60 0,78 -0,33	3,30 0,72 -0,43	3,64 0,79 -0,32	3,50 0,76 -0,37	3,00 0,65 -0,54
Basophils Blood, %	15	,454	4,3	,009	,168	0,30 1 0	0,20 0,67 -0,21	0,60 2,00 +0,62	0,45 1,52 +0,32	0,13 0,42 -0,36	0,11 0,37 -0,39
Stub Neutrophils Blood, %	17	,404	5,3	,004	,051	3,60 1 0	3,00 0,83 -0,56	3,90 1,08 +0,28	3,18 0,88 -0,39	2,50 0,69 -1,02	3,00 0,83 -0,56
Leukocytes Blood, 10^9/L	16	,444	4,5	,008	,136	12,7 1 0	12,7 1,01 +0,01	10,7 0,84 -0,33	12,9 1,02 +0,03	8,70 0,69 -0,67	11,4 0,90 -0,22
0-Lymphocytes Blood, %	10	,703	1,5	,232	,154	20,9 1 0	21,0 1,00 +0,02	16,6 0,79 -0,87	24,0 1,15 +0,63	22,2 1,06 +0,26	24,6 1,18 +0,75

The separating information contained in 35 variables is condensed into 5 canonical discriminant roots (Tables 5.7 and 5.8). The first root contains 62,0% of discriminatory opportunities, the second – 18,5%, the third – 7,7%, the fourth – 6,9%, the fifth – 4,9%.

Table 5.7. Tests χ^2 with sequential removal of roots

	Eigen-value	Cano-nical R	Wilks' Λ	Chi-Square	Degree freadom	p-level
0	27,15	,982	,00007	349	175	10^{-6}
1	8,12	,944	,00197	227	136	10^{-5}
2	3,38	,879	,01801	147	99	,001
3	3,02	,867	,07897	93	64	,011
4	2,15	,826	,31737	42	31	,091

Table 5.8 shows standardized and non-standardized coefficients and constants for discriminant variables.

Table 5.8. Standardized and non-standardized coefficients and constants for canonical variables

Variables	Standardized Coefficients					Raw Coefficients				
	Root 1	Root 2	Root 3	Root 4	Root 5	Root 1	Root 2	Root 3	Root 4	Root 5
Calcium Pla	-,204	2,370	,010	-,586	,676	-,2634	3,054	,0132	-,7551	,8705
Micr C Neu.	,542	1,163	1,602	-,514	-,365	,4719	1,012	1,394	-,4472	-,3172
Phosphate Pl	1,242	2,643	1,539	1,884	-,597	2,452	5,216	3,037	3,719	-1,178
Sodium Exc	-1,266	-,385	-,250	,365	-1,061	-,0077	-,0023	-,0015	,0022	-,0065
Entropy LC	4,786	-1,896	,601	-,652	-1,081	,157	-,062	,020	-,021	-,036
B-Lymphoc	-2,423	-,110	-,604	-,133	,737	-,8017	-,0363	-,1999	-,0441	,2437
Amylase Ur	-1,110	-,593	,095	-,194	-,461	-,0292	-,0156	,0025	-,0051	-,0121
Calcium Ur	,961	-1,050	,641	1,337	-,474	1,157	-1,264	,7723	1,611	-,5714
Neutroph Sp	1,637	-,049	,692	-,134	-,119	,8312	-,0249	,3513	-,0679	-,0604
Potassium Pl	1,048	,031	,099	,013	,884	1,437	,0429	,1358	,0179	1,2124
Thymus MI	,396	,505	-,261	,968	,082	,049	,063	-,032	,120	,010
Eosinophils	1,580	,151	-,520	,531	,594	,8236	,0788	-,2712	,2767	,3093
Testosterone	-,362	,806	-,484	,451	,192	-,1819	,4054	-,2434	,2269	,0963
Lymphoc Th	-2,435	,501	,134	,052	,761	-,9356	,1925	,0515	,0201	,2924
Parathyrin	,146	,070	2,026	2,022	-1,167	,2562	,1226	3,567	3,560	-2,055
Glom Filtrat	,582	-,853	,278	,183	-,311	,0072	-,0105	,0034	,0023	-,0038
MDA Plasm	1,290	-1,207	-,209	-,052	,404	,0456	-,0426	-,0074	-,0018	,0143
MDA Urine	-2,164	,704	1,459	-,394	,171	-,0696	,0227	,0469	-,0127	,0055
Mg Excret	-5,340	-1,260	,637	-,244	,658	-2,106	-,4968	,2514	-,0964	,2597
Entropy Spl	1,369	,291	,528	-,946	-,494	74,17	15,75	28,59	-51,25	-26,77
Basophils	-,464	1,489	,644	-,873	-,519	-,9432	3,026	1,308	-1,774	-1,054
Stub Neutro	-3,015	1,777	,200	-,035	-,029	-2,698	1,590	,1792	-,0312	-,0263
Leukocytes	1,895	-,651	-,037	-,556	,112	,4136	-,1421	-,0081	-,1213	,0244
Macroph Spl	1,662	-,319	,222	-,034	-,131	,9226	-,1768	,1233	-,0186	-,0729
Lymphoc Sp	2,046	,507	,403	-1,31	-,782	,8775	,2173	,1728	-,5627	-,3354
Phosphat Ur	,044	-,715	-,143	-1,54	,740	,0048	-,0771	-,0155	-,1658	,0799
Diene Co U	,767	-,081	-,750	,809	-,173	1,703	-,1806	-1,665	1,797	-,3836
K Erythroc	2,620	,504	-,264	,048	-,638	,4170	,0802	-,0421	,0077	-,1016
Retic ZAC	1,470	,129	,135	,148	-,244	,1349	,0119	,0124	,0136	-,0224
Creatin Excr	-3,814	-,599	,056	-,719	,843	-2,268	-,3563	,0335	-,4273	,5014
Uricosuria	2,809	-,360	-1,34	-,439	-,522	,8691	-,1115	-,4137	-,1358	-,1616
MMM U	-,999	-,072	-,221	-,463	-,037	-,0230	-,0017	-,0051	-,0107	-,0009
Calcitonin	,388	1,394	1,260	,809	,231	,3239	1,165	1,052	,6763	,1933
0-Lymphoc	-,833	-,847	-,602	,473	,499	-,1178	-,1199	-,0852	,0670	,0706
Glucose Pla	,398	,912	-,120	,095	-,756	,5143	1,179	-,1550	,1227	-,9771
	Constants					**-104,6**	**-37,57**	**-59,53**	**58,25**	**31,06**
	Discriminant Proportion, %					**62,0**	**18,5**	**7,7**	**6,9**	**4,9**

Based on the above parameters, both individual values of discriminant roots for each animal and average values for each of the six groups were calculated, followed by visualization of the localization of each rat and group in the information space of these roots.

In the plane of the first two roots, which contain 80,5% of recognizable information, there is a clear separation along the major root axis of the cluster of rats loaded with water **Sofia**, from other clusters (Fig. 5.1 and 5.2).

This extreme localization of this cluster along the axis of the first root (centroid: -9,62) reflects the **minimum** for sampling parameters that correlate with the root **positively** (microbial count of neutrophils, blood content of 0-lymphocytes, plasma levels of malonic dialdehyde and potassium, uricosuria as well as the mass index of the thymus) in combination with the **maximum** values of **negatively** correlated with the root parameters (magniuria, entropy of the leukocytogram and its content of rod-shaped neutrophils and basophils, content in the immunocytogram of B-lymphocytes, phosphatemia, as well as the concentration of creatinine and malonic dialdehyde in the urine) (Table 5.9). The opposite extreme zone of the axis is occupied by the **Hertsa** water cluster.

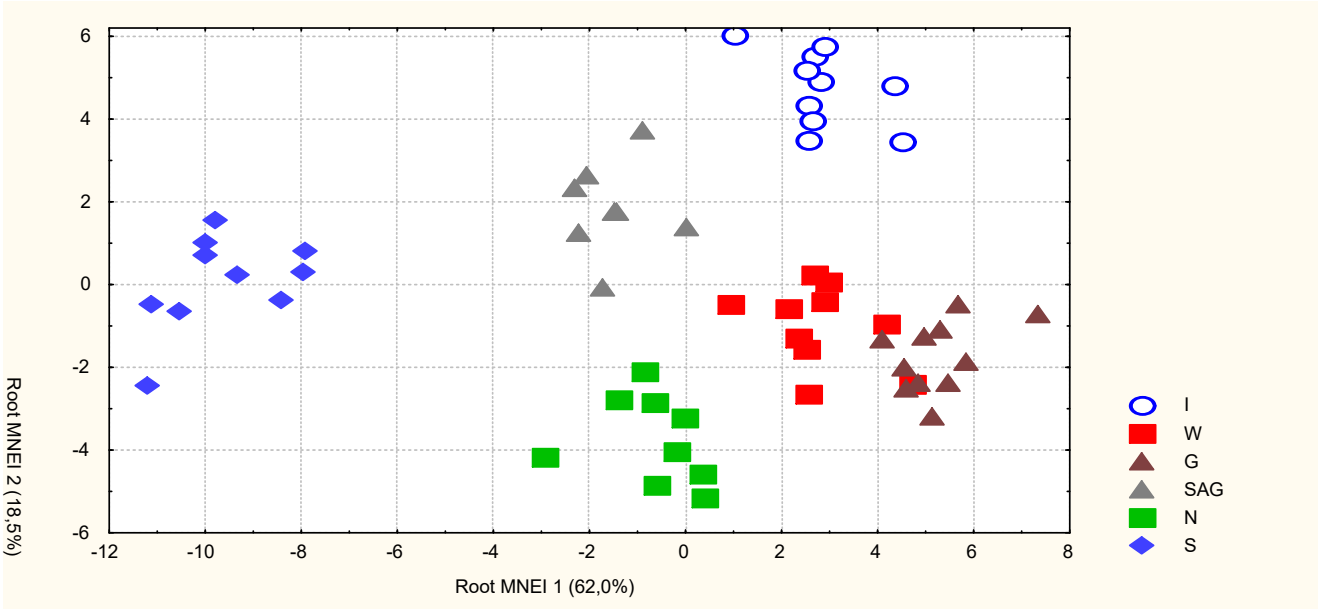


Fig. 5.1. Individual values of the first and second roots of metabolic, endocrine and immune parameters in intact rats (I) and loaded with daily water (W), water Naftussya (N), Sofia (S), Hertsa (G) and its artificial salt analogue (SAG)

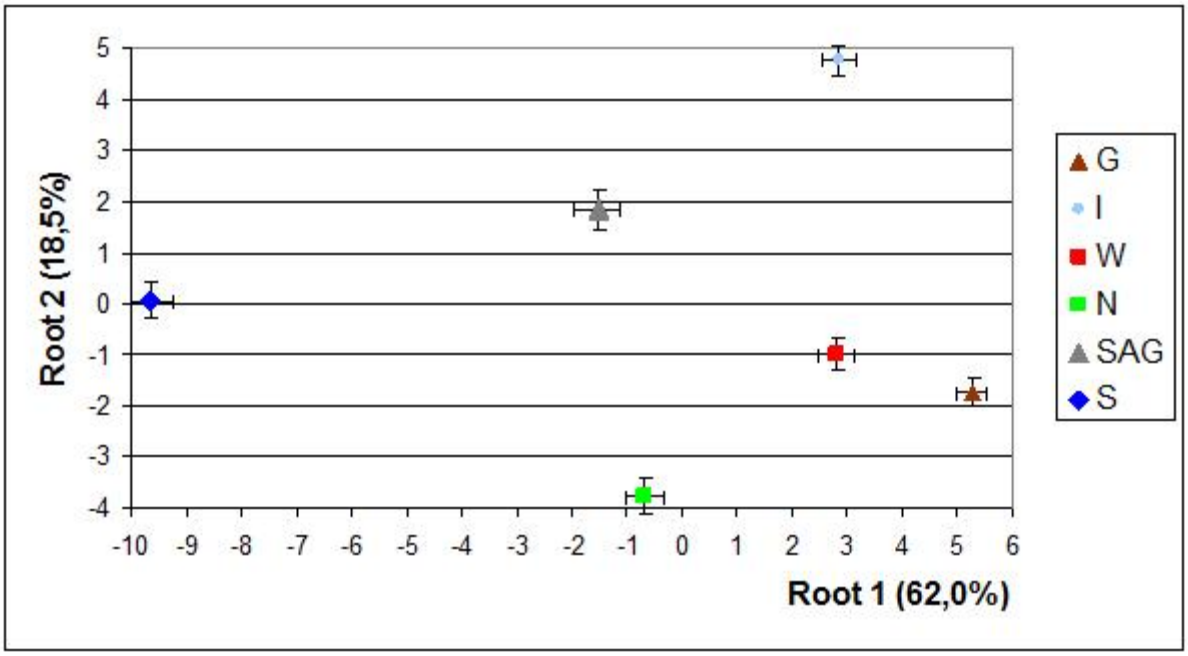


Fig. 5.2. Average values of the first and second roots of metabolic, endocrine and immune parameters in intact rats (I) and loaded with daily water (W), water Naftussya (N), Sofia (S), Hertsa (G) and its artificial salt analogue (SAG)

Along the axis of the second root, a cluster of rats receiving **Naftussya** water (centroid: -3,75) is located separately, which reflects the **minimum** values of the parameters correlating with the root **positively** (content of

eosinophils in the blood, T- and B-lymphocytes in the spleen), erythrocyte kalihistia and natriuria), instead of the **maximum** values of **negatively** correlated with the root parameters (calcium concentration in urine and associated calcitonin activity, as well as the entropy of the splenocytogram and the content of macrophages in it).

Along the axis of the third root, rats that received **fresh** water (centroid: -3,39) are separated (Figs. 5.3 and 5.4). This reflects the **maximum** level of plasma testosterone in the sample in combination with the **minimum** level of plasma calcium and neutrophils in the splenocytogram.

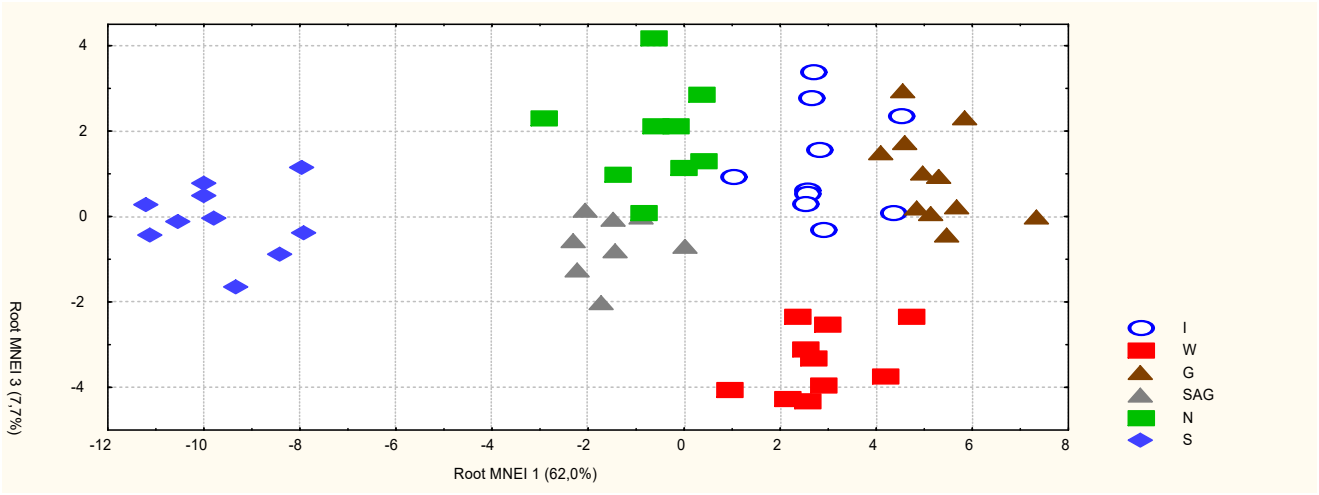


Fig. 5.3. Individual values of the first and third roots of metabolic, endocrine and immune parameters in intact rats (I) and loaded with daily water (W), water Naftussya (N), Sofia (S), Hertsa (G) and its artificial salt analogue (SAG)

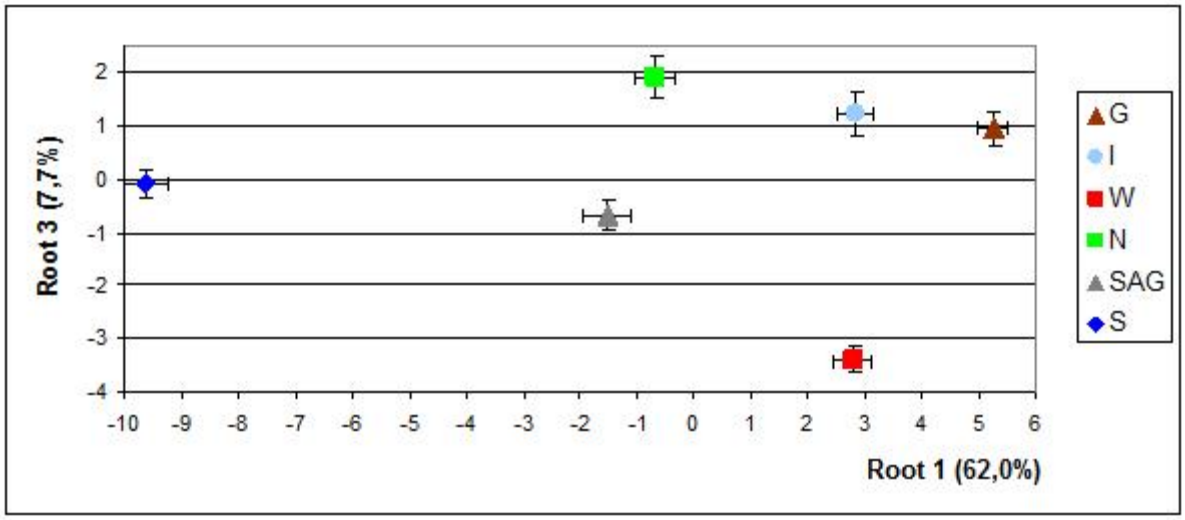


Fig. 5.4. Average values of the first and third roots of metabolic, endocrine and immune parameters in intact rats (I) and loaded with daily water (W), water Naftussya (N), Sofia (S), Hertsa (G) and its artificial salt analogue (SAG)

Finally, a cluster of rats loaded with a salt analogue of Hertsa water is separated from others along the axis of the fourth root (Fig. 5.5).

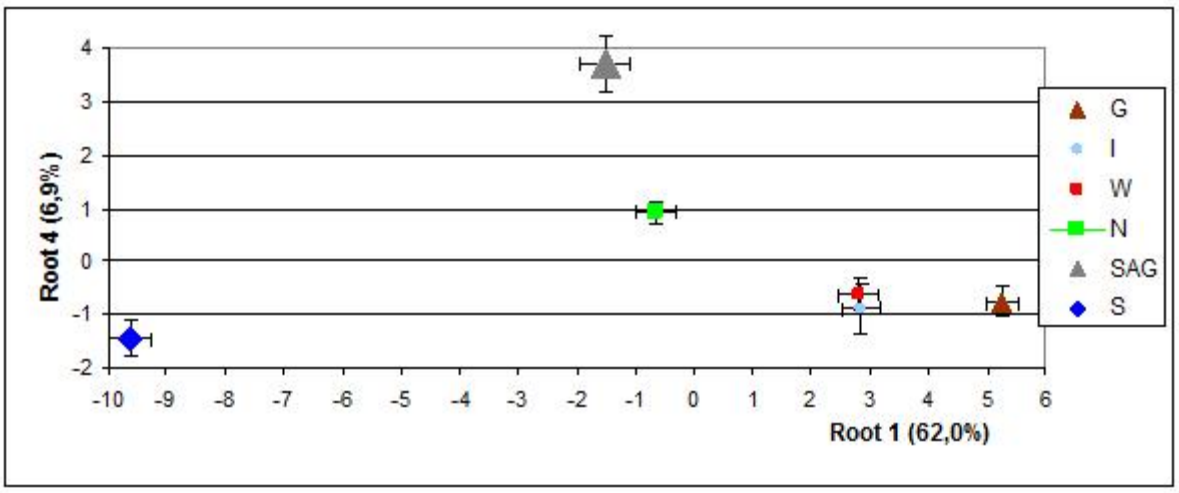


Fig. 5.5. Average values of the first and fourth roots of metabolic, endocrine and immune parameters in intact rats (I) and loaded with daily water (W), water Naftussya (N), Sofia (S), Hertsa (G) and its artificial salt analogue (SAG)

This localization reflects the **maximum** levels of glomerular filtration, parathyroid activity, glucose and diene conjugates in plasma aa well as reticulocytes and lymphocytes in the thymocytogram, which correlate with the root **directly**, combined with levels of lymphocytes in the blood aa well as amylase, middle mass molecules and phosphates in the urine associated with the root (Table 5.9).

Table 5.9. Factor structure of the matrix (correlations between variables and canonical roots) and average values of roots and variables

	Root 1	Root 2	Root 3	Root 4	Root 5	S	SAH	N	W	I	H
Root 1 (62,0%)						**-9,62**	-1,52	-1,52	2,80	2,85	**5,26**
Microb Count Neu	**,133**	,025	,017	-,068	,087	**6,4**	7,3	7,8	8,3	8,6	8,5
MDA Plasma	**,058**	-,102	-,094	-,035	,059	**57**	62	80	91	63	81
0-Lymphocytes	**,056**	-,057	,072	,094	,018	**16,6**	22,1	24,6	21,0	20,9	24,0
Uricosuria	**,049**	,017	-,081	,072	-,011	**3,8**	6,5	4,9	6,7	5,7	6,0
K Plasma	**,047**	,090	,038	,099	,238	**3,12**	3,82	3,86	3,71	4,23	3,35
Thymus Mass Ind	**,038**	-,028	-,011	,026	,057	**25**	28	30	30	29	29
Mg Excretion	**-,102**	,001	,064	,158	,021	**5,98**	5,85	5,07	2,65	3,30	2,51
Entropy LeukoCG	**-,072**	,014	,113	-,134	-,095	**338**	299	307	292	310	310
Creatinine Excret	**-,055**	-,044	-,041	-,033	-,031	**8,12**	7,01	7,16	7,15	6,41	6,83
Phosphate Plasma	**-,053**	,053	,000	-,048	,047	**1,22**	0,92	0,88	0,87	1,04	0,72
B-Lymphocytes	**-,045**	,042	-,110	-,160	-,022	**17,9**	14,8	14,2	16,7	16,0	15,1
Stub Neutrophils	**-,034**	,039	,057	-,198	-,000	**3,90**	2,50	3,00	3,00	3,60	3,18
MDA Urine	**-,029**	,015	,086	-,079	-,049	**102**	81	87	77	92	91
Basophils	**-,028**	,010	,028	-,152	-,152	**0,60**	0,13	0,11	0,20	0,30	0,45
Root 2 (18,5%)						0,07	1,84	**-3,75**	-1,00	4,75	-1,74
Eosinophils	,024	**,081**	,009	-,043	,009	3,30	3,50	**3,00**	3,60	4,60	3,64
Lymphocytes Spl	,049	**,073**	-,116	-,060	-,198	47,7	48,5	**46,0**	49,0	48,7	49,4
K Erythrocytes	-,027	**,066**	-,071	,058	-,087	88,5	90,1	**83,9**	86,9	87,0	85,8
Na Excretion	-,006	**,047**	,018	,106	-,275	175	282	**66**	89	135	225
Calcium Urine	,011	**-,112**	,123	,011	,147	2,07	1,93	**3,05**	2,15	2,10	2,32
Macrophages Spl	,010	**-,089**	-,005	-,095	,075	8,3	7,3	**9,0**	8,8	7,9	8,5
Entropy SplenoCG	-,034	**-,039**	,134	,044	,169	615	610	**627**	605	613	606
$(Cau•Pu/Pp•Cap)^{0,2}{}_{5}$	,032	**-,082**	,016	,124	-,040	2,13	4,00	**3,61**	3,08	2,35	3,24
Root 3 (7,7%)						-0,08	-0,66	1,91	**-3,39**	1,23	0,95
Testosterone	-,033	-,018	**-,188**	-,020	,020	5,53	4,76	4,11	**5,98**	3,93	3,98
Neutrophils Spleen	,001	-,039	**,182**	,040	,039	12,7	12,8	14,1	**11,8**	13,0	13,2
Calcium Plasma	-,019	,197	**,175**	,180	-,006	2,57	3,36	2,44	**1,88**	3,35	2,32
Root 4 (6,9%)						-1,44	**3,71**	0,94	-0,63	-0,90	-0,76

Glomerulary Filtrat	-,006	-,008	,027	**,201**	-,205	112	**194**	109	86,5	85,9	142
(Cap•Pu/Pp•Cau)^{0,2} $_{5}$	,027	,088	,057	**,142**	-,104	2,69	**4,11**	2,86	2,70	3,51	3,33
Glucose Plasma	-,015	-,033	-,135	**,133**	-,009	5,31	**5,76**	5,32	5,61	4,95	5,15
DC Urine	-,034	,121	-,067	**,098**	-,126	1,90	**2,14**	1,45	1,70	1,86	1,66
Reticular Zone AC	-,003	-,000	,021	**,084**	,023	42,0	**46,3**	45,0	42,0	42,8	42,1
Lymphocytes Thy	,009	,062	,029	**,070**	,093	68,6	**70,0**	69,4	69,0	70,0	68,5
Leukocytes Blood	,040	-,010	-,001	**-,143**	,032	10,7	**8,7**	11,4	12,8	12,7	12,9
Amylase Urine	,008	-,057	,007	**-,141**	,012	210	**181**	210	212	202	215
MMM Urine	,029	,031	-,042	**-,113**	,079	159	**147**	159	181	182	165
Phosphates Urine	,017	-,011	,017	**-,068**	,076	6,13	**5,89**	6,38	6,35	6,39	6,26

In general, in the information space of discriminant roots, all six clusters are clearly demarcated, ie differ from each other in the constellation of 35 metabolic, immune and endocrine parameters, documented by calculating the distances of Mahalanobis between them (Table 5.10).

Table 5.10. Squares of Mahalanobis distances between clusters and values F (df=35) and p

Groups	I	SAH	W	N	S	H
Intact rats (I)	0 F p	63 2,45 **,023**	61 2,70 **,014**	100 4,17 **,001**	204 9,06 **10^{-5}**	64 3,01 **,008**
Salt Analogue of Hertsa (SAG)	63 2,45 **,023**	0 F p	63 2,45 ,023	61 2,25 **,035**	107 4,16 **,001**	92 3,75 **,002**
Daily Water (W)	61 2,70 **,014**	63 2,45 ,023	0 F p	57 2,39 **,026**	189 8,41 **10^{-5}**	38 1,79 ,096
Water Naftussya (N)	100 4,17 **,001**	61 2,25 **,035**	57 2,39 **,026**	0 F p	124 5,20 **10^{-3}**	66 2,89 **,01**
Water Sofiya (S)	204 9,06 **10^{-5}**	107 4,16 **,001**	189 8,41 **10^{-5}**	124 5,20 **10^{-3}**	0 F p	254 11,9 **10^{-5}**
Water Hertsa (G)	64 3,01 **,008**	92 3,75 **,002**	38 1,79 ,096	66 2,89 **,01**	254 11,9 **10^{-5}**	0 F p

The use of classification functions (Table 5.11) allows retrospective identification of all six clusters without error (Table 5.12).

Table 5.11. Coefficients and constants for classification functions

Variables currently in the model	I p=,172	W p=,172	H p=,190	SAH p=,138	N p=,155	S p=,172
Calcium Plasma, mM/L	52,09	34,14	28,82	39,12	26,47	39,98
Microbian Count of Neutrophils, Bacter/Phagocyte	253,0	240,6	248,1	243,9	242,6	241,3
Phosphate Plasma, mM/L	808,9	766,0	784,3	796,9	763,9	750,0
Sodium Excretion, µM/24h•100 g Body Mass	-1,227	-1,204	-1,209	-1,160	-1,183	-1,108
Entropy of Leukocytogram •10^{3}	25,06	25,31	25,94	24,48	24,98	23,43
B-Lymphocytes Blood, %	-58,46	-57,34	-60,86	-55,17	-55,34	-48,42
Amylase Urine, mg/h•mL	-,905	-,824	-,837	-,736	-,688	-,447
Calcium Urine, mM/L	41,86	46,03	54,63	47,59	51,51	32,45
Neutrophils Spleen, %	170,9	169,4	173,2	166,5	168,3	160,4
Potassium Plasma, mM/L	154,4	153,2	153,7	145,3	150,2	134,0
Thymus Mass Index, mg/100 g Body Mass	2,239	2,056	1,944	2,437	1,741	1,293
Eosinophils Blood, %	10,36	11,14	10,98	7,698	7,402	-,606
Testosterone, nM/L	-34,36	-35,51	-37,63	-33,43	-36,82	-33,95
Lymphocytes Thymus, %	-77,69	-79,02	-82,11	-74,75	-75,70	-67,49
(Cap•Pu/Pp•Cau)^{0,25} as Parathyroid Activity	381,6	365,8	387,4	394,0	386,8	374,8
Glomerulary Filtration, µL/min•100 g Body Mass	-,142	-,097	-,046	-,132	-,075	-,182

Malonic Dialdehyde Plasma, µM/L	2,029	2,303	2,372	1,930	2,235	1,646
Malonic Dialdehyde Urine, µM/L	-6,101	-6,449	-6,447	-6,021	-6,036	-5,403
Magnesium Excreti-on, µM/24h•100 g Body Mass	-220,9	-219,2	-223,7	-211,7	-209,1	-193,1
Entropy of Splenocytogram •10^3	40,78	40,55	40,93	40,18	40,29	39,82
Basophils Blood, %	217,3	193,6	198,1	204,1	191,6	215,9
Stub Neutrophils Blood, %	-341,9	-351,7	-358,7	-335,1	-345,9	-315,8
Leukocytes Blood, 10^9/L	35,37	36,17	37,20	33,38	34,92	30,910
Macrophages Spleen, %	190,4	190,8	193,9	186,7	188,6	179,7
Lymphocytes Spleen, %	376,4	374,2	378,0	369,7	370,3	365,1
Phosphates Urine, mM/L	2,204	2,660	2,448	1,511	2,598	2,476
Diene conjugates Urine, E^{232}/mL	21,18	30,36	28,34	26,45	18,56	2,651
Potassium Erythrocytes, mM/L	60,92	60,65	61,73	59,19	58,67	55,57
Reticular Zone of Adrenal Cortex, µM	14,45	14,32	14,76	13,91	13,89	12,73
Creatinine Excretion, µM/24h•100 g Body Mass	-213,3	-211,5	-218,1	-205,4	-202,6	-184,0
Uric Acid Excretion, µM/24h•100 g Body Mass	90,45	92,94	93,9	87,45	87,67	81,02
Middle Mass Molecules Urine, units	-,783	-,751	-,824	-,715	-,711	-,474
(Cau•Pu/Pp•Cap)$^{0.25}$ as Calcitonin Activity	166,4	155,0	158,8	162,4	157,5	154,9
0-Lymphocytes Blood, %	-13,52	-12,42	-13,21	-12,33	-11,96	-11,54
Glucose Plasma, mM/L	259,7	253,8	256,4	256,9	247,1	249,6
Constants	-28998	-28484	-29078	-28106	-28210	-27554

Table 5.12. Classification matrix.
Observed (rows) and predicted (columns) classifications

Groups	Percent correct	I p=,172	W p=,172	H p=,190	SAH p=,138	N p=,155	S p=,172
Intact	100	**10**	0	0	0	0	0
Daily Water	100	0	**10**	0	0	0	0
Hertsa	100	0	0	**11**	0	0	0
Salt Analogue Hertsa	100	0	0	0	**8**	0	0
Naftussya	100	0	0	0	0	**9**	0
Sofiya	100	0	0	0	0	0	**10**
Total	100	10	10	11	8	9	10

CHAPTER 6

FACTOR AND CANONICAL ANALYSIS OF INFORMATION FIELD OF METABOLIC AND NEUROENDOCRINE-IMMUNE PARAMETERS AT FEMALE RATS

6.1. Factor analysis

According to the theory of factor analysis (Kim, Mueller, 1989), it is believed that the observed parameters (variables) are a linear combination of some latent (hypothetical, unobservable) factors. In other words, factors are hypothetical, not directly measurable, hidden variables in terms of which measurable variables are described. Some of the factors are allowed to be common to two or more variables, others are specific to each parameter separately. Characteristic (unique) factors are orthogonal to each other, ie do not contribute to the covariance between variables. In other words, only general factors, the number of which is much less than the number of variables, contribute to the covariance between them. The latent factor structure can be accurately identified by studying the resulting covariance matrix. In practice, it is impossible to obtain the exact structure of the factor model, you can only find estimates of the parameters of the factor structure. Therefore, according to the principle of postulate of parsimony, adopt a model with a minimum number of common factors.

One of the methods of factor analysis is the analysis of the principal components. Principal components (PCs) are linear combinations of observable variables that have orthogonal properties, ie they are natural orthogonal functions. Thus, PCs are the opposite of general factors, since the latter are hypothetical and are not expressed through a combination of variables, while PCs are linear functions of the observed variables.

The essence of the PC method is the linear transformation and **condensation** of initial information. Based on the correlation matrices, a system of orthogonal., linearly independent functions nominated by eigenvectors is determined, which correspond to the system of independent random variables nominated by the eigenvalues of the correlation matrix (λ). The first few eigenvalues of the correlation matrix exhaust the main part of the total variance of the field, so when analyzing the decomposition results, special attention is paid to the first eigenvalues and their corresponding components. And since large-scale processes, which are the functional systems of the body, are characterized by a large variance, it is fair to assume that they are reflected in the first components.

PC analysis is a method of converting a given sequence of observed variables into another sequence of variables. The method of obtaining the directions of the major axes is based on finding the eigenvalues and correlation vectors (covariances). The eigenvalue (λ) is the most important characteristic of the matrix (R); used in the decomposition of the covariance matrix and at the same time - as a criterion for determining the number of selected factors and as a measure of variance corresponding to this factor. The eigenvector (V) is a vector associated with the corresponding eigenvalue and is obtained in the process of isolating primary factors. These vectors, presented in normalized form, are **factor loads**. The relationship between these characteristics is expressed by the equation: $RV = \lambda V$.

The first eigenvalue represents the value of the variance corresponding to the first major axis, the second - the second, etc. The sum of eigenvalues is equal to the number of variables, and the fraction of variance corresponding to a given direction or PC is obtained by dividing the eigenvalue by the number of variables. The task of the PC is to **explain the maximum fraction of the variance** of observations, and the task of general factors is to explain the correlations between variables.

In the n-dimensional factor space, the first PC is a representation of points (data) along the selected main axis, it reproduces the maximum fraction of the variance of the experimental data. If you describe each point in the new coordinate system, the loss of information does not occur. In the case of a linear relationship between variables, the first PC contains all the information to describe each point, but if the variables are independent, the main axis is missing, and PC analysis does not contribute to even minimal compression of observation results. If there is a more or less close relationship between the variables, the rest of the information is contained in subsequent PCs, with the axis of the second PC perpendicular to the axis of the first PC and along it is a smaller part of the data, ie the second PC reproduces the next even less information is contained along the axis of the third PC, perpendicular to the first two, etc. It is believed that to study the factor structure of the studied field can be limited to the number of PC, the total contribution of which to the total variance of the original data exceeds 2/3. Another approach to determining the amount of PC is to use the Kaiser's ($\lambda>1$) and Cattell's criteria (for the maximum deceleration of the eigenvalue λ, graphically visualized) (Kim, Mueller, 1986).

At the first stage of factor analysis (PC method) it was found that the dispersion of the information field of 100 parameters (of which 5 are **integral.,** 20 reflect metabolism of **electrolytes**, 23 - **non-electrolytes**, 13 - **neuroendocrine regulation**, 19 - immune parameters of **blood**, 10 - **thymus** and 10 - **spleen**) is absorbed by 20 factors (Fig. 6.1).

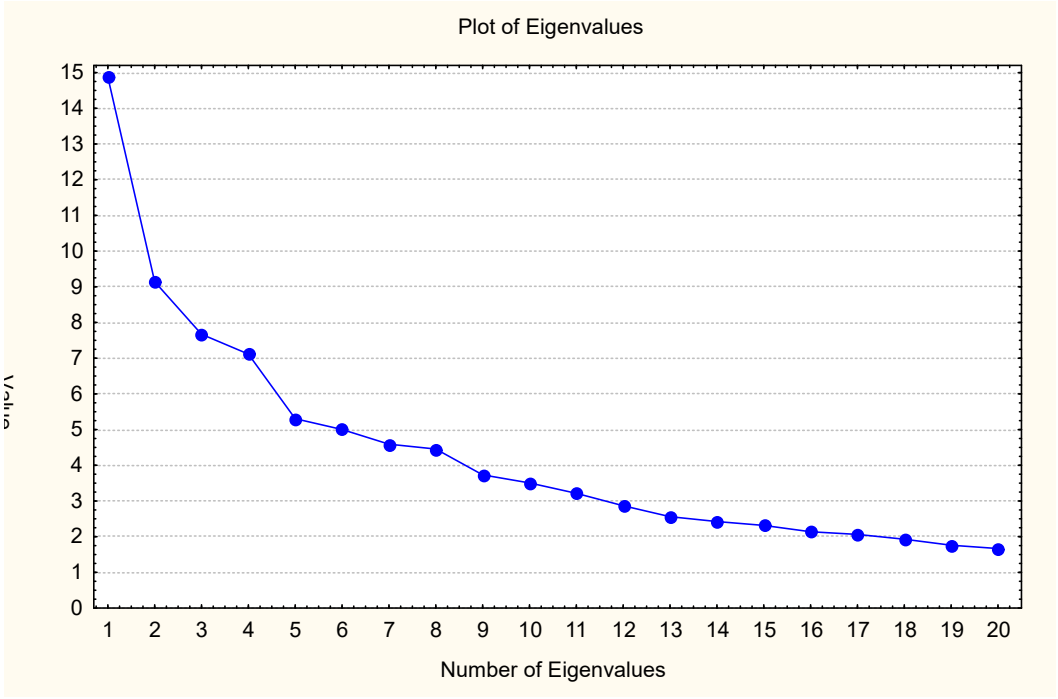


Fig. 6.1. Values of eigenvalues of principal components

Applying the Cattel's approach, we limit the number of analyzed factors to twelve (Table 6.1), the total contribution of which to the total variance of the initial data is 66.2%, ie just reaches the required critical level.

Table 6.1. The values of the eigenvalues of the principal components and the fraction of the dispersion absorbed by them. Extraction: Principal components

PC	Eigen-values	% total Variance	Cumul. Eigenval	Cumul. %
1	14,88	13,8	14,88	13,8
2	9,14	8,5	24,02	22,2
3	7,67	7,1	31,69	29,3
4	7,12	6,6	38,80	35,9
5	5,31	4,9	44,11	40,8
6	5,00	4,6	49,11	45,5
7	4,59	4,3	53,70	49,7
8	4,45	4,1	58,15	53,8
9	3,73	3,5	61,88	57,3
10	3,51	3,2	65,39	60,5
11	3,21	3,0	68,60	63,5
12	2,87	2,7	71,47	66,2

The factor structure is considered the simplest if all variables have a single factor complexity, ie when each variable has a non-zero load on only one common factor. If there are at least two factors, then each row contains only one non-zero element, each column has several zeros, for each pair of columns zero elements do not match.

However, such a simple structure for real data is unattainable. The simplicity of the structure is determined if for each factor there are at least three variables that have a significant load on this factor. In the orthogonal case, a simple structure is given by a set of points that have nonzero loads on only one factor (axis). To achieve a simpler interpretation of solutions, the concept of oblique (non-orthogonal) factors is used, which makes it possible to better represent clusters of variables without abandoning the orthogonality (independence) of factors.

In order to find the factor mapping matrix closest to the simplest ideal structure, the procedure of **orthogonal rotation** by quartimax, varimax and equamax methods is performed.

Varimax is a method of obtaining an orthogonal solution, which is reduced to simplifying the factor structure using the criterion of minimizing the column of the factor mapping matrix; quartimax is a criterion for obtaining an orthogonal solution, which is reduced to simplifying the description of rows of the matrix, and equamax combines the properties of the first two, therefore, at the next stage of factor analysis, the orthogonal

rotation procedure was performed by us using the Equamax normalized method in order to find the factor mapping matrix closest to the simplest ideal structure.

The result of factor analysis by the PC method of the field of variables is shown in Table 6.2, which is, in essence, a matrix of factor mapping, the elements of which are **factor loads** - correlation coefficients between factors (PC) and variables.

It is known that one PC combines indicators (variables) that are maximally related to each other and minimally related to other indicators. Therefore, we used factor analysis as a heuristic method of selection among the registered variables of clusters, since the found structures are considered as hypotheses that reflect in the obtained data some trends in the accumulation of variables in clusters (Kim, Mueller, 1986).

After orthogonal rotation in the PC there were slight changes in the proportions of the absorbed dispersion, except for the eighth PC, the share of which doubled - from 4,1% to 8,0%. Therefore, when characterizing PC elements, we will, for convenience, adhere, with one exception, to the primary priority.

The first PC, by definition, explains the maximum proportion of variability – 9,7%, and reflects, on the one hand, the state of thyroid and glucocorticoid functions, on the other - the processes of magnesium, uric acid and phosphate metabolism, on the third - phagocytosis and killing microbes (at least gram-positive) by monocytes/macrophages and neutrophils/microphages, as well as natural killers of blood. This immediately suggests a connection between these processes. At the same time the negative sign of loadings on PC from parameters of phagocytosis of microphages and uric acid metabolism draws attention.

The second PC, which absorbs 7,4% of the dispersion, is interpreted as excretory, as it receives positive factor loads from daily diuresis and urinary excretion of electrolytes (phosphates, mainly HPO_4^{2-}, as rat urine is alkaline; calcium and potassium) and nitrogen metabolites (creatinine and urea, as well as its concentration in the urine). This constellation is combined with the activity of urinary catalase.

Table 6.2. Factor loads (after Equamax normalized rotation). Load clusters that determine oblique factors for hierarchical analysis of parameters. Extraction: Principal components. Marked loadings are >0,700

	1	2	3	4	5	6	7	8	9	10	11	12
Monocytes	**0,929**	0,009	0,093	0,025	0,003	0,035	0,086	-0,122	0,002	-0,026	0,002	0,068
Triiodothyro	**0,910**	-0,024	0,086	0,063	0,072	0,045	0,019	-0,218	0,002	-0,095	-0,032	0,106
Natur Killer	**0,901**	-0,062	0,097	0,041	0,053	0,005	0,064	-0,098	0,024	-0,032	-0,021	0,154
MicCo Neu	**-0,885**	0,034	0,035	0,092	0,122	0,080	-0,041	0,226	-0,016	0,145	0,005	0,003
Mg Excret	**0,772**	-0,231	0,237	0,223	0,164	0,038	0,002	0,027	-0,090	0,047	0,077	-0,062
Mg Urine	**0,763**	-0,260	0,153	0,332	0,173	0,109	0,057	0,014	-0,032	0,015	-0,113	-0,071
Fascic ZAC	**0,717**	-0,003	0,058	0,131	0,001	0,085	0,040	-0,146	0,200	-0,134	-0,054	0,064
Pi Plasma	**0,700**	-0,054	0,047	0,119	0,001	0,047	0,020	-0,076	0,017	-0,151	-0,026	0,116
Ph I Neutr	**-0,693**	-0,160	0,054	0,065	0,189	0,329	-0,023	0,038	-0,183	0,143	0,048	-0,107
UrAc Urine	**-0,668**	-0,258	0,072	0,059	0,194	0,155	-0,039	0,128	-0,060	0,085	0,234	0,355
MicCo Mon	**0,613**	-0,033	0,116	0,105	0,092	0,142	-0,102	0,039	-0,258	0,095	0,129	0,204
UrAc Excr	**-0,502**	**-0,461**	0,109	0,112	0,255	0,134	-0,039	0,058	-0,146	0,117	0,169	**0,446**
UrAc Plasm	**-0,402**	0,117	0,120	0,276	0,085	0,220	-0,235	0,173	-0,074	0,100	-0,040	0,330
Diuresis	0,345	**0,911**	-0,035	0,016	0,030	-0,045	0,015	0,212	-0,144	0,047	0,033	0,148
Pi Exctetion	0,252	**0,889**	-0,037	0,131	0,005	0,121	-0,006	0,280	-0,093	0,000	-0,024	0,147
Creatin Exc	0,240	**0,856**	-0,096	0,108	0,087	-0,227	0,072	0,185	-0,095	0,048	-0,083	-0,057
Urea Excret	0,200	**0,854**	-0,119	0,016	0,158	-0,088	0,113	0,233	-0,079	0,072	-0,107	-0,041
Ca Excretio	0,190	**0,739**	-0,047	0,187	0,052	-0,043	0,091	**0,366**	-0,010	0,059	-0,072	**-0,307**
Urea Urine	0,182	**0,660**	-0,096	0,063	0,136	-0,172	0,246	-0,010	0,289	-0,045	-0,110	0,175

K Excretion	0,178	0,553	0,108	0,101	0,073	0,019	0,229	0,219	0,360	0,247	0,031	0,411
Katalase Ur	0,176	0,519	0,142	0,284	0,133	0,150	0,053	0,341	0,106	0,040	0,166	0,028
Na Urine	0,268	0,299	0,930	0,015	0,031	0,002	0,158	0,155	0,059	0,026	0,037	0,004
Osmolal Ur	0,301	0,299	0,919	0,103	0,045	0,070	0,101	0,110	0,014	0,099	0,047	0,101
Cl Urine	0,326	0,302	0,905	0,001	0,045	0,006	0,043	0,149	0,108	0,100	0,016	0,125
Cl Excretio	0,438	0,313	0,892	0,053	0,028	0,036	0,005	0,009	0,064	0,075	0,005	0,131
Na Excret	0,374	0,073	0,815	0,018	0,049	0,040	0,094	0,248	0,068	0,057	0,018	0,009
MCA P&U	0,159	0,403	0,807	0,159	0,096	0,065	0,126	0,333	0,009	0,029	0,026	0,140
Glucosae P	0,158	0,342	0,415	0,055	0,149	0,080	0,296	0,122	0,244	0,096	0,092	0,143
Creatinine P	0,157	0,289	0,293	0,829	0,156	0,144	0,079	0,168	0,062	0,042	0,052	0,179
Urea Plasm	0,144	0,267	0,283	0,748	0,030	0,075	0,199	0,055	0,074	0,253	0,000	0,149
Killing IN	0,267	0,280	0,346	0,620	0,349	0,137	0,169	0,034	0,075	0,084	0,250	0,037
Glomer Filt	0,177	0,536	0,037	0,575	0,013	0,073	0,108	0,204	0,021	0,160	0,109	0,181
Corticoster	0,136	0,216	0,233	0,416	0,128	0,050	0,199	0,204	0,119	0,281	0,188	0,101
Spleen MI	0,130	0,214	0,198	0,405	0,036	0,180	0,001	0,359	0,095	0,229	0,325	0,289
Reticu ZAC	0,126	0,202	0,197	0,396	0,378	0,145	0,187	0,075	0,134	0,038	0,012	0,186
Lymphoc	0,136	0,220	0,235	0,435	0,879	0,011	0,032	0,033	0,068	0,086	0,175	0,008
Segm Neut	0,249	0,217	0,261	0,345	0,851	0,068	0,060	0,027	0,047	0,005	0,153	0,042
Stub Neutro	0,258	0,223	0,333	0,349	0,741	0,002	0,047	0,225	0,045	0,144	0,088	0,085
Entro LCG	0,627	0,031	0,029	0,024	0,664	0,026	0,095	0,059	0,075	0,042	0,190	0,013
PTA P&U	0,126	0,192	0,175	0,342	0,377	0,758	0,031	0,146	0,070	0,052	0,109	0,335
CTA P&U	0,120	0,134	0,142	0,254	0,279	0,738	0,048	0,234	0,058	0,067	0,066	0,253
Pi Urine	0,143	0,226	0,236	0,451	0,002	0,540	0,045	0,261	0,157	0,059	0,221	0,004
Mg Plasma	0,235	0,208	0,248	0,303	0,317	0,485	0,189	0,333	0,194	0,070	0,074	0,125
Neutroph S	0,101	0,123	0,131	0,232	0,257	0,476	0,207	0,209	0,247	0,025	0,011	0,016
MDA Plasma	0,235	0,206	0,232	0,282	0,261	0,475	0,225	0,158	0,040	0,080	0,146	0,142
Cholesterol	0,099	0,109	0,124	0,222	0,216	0,374	0,004	0,352	0,285	0,153	0,051	0,045
Testosterone	0,181	0,129	0,182	0,198	0,156	0,329	0,190	0,107	0,038	0,274	0,121	0,127
AMo HRV	0,079	0,101	0,099	0,199	0,193	0,183	0,789	0,222	0,018	0,131	0,058	0,068
Mode HRV	0,189	0,154	0,183	0,228	0,171	0,334	0,756	0,225	0,009	0,128	0,113	0,017
MxDMnHRV	0,193	0,168	0,190	0,255	0,184	0,359	0,753	0,101	0,144	0,015	0,077	0,046
DC Plasma	0,197	0,173	0,195	0,276	0,213	0,373	0,687	0,003	0,157	0,051	0,173	0,040
Na Plasma	0,076	0,101	0,083	0,188	0,181	0,177	0,548	0,134	0,164	0,121	0,052	0,229
DC Urine	0,209	0,184	0,210	0,278	0,221	0,411	0,477	0,098	0,035	0,011	0,075	0,079
Cl Plasma	0,068	0,100	0,080	0,181	0,176	0,161	0,458	0,183	0,167	0,297	0,017	0,165

Macrophag S	0,058	0,080	0,079	0,176	0,170	0,153	**0,454**	**-0,438**	0,047	-0,091	-0,189	-0,034
PlasmocytesS	0,093	0,107	0,120	0,214	0,207	0,280	0,215	**0,773**	0,092	-0,073	-0,071	-0,255
Creatinine U	-0,140	-0,079	-0,123	-0,125	-0,100	-0,153	-0,080	**-0,759**	-0,044	-0,035	-0,049	-0,141
MMM Urine	-0,054	-0,078	-0,078	-0,158	-0,166	-0,151	**-0,363**	**-0,651**	-0,018	-0,002	-0,092	-0,107
Canal Reabs	-0,145	-0,092	-0,130	-0,135	-0,108	-0,187	-0,082	**-0,643**	-0,047	-0,069	-0,064	-0,069
Epithelioc T	-0,148	-0,093	-0,144	-0,146	-0,127	-0,191	-0,095	**-0,620**	-0,028	-0,084	-0,053	-0,096
Na Erythroc	-0,050	-0,074	-0,077	-0,153	-0,161	-0,149	-0,301	**-0,600**	-0,076	-0,099	-0,173	-0,149
K Urine	-0,152	-0,093	-0,152	-0,151	-0,136	-0,195	-0,113	**-0,596**	-0,294	-0,179	-0,057	**0,342**
LymBlaste S	0,045	0,069	0,074	0,142	0,148	0,145	0,271	**0,566**	-0,070	-0,211	-0,014	-0,034
Katalase Plas	-0,042	-0,068	-0,064	-0,140	-0,138	-0,140	-0,250	**-0,495**	-0,080	-0,047	-0,212	-0,218
Bilirubine Pl	-0,027	-0,059	-0,062	-0,136	-0,120	-0,127	-0,231	**-0,466**	-0,121	-0,122	-0,032	-0,124
MMM Plasm	-0,026	-0,053	-0,055	-0,126	-0,090	-0,095	-0,213	**-0,432**	-0,089	-0,191	-0,114	-0,021
LymBlaste T	-0,021	-0,053	-0,051	-0,123	-0,082	-0,086	-0,194	**-0,420**	-0,037	-0,310	-0,169	-0,071
Amylase Pla	-0,161	-0,095	-0,153	-0,153	-0,141	-0,197	-0,117	**-0,470**	-0,058	-0,035	-0,153	-0,018
MDA Urine	-0,162	-0,096	-0,161	-0,154	-0,146	-0,219	-0,119	**-0,438**	-0,056	-0,098	-0,008	-0,067
K Erythroc	-0,165	-0,108	-0,178	-0,157	-0,151	-0,234	-0,170	**-0,437**	-0,184	-0,255	-0,271	-0,039
Amylase Ur	-0,179	-0,116	-0,178	-0,195	-0,155	-0,272	-0,230	**-0,419**	**0,396**	-0,031	-0,033	-0,151
Reticulocyt T	-0,182	-0,146	-0,182	-0,216	-0,158	**-0,333**	-0,268	**-0,367**	-0,174	-0,040	-0,113	-0,193
Hassal Cor T	-0,111	-0,071	-0,079	-0,087	-0,076	-0,090	-0,059	-0,184	**-0,628**	**-0,304**	-0,042	-0,193
Reticulocyt S	-0,112	-0,077	-0,087	-0,095	-0,087	-0,115	-0,063	-0,216	**-0,625**	-0,074	-0,055	-0,035
Thymus MI	0,021	0,052	0,047	0,118	0,081	0,085	0,191	**0,356**	**0,598**	-0,082	-0,079	-0,170
Body Mass	-0,121	-0,077	-0,094	-0,109	-0,095	-0,143	-0,067	-0,286	**-0,558**	-0,094	-0,020	-0,058
Glomer ZAC	0,019	0,050	0,036	0,115	0,078	0,084	0,188	0,278	**0,534**	-0,064	-0,175	-0,106
Leukocytes	0,013	0,046	0,034	0,112	0,077	0,066	0,161	0,270	**0,467**	-0,210	-0,040	-0,070
LymCytes S	0,013	0,042	0,029	0,099	0,062	0,044	0,155	0,242	**0,443**	-0,054	-0,262	-0,242
FibroblastesS	-0,125	-0,078	-0,114	-0,110	-0,099	-0,151	-0,074	**-0,346**	**-0,358**	-0,030	-0,264	-0,067
EntropyTCG	-0,086	-0,056	-0,051	-0,037	-0,049	-0,032	-0,035	-0,144	-0,206	**-0,686**	-0,103	-0,235
LymCytes T	0,026	0,059	0,061	0,134	0,100	0,096	0,227	**0,442**	0,095	**0,558**	-0,143	-0,258
PlasmocytesT	-0,086	-0,056	-0,052	-0,048	-0,055	-0,059	-0,039	-0,158	-0,213	**-0,544**	-0,091	-0,108
EndotheliocT	-0,095	-0,061	-0,054	-0,068	-0,061	-0,076	-0,058	-0,163	-0,215	**-0,469**	**0,311**	-0,138
Macrophag T	-0,107	-0,070	-0,066	-0,072	-0,072	-0,088	-0,059	-0,176	**0,330**	**-0,441**	-0,135	-0,103
Ca Plasma	0,010	0,041	0,025	0,097	0,061	0,040	0,151	0,173	0,268	**0,402**	-0,065	-0,064
K Plasma	0,006	0,027	0,023	0,094	0,058	0,035	0,139	0,166	0,214	**0,391**	-0,232	-0,053
Eosinophils	-0,013	-0,022	-0,015	-0,093	-0,047	-0,031	-0,114	-0,131	-0,117	**0,351**	-0,025	-0,201

0 Lymphocyt	-0,085	-0,054	-0,050	-0,030	-0,044	-0,027	-0,026	0,119	-0,138	-0,130	**-0,876**	-0,115
B Lymphocyt	-0,019	0,013	0,014	0,092	0,046	0,031	0,093	0,116	0,115	**0,313**	**0,753**	0,022
T cytolytic L	-0,025	0,001	0,011	0,067	0,045	0,027	0,087	0,110	0,095	0,184	**0,652**	0,191
T helper Lym	-0,038	-0,003	0,007	0,066	0,037	0,022	0,081	0,101	0,083	0,104	**0,530**	**-0,490**
SOD	-0,040	-0,008	0,003	0,055	0,020	0,021	0,080	0,077	0,071	0,074	**0,421**	-0,153
Basophils	-0,078	-0,049	-0,040	-0,030	-0,039	-0,018	-0,024	0,110	0,129	0,125	**-0,329**	-0,034
Adrenals MI	-0,064	-0,038	-0,034	-0,013	-0,027	-0,007	-0,013	0,064	0,077	0,077	**-0,244**	-0,174
Ca Urine	-0,047	-0,022	-0,008	0,046	-0,017	-0,013	0,068	0,045	-0,035	-0,033	-0,008	**-0,639**
Entropy ICG	-0,063	-0,031	-0,029	0,000	-0,023	-0,004	-0,025	0,019	-0,044	-0,067	-0,171	**0,597**
Eosinophils S	-0,053	-0,024	-0,017	-0,031	-0,011	-0,011	-0,068	-0,025	-0,019	-0,014	-0,049	**-0,487**
Entropy SCG	-0,072	-0,047	-0,034	-0,017	-0,029	-0,012	-0,004	0,087	-0,085	-0,080	-0,287	**-0,458**
PhI Mon	-0,055	-0,025	-0,021	-0,025	-0,019	-0,008	-0,039	-0,021	-0,001	-0,019	-0,122	**0,322**
Expl. Var	***10,42***	***8,03***	***7,17***	***5,27***	***4,57***	***5,32***	***5,02***	***8,60***	***4,41***	***3,77***	***4,03***	***4,84***
Prp. Total	***0,097***	***0,074***	***0,066***	***0,049***	***0,042***	***0,049***	***0,047***	***0,080***	***0,041***	***0,035***	***0,037***	***0,045***

It should be noted that there are significant factor loads on this PC from uric acid excretion and glomerular filtration, but these variables are formally attributed to other PCs.

The third PC (6,6% variability) reflects the inverse osmolality of urine, determined to the greatest extent, judging by the factor loads, the concentration of sodium and chloride ions in it, which, in turn, is determined by their excretion. Naturally, the mineralocorticoid activity, calculated from the content in plasma and urine of sodium and chloride ions, gives the opposite sign of the load. Localization in this information field of glycemia is unexpected.

The fourth PC explains 4,9% of the variance and reflects, on the one hand, corticosteronemia and the thickness of the reticular zone of the adrenal cortex, which in females is a source of androgens and testosterone, on the other hand - glomerular filtration and plasma creatinine and urea levels, ie markers of renal depurative function, and on the third hand - the mass index of the spleen and the index of the killing of bacteria by neutrophils as possible targets of these hormones and metabolites.

The fifth PC (4,2% variability) includes most elements of the peripheral blood leukocytogram, as well as the entropy of the leukocytogram.

The sixth PC, explaining 4,9% of the variance, reflects the content of phosphate in the urine, which is known to be subject to unidirectional exposure to both parathyroid hormone and calcitonin. In contrast, testosterone puts a negative load on the PC, as do plasma levels of magnesium and malonic dialdehyde, while other possible targets for hormones, such as plasma cholesterol and spleen neutrophils, put a positive load on the PC.

Seventh PC (4,7% variability) characterizes directly sympathetic tone and plasma levels of sodium and chloride as well as macrophages in the spleen, while inverse - vagal tone, humoral regulatory channel and the content of diene conjugates in plasma and urine.

The ninth PC (4,1% dispersion) receives positive loads from the thickness of the glomerular zone of the adrenal cortex, producing mineralocorticoids, on the one hand, and the content of lymphocytes in the spleen, total leukocytes in the blood, and thymus mass relative to body weight (which is also presented in this PC, but inverse) - on the other hand. Instead, the negative loads come from the content of Hassall's corpuscles in the thymus as well as reticulocytes and fibroblasts in the spleen.

The tenth PC (3,5% dispersion) receives positive loads from plasma calcium and potassium as well as eosinophils in the blood and T-lymphocytes in the thymocytogram, while negative - from the entropy of the thymocytogram and the proportions of its minor elements - plasma cells, endothelial cells and macrophages.

The eleventh PC absorbs 3,7% of variability and receives positive loads from the levels in the immunocytogram of blood B-lymphocytes, T-cytolytic and T-helper lymphocytes, while negative - from the levels of 0-lymphocytes and basophils. This composition of PC is supplemented by the activity of superoxide dismutase of erythrocytes and the mass index of the adrenal glands.

The twelfth PC (4,5% dispersion) reflects inverse the concentration of calcium in the urine, which decreases under the influence of parathyroid hormone, while calcitonin increases it. The entropy of the splenocytogram and

the share of eosinophils in it are directed to calcium loads, while the entropy of the thymocytogram and the phagocytic index of blood monocytes give the opposite loads.

Finally, consider the composition of the eighth PC, which after rotation became the second in weight. It receives negative factor loads from the concentration of creatinine, malonic dialdehyde, amylase and potassium in the urine, as well as plasma amylase and potassium in erythrocytes. This composition is supplemented by plasma cells of the spleen and lymphoblasts of the spleen and thymus. Instead, plasma catalase and bilirubin, plasma and urine medium mass molecules, aa well as erythrocyte sodium exert positive loads on PC, as do thymic epitheliocytes and reticulocytes, as well as splenic macrophages.

In the next step, the obtained correlation matrix for oblique factors was further analyzed to identify the set of orthogonal factors that divide the variability in variables into that related to total variance (secondary factors) and individual variances related to clusters or similar variables (primary factors).

Three generals (secondary) hypothetical, ie not directly measured, factors were revealed (Table 6.3). In this case, each of the common factors includes a constellation of indicators of neuroendocrine regulation, metabolism and immunity, interconnected by bilateral causal relationships.

Table 6.3. Load on common (S) factors

Variables	S 1	S 2	S 3
Macrophages Spleen	**-,55**	,04	,06
Lymphoblastes Spleen	**,55**	,04	,02
Plasmocytes Spleen	**,53**	-,00	,27
Epitheliocytes Thymus	**-,53**	-,28	-,12
AMo HRV as Sympathetic tone	**-,52**	,01	-,03
1/Mode HRV as Catecholamines	**-,52**	,08	,03
Na Erythrocytes	**,52**	,01	,16
Creatinine Urine	**-,51**	-,13	-,05
Triiodothyronine	**-,48**	,12	-,24
Mineralocorticod Activity	**-,48**	,06	,03
Na Excretion	**,48**	,10	-,03
Microbial Count Neutrophils	**,47**	-,13	,19
Lymphocytes T	**,46**	,36	-,05
MxDMn HRV as Vagal tone	**,45**	-,21	-,04
Amylase Plasma	**-,45**	-,10	,13
Monocytes Blood	**-,42**	,13	-,17
Natural Killers Blood	**-,42**	,15	-,24
K Urine	**-,41**	-,05	-,17
EntropyThymocytogram	**-,40**	-,32	,09
Na Urine	**,38**	-,09	-,07
Katalase Plasma	**,38**	,20	,36
Katalase Urine	**,36**	,26	,27
Mg Urine	**-,36**	-,04	,11
MMM Urine	**,35**	,23	,02
Pi Plasma	**-,34**	,07	-,11
Calcitonin Activity	**,33**	-,02	,17
Cholesterol Plasma	**,32**	,00	,03
MDA Urine	**-,32**	-,21	-,01
Urea Urine	**,31**	,17	,09
Cl Excretion	**,30**	,11	-,16
Phagocytic Index Neutrophils	**,30**	-,22	,17
Hassall's Corpuscles Thymus	**-,30**	,15	,27
Testosterone	**-,28**	,01	,09
Lymphocytes Blood	,15	**-,47**	,16
Entropy Leukocytogram	-,33	**,45**	-,17
Diuresis	,25	**,44**	,00
Pi Excretion	,34	**,41**	,07
Rod-shaped Neutrophils Blood	-,01	**,40**	-,04
Segmented Neutrophils Blood	-,05	**,38**	-,12
Urea Excretion	,37	**,38**	,06
Amylase Urine	-,33	**-,37**	,07
0-Lymphocytes Blood	,00	**,36**	-,10

Creatinine Excretion	-,05	**,35**	-,04
K Excretion	-,09	**,34**	-,11
Thymus Mass Index	-,18	**-,34**	-,11
B-Lymphocytes Blood	,16	**-,33**	,07
Uric acid Urine	,10	**-,33**	-,05
Neutrophils Spleen	,01	**-,32**	-,01
Fibroblastes Spleen	-,16	**,30**	,07
T-cytolytic Lymphocytes Blood	,04	**-,29**	-,14
Creatinine Plasma	-,06	,06	**,59**
Ca Urine	,24	,03	**,58**
Urea Plasma	-,22	,09	**,49**
Canalicular Reabsorbtion	-,33	-,10	**-,46**
Glomerular Filtration	,11	,20	**-,43**
Entropy Splenocytogram	,10	,21	**,41**
T-helper Lymphocytes Blood	,08	-,27	**,39**
Ca Excretion	,33	,33	**,36**
Lymphocytes Spleen	,14	-,19	**-,35**
Spleen Mass Index	-,21	,12	**,32**
Parathyroid Activity	,25	-,02	**-,31**
Corticosterone	,00	,20	**,29**

The first common factor reflects the inverse neuro-hormonal block: sympathetic tone, circulating catecholamines, levels of thyroid, mineralocorticoid and androgenic functions in combination with the concentration of potassium, magnesium and creatinine in urine and phosphates, amylase and malonic dialdehyde in plasma, as well as the content of natural killers and monocytes in blood, macrophages in spleen, epitheliocytes and Hassall's body in the thymus as well as the entropy of the thymocytogram. Instead, positive factor loads give vagal tone and calcitonin activity, on the one hand, the concentration of sodium in erythrocytes and urine, excretion of sodium and chloride, urinary concentration of magnesium and urea as well as cholesterolemia - on the other hand; the content of T-lymphocytes in the thymus and lymphoblasts and plasma cells in the spleen - on the third side.

The second common factor concerns, first of all, the entropy of the leukocytogram and its neutrophils, as well as 0-lymphocytes of blood and spleen fibroblasts in combination with daily diuresis and urinary excretion of phosphates, urea and creatinine, which give a positive load. This factor receives opposite loads from total lymphocytes and their B- and Tc-populations, thymus mass, neutrophil content in the spleen - on the one hand, and amylase and uric acid concentrations in urine - on the other hand.

Finally, the third common factor reflects the reciprocal relationship between glomerular filtration and tubular water reabsorption, on the one hand, and plasma creatinine and urea levels in combination with calciumuria, on the other. The immune block reflects the level of T-helpers in the blood, the mass of the spleen and its entropy (directly), as well as the content of lymphocytes (inverse). Corticosterone and calcitonin give the opposite load factor.

Thus, information on the neuroendocrine-immune and metabolic status of rats is condensed into three common factors, which include parameters between which there are apparently causal relationships. Testing this assumption was the object of the next section.

6.2. Canonical analysis

We have previously shown that drinking mineral water has a significant effect on the neuroendocrine, metabolic and immune parameters of healthy rats. A priori effect of mineral waters on metabolism and immunity is realized through nervous and hormonal mechanisms. 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.

At the first stage, a matrix of neuroendocrine-metabolic correlations was created (Table 6.4).

Table 6.4. Matrix of correlations between neuroendocrine and metabolic parameters of rats

Variables	Symp tone	Glom ZAC	Fasc ZAC	Retic ZAC	Adre Mass	T_3	Mo-de	Vag tone	Corti-coster	Testo-sterone
Na Urine	**-,39**	-,15	-,03	-,24	-,16	-,23	,24	,21	,03	,07
Cl Urine	-,22	-,18	,05	-,20	-,20	-,08	,06	,01	,02	,12
K Urine	**,29**	-,08	-,02	,06	-,09	,10	**-,28**	**-,30**	,02	,05
Mg Urine	,24	,03	**,46**	**,41**	,06	**,68**	-,20	-,18	,12	,10
Ca Urine	-,11	-,00	-,25	-,07	,17	**-,38**	,11	,04	**,35**	-,10
Pi Urine	-,02	,14	-,15	-,14	,17	-,23	,09	,05	,14	-,01
Urea Urine	-,18	,07	-,11	-,13	,03	-,11	,18	,23	,05	-,05
Creatinine Urine	**,30**	,13	,23	,11	-,03	**,28**	-,25	-,15	-,06	**,28**
Uric acid Urine	-,07	,07	**-,50**	,04	-,21	**-,54**	,14	,25	-,08	-,16
Amylase Urine	**,28**	**,30**	,18	,10	,16	-,03	**-,29**	-,19	-,06	,11
MMM Urine	-,07	-,12	-,20	-,16	,21	**-,26**	,09	-,03	,14	-,07
Katalase Urine	-,20	**-,33**	**-,28**	-,21	,15	**-,33**	,19	,05	,17	,08
MDA Urine	,01	,00	-,03	**,30**	-,19	,15	-,04	-,09	,00	,13
DC Urine	**-,33**	,04	,03	,17	-,25	,07	**,33**	,22	-,17	,07
Osmolality Urine	**-,29**	-,14	-,05	-,24	-,18	-,18	,14	,12	,05	,08
Na Excretion	**-,31**	**-,26**	-,08	**-,27**	-,01	-,24	,23	,23	,00	-,01
Cl Excretion	-,21	**-,28**	,04	**-,27**	-,10	-,11	,10	,07	,01	,00
K Excretion	,15	-,22	,00	,01	-,02	,02	-,14	-,24	,11	-,13
Mg Excretion	,15	-,03	**,45**	**,45**	,02	**,69**	-,13	-,16	,10	,01
Ca Excretion	-,07	-,18	-,23	-,12	,23	**-,29**	,07	,02	,21	-,07
Pi Excretion	-,07	-,17	-,12	-,09	,11	-,15	,10	,03	,08	-,13
Creatinine Excretion	,09	-,08	,18	,08	,02	,20	-,09	-,10	,04	,01
Urea Excretion	-,14	-,12	-,14	-,16	,11	-,14	,13	,12	,03	-,05
Uric acid Excretion	-,08	-,19	**-,44**	,06	-,12	**-,50**	,18	,20	-,00	-,20
Diurese	-,08	-,21	-,02	-,01	,07	-,03	,08	,02	,04	-,13
Canalicular Reabsorbtion	,25	,22	**,27**	,01	-,04	**,33**	-,14	-,02	**-,42**	,14
Glomerular Filtration	-,03	,11	**,27**	-,04	-,07	,20	,09	,12	-,23	-,10
Creatinine Plasma	-,02	-,19	-,15	,13	,05	-,12	-,09	-,17	**,49**	,03
Na Erythrocytes	**-,31**	-,19	-,09	-,14	-,06	**-,37**	,22	**,26**	,02	,04
K Erythrocytes	,15	,03	,16	,07	-,10	,14	-,20	-,13	,03	,10
Na Plasma	**,32**	,06	-,03	,02	,01	-,05	**-,28**	-,16	,08	,14
K Plasma	-,01	-,21	-,16	-,08	,03	**-,34**	,06	-,07	,13	-,09
Mg Plasma	-,24	,10	-,16	,14	-,10	-,14	,16	,22	,15	,13
Ca Plasma	-,01	,16	**,31**	,16	-,06	**,36**	,03	-,05	-,03	-,08
Pi Plasma	,20	,08	**,47**	**,29**	-,04	**,65**	-,16	-,19	,06	,12
Cl Plasma	,19	,06	-,04	,03	-,02	-,08	-,16	-,08	,09	,10
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**	**,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 Plasma	-,15	-,02	-,00	,01	,18	-,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, excretion and blood concentration. The program identified 6 pairs of canonical roots. Neuroendocrine root was taken as causal., and metabolic - as consequential.

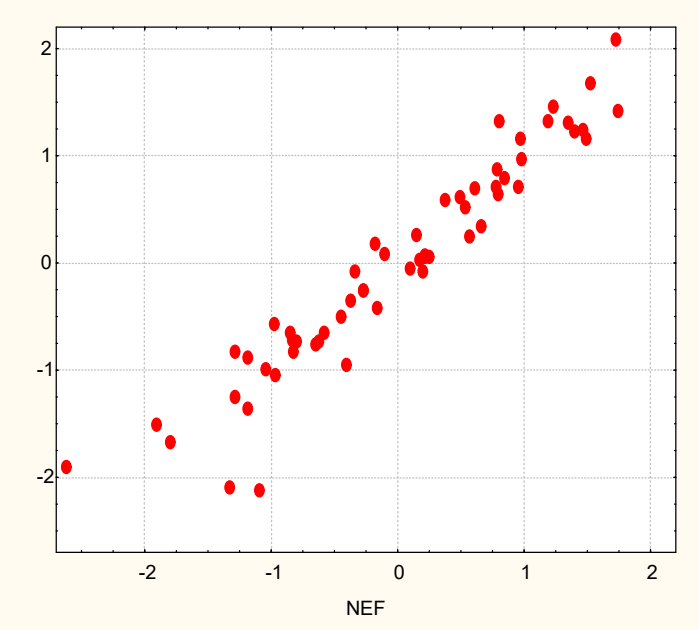
The factor structure of the first neuroendocrine radical is formed, in descending order of load, triiodothyronine, the thickness of the fascicular and reticular zones of the adrenal cortex as well as testosterone (Table 6.5).

Table 6.5. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and concentration or activity in the urine of metabolites

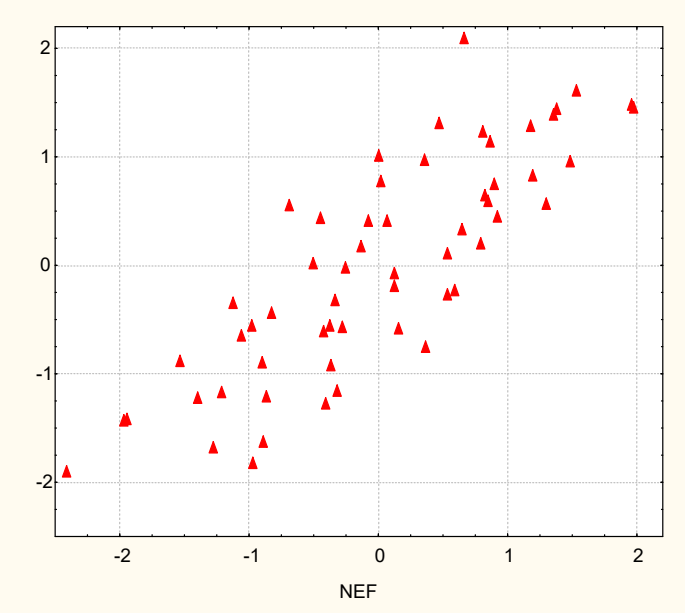
Neuroendocrine factors	Root 1	Root 2
Triiodothyronine	**,973**	,015
Fascicular ZAC	**,712**	,172
Reticular ZAC	**,326**	-,245
Testosterone	**,268**	,169
Sympathetic tone	**,286**	**,639**
Catecholamines (1/Mode)	**,285**	**,585**
Adrenals Mass Index	,029	**,562**
Glomerular ZAC	-,035	**,274**
Corticosterone	-,045	**,166**
Vagal tone	-,283	**-,585**
Metabolic parameters	Root 1	Root 2
Uric acid Urine Concentr	**-,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 Concent	**,317**	,155
Malonic dyaldehid 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**

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. 6.2 above).

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. 6.2 below).



R=0,957; R^2=0,916; $\chi^2_{(160)}$=286; $p<10^{-6}$; Λ Prime=0,001



R=0,831; R^2=0,690; $\chi^2_{(135)}$=178; p=0,007; Λ Prime=0,017

Fig. 6.2. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and urinary concentrations of metabolites (Y-axis)

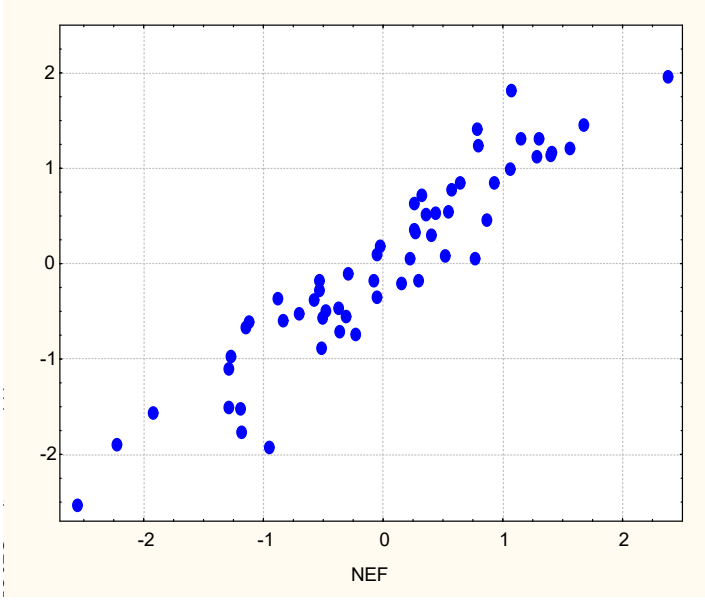
Canonical analysis of neuroendocrine-excretory connections revealed the following (Table 6.6).

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. 6.3 above).

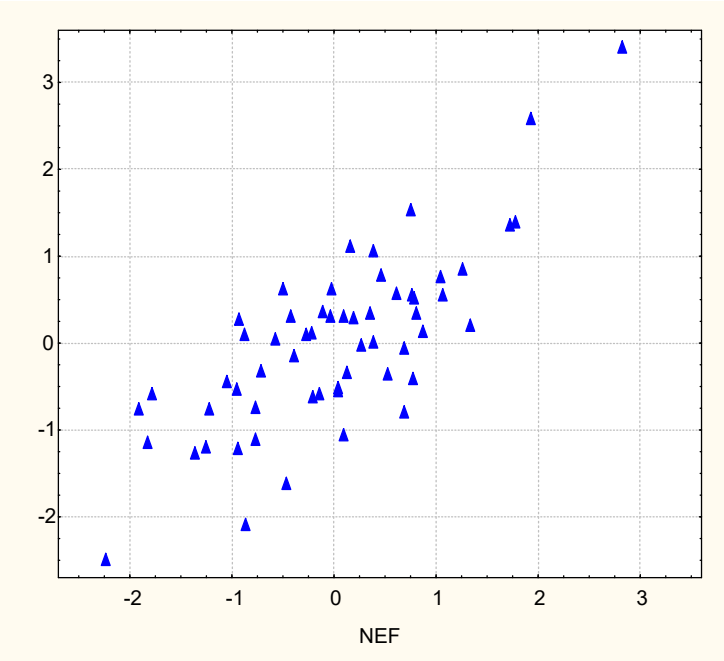
The second neuroendocrine radical is represented **directly** by sympathetic tone, thickness of the glomerular zone of the adrenal cortex and their mass, while **inverse** by corticosteronemia. The metabolic canonical radical receives significant negative factor loads from urinary excretion of chloride 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. 6.3 below).

Table 6.6. 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 Reabsorbtion	,397	**,382**



R=0,940; R^2=0,883; $\chi^2_{(130)}$=237; p<10^{-6}; Λ Prime=0,005



R=0,772; R^2=0,596; $\chi^2_{(108)}$=141; p=0,018; Λ Prime=0,044

Fig. 6.3. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and indicators of renal excretion (Y-axis)

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 6.7).

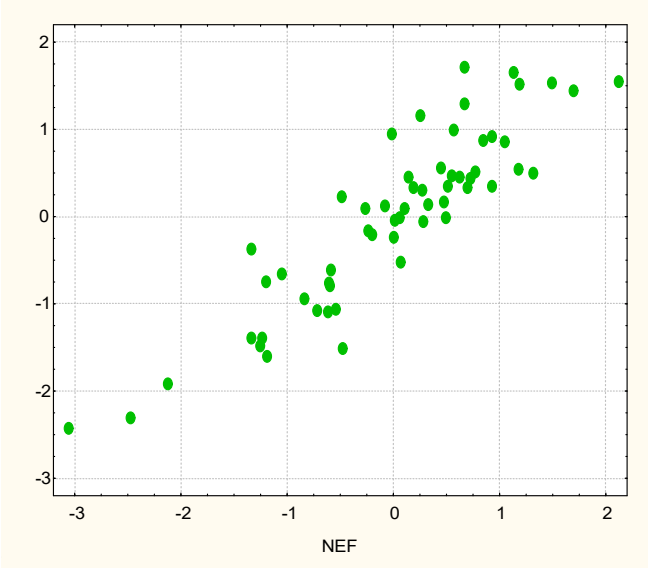
Table 6.7. Factor structure of two pairs of canonical roots, which represent neuroendocrine parameters and blood concentration of metabolites

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	Root 1	Root 2
Bilirubine	**-,516**	**-,346**
Malonic dyaldehid	**-,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**

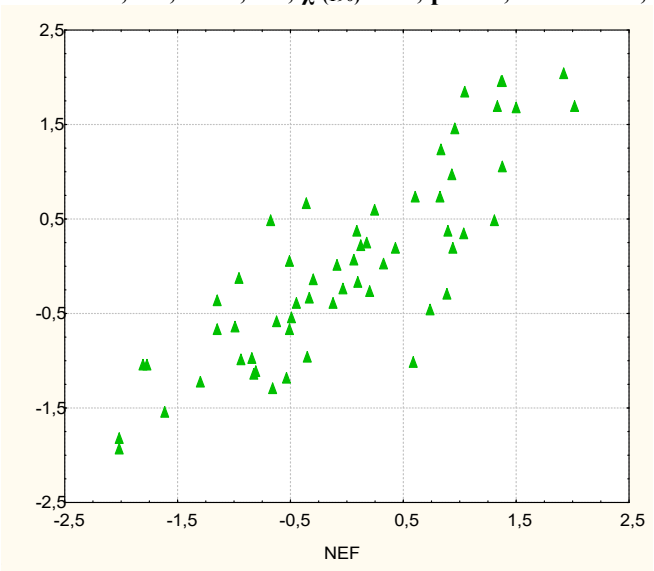
Mg	-,102	**,490**
Uric acid	-,206	**,358**
Glucose	-,010	**,220**
Urea	-,009	**-,108**

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. 6.4 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 6.7). As a result, the determination of neuroendocrine factors of this set of blood plasma metabolites is 72% (Fig. 6.4 below).



R=0,901; R^2=0,813; $\chi^2_{(190)}$=282; p<10^{-4}; Λ Prime=0,001



R=0,851; R^2=0,723; $\chi^2_{(152)}$=212; p=0,006; Λ Prime=0,006

Fig. 6.4. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and metabolic parameters of blood (Y-axis)

Following the accepted algorithm, a matrix of noteworthy correlations between neuroendocrine indicators, on the one hand, and immunity indicators, on the other, was first created (Table 6.8).

Table 6.8. Matrix of correlations between neuroendocrine and immune parameters

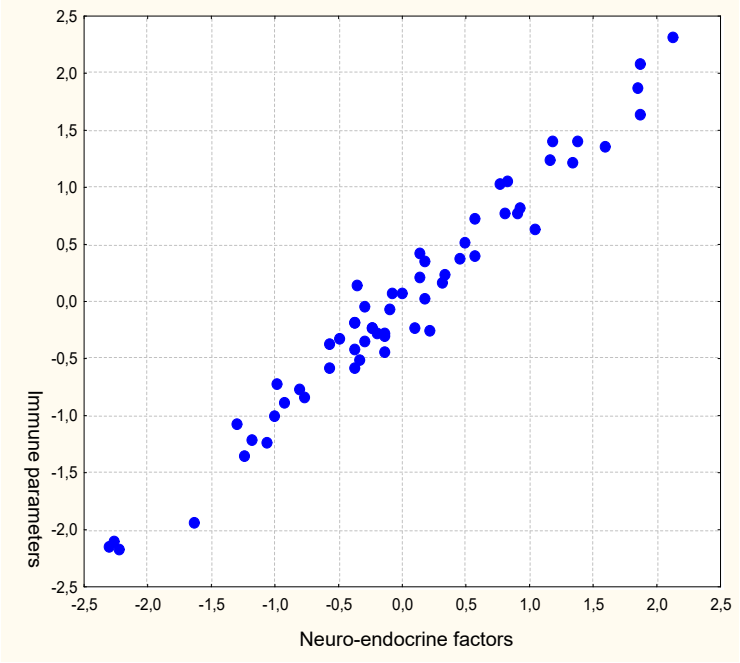
r	HL CG	HI CG	HT CG	FN N	FI M	FN M	FI N	Lb S	Pla S	RetS	Fib S	Mac S	Thy mus	Lc T	Ret T	Epi T	Mo-noc	Ly-mph	NK	Th	Tc	B
T_3				**-,89**	,22	**,47**	**-,60**	**-,27**	**-,40**					-,24		**,36**	**,87**	-,23	**,90**			-,21
AMo			**,28**	**-,28**				**-,31**	**-,31**			**,69**		**-,30**		**,29**	**,29**		**,30**			
CA	**-,28**		,23	-,24				**-,34**	-,22			**,65**		**-,26**		**,31**	**,30**		**,27**			-,24
DX	,25	**,28**		**,27**		-,24	,24	**,30**				**-,43**		0,24		-,22	**-,33**	,24	**-,29**			,22
Med				**,28**				**,53**	**,52**			**-,29**	**-,38**	,23		**-,37**	**-,35**		**-,28**			
CTA	-,25			,24			**,28**	**,41**	**,35**			**-,29**				**-,36**	-,21		-,23			
PTA												-,25					**,29**		**,29**	-,22		
MC		,23		**-,34**					**-,30**	-,22			,24				**,28**		**,28**		,20	,20
Glo					-,21					-,25	**-,32**		**,46**								,24	
Cort	**-,27**	**-,28**																			-,22	**-,27**
Fasc				**-,61**		**,36**	**-,37**		-,25						,19	,23	**,63**		**,56**			-,23
Test			,23	**-,28**								**,32**										-,22
Ret				**-,26**									**,39**				**,31**		**,29**			

For further consideration, two pairs of significantly related pairs of canonical radicals were selected (Table 6.9). 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 object to the types of **upregulation**/**downregulation**. The degree of neuroendocrine immunomodulation is very significant – 96,5%.

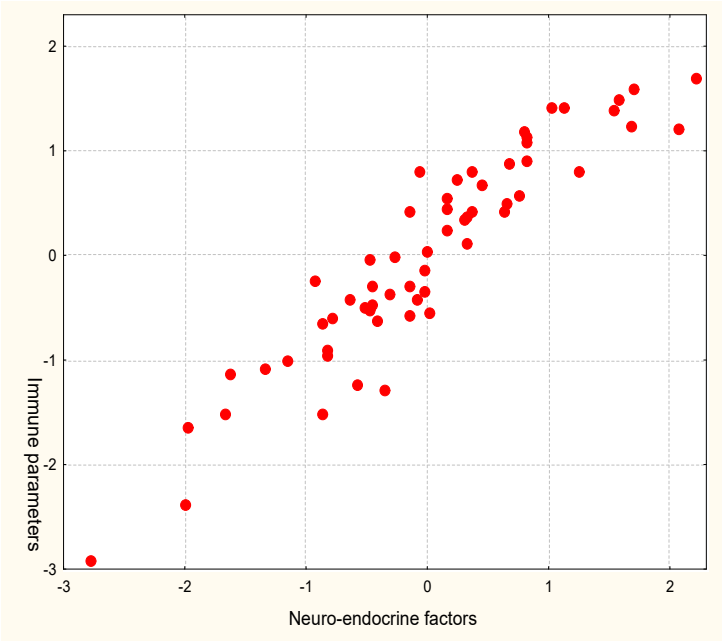
Table 6.9. Factor structure of two pairs of canonical roots, which represent neuroendocrine and immune parameters

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**	0,303
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
Epitheliocytes Thymus	**0,435**	-0,214
EntropyThymocytogram	**0,256**	-0,287
Reticulocytes Thymus	**0,213**	0,078
Phagocytic Index Monocytes	**0,185**	0,201

Reticulocytes Spleen	**0,139**	0,172
Microbial Count Neutrophils	**-0,908**	-0,144
Phagocytic Index Neutrophils	**-0,610**	-0,275
Plasmocytes Spleen	**-0,510**	0,223
Lymphoblastes 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; R^2=0,965; $\chi^2_{(345)}$=533; p<10^{-6}; Λ Prime=10^{-6}



R=0,933; R^2=0,871; $\chi^2_{(308)}$=400; p<10^{-3}; Λ Prime=0,00004

Fig. 6.5. Canonical correlation between indicators of neuroendocrine regulation (X-axis) and immunity (Y-axis)

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%.

CHAPTER 7

RELATIONSHIPS BETWEEN COMPONENTS OF THE CHEMICAL COMPOSITION OF WATER-SALT SOLUTIONS AND PARAMETERS OF NEUROENDOCRINE REGULATION, METABOLISM AND IMMUNITY

Identification of parameters of neuroendocrine regulation and metabolism, changes which are specific after water-salt loads of different chemical composition, provides grounds for detailed analysis of correlations between components of chemical composition of water-salt solutions, on the one hand, and parameters of neuroendocrine regulation, metabolism and immunity on the other hand.

As a preamble, we present a matrix of relationships between the components of the chemical composition of liquids used for water-salt loads (Table 7.1).

Table 7.1. Matrix of correlations between components of chemical composition of liquids

	Corg	Norg	H_2SiO_3	H_3BO_3	Br^-	J^-	F^-	Na^+	Cl^-	HCO_3^-	Ca^{2+}	Mg^{2+}	SO_4^{2-}	K^+
Corg	1,00	-0,12	0,81	0,93	0,84	0,93	0,16	0,31	0,34	0,07	-0,26	0,19	-0,32	0,22
Norg	-0,12	1,00	0,07	-0,06	-0,12	-0,06	-0,05	0,11	0,03	0,69	0,82	0,59	0,93	-0,85
H_2SiO_3	0,81	0,07	1,00	0,58	0,53	0,58	0,21	-0,23	-0,22	0,25	-0,32	-0,09	-0,22	-0,19
H_3BO_3	0,93	-0,06	0,58	1,00	0,93	1,00	0,20	0,59	0,61	-0,01	-0,01	0,38	-0,15	0,33
Br^-	0,84	-0,12	0,53	0,93	1,00	0,93	0,55	0,41	0,42	-0,31	0,04	0,12	-0,12	0,45
J^-	0,93	-0,06	0,58	1,00	0,93	1,00	0,20	0,59	0,61	-0,02	-0,01	0,38	-0,15	0,34
F^-	0,16	-0,05	0,21	0,20	0,55	0,20	1,00	-0,30	-0,31	-0,66	0,18	-0,51	0,12	0,29
Na^+	0,31	0,11	-0,23	0,59	0,41	0,59	-0,30	1,00	1,00	0,16	0,37	0,82	0,17	0,25
Cl^-	0,34	0,03	-0,22	0,61	0,42	0,61	-0,31	1,00	1,00	0,11	0,29	0,78	0,08	0,32
HCO_3^-	0,07	0,69	0,25	-0,01	-0,31	-0,02	-0,66	0,16	0,11	1,00	0,26	0,67	0,42	-0,83
Ca^{2+}	-0,26	0,82	-0,32	-0,01	0,04	-0,01	0,18	0,37	0,29	0,26	1,00	0,57	0,96	-0,44
Mg^{2+}	0,19	0,59	-0,09	0,38	0,12	0,38	-0,51	0,82	0,78	0,67	0,57	1,00	0,51	-0,34
SO_4^{2-}	-0,32	0,93	-0,22	-0,15	-0,12	-0,15	0,12	0,17	0,08	0,42	0,96	0,51	1,00	-0,66
K^+	0,22	-0,85	-0,19	0,33	0,45	0,34	0,29	0,25	0,32	-0,83	-0,44	-0,34	-0,66	1,00

First of all, it is noteworthy that there is no correlation between the content of organic carbon and nitrogen in liquids. The content of organic carbon is very closely related to the content of trace elements in liquids - orthoboric and metasilicic acids as well as bromide and iodide. Instead, the level of organic nitrogen is associated with electrolytes - sulfate, potassium, calcium, bicarbonate and magnesium.

First, let's find out the physiological role of organic matter, then trace elements, and finally electrolytes.

7.1. Relationships between the content of organic nitrogen and carbon in liquids and neuroendocrine parameters

Screening found that among the neuroendocrine parameters, only four significantly correlated with the organic components of fluids (Table 7.2).

Table 7.2. Matrix of correlations between the content of organic nitrogen and carbon in liquids and neuroendocrine parameters after weekly water-salt loads

Neuroendocrine Variables	Norg	Corg
Triiodothyronin, nM/L	**,45**	**-,33**
Fascicular Zone of Adrenal Cortex, µM	**,36**	-,14
$(Pp•Cap)^{-0,5}$ as Calcitonin Activity	**-,34**	,13
$(Cau•Pu/Pp•Cap)^{0.25}$ as Calcitonin Activity	-,25	,06
$(Nap/Kp)^{0.5}$ as Mineralocorticoid Activity	,27	-,04
Glomerular Zone of Adrenal Cortex, µM	-,22	,19
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	-,21	-,12
$(Cap•Pu/Pp•Cau)^{0.25}$ as Parathyroid Activity	-,20	-,24
Adrenals Mass Index, %	,07	**,34**
Corticosterone, nM/L	,02	,27
AMo HRV as Sympathetic Tone, %	,01	,25
Testosterone, nM/L	,08	-,25

Variative Swing HRV as Vagal Tone, msec	,00	-,20
$(Nap•Ku/Kp•Nau)^{-0,25}$ as Mineralocorticoid Activity	,06	,19
Moda HRV, msec	,08	-,15
Reticular Zone of Adrenal Cortex, µM	-,03	-,01

Note. For a sample of 48 animals, the critical value of the modulus of the correlation coefficient |r| for p<0,05 (t>2,0) is **0,28**, for p<0,01 (t>2,7) **0,38**, for p<0,001 (t>3,5) **0,48**.

In particular, it is plasma triiodothyronine, the level of which correlates positively with organic nitrogen and negatively with organic carbon. The thickness of the fascicular zone of the adrenal cortex and calcitonin (both morning and daily) and morning mineralocorticoid activity correlate with the first component, and with the second - the mass index of the adrenal glands and, on the verge of significance, plasma corticosterone and testosterone. Among the parameters of the autonomic nervous system, only the sympathetic tone marker (AMo) correlates insignificantly with organic carbon.

Despite this structure of the correlation matrix, the results of regression analysis with stepwise exclusion to reach the maximum Adjusted R^2 in the model included some endocrine parameters with very small modules of their correlation coefficients with both organic nitrogen (Table 7.3) and carbon (Table 7.4), instead, the parameters with significant modules were outside the regression models.

Table 7.3. Result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of organic nitrogen in fluids

Nitrogen Organic (mg/L) as Inependent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
Dependent Variables	r		Intercpt	-,921	,480	-1,92	,062
Triiodothyronin, nM/L	**,45**	,487	,142	,411	,119	3,44	,001
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	,27	,239	,137	,104	,060	1,75	,088
$(Cap/Pp)^{0,5}$ as Parathyrin Activity	-,21	,445	,310	,223	,156	1,44	,158
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyroid Activity	-,20	-,522	,293	-,259	,145	-1,78	,082
	R=0,543; R^2=0,295; Adjusted R^2=0,229; $F_{(4,4)}$=4,5; p=0,004						

Table 7.4. Result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of organic carbon in fluids

Carbon Organic (mg/L) as Inependent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(44)}$	p-level
Dependent Variables	r		Intercpt	30,92	14,96	2,07	,045
Triiodothyronin, nM/L	**-,33**	-,291	,141	-10,25	4,96	-2,07	,045
Testosterone, nM/L	-,25	-,167	,142	-,964	,820	-1,18	,246
Glomerular Zone of Adrenal Cortex, µM	,19	,150	,139	,052	,048	1,08	,287
	R=0,407; R^2=0,165; Adjusted R^2=0,109; $F_{(3,4)}$=2,9; p=0,045						

At the final stage, the canonical correlation between the content of organic nitrogen and carbon in liquids, accepted as a factor trait (argument), and endocrine parameters, adopted as a resultant trait (function), was analyzed.

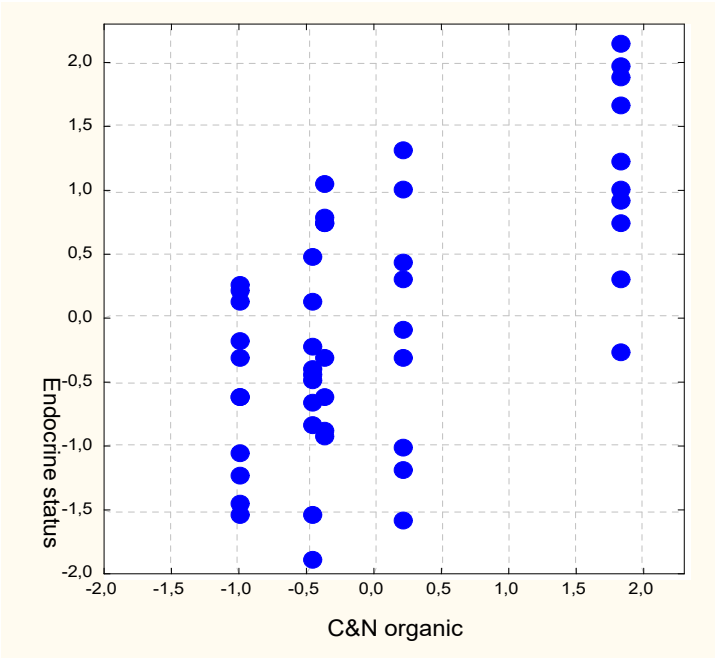
The chemical canonical root correlates very closely with organic nitrogen, but only moderately and inversely with organic carbon. Endocrine root receives positive factor loads from plasma levels of triiodothyronine and testosterone as well as morning mineralocorticoid activity, while negative loads from basal mineralocorticoid activity (estimated by the thickness of the glomerular zone of the adrenal cortex), as well as parathyroid activity, both morning and average daily (Table 7.5).

Table 7.5. Factor structure of canonical correlation between roots, which represent the content of organic nitrogen and carbon in liquids and endocrine parameters

Chemic factors	Root
Nitrogen Organic, mg/L	**,926**
Carbon Organic, mg/L	**-,490**
Endocrine parameters	Root
Triiodothyronine, nM/L	**,834**
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	**,335**
Testosterone, nM/L	**,213**

Glomerular Zone of Adrenal Cortex, µM	**-,389**
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	**-,351**
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyroid Activity	**-,307**

Taken together, both markers of organic matter determine endocrine status by 39% (Fig. 7.1).



R=0,626; R^2=0,391; $\chi^2_{(12)}$=26; p=0,011; Λ Prime=0,544
Fig. 7.1. Canonical correlation between roots, which represent the content of organic nitrogen and carbon in liquids (X axis) and endocrine parameters (Y axis)

7.2. Relationships between the content of organic carbon and nitrogen in liquids and metabolic parameters

It was found (Table 7.6) that the content of organic carbon in liquids is significantly negatively correlated with the concentration of diene conjugates in the daily urine and the excretion (but not with the concentration) of magnesium. Instead, the content of organic nitrogen in liquids correlates positively with the excretion (and concentration) of magnesium, as well as with the excretion and concentration of uric acid.

Table 7.6. Matrix of correlations between the content of organic carbon and nitrogen in liquids and metabolic parameters of daily urine after weekly water-salt loads

Concentration	**Corg**	**Norg**
Diene conjugates Urine, E^{232}/mL	**-,46**	,03
Magnesium Urine, mM/L	-,24	**,47**
Uric Acid Urine, mM/L	,18	**-,29**
Creatinine Urine, mM/L	-,13	,26
Urea Urine, mM/L	,18	,00
Amylase Urine, mg/h•mL	,22	,08
Malonic Dialdehyde Urine, µM/L	,06	,25
Katalase Urine, nM/h•mL	-,11	-,13
Excretion		
Magnesium Excretion, µM/24h•100 g	**-,33**	**,30**
Uric Acid Excretion, µM/24h•100 g	,02	**-,40**
Creatinine Excretion, µM/24h•100 g	-,18	,03
Urea Excretion, µM/24h•100 g	,10	-,13
Phosphates Excretion, µM/24h•100 g	-,02	-,16

Among the metabolic parameters of blood (Table 7.7) significantly correlate with the content of organic nitrogen in liquids only phosphate and diene conjugates, noteworthy borderline relative in importance indicators of malonic dialdehyde, potassium, amylase, chloride and uric acid of plasma, as well as sodium of erythrocytes.

In contrast, no metabolic parameter in the blood correlates significantly with the content of organic carbon in liquids, but phosphate and diene conjugates should be considered.

Table 7.7. Matrix of correlations between the content of organic carbon and nitrogen in liquids and metabolic parameters of blood after weekly water-salt loads

Level of	Norg	Corg
Phosphate Plasma, mM/L	**,29**	-,22
Diene conjugates Plasma, E^{232}/mL	**-,28**	-,21
Malonic Dialdehyde Plasma, µM/L	-,26	,15
Potassium Plasma, mM/L	-,26	-,12
Amylase Plasma, mg/h•mL	,25	,13
Chloride Plasma, mM/L	-,22	-,07
Sodium Erythrocytes, mM/L	-,21	-,10
Uric Acid Plasma, µM/L	-,20	,20
Calcium Plasma, mM/L	,02	-,18
Magnesium Plasma, mM/L	-,06	-,13
Potassium Erythrocytes, mM/L	,02	-,19
Sodium Plasma, mM/L	-,18	-,01
Creatinine Plasma, µM/L	,16	-,15
Urea Plasma, mM/L	,16	-,07
Glucose Plasma, mM/L	-,10	-,19
Middle Mass Molecules Plasma, units	-,15	-,09
Cholesterol Plasma, mM/L	,10	,14
Superoxide Dismutase Erythrocytes, un/mL	,17	,09

Constellations of metabolic parameters, which by step exclusion remained in regression models, are given in Tables 7.8 and 7.9.

Table 7.8. Result of regression analysis with step-by-step exclusion of metabolic parameters in relation to the content of organic carbon in liquids

Carbon Organic (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(37)}$	p-level
Dependent Variables	r		Intercpt	45,22	24,44	1,85	,072
Diene conjugates Urine, E^{232}/mL	**-,46**	-,302	,122	-8,675	3,497	-2,48	,018
Magnesium Excretion, µM/24h•100 g	**-,33**	,861	,443	3,653	1,879	1,94	,059
Magnesium Urine, mM/L	-,25	-,944	,391	-6,849	2,838	-2,41	,021
Potassium Erythrocytes, mM/L	-,19	-,287	,135	-,575	,270	-2,13	,040
Creatinine Excretion, µM/24h•100 g	-,18	-,618	,263	-1,595	,679	-2,35	,024
Middle Mass Molecules Plasma, units	-,19	-,210	,140	-36,18	24,08	-1,50	,141
Amylase Urine, mg/h•mL	,22	,407	,172	,149	,063	2,37	,023
Uric Acid Plasma, µM/L	,20	,225	,140	,006	,004	1,61	,116
Urea Urine, mM/L	,18	,353	,144	,091	,037	2,46	,019
Amylase Plasma, mg/h•mL	,18	,227	,156	,084	,058	1,45	,154
	R=0,697; R^2=0,486; Adjusted R^2=0,347; $F_{(10,4)}$=3,50; p=0,0025						

Table 7.9. Result of regression analysis with step-by-step exclusion of metabolic parameters in relation to the content of organic nitrogen in liquids

Nitrogen Organic (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(42)}$	p-level
Dependent Variables	r		Intercpt	-,3492	,2666	-1,31	,197
Magnesium Urine, mM/L	**,47**	,345	,134	,0600	,0232	2,59	,013
Creatinine Urine, mM/L	,26	,236	,134	,0431	,0246	1,75	,087
Malonic Dialdehyde Urine, µM/L	,28	,225	,136	,0024	,0015	1,65	,106
Uric Acid Excretion, µM/24h•100 g	**-,40**	-,341	,142	-,0381	,0158	-2,41	,020
Phosphates Excretion, µM/24h•100 g	-,16	,218	,150	,013	,009	-1,46	,152
	R=0,621; R^2=0,385; Adjusted R^2=0,312; $F_{(5,4)}$=5,3; p=0,0008						

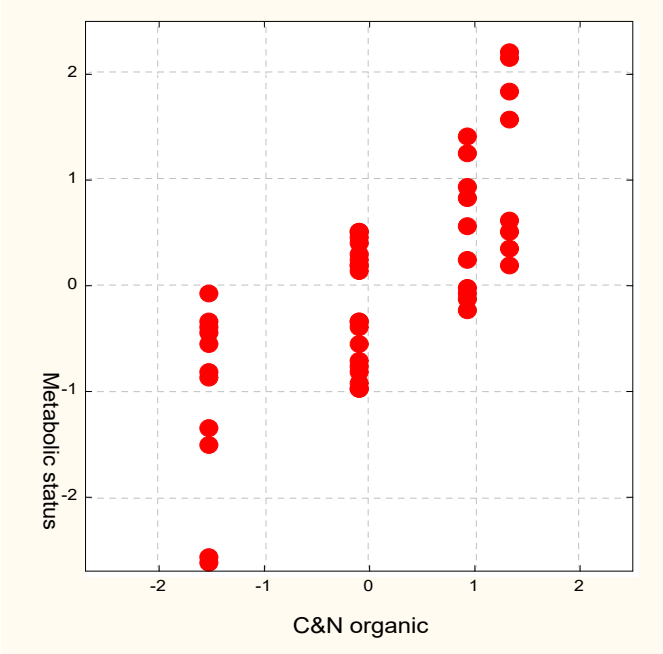
Using a similar algorithm, the next step revealed two equivalent pairs of canonical roots. In this case, the causal canonical root of the first pair, judging by the factor load, represents mainly organic carbon, while the factor load on it from organic nitrogen is much weaker, but with the same sign (Table 7.10).

Table 7.10. Factor structure of the canonical correlation between the first pair of roots, which represent the content of organic carbon and nitrogen in liquids and metabolic parameters

Chemic factors	Root 1
Carbon Organic (mg/L)	**-,932**
Nitrogen Organic (mg/L)	**-,245**
Metabolic parameters	Root 1
Diene conjugates Urine, E^{232}/mL	**,353**
Middle Mass Molecules Plasma, units	**,351**
Creatinine Excretion, µM/24h•100 g	**,296**
Magnesium Excretion, µM/24h•100 g	**,288**
Potassium Erythrocytes, mM/L	**,250**
Uric Acid Excretion, µM/24h•100 g	,169
Phosphates Excretion, µM/24h•100 g	,115
Magnesium Urine, mM/L	,094
Creatinine Urine, mM/L	,048
Amylase Plasma, mg/h•mL	**-,390**
Amylase Urine, mg/h•mL	**-,309**
Urea Urine, mM/L	**-,236**
Malonic Dialdehyde Urine, µM/L	**-,234**
Uric Acid Plasma, µM/L	-,174

Both organic components of the chemical composition of loading fluids have a **negative** effect on the level of 9 metabolic parameters (concentration in the urine of diene conjugates, magnesium and creatinine, excretion of creatinine, magnesium, uric acid and phosphates, the level of medium molecules in plasma and potassium in erythrocytes). In contrast, the other 5 parameters (plasma and urine amylase activity, urea and malonic dialdehyde concentration in urine, uric acid plasma level) have a **positive** effect on organic components.

In total, the organic components of the chemical composition of the loading fluids determine their effect on these metabolic parameters by 53% (Fig. 7.2).



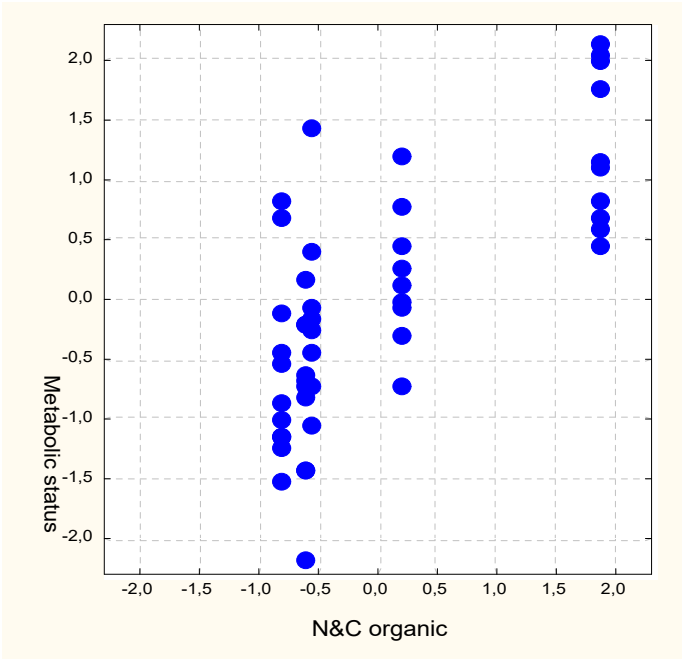
R=0,728; R^2=0,530; $\chi^2_{(28)}$=57; p=0,0009; Λ Prime=0,225

Fig. 7.2. Canonical correlation between the first pair of roots, which represent the content of organic carbon and nitrogen in liquids (X-axis) and metabolic parameters (Y-axis)

In contrast, in the second pair, the causal canonical root represents mainly organic nitrogen, and the factor load on it from organic carbon is not only much weaker, but also with the opposite sign (Table 7.11). Organic components of chemical composition have opposite effects on individual metabolic parameters, and together determine the effect of loading fluids on metabolism by 52% (Fig. 7.3).

Table 7.11. Factor structure of the canonical correlation between the second pair of roots, which represent the content of organic carbon and nitrogen in liquids and metabolic parameters

Chemic factors	Root 2
Nitrogen Organic (mg/L)	**,970**
Carbon Organic (mg/L)	**-,363**
Metabolic parameters	Root 2
Magnesium Urine, mM/L	**,690**
Magnesium Excretion, µM/24h•100 g	**,506**
Creatinine Urine, mM/L	**,380**
Malonic Dialdehyde Urine, µM/L	**,340**
Amylase Plasma, mg/h•mL	**,326**
Diene conjugates Urine, E^{232}/mL	,106
Potassium Erythrocytes, mM/L	,085
Amylase Urine, mg/h•mL	,072
Creatinine Excretion, µM/24h•100 g	,072
Uric Acid Excretion, µM/24h•100 g	**-,528**
Uric Acid Plasma, µM/L	**-,328**
Phosphates Excretion, µM/24h•100 g	**-,206**
Middle Mass Molecules Plasma, units	-,188
Urea Urine, mM/L	-,063



R=0,722; R^2=0,521; $\chi^2_{(13)}$=28; p=0,0081; Λ Prime=0,479

Fig. 7.3. Canonical correlation between the second pair of roots, which represent the content of organic carbon and nitrogen in liquids (X-axis) and metabolic parameters (Y-axis)

7.3. Relationships between the content of organic carbon and nitrogen in liquids and immune parameters

Two regression models were built on the basis of the correlation matrix (Table 7.12).

Table 7.12. Matrix of correlations between the content of organic nitrogen and carbon in liquids and the immune parameters of the thymus, spleen and blood after weekly water-salt loads

Immune parameters	Nor	Cor
Thymus Mass Index, mg/100 g BM	-0,22	0,09
Lymphocytes of Thymus, %	-0,08	-0,12
Lymphoblastes of Thymus, %	0,01	0,14
Reticulocytes of Thymus, %	0,04	0,01
Epitheliocytes of Thymus, %	0,11	-0,00
Endotheliocytes of Thymus, %	-0,18	0,23
Plasmocytes of Thymus, %	0,14	0,10
Macrophages of Thymus, %	-0,09	-0,12
Hassal corpuscles of Thymus, %	0,23	-0,08
Entropy of Thymocytogram	0,09	0,13
Spleen Mass Index, mg/100 g BM	0,21	0,05
Lymphoblastes of Spleen, %	-0,18	0,10
Lymphocytes of Spleen, %	-0,24	0,15
Plasmocytes of Spleen, %	-0,09	0,04
Reticulocytes of Spleen, %	0,19	**-0,28**
Fibroblastes of Spleen, %	0,16	**-0,31**
Macrophages of Spleen, %	0,04	0,12
Microphages of Spleen, %	0,08	0,15
Eosinophiles of Spleen, %	0,13	-0,10
Entropy of Splenocytogram	0,21	-0,10
Leukocytes of Blood, 10^9/L	-0,06	0,22
Monocytes of Blood, %	**0,50**	**-0,35**
Lymphocytes of Blood, %	-0,14	**-0,31**
Eosinophiles of Blood, %	-0,08	0,04
Basophiles of Blood, %	0,26	0,13
Stub Neutrophils of Blood, %	**0,34**	0,04
Segmented Neutrophiles of Blood, %	-0,08	**0,45**
Entropy of Leukocytogram	**0,51**	0,03
Microbian Count of Neutrophils, B/Ph	**-0,51**	0,39
Killing Index of Neutrophils, %	0,19	0,14
Phagocytic Index of Neutrophils, %	**-0,37**	0,23
Phagocytic Index of Monocytes, %	-0,16	-0,26
Microbian Count of Monocytes, B/Ph	**0,30**	**-0,30**
Natural Killer Lymphocytes, %	**0,44**	**-0,32**
T-helper Lymphocytes, %	0,05	-0,04
T-cytolytic Lymphocytes, %	0,03	-0,07
B-Lymphocytes, %	0,25	-0,15
0-Lymphocytes, %	-0,26	0,20
Entropy of Immunocytogram	-0,10	-0,05

It was stated (Table 7.13) that organic nitrogen causes an increase in the relative content of natural killers and B-lymphocytes in the blood (accompanied by a decrease in the content of 0-lymphocytes), as well as rod-shaped neutrophils. Organic nitrogen acts on the microbial number of monocytes by the type of upregulation, while on their phagocytic activity - by the type of downregulation. In addition, this chemical factor causes an increase in spleen mass and entropy of the splenocytogram, adversely affecting the content of endothelial cells in the thymocytogram. The degree of determination of organic nitrogen of this immune constellation is 67%.

Table 7.13. Results of regression analysis with step-by-step exclusion of immune parameters in relation to the content of organic nitrogen in liquids

Nitrogen Organic (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(38)}$	p-level
Dependent Variables	r		Intercpt	-5,734	1,253	-4,58	10^{-4}
Natural Killer Lymphocytes, %	**0,44**	,533	,120	,0782	,0176	4,45	10^{-4}
Stub Neutrophils of Blood, %	**0,34**	,227	,102	,0585	,0262	2,23	,032
Microbian Count of Monocytes, B/Ph	**0,30**	,277	,121	,0437	,0191	2,29	,027
B-Lymphocytes, %	0,25	,775	,187	,0724	,0174	4,16	10^{-3}
Spleen Mass Index, mg/100 g BM	0,21	,236	,103	1,1962	,5199	2,30	,027
Entropy of Splenocytogram	0,21	,292	,105	4,8910	1,7565	2,78	,008
0-Lymphocytes, %	-0,26	,494	,191	,0193	,0075	2,58	,014
Endotheliocytes of Thymus, %	-0,18	-,255	,114	-,0802	,0359	-2,23	,031
Phagocytic Index of Monocytes, %	-0,16	-,327	,110	-,1104	,0369	-2,99	,005
	$R=0,818$; $R^2=0,669$; Adjusted $R^2=0,591$; $F_{(9,4)}=8,5$; $p<10^{-5}$						

The objects of regulatory influence of organic carbon, common to those of organic nitrogen, are the phagocytic index of blood monocytes and thymic endotheliocytes. The effect on the first parameter is the same suppressive, while on the second - enhancing. Maximum upregulation was found with respect to the intensity of neutrophil phagocytosis, whereas levels of pan-lymphocytes in the blood and fibroblasts and reticulocytes in the spleen are subject to downregulation. The degree of determination of organic carbon of this immune constellation is 48% (Table 7.14).

Table 7.14. Results of regression analysis with step-by-step exclusion of immune parameters in relation to the content of organic carbon in liquids

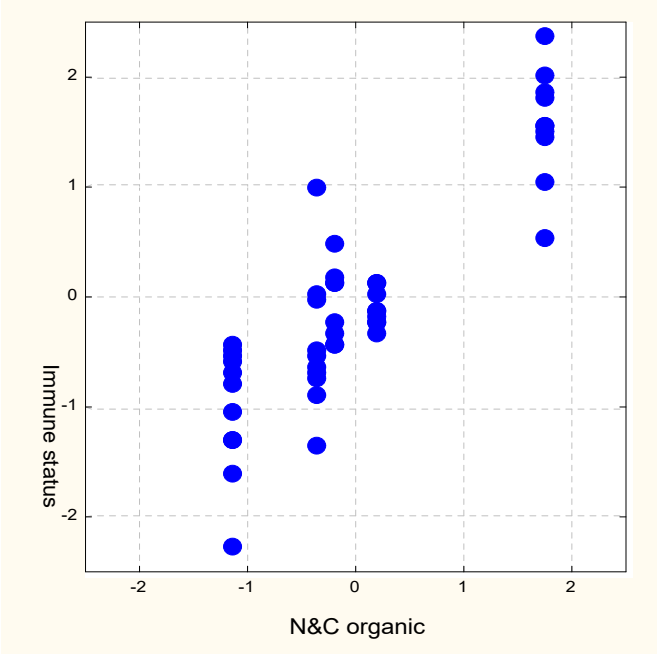
Carbogen Organic (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(41)}$	p-level
Dependent Variables	r		Intercpt	79,02	24,27	3,26	,002
Microbian Count of Neutrophils, B/Ph	**0,39**	,243	,126	2,596	1,350	1,92	,061
Endotheliocytes of Thymus, %	0,23	,229	,119	3,010	1,569	1,92	,062
Lymphocytes of Blood, %	**-0,31**	-,437	,116	-,749	,198	-3,78	10^{-3}
Fibroblastes of Spleen, %	**-0,31**	-,289	,122	-2,184	,922	-2,37	,023
Reticulocytes of Spleen, %	**-0,28**	-,230	,117	-1,674	,852	-1,97	,056
Phagocytic Index of Monocytes, %	-0,26	-,155	,115	-2,184	1,619	-1,35	,185
	$R=0,690$; $R^2=0,477$; Adjusted $R^2=0,400$; $F_{(6,4)}=6,2$; $p=0,0001$						

As a result of canonical analysis, two pairs of roots were identified. The chemical root of the first pair is represented as much as possible by organic nitrogen, while organic carbon gives a much smaller and opposite factor load (Table 7.15).

Table 7.15. Factor structure of the canonical correlation between the first pair of roots, which represent the content of organic nitrogen and carbon in liquids and immunity parameters

Chemic factors	Root 1
Nitrogen Organic (mg/L)	**,876**
Carbon Organic (mg/L)	**-,588**
Immune parameters	Root 1
Natural Killer Lymphocytes, %	**,583**
Microbian Count of Monocytes, B/Ph	**,441**
B-Lymphocytes, %	**,314**
Stub Neutrophils of Blood, %	**,292**
Entropy of Splenocytogram	**,249**
Spleen Mass Index, mg/100 g BM	**,163**
0-Lymphocytes, %	**-,351**
Endotheliocytes of Thymus, %	**-,296**
Phagocytic Index of Monocytes, %	**-,008**
Microbian Count of Neutrophils, B/Ph	**-,691**
Reticulocytes of Spleen, %	**,334**
Fibroblastes of Spleen, %	**,315**
Lymphocytes of Blood, %	**,042**

The immune root is represented by parameters that are subject to **activating** (NK and B lymphocytes, rod-shaped neutrophils, intensity of monocyte phagocytosis, spleen mass and splenocytogram entropy) or **suppressor** (blood 0-lymphocytes, thymus endothelial cells, activity of phagocytosis of monocytes) influence of organic nitrogen and **activating** (intensity of phagocytosis of neutrophils) or **suppressive** (reticulocytes and fibroblasts of the spleen and lymphocytes of blood) influence of organic carbon. The degree of determination of organic matter of this immune constellation reaches 77% (Fig. 7.4).



R=0,878; R^2=0,772; $\chi^2_{(26)}$=80; p<10^{-6}; Λ Prime=0,130

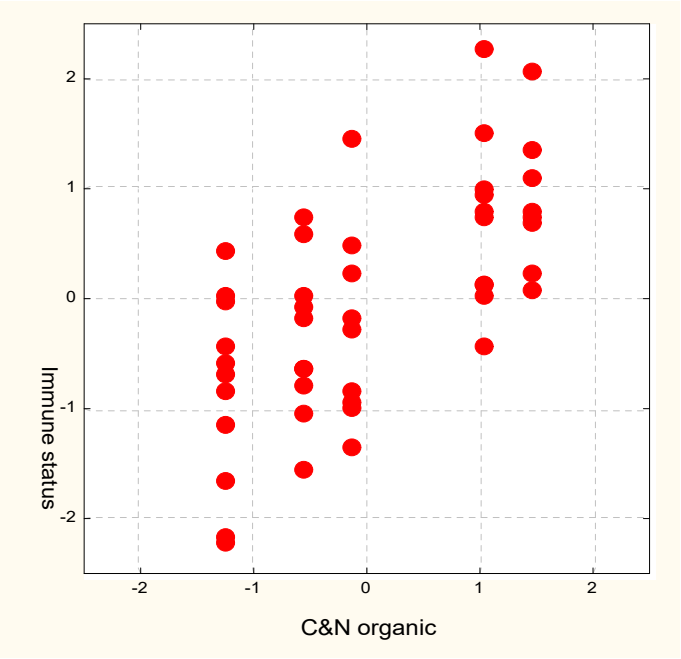
Fig. 7.4. Canonical correlation between the first pair of roots, which represent the content of organic nitrogen and carbon in liquids (X axis) and immunity parameters (Y axis)

The chemical root of the second pair is represented to the maximum extent by organic carbon, and organic nitrogen gives much less, but unidirectional factor load (Table 7.16).

Table 7.16. Factor structure of the canonical correlation between the second pair of roots, which represent the content of organic nitrogen and carbon in liquids and immunity parameters

Chemic factors	Root 2
Carbon Organic (mg/L)	**-,809**
Nitrogen Organic (mg/L)	**-,483**
Immune parameters	Root 2
Lymphocytes of Blood, %	**,548**
Phagocytic Index of Monocytes, %	**,488**
Fibroblastes of Spleen, %	**,272**
Reticulocytes of Spleen, %	**,208**
Endotheliocytes of Thymus, %	**-,138**
Microbian Count of Neutrophils, B/Ph	**-,063**
Stub Neutrophils of Blood, %	**-,369**
Spleen Mass Index, mg/100 g BM	**-,257**
Microbian Count of Monocytes, B/Ph	**,138**

The immune root is represented mainly by parameters subject to suppressive effects of organic **carbon** (blood lymphocytes, splenic reticulocytes and fibroblasts as well as activity of monocyte phagocytosis) or **nitrogen** (intensity of monocyte phagocytosis), as well as those activated by **carbon** (thymic endotheliocytes and the intensity of phagocytosis of neutrophils) or **nitrogen** (rod-shaped neutrophils in the blood and spleen mass) (Table 7.16). However, the determining influence of organic substances on such an immune constellation is much weaker - only 43% (Fig. 7.5).



R=0,657; R^2=0,431; $\chi^2_{(12)}$=22; p=0,037; Λ Prime=0,569

Fig. 7.5. Canonical correlation between the second pair of roots, which represent the content of organic nitrogen and carbon in liquids (X axis) and immunity parameters (Y axis)

7.4. Relationships between trace elements fluid content and neuroendocrine parameters

According to the correlation matrix (Table 7.17), the trace elements found in mineral waters are only moderately and weakly related to the neuroendocrine parameters of the animals consuming them.

Table 7.17. Matrix of correlations between the content of trace elements in fluids and neuroendocrine parameters after weekly water-salt loads

Neuroendocrine parameters	F^-	H_2SiO_3	Br^-	H_3BO_3	J^-
Variative Swing HRV as Vagal Tone, msec	-,06	-,18	,01	,03	,03
Moda HRV, msec	-,18	-,12	-,11	-,06	-,06
AMo HRV as Sympathetic Tone, %	,17	,26	,01	-,04	-,04
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	,17	-,07	,18	,15	,15
$(Nap•Ku/Kp•Nau)^{0,25}$ as Mineralocorticoid Activity	,19	**,28**	-,08	-,16	-,16
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	**-,30**	-,21	-,02	,09	,09
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyroid Activity	-,23	**-,29**	,01	,09	,09
$(Pp•Cap)^{-0,5}$ as Calcitonin Activity	,16	,02	,21	,17	,17
$(Cau•Pu/Pp•Cap)^{0,25}$ as Calcitonin Activity	-,13	-,03	,02	,07	,07
Triiodothyronine, nM/L	-,27	,01	**-,31**	-,23	-,23
Testosterone, nM/L	,19	,04	-,09	-,20	-,20
Corticosterone, nM/L	**-,29**	,25	-,24	-,14	-,14
Glomerular Zone of Adrenal Cortex, μM	-,01	-,03	,12	,14	,13
Fascicular Zone of Adrenal Cortex, μM	-,13	-,01	-,22	-,19	-,19
Reticular Zone of Adrenal Cortex, μM	-,14	,01	-,11	-,08	-,08
Adrenals Mass Index, %	,07	**,36**	-,08	-,11	-,11

Fluoride has the most significant hormone-modulating effect, reducing parathyroid activity and plasma levels of corticosterone and triiodothyronine, while increasing mineralocorticoid activity and plasma levels of testosterone (Table 7.18).

Table 7.18. The result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of fluoride in fluids

Ftoride (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(42)}$	p-level
Dependent Variables	r		Intercpt	1,703	,3488	4,88	10^{-4}
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	**-,30**	-,343	,125	-,1886	,0686	-2,75	,009
Corticosterone, nM/L	**-,29**	-,329	,117	-,0006	,0002	-2,81	,008
Triiodothyronine, nM/L	-,27	-,505	,128	-,4659	,1185	-3,93	10^{-3}
$(Nap•Ku/Kp•Nau)^{0,25}$ as MC Activity	,19	,259	,125	,0867	,0419	2,07	,044
Testosterone, nM/L	,19	,237	,121	,0359	,0184	1,96	,057
	R=0,652; R^2=0,425; Adjusted R^2=0,357; $F_{(5,4)}$=6,2; p=0,0002						

The hormone-modulating effect of meta-silicic acid is of the same considerable force, but has a enhancing character and applies to other neuroendocrine constellations. In particular, it causes an increase in adrenal mass and increase their mineralocorticoid and glucocorticoid activity, as well as sympathetic tone, reciprocally reducing the vagal tone (Table 7.19).

Table 7.19. The result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of metasilicic acid in fluids

H_2SiO_3 (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(42)}$	p-level
Dependent Variables	r		Intercpt	-14,4	4,61	-3,13	,003
Adrenals Mass Index, %	**,36**	,379	,129	30,53	10,36	2,95	,005
$(Nap•Ku/Kp•Nau)^{0,25}$ as MCA	**,28**	,262	,144	,885	,486	1,82	,076
AMo HRV as Sympathetic Tone, %	,26	,371	,218	,052	,030	1,70	,097
Corticosterone, nM/L	,25	,360	,151	,007	,003	2,39	,021
Variative Swing HRV as Vagal Tone, msec	-,18	,383	,231	,027	,016	1,65	,105
	R=0,568; R^2=0,323; Adjusted R^2=0,242; $F_{(5,4)}$=4,0; p=0,005						

The degree of hormone-modulating effect of bromide passes to the category of moderate, but statistically significant. It is manifested in a decrease in plasma levels of triiodothyronine and corticosterone and increased mineralocorticoid activity (Table 7.20).

Table 7.20. The result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of bromide in fluids

Bromide (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(44)}$	p-level
Dependent Variables	r		Intercpt	16,151	12,295	1,31	,196
Triiodothyronine, nM/L	**-,31**	-,345	,138	-7,749	3,095	-2,50	,016
Corticosterone, nM/L	-,24	-,203	,140	-,009	,006	-1,45	,154
$(Nap/Kp)^{0.5}$ as Mineralocorticoid Activity	,18	,191	,142	2,217	1,647	1,35	,185
	R=0,434; R^2=0,189; Adjusted R^2=0,133; $F_{(3,4)}$=3,4; p=0,026						

While the connections with the endocrine parameters of orthoboric acid and iodide are weak and statistically insignificant (Tables 7.21 and 7.22).

Table 7.21. The result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of orthoboric acid in fluids

H_3BO_3 (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
Dependent Variables	r		Intercpt	39,78	16,47	2,42	,020
Triiodothyronine, nM/L	-,23	-,195	,146	-9,56	7,18	-1,33	,190
Testosterone, nM/L	-,20	-,160	,146	-1,29	1,18	-1,09	,282
	R=0,276; R^2=0,076; Adjusted R^2=0,035; $F_{(2,5)}$=1,9; p=0,168						

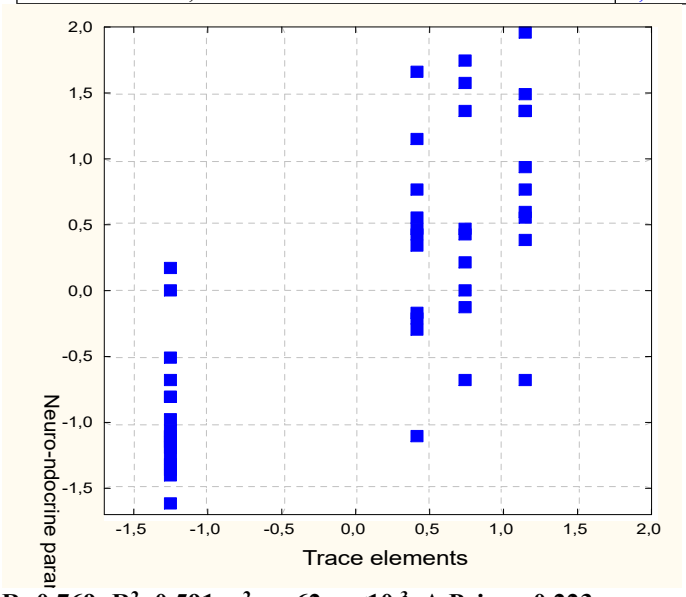
Table 7.22. The result of regression analysis with stepwise exclusion of endocrine parameters in relation to the content of jodide in fluids

Jodide (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
Dependent Variables	r		Intercpt	6,147	2,556	2,41	,020
Triiodothyronine, nM/L	-,23	-,194	,146	-1,478	1,114	-1,33	,191
Testosterone, nM/L	-,20	-,159	,146	-,199	,183	-1,09	,282
	$R=0,275$; $R^2=0,076$; Adjusted $R^2=0,035$; $F_{(2,5)}=1,8$; $p=0,169$						

The combined modulating effect of trace elements of mineral waters on the neuroendocrine parameters of the animals consuming them is much stronger than the partial effects: the degree of determination reaches 59% (Table 7.23 and Fig. 7.6). At the same time, the maximum factor load on the micronutrient canonical root is given by fluoride, less by meta-silicic acid and bromide, while the loads from orthoboric acid and iodide are negligible.

Table 7.23. Factor structure of canonical correlation between roots, which represent the content of microelements in fluids and neuroendocrine parameters

Trace elements	R
F^-	**-,519**
H_2SiO_3	**-,430**
Br^-	**-,291**
H_3BO_3	-,120
J^-	-,118
Neuroendocrine parameters	R
Triiodothyronine, nM/L	**,697**
Testosterone, nM/L	,031
AMo HRV as Sympathetic Tone, %	**-,354**
Adrenals Mass Index, %	**-,333**
$(Nap•Ku/Kp•Nau)^{0,25}$ as Mineralocorticoid Activity	**-,281**
Corticosterone, nM/L	-,083



$R=0,769$; $R^2=0,591$; $\chi^2_{(30)}=62$; $p<10^{-3}$; Λ Prime=0,223

Fig. 7.6. Canonical correlation between roots, which represent the content of trace elements in fluids (X-axis) and neuro-endocrine parameters (Y-axis)

Neuroendocrine root reflects the inhibitory effect of trace elements on plasma levels of triiodothyronine and, to a lesser extent, testosterone on the one hand, and the enhancing effect on sympathetic tone, adrenal mass and their mineralocorticoid aa well as, to a lesser extent, glucocorticoid activity.

8.5. Relationships between trace element fluid content and metabolic parameters

Following the accepted algorithm, we first present a correlation matrix for trace elements of mineral waters and metabolic parameters of urine and blood (Table 7.24).

Table 7.24. Matrix of correlations between the content of trace elements in fluids and metabolic parameters of urine and blood after weekly water-salt loads

Urine metabolites	**H_2SiO_3**	**H_3BO_3**	**Br^-**	**J^-**	**F^-**
Chloride Urine, mM/L	**-,35**	,22	,19	,23	-,04
Sodium Urine, mM/L	**-,33**	,20	,14	,20	-,10
Potassium Urine, mM/L	-,00	,11	,19	,11	,27
Magnesium Urine, mM/L	,23	-,23	**-,29**	-,23	-,19
Calcium Urine, mM/L	**,31**	-,02	-,07	-,02	-,11
Phosphate Urine, mM/L	,12	,02	,05	,02	,10
Urea Urine, mM/L	-,16	,20	,14	,20	-,10
Creatinine Urine, mM/L	,08	-,10	-,08	-,10	,03
Uric Acid Urine, mM/L	-,05	,15	,25	,15	**,31**
Amylase Urine, mg/h•mL	,15	,15	,20	,14	,23
Diene conjugates Urine, E^{232}/mL	**-,35**	-,10	-,12	-,10	-,13
Malonic Dialdehyde Urine, µM/L	,01	,10	,06	,10	-,04
Katalase Urine, nM/h•mL	-,06	-,10	-,10	-,09	-,07
Middle Mass Molecules Urine, units	,06	,02	,11	,02	,25
Osmolality Urine, mOsm/L	**-,37**	**,28**	,24	**,28**	-,01
Sodium Excretion, µM/24h•100 g	**-,39**	,18	,11	,19	-,16
Chloride Excretion, µM/24h•100 g	**-,42**	,19	,13	,19	-,12
Potassium Excretion, µM/24h•100 g	-,02	-,09	-,07	-,09	-,01
Magnesium Excretion, µM/24h•100 g	,09	**-,29**	**-,40**	**-,29**	**-,37**
Calcium Excretion, µM/24h•100 g	-,00	,05	-,04	,05	-,23
Phosphates Excretion, µM/24h•100 g	-,18	-,00	-,08	-,00	-,23
Urea Excretion, µM/24h•100 g	-,23	,14	,06	,14	-,17
Uric Acid Excretion, µM/24h•100 g	-,17	,02	,07	,02	,07
Creatinine Excretion, µM/24h•100 g	-,11	-,18	-,27	-,18	**-,35**
Plasma metabolites	**H_2SiO_3**	**H_3BO_3**	**Br^-**	**J^-**	**F^-**
Chloride Plasma, mM/L	,07	-,13	-,08	-,13	,06
Sodium Plasma, mM/L	,14	-,08	-,01	-,08	,17
Potassium Plasma, mM/L	,11	-,21	-,22	-,21	-,13
Calcium Plasma, mM/L	-,23	-,10	-,26	-,10	**-,49**
Phosphate Plasma, mM/L	,08	-,17	-,16	-,17	-,02
Magnesium Plasma, mM/L	,14	-,16	-,10	-,16	,11
Potassium Erythrocytes, mM/L	-,21	-,08	-,08	-,08	-,05
Sodium Erythrocytes, mM/L	-,25	-,03	-,00	-,03	,00
Creatinine Plasma, µM/L	**,29**	-,22	-,20	-,22	-,01
Urea Plasma, mM/L	,27	-,13	-,12	-,13	,03
Uric Acid Plasma, µM/L	**-,28**	,25	,25	,25	,07
Bilirubine Plasma, µM/L	,07	,04	,09	,04	,13
Glucose Plasma, mM/L	-,05	-,23	-,20	-,23	-,04
Cholesterol Plasma, mM/L	,16	-,06	-,04	-,06	,04
Middle Mass Molecules Plasma, units	,05	*-,20*	-,09	-,20	,19
Diene conjugates Plasma, E^{232}/mL	**-,30**	,20	,18	,20	-,02
Malonic Dialdehyde Plasma, µM/L	,11	,06	,15	,06	,24
Superoxide Dismutase Erythrocyte, un/mL	,18	-,17	-,14	-,17	,04
Katalase Plasma, nM/h•mL	,11	-,14	-,12	-,14	,00
Amylase Plasma, mg/h•mL	,04	,20	,20	,20	,11
Others parameters	**H_2SiO_3**	**H_3BO_3**	**Br^-**	**J^-**	**F^-**
Diurese, mL/24h•100 g	-,22	-,06	-,15	-,06	**-,32**
Glomerulary Filtration, µL/min•100 g	**-,28**	,08	-,02	,08	-,27
Canalicular Reabsorbtion, %	-,20	,17	,17	,17	,06

Regression-based regression models show that fluoride has the maximum metabolic effect (Table 7.25). It is aimed primarily at reducing the concentration of calcium in plasma and its excretion in the daily urine. However, the concentration of potassium and medium-weight molecules in the urine increases.

Table 7.25. The result of regression analysis with step-by-step exclusion of metabolic parameters in relation to the content of fluoride in fluids

Ftoride (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(43)}$	p-level
Dependent Variables	r		Intercpt	,4347	,2705	1,61	,115
Calcium Plasma, mM/L	**-,49**	-,480	,117	-,1908	,0465	-4,10	,0002
Calcium Excretion, µM/24h•100 g	-,23	-,288	,139	-,0383	,0184	-2,08	,044
Potassium Urine, mM/L	,27	,262	,131	,0021	,0011	1,99	,052
Middle Mass Molecules Urine, units	,25	,311	,126	2,489	1,012	2,46	,018
	R=0,670; R^2=0,449; Adjusted R^2=0,398; $F_{(4,4)}$=8,8; p=0,00003						

The severity of the metabolotropic effect of metasilicic acid, judging by the integral coefficients, is similar (Table 7.26). However, the main target is sodium chloride, the urinary concentration and excretion of which are reduced. This is accompanied by a decrease in the concentration of diene conjugates in plasma and potassium in erythrocytes and an increase in creatinine.

Table 7.26. The result of regression analysis with step-by-step exclusion of metabolic parameters in relation to the content of metasilicic acid in liquids

H_2SiO_3 (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(40)}$	p-level
Dependent Variables	r		Intercpt	21,67	6,11	3,55	,001
Chloride Excretion, µM/24h•100 g	**-,42**	,888	,605	,021	,014	1,47	,150
Sodium Excretion, µM/24h•100 g	**-,39**	-1,489	,607	-,027	,011	-2,45	,019
Chloride Urine, mM/L	**-,35**	-1,294	,601	-,049	,023	-2,15	,038
Sodium Urine, mM/L	**-,33**	1,447	,595	,052	,022	2,43	,020
Diene conjugates Plasma, E^{232}/mL	**-,30**	-,310	,127	-2,383	,974	-2,45	,019
Potassium Erythrocytes, mM/L	-,21	-,340	,132	-,181	,070	-2,59	,013
Creatinine Plasma, µM/L	**,29**	,309	,130	29,14	12,28	2,37	,023
	R=0,672; R2=0,451; Adjusted R^2=0,355; $F_{(7,4)}$=4,7; p=0,0006						

A similar metabolotropic effect of bromide is directed to another constellation (Table 7.27). It is manifested in a decrease in urinary magnesium concentration, creatinine excretion and its concentration in plasma in combination with an increase in plasma urate concentration and amylase activity, and in urine - chloride concentration and urine osmolality.

Table 7.27. The result of regression analysis with stepwise exclusion of metabolic parameters in relation to the content of bromide in liquids

Bromide (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(40)}$	p-level
Dependent Variables	r		Intercpt	-5,99	8,63	-,69	,491
Magnesium Urine, mM/L	**-,29**	-,232	,155	-1,075	,716	-1,50	,141
Creatinine Excretion, µM/24h•100 g	-,27	-,319	,131	-,526	,216	-2,43	,020
Creatinine Plasma, µM/L	-,20	-,228	,146	-51,7	33,2	-1,56	,127
Uric Acid Plasma, µM/L	,25	,258	,146	,005	,003	1,77	,085
Osmolality Urine, mOsm/L	,24	,737	,411	,031	,017	1,79	,080
Amylase Plasma, mg/h•mL	,20	,397	,138	,094	,033	2,87	,007
Chloride Urine, mM/L	,19	-,713	,414	-,066	,038	-1,72	,093
	R=0,625; R^2=0,391; Adjusted R^2=0,284; $F_{(7,4)}$=3,7; p=0,004						

The content of orthoboric acid in mineral waters is also associated with an increase in plasma urate concentration and amylase activity and a decrease in creatinine excretion and plasma concentration. An additional manifestation of the metabolic effect is an increase in the concentration of urea in the urine (Table 7.28).

Table 7.28. The result of regression analysis with step-by-step exclusion of metabolic parameters in relation to the content of orthoboric acid in liquids

H_3BO_3 (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(42)}$	p-level
Dependent Variables	r		Intercpt	-23,4	16,2	-1,44	,156
Uric Acid Plasma, µM/L	,25	,340	,140	,013	,005	2,44	,019
Amylase Plasma, mg/h•mL	,20	,397	,138	,205	,071	2,87	,006
Urea Urine, mM/L	,20	,355	,134	,127	,048	2,65	,011
Creatinine Plasma, µM/L	-,22	-,230	,134	-113,8	66,3	-1,72	,093
Creatinine Excretion, µM/24h•100 g	-,18	-,281	,133	-1,010	,479	-2,11	,041
	$R=0,580$; $R^2=0,337$; Adjusted $R^2=0,258$; $F_{(5,4)}=4,3$; $p=0,003$						

In contrast, mineral water iodide is weakly and statistically insignificantly associated with metabolic parameters (Table 7.29).

Table 7.29. The result of regression analysis with step-by-step exclusion of metabolic parameters in relation to the content of iodide in liquids

Jodide (mg/L) as Independent Variable		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(45)}$	p-level
Dependent Variables	r		Intercpt	-,8491	1,1828	-,72	,477
Osmolality Urine, mOsm/L	**,28**	,238	,144	,0033	,0020	1,65	,106
Uric Acid Plasma, µM/L	,25	,196	,144	,0012	,0009	1,36	,180
	$R=0,341$; $R^2=0,117$; Adjusted $R^2=0,077$; $F_{(2,4)}=3,0$; $p=0,061$						

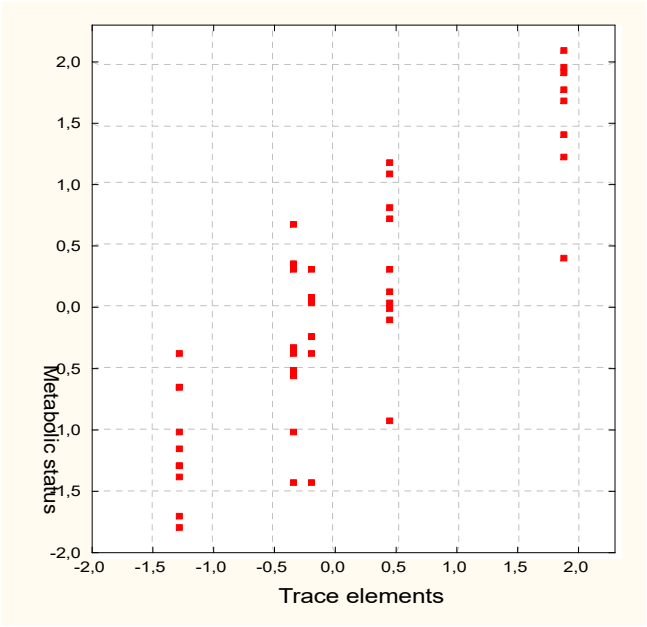
Canonical correlation analysis shows that among the considered microelements the maximum metabolic activity, judging by the factor loads on the root, is shown by fluoride, much less active metasilicic acid and bromide, while the activity of orthoboric acid and iodide is minimal (Table 7.30).

The vast majority of metabolic parameters are affected by trace elements by type of downregulation. These include plasma concentrations of calcium and diene conjugates and concentration of potassium in erythrocytes, urinary excretion of creatinine, calcium, chloride and sodium, as well as urine osmolality and closely related concentrations of the latter two electrolytes, as well as urea and magnesium. Instead, the concentration of potassium and medium-weight molecules in the urine increases, as do the plasma concentrations of creatinine and amylase activity.

Table 7.30. Factor structure of canonical correlation between roots, which represent the content of trace elements in fluids and metabolic parameters

Trace elements	Root 1
F^-	**-,816**
H_2SiO_3	**-,447**
Br^-	**-,426**
H_3BO_3	-,161
J^-	-,159
Metabolic parameters	Root 1
Calcium Plasma, mM/L	**,657**
Creatinine Excretion, µM/24h•100 g	**,461**
Chloride Excretion, µM/24h•100 g	**,379**
Sodium Excretion, µM/24h•100 g	**,365**
Sodium Urine, mM/L	**,283**
Chloride Urine, mM/L	**,267**
Potassium Erythrocytes, mM/L	**,261**
Magnesium Urine, mM/L	**,233**
Osmolality Urine, mOsm/L	**,230**
Calcium Excretion, µM/24h•100 g	,131
Urea Urine, mM/L	,125
Diene conjugates Plasma, E^{232}/mL	,096
Middle Mass Molecules Urine, units	**-,297**
Potassium Urine, mM/L	**-,233**
Amylase Plasma, mg/h•mL	-,106
Creatinine Plasma, µM/L	-,089

Taken together, these trace elements of mineral waters have a strong metabolic effect on the animals consuming them, the quantitative estimate of which reaches 72% (Fig. 7.7).



R=0,850; R^2=0,723; $\chi^2_{(80)}$=105; p=0,030; Λ Prime=0,053
Fig. 7.7. Canonical correlation between roots, which represent the content of trace elements in liquids (X-axis) and metabolic parameters (Y-axis)

8.6. Relationships between the content of trace elements in fluids and the immune parameters of the thymus, spleen and blood

The correlation matrix for trace elements of mineral waters and immune parameters of animals has the following form (tab. 7.31).

Table 7.31. Matrix of correlations between the content of trace elements in fluids and the immune parameters of the thymus, spleen and blood after weekly water-salt loads

Immune parameters	H_2SiO_3	H_3BO_3	Br^-	J^-	F^-
Thymus Mass Index, mg/100 g BM	0,12	0,01	0,03	0,01	0,04
Lymphocytes of Thymus, %	-0,10	-0,13	**-0,16**	-0,13	-0,14
Lymphoblastes of Thymus, %	**0,28**	0,04	0,05	0,04	0,09
Reticulocytes of Thymus, %	-0,00	0,04	0,07	0,04	0,10
Epitheliocytes of Thymus, %	-0,06	0,05	0,03	0,05	-0,03
Endotheliocytes of Thymus, %	**0,30**	0,11	0,08	0,11	-0,01
Plasmocytes of Thymus, %	0,14	0,10	0,16	0,10	**0,22**
Macrophages of Thymus, %	**-0,25**	-0,02	0,02	-0,02	0,04
Hassal corpuscles of Thymus, %	-0,03	-0,05	-0,07	-0,05	-0,04
Entropy of Thymocytogram	0,13	0,14	0,18	0,14	0,17
Spleen Mass Index, mg/100 g BM	0,15	0,00	-0,06	0,00	-0,13
Lymphoblastes of Spleen, %	-0,03	0,13	0,10	0,13	-0,04
Lymphocytes of Spleen, %	-0,08	0,26	0,34	0,26	0,26
Plasmocytes of Spleen, %	0,07	0,01	0,02	0,00	0,03
Reticulocytes of Spleen, %	**-0,16**	**-0,28**	**-0,27**	**-0,28**	-0,06
Fibroblastes of Spleen, %	**-0,26**	**-0,27**	**-0,28**	**-0,27**	-0,12
Macrophages of Spleen, %	**0,25**	0,03	0,08	0,03	**0,16**
Microphages of Spleen, %	0,20	0,07	-0,05	0,07	**-0,24**
Eosinophiles of Spleen, %	-0,03	-0,12	**-0,16**	-0,12	-0,14

Entropy of Splenocytogram	0,10	-0,20	-0,29	-0,20	**-0,27**
Monocytes of Blood, %	-0,30	-0,26	-0,34	-0,26	-0,27
Leukocytes of Blood, 10^9/L	0,26	**0,18**	**0,25**	**0,18**	0,27
Lymphocytes of Blood, %	**-0,22**	-0,34	**-0,31**	-0,34	-0,06
Stub Neutrophils of Blood, %	0,09	0,08	0,11	0,08	0,14
Segmented Neutrophiles of Blood, %	0,34	**0,43**	**0,40**	**0,43**	0,11
Eosinophiles of Blood, %	-0,03	0,07	0,10	0,07	0,08
Basophiles of Blood, %	0,06	**0,22**	**0,22**	**0,22**	0,13
Entropy of Leukocytogram	0,03	0,10	0,04	0,10	-0,05
Natural Killer Lymphocytes, %	**-0,30**	-0,22	-0,31	-0,22	**-0,30**
T-helper Lymphocytes, %	-0,02	-0,04	-0,02	-0,04	0,04
T-cytolytic Lymphocytes, %	-0,12	-0,01	0,05	-0,01	0,15
B-Lymphocytes, %	-0,13	-0,06	0,04	-0,06	**0,26**
0-Lymphocytes, %	**0,20**	0,11	0,05	0,11	-0,11
Entropy of Immunocytogram	-0,15	-0,01	-0,04	-0,01	-0,11
Microbian Count of Neutrophils, B/Ph	0,36	0,28	**0,34**	0,28	0,25
Phagocytic Index of Monocytes, %	**-0,29**	-0,23	-0,25	-0,23	-0,17
Microbian Count of Monocytes, B/Ph	-0,29	-0,22	-0,26	-0,22	-0,17
Killing Index of Neutrophils, %	0,10	0,16	0,09	0,16	-0,11
Phagocytic Index of Neutrophils, %	0,16	0,19	0,24	0,19	0,18

Comparative analysis of regression models showed that bromide has the most significant immunotropic activity (Table 7.32). It is manifested in the activating effect on the intensity of phagocytosis of blood neutrophils, the content of leukocytes in general and the share in the leukocytogram of basophils, while the share of lymphocytes in the leukocytogram and thymocytogram decreases, as well as the share in splenocytogram of fibroblasts, reticulocytes and eosinophils. The degree of immunomodulatory determination is 65.5%.

Table 7.32. The result of regression analysis with step-by-step exclusion of immune parameters in relation to the content of bromide in liquids

Bromide (mg/L) as Independent Variable		**Beta**	**St. Err.**	**B**	**St. Err.**	**$t_{(39)}$**	**p-level**
Dependent Variables	r		**Intercpt**	129,6	27,98	4,63	10^{-4}
Microbian Count of Neutrophils, B/Ph	**0,34**	0,330	0,107	2,252	0,729	3,09	0,004
Leukocytes of Blood, 10^9/L	0,25	0,230	0,099	0,426	0,183	2,34	0,025
Basophiles of Blood, %	0,22	0,385	0,103	6,023	1,602	3,76	0,001
Lymphocytes of Blood, %	**-0,31**	-0,488	0,099	-0,533	0,108	-4,95	10^{-6}
Fibroblastes of Spleen, %	**-0,28**	-0,320	0,105	-1,546	0,506	-3,06	0,004
Reticulocytes of Spleen, %	-0,27	-0,279	0,102	-1,293	0,471	-2,74	0,009
Eosinophiles of Spleen, %	-0,16	-0,168	0,104	-1,741	1,082	-1,61	0,116
Lymphocytes of Thymus, %	-0,16	-0,368	0,104	-1,142	0,323	-3,54	0,001
	$R=0,810$; $R^2=0,655$; Adjusted $R^2=0,585$; $F_{(8,4)}=9,3$; $p<10^{-5}$						

Metasilicic acid has a less noticeable immunomodulatory effect (coefficient of determination is 53%) (Table 7.33). It is manifested in the suppressive effect on the level in the blood of natural killers and total lymphocytes, the activity of phagocytosis of blood monocytes, the content in the spleen of fibroblasts and reticulocytes, and in the thymus - macrophages. Instead, the content of endothelial cells and lymphoblasts in the thymus, of macrophages in the spleen, and of 0-lymphocytes in the blood decreases.

Table 7.33. The result of regression analysis with step-by-step exclusion of immune parameters in relation to the content of metasilicic acid in liquids

H_2SiO_3 (mg/L) as Independent Variable		Beta	St. Err.	B	St. Err.	$t_{(37)}$	p-level
Dependent Variables	r		**Intercpt**	15,903	9,100	1,75	0,089
Natural Killer Lymphocytes, %	**-0,30**	-0,164	0,128	-0,286	0,224	-1,28	0,209
Phagocytic Index of Monocytes, %	**-0,29**	-0,171	0,123	-0,687	0,494	-1,39	0,173
Fibroblastes of Spleen, %	-0,26	-0,277	0,122	-0,597	0,263	-2,27	0,029
Macrophages of Thymus, %	-0,25	-0,154	0,122	-0,513	0,405	-1,26	0,214
Lymphocytes of Blood, %	-0,22	-0,196	0,128	-0,096	0,062	-1,54	0,133
Reticulocytes of Spleen, %	-0,16	-0,194	0,122	-0,401	0,252	-1,59	0,120
Endotheliocytes of Thymus, %	**0,30**	0,235	0,125	0,877	0,466	1,88	0,067
Lymphoblastes of Thymus, %	**0,28**	0,226	0,134	0,785	0,464	1,69	0,099
Macrophages of Spleen, %	0,25	0,335	0,126	0,644	0,242	2,67	0,011
0-Lymphocytes, %	0,20	0,154	0,125	0,071	0,058	1,23	0,228
	R=0,729; R^2=0,532; Adjusted R^2=0,405; $F_{(10,4)}$=4,2; p=0,0006						

The immunomodulatory effect of orthoboric acid (Table 7.34) and iodide (Table 7.35) was almost similar in strength. These micronutrients equally contribute to the increase in the content of leukocytes in the blood and the share in the leukocytogram of segmental neutrophils and basophils and reduce the content in the splenocytogram of reticulocytes and fibroblasts.

Table 7.34. The result of regression analysis with step-by-step exclusion of immune parameters in relation to the content of orthoboric acid in liquids

H_3BO_3 (mg/L) as Independent Variable		Beta	St. Err.	B	St. Err.	$t_{(42)}$	p-level
Dependent Variables	r		**Intercpt**	33,42	21,61	1,55	0,129
Segmented Neutrophiles of Blood, %	**0,43**	0,467	0,110	1,291	0,303	4,26	10^{-4}
Basophiles of Blood, %	0,22	0,419	0,116	14,293	3,965	3,61	0,001
Leukocytes of Blood, 10^9/L	0,18	0,191	0,113	0,772	0,457	1,69	0,098
Reticulocytes of Spleen, %	**-0,28**	-0,250	0,112	-2,528	1,137	-2,22	0,032
Fibroblastes of Spleen, %	-0,27	-0,387	0,116	-4,077	1,224	-3,33	0,002
	R=0,707; R^2=0,500; Adjusted R^2=0,440; $F_{(5,4)}$=8,4; p<10^{-5}						

Table 7.35. The result of regression analysis with step-by-step exclusion of immune parameters in relation to the content of iodide in liquids

Jodide (mg/L) as Independent Variable		Beta	St. Err.	B	St. Err.	$t_{(42)}$	p-level
Dependent Variables	r		**Intercpt**	5,180	3,355	1,54	0,130
Segmented Neutrophiles of Blood, %	**0,43**	0,467	0,110	0,200	0,047	4,26	10^{-4}
Basophiles of Blood, %	0,22	0,419	0,116	2,217	0,615	3,60	0,001
Leukocytes of Blood, 10^9/L	0,18	0,190	0,113	0,119	0,071	1,68	0,099
Reticulocytes of Spleen, %	**-0,28**	-0,250	0,112	-0,392	0,176	-2,22	0,032
Fibroblastes of Spleen, %	-0,27	-0,387	0,116	-0,632	0,190	-3,32	0,002
	R=0,707; R^2=0,499; Adjusted R^2=0,440; $F_{(5,4)}$=8,4; p<10^{-5}						

Minimal immunomodulatory activity (coefficient of determination is 34%) was found in fluoride, which has a suppressive effect on the level of natural killers in the blood and microphages in the spleen as well as the entropy of the splenocytogram, while enhancing effect on content in spleen of macrophages, in blood of B-lymphocytes and in thymus of plasmocytes (Table 7.36).

Table 7.36. The result of regression analysis with step-by-step exclusion of immune parameters in relation to the content of fluoride in liquids

Ftoride (mg/L) as Independent Variable		Beta	St. Err.	B	St. Err.	$t_{(41)}$	p-level
Dependent Variables	r		**Intercpt**	3,870	1,534	2,52	0,016
Natural Killer Lymphocytes, %	**-0,30**	-0,252	0,133	-0,040	0,021	-1,90	0,064
Entropy of Splenocytogram	-0,27	-0,287	0,129	-5,271	2,370	-2,22	0,032
Microphages of Spleen, %	-0,24	-0,194	0,131	-0,030	0,020	-1,48	0,146
B-Lymphocytes, %	0,26	0,212	0,133	0,022	0,014	1,60	0,118
Plasmocytes of Thymus, %	0,22	0,205	0,130	0,080	0,051	1,57	0,124
Macrophages of Spleen, %	0,16	0,238	0,134	0,042	0,024	1,78	0,083
	R=0,582; R^2=0,339; Adjusted R^2=0,242; $F_{(6,4)}$=3,5; p=0,007						

According to the canonical correlation analysis, the most active immunotropic trace element is metasilicic acid, bromide, orthoboric acid and iodide, which are slightly inferior in factor loads, while fluoride lags far behind (Table 7.37).

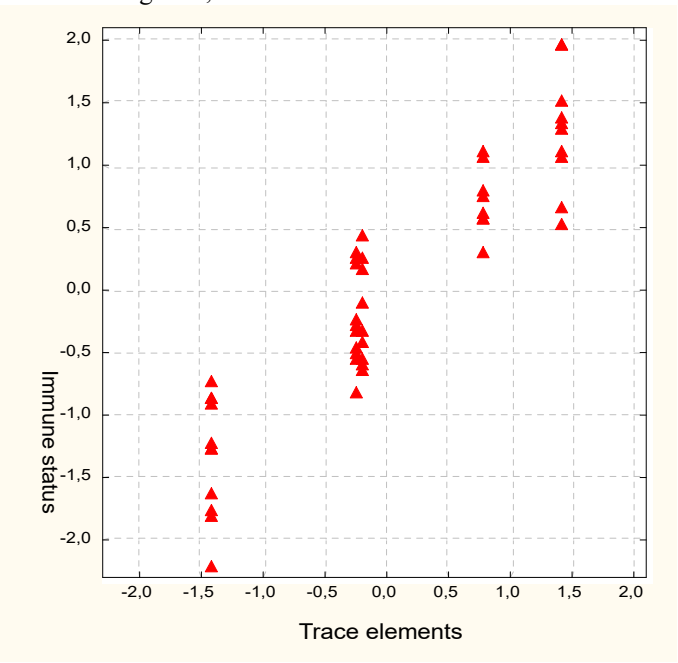
Table 7.37. Factor structure of the canonical correlation between the roots, which represent the content of trace elements in the fluids and the immune parameters of the thymus, spleen and blood

Trace elements	R
H_2SiO_3	**-0,737**
Br^-	**-0,689**
H_3BO_3	**-0,674**
J^-	**-0,672**
F^-	**-0,287**
Immune parameters	R
Microbian Count of Neutrophils, B/Ph	**-0,674**
Segmented Neutrophiles of Blood, %	**-0,414**
Endotheliocytes of Thymus, %	**-0,345**
0-Lymphocytes, %	**-0,321**
Leukocytes of Blood, 10^9/L	**-0,284**
Lymphoblastes of Thymus, %	**-0,201**
Macrophages of Spleen, %	-0,162
Microphages of Spleen, %	-0,083
Plasmocytes of Thymus, %	-0,050
Natural Killer Lymphocytes, %	**0,578**
Fibroblastes of Spleen, %	**0,361**
Reticulocytes of Spleen, %	**0,322**
B-Lymphocytes, %	**0,246**
Phagocytic Index of Monocytes, %	0,170
Lymphocytes of Blood, %	0,161
Eosinophiles of Spleen, %	0,154
Entropy of Splenocytogram	0,154
Macrophages of Thymus, %	0,109
Lymphocytes of Thymus, %	0,065
Basophiles of Blood, %	0,056

Among the parameters of immunity to stimulating effects subordinates are primarily responsible for phagocytosis: the microbial count of neutrophils, their share in the leukocytogram and the total content of leukocytes in the blood, which together reflects its bactericidal ability. Endothelial cells, lymphoblasts and plasma cells of the thymus and macrophages and microphages of the spleen are also object to stimulation. Instead, natural killers, B-lymphocytes and basophils of the blood, fibroblasts, reticulocytes and eosinophils of the spleen (and the associated entropy of the splenocytogram) as well as macrophages and lymphocytes of the

thymus, are object to suppression. The increase in the proportion of 0-lymphocytes in the immunocytogram of the blood reflects, in essence, the immunosuppressive effect of trace elements.

Taken together, these trace elements determine the immune status of animals by 84% (Fig. 7.8).



R=0,919; R^2=0,844; $\chi^2_{(100)}$=133; p=0,016; Λ Prime=0,020

Fig. 7.8. Canonical correlation between roots, which represent the content of trace elements in fluids (X-axis) and immune parameters (Y-axis)

7.7. Relationships between electrolytes fluid content and neuroendocrine parameters

Screening revealed that all seven mineral ions have significant connections with the neuroendocrine parameters of animals (Table 7.38).

Table 7.38. Matrix of correlations between electrolyte content in fluids and neuroendocrine parameters after weekly water-salt loads

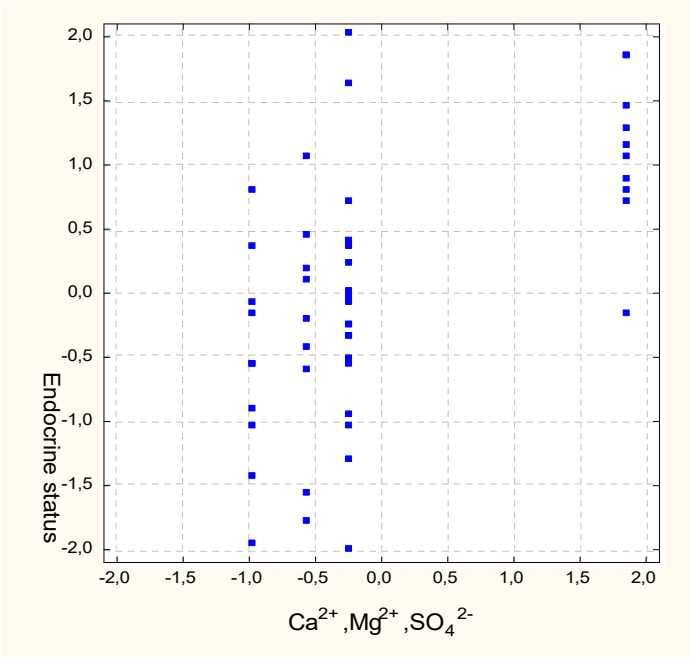
Neuroendocrine parameters	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Na^+	Cl^-	HCO_3^-	K^+
Variative Swing HRV as Vagal Tone, msec	,10	,13	,06	,18	,18	-,02	,07
Moda HRV, msec	,11	,18	,09	,17	,16	,10	-,03
AMo HRV as Sympathetic Tone, %	-,10	-,22	-,04	**-,28**	**-,28**	-,03	-,08
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	**,33**	,19	**,30**	,15	,13	,05	-,10
$(Nap•Ku/Kp•Nau)^{-0,25}$ as Mineralocorticoid Activity	-,03	-,25	,03	**-,32**	**-,32**	-,06	-,11
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	-,21	,13	-,25	,21	,23	,05	,17
$(Cap•Pu/Pp•Cau)^{0,25}$ as Parathyroid Activity	-,12	,13	-,19	,26	**,28**	-,03	,22
$(Cap•Pp)^{-0,5}$ as Calcitonin Activity	**-,35**	**-,28**	**-,35**	-,16	-,12	-,22	,24
$(Cau•Pu/Pp•Cap)^{0,25}$ as Calcitonin Activity	**-,30**	-,08	**-,30**	-,02	,01	-,02	,14
Triiodothyronine, nM/L	**,46**	**,41**	**,48**	,21	,16	**,36**	**-,38**
Testosterone, nM/L	,15	-,14	,15	-,12	-,13	-,17	,01
Corticosterone, nM/L	-,21	-,02	-,13	-,16	-,16	,26	-,20
Glomerular Zone of Adrenal Cortex, μM	-,23	-,09	-,24	-,02	,00	-,07	,14
Fascicular Zone of Adrenal Cortex, μM	**,38**	,26	**,38**	,14	,10	,20	-,25
Reticular Zone of Adrenal Cortex, μM	-,07	,01	-,06	,00	,00	,05	-,01
Adrenals Mass Index, %	-,11	-,20	-,02	**-,33**	**-,33**	,09	-,20

However, the program for canonical analysis included in the factor structure of the electrolyte root only calcium, sulfate and magnesium (Table 7.39). The structure of the effective root reflects the stimulating effect of these electrolytes on the plasma level of triiodothyronine and glucocorticoid as well as mineralocorticoid activity of the adrenal cortex despite the weak negative effect on their mass. Instead, calcitonin activity decreases under the influence of these electrolytes.

Table 7.39. Factor structure of canonical correlation between roots, which represent the content of electrolytes in fluids and neuro-endocrine parameters

Electrolytes	R
Ca^{2+}	**,943**
SO_4^{2-}	**,934**
Mg^{2+}	**,781**
Endocrine parameters	R
Triiodothyronine, nM/L	**,886**
Fascicular Zone of Adrenal Cortex, µM	**,669**
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	**,525**
$(Cap•Pp)^{-0,5}$ as Calcitonin Activity	**-,643**
Adrenals Mass Index, %	**-,188**

However, electrolyte determination of endocrine status is weak (33%) and on the verge of statistical significance (Fig. 7.9).



R=0,577; R^2=0,333; $\chi^2_{(15)}$=24,5; p=0,057; Λ Prime=0,561

Fig. 7.9. Canonical correlation between roots representing electrolyte content (X-axis) and endocrine parameters (Y-axis)

7.8. Relationships between electrolytes fluid content and metabolic parameters

Despite the very wide choice of the matrix of correlations between the content of electrolytes in the fluids and metabolic parameters of urine and blood (Table 7.40), the program for canonical analysis included in the factor structure of the electrolyte root only sulfate, sodium and chloride (Table 7.41).

Table 7.40. Matrix of correlations between fluid content of electrolytes and metabolic parameters of urine and blood after weekly water-salt loads

Urine metabolites	**Na^+**	**Cl^-**	**SO_4^{2-}**	**Ca^{2+}**	**Mg^{2+}**	**HCO_3^-**	**K^+**
Chloride Urine, mM/L	**,40**	**,39**	,15	,24	**,32**	,02	,10
Sodium Urine, mM/L	**,37**	**,37**	,07	,15	**,29**	,03	,11
Potassium Urine, mM/L	-,01	-,02	,16	,17	-,02	-,09	-,01
Magnesium Urine, mM/L	-,00	-,05	**,43**	**,35**	,27	**,40**	**-,48**
Calcium Urine, mM/L	-,23	-,22	-,11	-,21	-,09	,21	-,20
Phosphate Urine, mM/L	-,13	-,13	-,04	-,06	-,11	-,02	-,03
Urea Urine, mM/L	,21	,21	-,04	-,00	,18	,08	,05
Creatinine Urine, mM/L	,00	-,02	**,28**	,26	,11	,12	-,20
Uric Acid Urine, mM/L	-,14	-,11	-,26	-,23	**-,31**	**-,35**	**,30**

Amylase Urine, mg/h•mL	-,12	-,13	,06	,04	-,07	,00	-,08
Diene conjugates Urine, E^{232}/mL	**,32**	**,31**	,15	,23	,22	-,08	,13
Malonic Dialdehyde Urine, µM/L	,14	,12	,25	,24	,25	,22	-,20
Katalase Urine, nM/h•mL	-,01	,00	-,12	-,11	-,07	-,09	,11
Middle Mass Molecules Urine, units	-,15	-,15	-,03	-,04	-,20	-,18	,08
Osmolality Urine, mOsm/L	**,42**	**,41**	,13	,22	**,33**	,01	,12
Sodium Excretion, µM/24h•100 g	**,40**	**,41**	-,01	,08	**,29**	-,00	,19
Chloride Excretion, µM/24h•100 g	**,44**	**,44**	,09	,19	**,33**	,00	,16
Potassium Excretion, µM/24h•100 g	-,01	-,01	,03	,04	-,01	-,03	,01
Magnesium Excretion, µM/24h•100 g	,09	,07	**,29**	,24	**,29**	**,36**	**-,35**
Calcium Excretion, µM/24h•100 g	,04	,06	-,18	-,20	,05	,12	,01
Phosphates Excretion, µM/24h•100 g	,16	,17	-,16	-,13	,09	,01	,13
Urea Excretion, µM/24h•100 g	,23	,25	-,13	-,09	,15	,02	,15
Uric Acid Excretion, µM/24h•100 g	-,03	,00	**-,34**	**-,29**	-,24	**-,33**	**,37**
Creatinine Excretion, µM/24h•100 g	,16	,16	,01	,02	,18	,14	-,04
Plasma metabolites	**Na^+**	**Cl^-**	**SO_4^{2-}**	**Ca^{2+}**	**Mg^{2+}**	**HCO_3^-**	**K^+**
Chloride Plasma, mM/L	-,20	-,18	-,20	-,21	-,27	-,18	,13
Sodium Plasma, mM/L	-,26	-,25	-,17	-,20	**-,31**	-,20	,10
Potassium Plasma, mM/L	-,21	-,19	**-,28**	**-,32**	-,24	-,08	,09
Calcium Plasma, mM/L	**,33**	**,33**	,03	,06	**,36**	,23	-,03
Phosphate Plasma, mM/L	,02	-,01	**,32**	**,30**	,14	,14	-,23
Magnesium Plasma, mM/L	-,21	-,21	-,03	-,06	-,21	-,11	,00
Potassium Erythrocytes, mM/L	,18	,18	,11	,17	,12	-,07	,08
Sodium Erythrocytes, mM/L	,12	,13	-,11	-,04	-,04	-,22	,26
Creatinine Plasma, µM/L	-,23	-,25	,13	,05	-,08	,13	-,25
Urea Plasma, mM/L	-,21	-,22	,11	,04	-,07	,12	-,23
Uric Acid Plasma, µM/L	,20	,21	-,17	-,09	,03	-,17	**,29**
Bilirubine Plasma, µM/L	-,12	-,11	-,08	-,09	-,14	-,09	,05
Glucose Plasma, mM/L	-,06	-,05	-,07	-,06	-,13	-,15	,12
Cholesterol Plasma, mM/L	-,13	-,13	,07	,03	-,05	,06	-,13
Middle Mass Molecules Plasma, units	-,22	-,21	-,11	-,10	**-,32**	**-,29**	,16
Diene conjugates Plasma, E^{232}/mL	,21	,23	-,23	-,15	,03	-,17	**,31**
Malonic Dialdehyde Plasma, µM/L	-,26	-,23	-,27	**-,29**	**-,35**	-,24	,18
Superoxide Dismutase Erythrocyte, un/mL	-,14	-,16	,15	,11	-,04	,07	-,18
Katalase Plasma, nM/h•mL	-,17	-,16	-,11	-,14	-,18	-,07	,03
Amylase Plasma, mg/h•mL	,10	,08	,26	,25	,20	,17	-,19
Others parameters	**Na^+**	**Cl^-**	**SO_4^{2-}**	**Ca^{2+}**	**Mg^{2+}**	**HCO_3^-**	**K^+**
Diurese, mL/24h•100 g	,21	,23	-,14	-,10	,14	,04	,13
Glomerulary Filtration, µL/min•100 g	**,29**	**,31**	-,11	-,06	,21	,05	,14
Canalicular Reabsorbtion, %	,21	,20	,07	,13	,14	-,03	,08

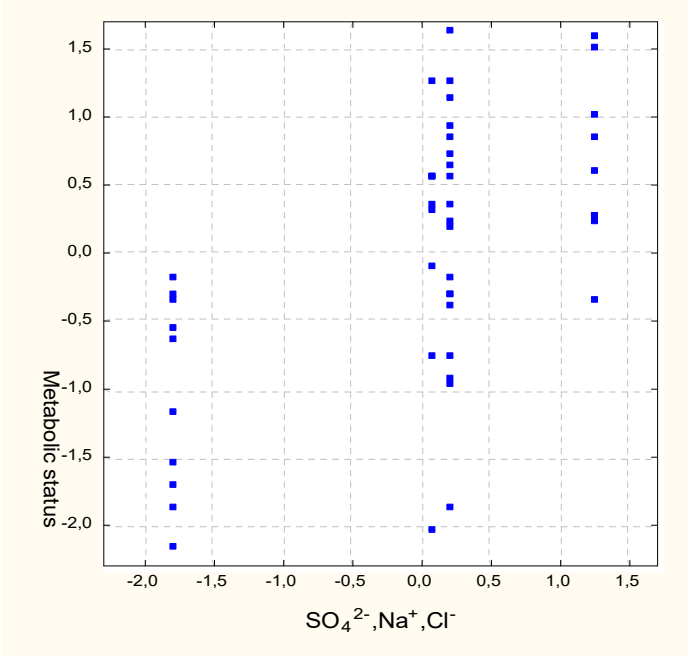
Table 7.41. Factor structure of canonical correlation between roots, which represent the content of electrolytes in liquids and metabolic parameters

Electrolytes	R
SO_4^{2-}	-0,882
Na^+	-0,440
Cl^-	-0,362
Metabolic parameters	**R**
Magnesium Urine, mM/L	-0,745
Osmolality Urine, mOsm/L	-0,329
Chloride Excretion, µM/24h•100 g	-0,288
Sodium Excretion, µM/24h•100 g	-0,162
Uric Acid Excretion, µM/24h•100 g	0,617

The metabolic root is represented, on the one hand, by the concentration of magnesium in the urine and the excretion of chloride and sodium with urine as well as its osmolality, which is created mainly by these ions

together with the urea, and on the other hand - the excretion of uric acid, which are object to positive and negative effects, respectively.

The electrolytes of the used liquids determine parameters of the urine weakly (36%), but statistically significant (fig. 7.10).



R=0,601; R^2=0,361; $\chi^2_{(15)}$=29,6; p=0,013; Λ Prime=0,498

Fig. 7.10. Canonical correlation between roots representing electrolyte content (X-axis) and metabolic parameters (Y-axis)

7.9. Relationships between electrolytes fluid content and immune parameters

The most numerous and strongest bonds of electrolytes of mineral waters were found in relation to the parameters of immunity (Table 7.42).

Table 7.42. Matrix of correlations between electrolyte fluid content and immune parameters of thymus, spleen and blood after weekly exercise

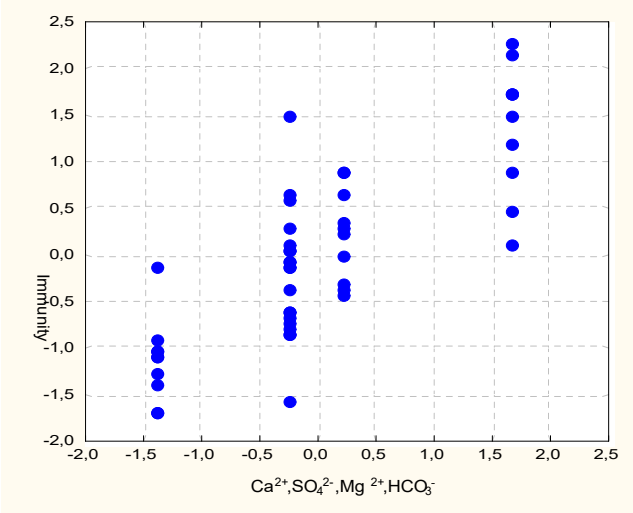
Immune parameters	Ca^{2+}	Mg^{2+}	SO_4^{2-}	HCO_3^-	Na^+	Cl^-	K^+
Thymus Mass Index, mg/100 g BM	-0,27	-0,21	-0,25	-0,12	-0,15	-0,13	0,13
Lymphocytes of Thymus, %	-0,09	-0,04	-0,08	0,01	-0,04	-0,03	0,01
Lymphoblastes of Thymus, %	-0,14	-0,15	-0,08	0,05	-0,23	-0,22	-0,10
Reticulocytes of Thymus, %	0,08	0,00	0,06	-0,06	0,02	0,02	0,02
Epitheliocytes of Thymus, %	0,15	0,16	0,12	0,06	0,15	0,14	-0,03
Endotheliocytes of Thymus, %	**-0,32**	-0,17	**-0,29**	0,00	-0,16	-0,14	0,05
Plasmocytes of Thymus, %	0,14	-0,01	0,14	-0,02	-0,05	-0,06	-0,07
Macrophages of Thymus, %	0,09	0,03	0,02	-0,18	0,16	0,16	0,19
Hassal corpuscles of Thymus, %	0,19	0,14	0,22	0,16	0,03	0,01	-0,20
Entropy of Thymocytogram	0,09	0,01	0,08	-0,02	0,01	0,00	-0,02
Spleen Mass Index, mg/100 g BM	0,06	0,13	0,12	0,28	-0,03	-0,05	-0,26
Lymphoblastes of Spleen, %	-0,11	0,03	-0,16	-0,10	0,15	0,16	0,21
Lymphocytes of Spleen, %	0,01	-0,04	-0,12	**-0,38**	0,23	0,24	**0,42**
Plasmocytes of Spleen, %	-0,11	-0,10	-0,10	-0,05	-0,08	-0,08	0,04
Reticulocytes of Spleen, %	0,16	0,01	0,21	0,12	-0,12	-0,14	-0,23
Fibroblastes of Spleen, %	0,18	0,07	0,20	0,10	-0,02	-0,04	-0,17
Macrophages of Spleen, %	-0,07	-0,16	-0,01	0,01	-0,22	-0,22	-0,09
Microphages of Spleen, %	-0,11	0,13	-0,05	0,29	0,01	0,01	-0,18

Eosinophiles of Spleen, %	0,06	0,08	0,10	0,17	-0,03	-0,05	-0,18
Entropy of Splenocytogram	-0,02	0,07	0,09	**0,37**	-0,17	-0,19	**-0,38**
Monocytes of Blood, %	**0,48**	**0,40**	**0,52**	**0,40**	0,16	0,11	**-0,45**
Leukocytes of Blood, 10^9/L	-0,08	-0,16	-0,08	-0,13	-0,12	-0,11	0,08
Lymphocytes of Blood, %	-0,12	-0,23	-0,09	-0,11	-0,22	-0,22	0,02
Rod-shaped Neutrophils of Blood, %	**0,32**	0,17	**0,34**	0,14	0,05	0,02	-0,23
Segmented Neutrophiles of Blood, %	-0,11	0,08	-0,15	-0,02	0,15	0,17	0,14
Eosinophiles of Blood, %	-0,01	-0,02	-0,04	-0,12	0,06	0,07	0,14
Basophiles of Blood, %	**0,31**	0,24	**0,28**	0,08	0,21	0,19	-0,09
Entropy of Leukocytogram	**0,46**	**0,42**	**0,48**	**0,36**	0,22	0,18	-0,37
Natural Killer Lymphocytes, %	**0,43**	**0,41**	**0,45**	**0,38**	0,20	0,16	-0,38
T-helper Lymphocytes, %	0,05	-0,02	0,06	-0,00	-0,04	-0,04	-0,04
T-cytolytic Lymphocytes, %	0,15	-0,00	0,11	-0,13	0,05	0,04	0,07
B-Lymphocytes, %	**0,37**	0,04	**0,35**	-0,07	0,01	-0,02	-0,09
0-Lymphocytes, %	**-0,35**	-0,11	**-0,34**	-0,01	-0,06	-0,03	0,13
Entropy of Immunocytogram	-0,03	0,05	-0,07	-0,05	0,13	0,13	0,12
Microbian Count of Neutrophils, B/Ph	**-0,52**	**-0,41**	-0,55	**-0,37**	-0,18	-0,13	0,43
Phagocytic Index of Monocytes, %	-0,09	-0,06	-0,10	-0,08	-0,00	0,01	0,10
Microbian Count of Monocytes, B/Ph	**0,33**	0,24	**0,34**	0,21	0,11	0,08	-0,25
Killing Index of Neutrophils, %	0,13	0,26	0,14	0,22	0,18	0,17	-0,14
Phagocytic Index of Neutrophils, %	**-0,32**	-0,25	**-0,36**	**-0,30**	-0,06	-0,02	**0,35**

In this situation, the factor structure of the electrolyte root included, in addition to calcium, sulfate and magnesium, also bicarbonate (Table 7.43). The latter is responsible for reducing the content of lymphocytes in the spleen. In contrast, divalent ions are responsible for increasing the relative levels of 0- and B-lymphocytes, rod-shaped neutrophils, monocytes, basophils and natural killers, as well as activating two phagocytosis parameters: microbial count of monocytes and killing index of neutrophils in combination with inhibition of activity and intensity of phagocytosis of neutrophils and a decrease in thymus mass and its endothelial cell content.

Table 7.43. Factor structure of canonical correlation between roots, which represent the content of electrolytes in fluids and immune parameters

Electrolytes	Root 1
Ca^{2+}	**0,937**
SO_4^{2-}	**0,834**
Mg^{2+}	**0,353**
HCO_3^-	**-0,119**
Immune parameters	**Root 1**
0-Lymphocytes, %	**0,468**
B-Lymphocytes, %	**0,451**
Rod-shaped Neutrophils of Blood, %	**0,381**
Monocytes of Blood, %	**0,378**
Microbian Count of Monocytes, B/Ph	**0,355**
Basophiles of Blood, %	**0,341**
Natural Killer Lymphocytes, %	**0,308**
Killing Index of Neutrophils, %	**0,178**
Entropy of Leukocytogram	**0,101**
Microbian Count of Neutrophils, B/Ph	**-0,432**
Thymus Mass Index, mg/100 g BM	**-0,301**
Endotheliocytes of Thymus, %	**-0,288**
Phagocytosis Index of Neutrophils, %	**-0,189**
Lymphocytes of Spleen, %	**0,216**



R=0,812; R^2=0,659; $\chi^2_{(56)}$=87; p=0,005; Λ Prime=0,110

Fig. 7.11. Canonical correlation between roots representing electrolyte content (X-axis) and immune parameters (Y-axis)

Taken together, these electrolytes determine the selected constellation of immune parameters by 66% (Fig. 7.11).

Thus, by canonical correlation analysis of the relationship between the chemical composition of irritating fluids, on the one hand, and indicators of metabolism and neuroendocrine-immune complex of animals - on the other hand, found that the content of organic nitrogen and carbon in liquids determines levels of endocrine parameters on 39%, metabolic - 53%, immune - 77%. The degree of determination by trace elements is 59%, 32% and 84%, while by the electrolyte 33%, 36% and 66%, respectively.

The results of this study, in our opinion, prove the significant role of organic substances in drinking mineral waters in their immunomodulatory action. We assume that the following groups of compounds (found in the Naftussya water) are immunomodulators: alkylbenzene, alkenylbenzene, esters of aromatic acids, alkyl phenols, polyaromatic hydrocarbons, alkylnaphthalenes, unidentified polyaromatic hydrocarbons, the share of which in the total mass is 38% (Dats'ko et al., 2008), therefore, the intake of substances in the body is about 7,9 µg/kg•day.

This assumption is based on the fact that cells within the immune system, such as lymphocytes (T cells and B cells), macrophages, dendritic cells, granulocytes, and natural killer cells express **aryl hydrocarbon receptor** (AhR). The expression of the AhR in a majority of immune cell types and the expression of multiple xenobiotic- or dioxin-responsive elements (XREs/DREs) in the promoter region of many genes that regulate the immune response demonstrates the importance of this receptor in immunological processes (reviews: Quintana, Sherr, 2013; Esser, Rannug, 2015; Avilla et al., 2020). The most interesting feature of AhR ligands is their ability to induce the alteration M1/M2 macrophage polarization (Climaco-Arvizu et al., 2016; Yang et al., 2020) as well as the differentiation of T-regulatory vs Th-17 cells (reviews: Prasad et al., 2020; Abdulla et al., 2021).

Researchers have discovered a wide range of AhR ligands, both natural and synthetic, including environmental contaminants, dietary compounds, microbial byproducts, and endogenous mediators. Typically, components of environmental pollutants: polycyclic aromatic hydrocarbons such as benzo(a)pyrene, anthracene, and 3-methylcholanthrene as well as halogenated aromatic hydrocarbons such as polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls (review: Busbee et al., 2013).

As we can see, the presence of AhR ligands is quite probable in the constellation of organic substances found in the waters.

The AhR was first discovered in the early 1970s for its ability to bind 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin with high affinity and induce a xenobiotic-metabolizing enzyme known as aryl hydrocarbon hydroxylase. Initially, the AhR was thought to be involved predominantly in the metabolism of environmental chemicals. Researchers discovered a mechanism by which the AhR can direct the metabolism of xenobiotics by increasing the levels of cytochrome P450 enzymes, which then causes biotransformation and increases the water solubility of foreign chemicals, which leads to their expulsion (review: Busbee et al., 2013).

Back in 1990, we found in an experiment that both native Naftussya water and the organic substances isolated from it reduce the duration of nembutal sleep (Ivassivka et al., 1990; Popovych et al., 1990), which indicates the induction of hydroxylases.

Recently, in clinical observation, we found that course drinking of stable water solution of the Ozokerite, extracted from the Boryslav's field, imitates favorable effects of Naftussya water on parameters of immune and

autonomous nervous systems at volunteers with their dysfunction (Popovych et al., 2016; Popovych, 2018; 2019).

CHAPTER 8

SIMILAR EFFECTS OF SULFATE-CHLORIDE SODIUM-MAGNESIUM MINERAL WATERS MYROSLAVA AND KHRYSTYNA OF TRUSKAVETS' SPA ON METABOLISM AND NEUROENDOCRINE-IMMUNE COMPLEX IN FEMALE RATS

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 chapter is the first in a series of experimental and clinical studies of their physiological activity in line with the concepts of neuroendocrine-immune complex and functional-metabolic continuum.

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 8.1.

Table 8.1. 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 experiment was performed according to a similar algorithm.

8.1. Neuroendocrine and metabolic effects

This chapter 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. 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 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 8.2 and 8.3). The rest of the registered parameters were outside the discriminant model (Tables 8.3-8.7).

Table 8.2. 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	4,23	3,54	3,38	0,157	0,673	7,28	0,003	0,355

Plasma, **mM/L**	1 0	0,84 -0,98	0,80 -1,21					
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 8.3. 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}

Table 8.4. 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}	3,63	3,63	3,50	0,103	0,973	0,41	0,668	0,582

as Calcitonin Activity	1 0	1,00 0,00	0,97 -0,15					
(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 8.5. 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	Partial Λ	F to enter	p-level	Tolerancy
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 8.6. 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 8.7. 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	58,0 1 0	58,2 1,00 +0,02	53,8 0,93 -0,39	0,106	0,998	0,03	0,972	0,602
Malondyaldehide Plasma, µM/L	63 1 0	79 1,25 +0,74	68 1,08 +0,24	0,105	0,992	0,11	0,896	0,205
Creatinine Plasma, µM/L	72,5 1 0	92 1,26 +0,79	76 1,05 +0,14	0,104	0,983	0,25	0,778	0,483
Bilirubin Plasma, µM/L	4,63 1 0	4,65 1,00 +0,01	4,34 0,94 -0,11	0,101	0,958	0,63	0,540	0,614
Urea Plasma, mM/L	7,42 1 0	9,46 1,27 +1,19	8,32 1,12 +0,53	0,105	0,994	0,08	0,918	0,034
Middle Mass Molecules Plasma,	154 1	175 1,14	129 0,84	0,099	0,940	0,93	0,406	0,112

units	0	+0,41	-0,48					
Glucose Plasma, mM/L	4,95 1 0	5,49 1,11 +0,49	5,39 1,09 +0,40	0,100	0,943	0,88	0,424	0,022
Amylase Activity Plasma, g/h•L	152 1 0	154 1,02 +0,10	159 1,05 +0,30	0,103	0,970	0,45	0,641	0,701
Uric Acid Plasma, µM/L	662 1 0	620 0,94 -0,12	787 1,19 +0,37	0,105	0,988	0,18	0,839	0,038
Diuresis, mL/24h•100 g Body Mass	1,44 1 0	1,48 1,03 +0,05	1,83 1,27 +0,43	0,103	0,976	0,36	0,699	0,226
Canalicular Reabsorbtion, %	98,7 1 0	98,6 1,00 -0,05	98,9 1,00 +0,23	0,104	0,986	0,20	0,817	0,453
$(Ca•UA/Mg•Cr)^{0,25}$ as Lithogenicity Urine Index	0,90 1 0	0,90 1,00 0,00	0,82 0,91 -0,31	0,100	0,948	0,79	0,462	0,684

The dividing information contained in 18 variables is condensed in 2 canonical discriminant roots (Table 8.8). 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 8.8 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. 8.1).

Table 8.8. 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 8.9 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. 8.1) reflects their maximally elevated levels of testosterone, circulating catecholamines, 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 (Polovynko IS, 2016; Polovynko IS et al., 2016; Gozhenko AI et al., 2019; Popovych IL et al., 2020).

Metabolic manifestations of chronic stress, apparently, are increased plasma levels of urea, creatinine and malonic dialdehyde as well as catalase activity in plasma and urine.

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

Table 8.9. 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 Catecholamines	**-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
Malonic dyaldehide 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**	0
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.

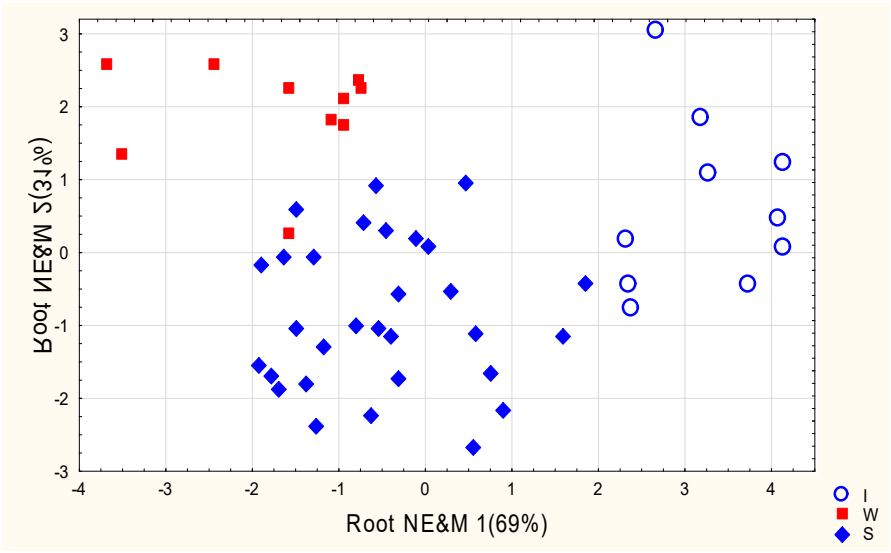


Fig. 8.1. Individual values of the first and second roots of the neuroendocrine and metabolic parameters in intact rats (I) and loaded with Daily water (W) and Salt waters (S)

Figure 8.2 illustrates the lack of differences between the two mineral waters in the set of discriminant variables.

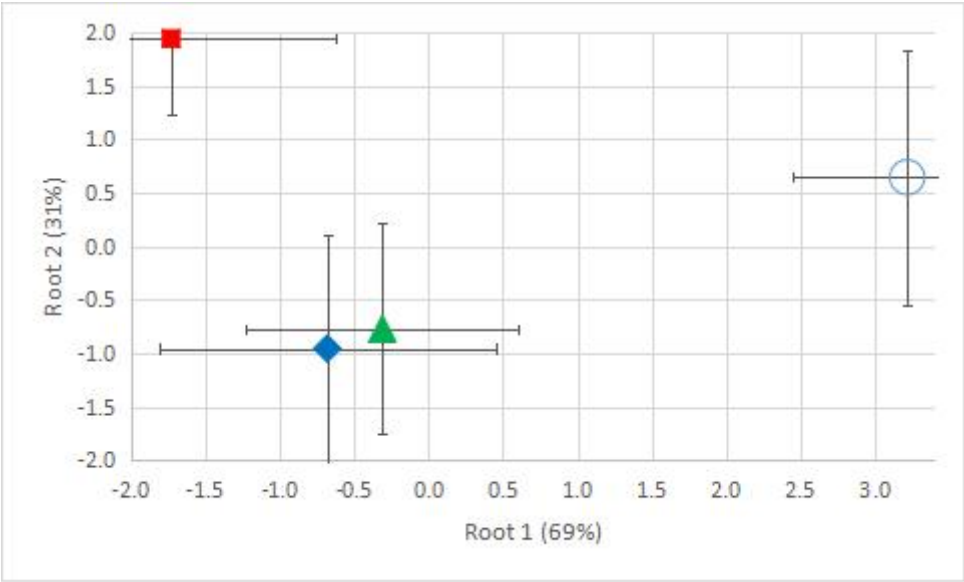


Fig. 8.2. Average values (Mean±SD) of the first and second roots of the neuroendocrine and metabolic parameters in intact rats (O) and loaded with Daily water and Salt waters Myroslava or Khrystyna

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 8.10).

Table 8.10. 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 8.11) enables the retrospective identification of intact rats unmistakable, and the other two groups - with a single error (Table 8.12).

Table 8.11. 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 8.12. 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

8.2. Immunotropic effects

Following the accepted algorithm, in the first stage of the analysis, both research groups were combined into the group "Salt Waters". The method of discriminant analysis revealed 12 parameters, according to which the immune status of animals loaded with mineral waters and tap water, as well as intact, differ significantly from each other.

Two parameters of **thymocytogram** and **splenocytogram**, 7 parameters of **leukocytogram and phagocytosis**, and also parameter of **immunocytogram** of blood were recognizable (Tables 8.13-8.14).

Table 8.13. Discriminant Function Analysis Summary
Step 12, N of Variables currently in the model: 12; Grouping: 3 groups
Wilks' Lambda: 0,2735; approx. $F_{(25)}$=2,74; p=0,0005

Variables currently in the model	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Salt Waters (30)	Daily Water (10)	Wilks' Λ	Par-tial Λ	F-re-move (2,36)	p-level	Tole-rancy
Microbial Count Neutrophils, Bacteria/Phagocyte	8,61 0	7,40,86 -0,65	8,20,95 -0,21	0,525	0,521	16,5	10^{-5}	0,104
Monocytes Blood, %	4,801 0	5,101,06 +0,10	4,200,88 -0,20	0,329	0,832	3,64	0,036	0,133
Phagocytic Index Neutrophils, %	69,51 0	69,10,99 -0,10	71,91,03 +0,56	0,406	0,674	8,69	0,001	0,259
Eosinophils Blood, %	4,601 0	3,630,79 -0,32	3,800,83 -0,27	0,295	0,926	1,44	0,250	0,754
Plasmocytes Thymus, %	1,801 0	1,971,09 +0,21	2,441,36 +0,82	0,377	0,726	6,81	0,003	0,513
Macrophages Spleen, %	7,901 0	8,131,03 +0,15	9,101,15 +0,75	0,379	0,721	6,96	0,003	0,604
Entropy Leukocytogram	0,5961 0	0,5710,96 -0,42	0,5570,94 -0,66	0,284	0,963	0,69	0,507	0,825
Phagocytic Index Monocytes %	2,901 0	2,830,98 -0,10	2,750,95 -0,21	0,300	0,910	1,77	0,184	0,656
NK Lymphocytes Blood, %	15,61 0	16,31,04 +0,25	14,80,95 -0,30	0,299	0,915	1,67	0,203	0,124
Lymphocytes Thymus, %	70,31 0	68,80,98 -0,61	69,30,99 -0,43	0,311	0,880	2,45	0,101	0,587
Basophiles Blood, %	0,301 0	0,431,44 +0,28	0,301,00 0,00	0,306	0,893	2,15	0,131	0,561
Reticulocytes Spleen, %	14,31 0	15,11,05 +0,41	14,81,03 +0,26	0,303	0,903	1,93	0,160	0,653

Table 8.14. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Microbial Count Neutrophils, Bac/Phag	3,95	0,026	0,856	3,95	0,026
Monocytes Blood, %	5,07	0,010	0,701	4,46	0,002
Phagocytic Index Neutrophils, %	3,19	0,051	0,614	4,14	0,001
Eosinophiles Blood, %	2,69	0,079	0,547	3,87	0,001
Plasmocytes Thymus, %	2,32	0,111	0,494	3,64	10^{-4}
Macrophages Spleen, %	2,58	0,087	0,440	3,55	10^{-4}
Entropy Leukocytogram	1,74	0,188	0,405	3,34	10^{-4}
Phagocytic Index Monocytes, %	1,53	0,230	0,377	3,15	10^{-4}
NK Lymphocytes Blood, %	1,79	0,180	0,345	3,04	10^{-4}
Lymphocytes Thymus, %	1,25	0,297	0,324	2,88	10^{-4}
Basophiles Blood, %	1,27	0,293	0,303	2,75	0,001
Reticulocytes Spleen, %	1,93	0,160	0,274	2,74	0,001

The rest of the registered immunity parameters turned out to be outside the discriminant model, despite the fact that some of them carry identifying information (Tables 8.15-8.18).

Table 8.15. Immune Variables of Thymus currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Salt Waters (30)	Daily Water (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Thymus Mass Index, mg/100g Body Mass	28,51 0	27 0,96 -0,10	32 1,14 +0,34	0,263	0,963	0,67	0,520	0,695
Epitheliocytes Thymus, %	8,80 0	9,67 1,10 +0,44	8,79 1,00 -0,01	0,272	0,993	0,12	0,884	0,392
Lymphoblastes Thymus, %	7,40 0	6,93 0,94 -0,55	7,22 0,98 -0,21	0,261	0,953	0,87	0,430	0,763
Reticulocytes Thymus, %	4,70 0	4,83 1,03 +0,08	4,44 0,95 -0,15	0,273	0,997	0,04	0,956	0,674
Endotheliocytes Thymus, %	2,60 0	2,50 0,96 -0,10	3,00 1,15 +0,41	0,263	0,962	0,69	0,506	0,733
Macrophages Thymus, %	2,70 0	3,23 1,20 +0,40	3,00 1,11 +0,22	0,267	0,974	0,46	0,636	0,756
Hassal's corpuscles Thymus, %	1,70 0	2,02 1,19 +0,59	1,83 1,08 +0,25	0,267	0,977	0,41	0,667	0,385
Entropy Thymocytogram	0,538 0	0,559 1,04 +0,60	0,560 1,04 +0,61	0,269	0,985	0,26	0,769	0,043

Table 8.16. Immune Variables of Spleen currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Salt Waters (30)	Daily Water (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Spleen Mass Index, mg/100g Body Mass	312 0	289 0,93 -0,23	294 0,94 -0,18	0,263	0,961	0,71	0,497	0,647
Lymphocytes Spleen, %	48,7 0	48,5 1,00 -0,07	48,2 0,99 -0,18	0,270	0,988	0,22	0,804	0,576
Lymphoblastes Spleen, %	3,90 0	4,20 1,08 +0,25	3,80 0,97 -0,08	0,264	0,966	0,61	0,547	0,569
Plasmocytes Spleen, %	2,50 0	1,77 0,71 -0,46	2,00 0,80 -0,32	0,268	0,979	0,38	0,688	0,589
Fibroblastes Spleen, %	8,20 0	7,97 0,97 -0,11	7,90 0,96 -0,14	0,271	0,992	0,14	0,872	0,758
Microphages Spleen, %	13,0 0	12,9 0,99 -0,05	12,8 0,98 -0,14	0,269	0,983	0,31	0,736	0,654
Eosinophils Spleen, %	1,50 0	1,43 0,96 -0,06	1,40 0,93 -0,09	0,270	0,985	0,26	0,774	0,669

Entropy Splenocytogram	0,753 1 0	0,750 1,00 -0,12	0,750 1,00 -0,11	0,273	0,999	0,02	0,976	0,866

Table 8.17. Immune Variables of Blood currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	**Salt Waters (30)**	**Daily Water (10)**	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Blast Transformation T- Lymphocytes Blood, %	78,8 1 0	75,1 0,95 -0,52	78,5 1,00 -0,04	0,269	0,982	0,32	0,727	0,636
T helper Lymphocytes Blood, %	31,5 1 0	30,6 0,97 -0,28	30,5 0,97 -0,32	0,271	0,990	0,18	0,838	0,782
T cytolytic Lymphocytes Blood, %	16,0 1 0	16,2 1,01 +0,07	15,8 0,99 -0,08	0,269	0,984	0,28	0,755	0,700
B Lymphocytes Blood, %	16,0 1 0	16,1 1,00 +0,02	16,7 1,04 +0,24	0,269	0,985	0,26	0,770	0,613
Plasmocytes Blood, %	0,47 1 0	0,85 1,82 +0,83	0,86 1,84 +0,85	0,268	0,978	0,39	0,680	0,753
0-Lymphocytes Blood, %	22,2 1 0	21,4 0,96 -0,13	23,5 1,06 +0,21	0,269	0,985	0,27	0,763	0,888
Entropy Immunocytogram	0,874 1 0	0,883 1,01 +0,51	0,887 1,02 +0,76	0,273	0,999	0,02	0,980	0,680

Table 8.18. Variables of Leukocytogram and Phagocytosis currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	**Salt Waters (30)**	**Daily Water (10)**	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Leukocytes Blood, 10^9/L	12,68 1 0	11,02 0,87 -0,28	12,55 0,99 -0,02	0,261	0,955	0,83	0,446	0,734
Pan Lymphocytes Blood, %	60,7 1 0	59,4 0,98 -0,14	61,1 1,01 +0,04	0,263	0,963	0,67	0,518	0,667
Rod-shaped Neutrophils Blood, %	3,60 1 0	3,23 0,90 -0,34	3,20 0,89 -0,37	0,271	0,992	0,14	0,870	0,777
Polymorphonuclear Neutrophils Blood, %	26,0 1 0	28,1 1,08 +0,31	27,4 1,05 +0,21	0,260	0,949	0,93	0,402	0,734
Killing Index Neutrophils, %	50,7 1 0	54,6 1,08 +0,62	51,9 1,02 +0,19	0,259	0,947	0,99	0,383	0,790
Microbial Count Monocytes, Bacteria/Phagocyte	5,0 1 0	4,9 0,97 -0,07	3,8 0,76 -0,64	0,271	0,992	0,14	0,866	0,345

The dividing information contained in 12 variables is condensed in 2 canonical discriminant roots (Table 8.19). The major root contains 75% of discriminative opportunities (r*=0,770; Wilks' Λ=0,274; $\chi^2_{(24)}$=54; p=0,0005) and the minor root 25% (r*=0,572; Wilks' Λ=0,673; $\chi^2_{(11)}$=16; p=0,125).

At the next stage, using raw coefficients and constants (Table 8.19), individual values of discriminant roots were calculated, which allowed to visualize each rat in the information field of these roots (Fig. 8.3).

Table 8.19. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Microbial Count Neutrophils, Bac/Phag	-2,730	-0,734	-2,080	-0,559
Monocytes Blood, %	-1,058	-1,353	-0,437	-0,558
Phagocytic Index Neutrophils, %	1,435	-0,337	0,372	-0,087
Eosinophils Blood, %	-0,274	-0,405	-0,135	-0,199
Plasmocytes Thymus, %	0,903	-0,397	1,192	-0,525
Macrophages Spleen, %	0,735	-0,656	0,407	-0,363
Entropy Leukocytogram	-0,275	-0,019	-4,408	-0,312
Phagocytic Index Monocytes, %	0,443	-0,250	0,5118	-0,288
NK Lymphocytes Blood, %	-0,840	0,897	-0,388	0,414
Lymphocytes Thymus, %	0,529	-0,340	0,211	-0,135
Basophiles Blood, %	-0,517	0,312	-0,963	0,580
Reticulocytes Spleen, %	0,499	-0,039	0,268	-0,021
	Constants		-23,83	21,85
	Eigenvalues		1,459	0,487
Cumulative Proportions			0,750	1

Localization in the extreme right zone of the axis of the first root of rats loaded with tap water reflects the maximum increase in immune parameters that represent the root **directly**, and the maximum decrease in **inversely** correlated with the root parameters (Table 8.20).

In contrast, in rats of both experimental groups, these immune parameters did not differ significantly from normal or deviated to a lesser extent.

Since the control and intact animals received the same daily fresh water, the detected changes in immune parameters, apparently due to the adverse stress from the introduction of the tube into the stomach (Polovynko IS, 2016; Polovynko IS et al., 2016; Gozhenko AI et al., 2019; Popovych IL et al., 2020). Both mineral waters prevent or minimize the immunotropic effects of stress.

The other constellation of immune parameters was not affected at all or to a lesser extent by stress factors. Instead, they **decrease** or **increase** under the influence of mineral waters. This situation is illustrated by the top position of the rats loaded by them along the axis of the second root.

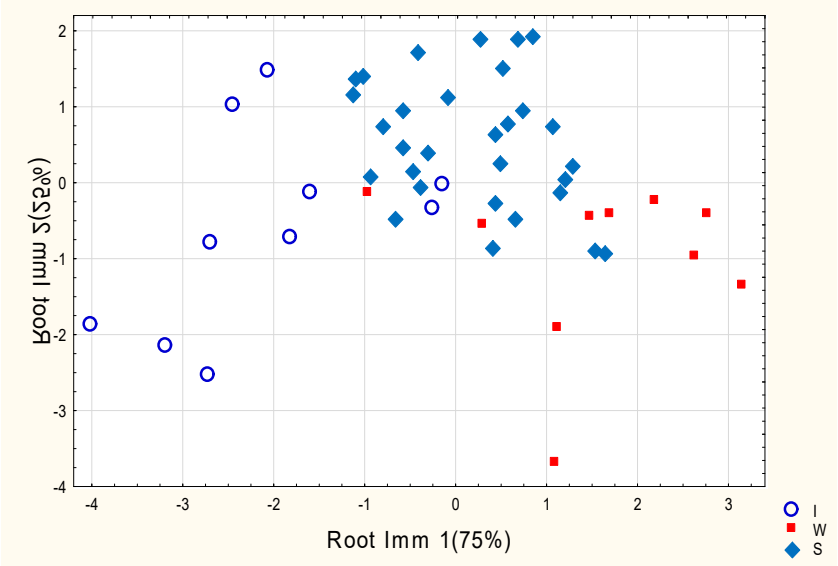


Fig. 8.3. Individual values of the first and second roots of the immune parameters in intact rats (I) and loaded with Daily water (W) and Salt waters (S)

Table 8.20. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots		Intact Rats (10)	Salt Waters (30)	Daily Water (10)
Root 1 (75%)	**R1**	**R2**	-2,09	+0,18	**+1,54**
Plasmocytes Thymus	**0,197**	-0,197	0	+0,21	**+0,82**
Macrophages Spleen	**0,160**	-0,204	0	+0,15	**+0,75**
Phagocytic Index Neutrophils	**0,131**	-0,356	0	-0,10	**+0,56**
Endotheliocytes Thymus			0	-0,10	**+0,41**
Entropy Immunocytogram			0	+0,51	**+0,76**
Entropy Leukocytogram	**-0,170**	-0,006	0	-0,42	**-0,66**
Phagocytic Index Monocytes	**-0,045**	0,022	0	-0,10	**-0,21**
Microbial Count Monocytes			0	-0,07	**-0,64**
Root 2 (25%)	**R1**	**R2**	-0,61	**+0,54**	-1,02
Microbial Count Neutrophils	-0,143	**-0,533**	0	**-0,65**	-0,21
Lymphocytes Thymus	-0,138	**-0,229**	0	**-0,61**	-0,43
Blast Transformation T- Lymphocytes			0	**-0,52**	-0,04
Eosinophiles Blood	-0,126	**-0,163**	0	**-0,32**	-0,27
Plasmocytes Spleen			0	**-0,46**	-0,32
Lymphoblastes Thymus			0	**-0,55**	-0,21
NK Lymphocytes Blood	-0,062	**0,400**	0	**+0,25**	-0,30
Monocytes Blood	-0,046	**0,198**	0	**+0,10**	-0,20
Basophiles Blood	0,020	**0,176**	0	**+0,28**	0,00
Epitheliocytes Thymus			0	**+0,44**	-0,01
Reticulocytes Spleen	0,093	**0,173**	0	**+0,41**	+0,26
Killing Index Neutrophils			0	**+0,62**	+0,19
Hassal's corpuscles Thymus			0	**+0,59**	+0,25

Both mineral waters have almost the same integral modulating effect on the listed immune parameters, as evidenced by the identity of the centroids of the first immune root and the absence of significant differences between the centroids of the second root (Fig. 8.4).

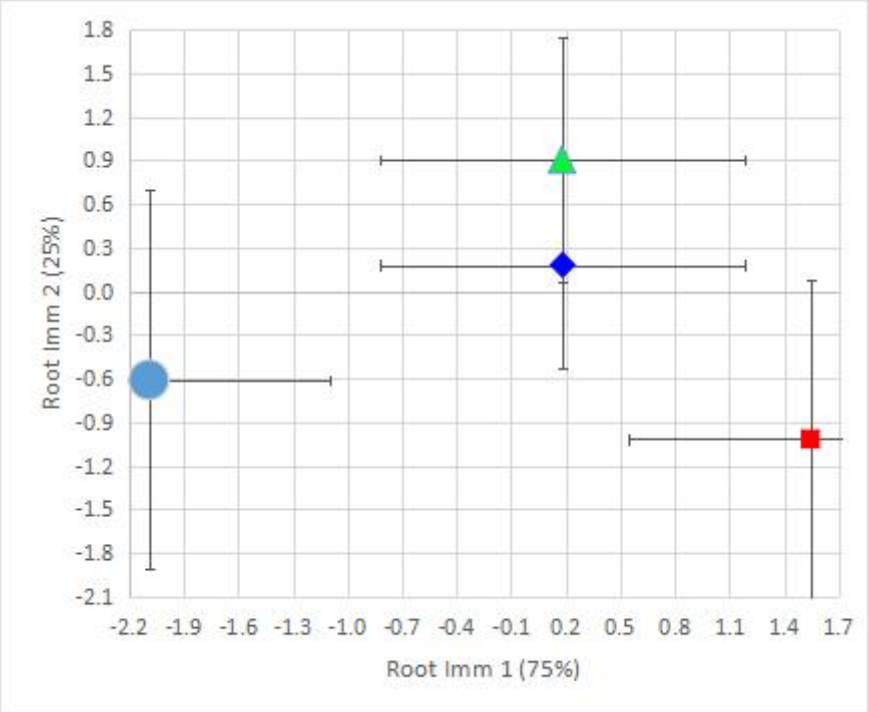


Fig. 8.4. Average values (Mean±SD) of the first and second roots of the immune parameters in intact rats (O) and loaded with Daily water and Salt waters Myroslava or Khrystyna

Despite the not very clear delineation of the three clusters, the differences between them are statistically significant (Table 8.21).

Table 8.21. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=12,4) and p-levels (under diagonal)

Groups	I (10)	DW (10)	SW (30)
Intact rats (I)	**0,0**	13,4	6,49
Daily Water (DW)	**4,27 ,0003**	**0,0**	4,29
Salt Waters (SW)	**3,11 ,004**	**2,05 ,048**	**0,0**

The application of the classifying functions (Table 10) enables the retrospective identification of intact rats with 3 errors, and the other two groups - with 2 errors. Total accuracy is 86% (Table 8.22).

Table 8.22. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact rats	**Daily Water**	**Salt Waters**
Microbial Count Neutrophils, Bac/Phag	-40,97	-48,30	-46,34
Monocytes Blood, %	-21,68	-23,04	-23,32
Phagocytic Index Neutrophils, %	22,76	24,14	23,50
Eosinophiles Blood, %	9,189	8,780	8,654
Plasmocytes Thymus, %	68,48	73,02	70,58
Macrophages Spleen, %	17,55	19,18	18,06
Entropy Leukocytogram	363,5	347,6	353,1
Phagocytic Index Monocytes, %	15,63	17,61	16,47
NK Lymphocytes Blood, %	26,28	24,70	25,88
Lymphocytes Thymus, %	21,93	22,75	22,25
Basophiles Blood, %	-69,75	-73,49	-71,27
Reticulocytes Spleen, %	20,34	21,32	20,92
Constants	-1958	-2053	-1984

Table 8.23. Classification Matrix

Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,20	DW p=,20	SW p=,60
Intact rats (I)	70,0	**7**	0	3
Daily Water (DW)	80,0	0	**8**	2
Salt Waters (SW)	93,3	0	2	**28**
Total	86,0	7	10	33

CHAPTER 9

PECULIARITIES OF EFFECTS OF SULFATE-CHLORIDE SODIUM-MAGNESIUM MINERAL WATERS MYROSLAVA AND KHRYSTYNA ON METABOLISM AND NEUROENDOCRINE-IMMUNE COMPLEX IN FEMALE RATS

Having clarified the general effects of mineral waters, consider their specific effects.

9.1. Neuro-endocrine and metabolic effects

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 was used. The forward stepwise program included 24 parameters in the model (Tables 9.1 and 9.2), 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 9.3-9.5).

Table 9.1. 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)	Myroslava (15)	Khrystyna (15)	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
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,	193 1	207 1,07	182 0,94	185 0,96	0,040	0,628	4,53	0,012	0,307

µM	0	+0,29	-0,25	-0,18					
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
Malonic dialdehyde 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 9.2. 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}
Malonic dialdehyde Urine	1,21	0,327	0,029	2,38	10^{-3}
Uric Acid Excretion	1,17	0,342	0,025	2,34	10^{-3}

Table 9.3. 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)	Myro slava (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 9.4. Discriminant Function Analysis Summary. Electrolytic variables currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Intact rats	Daily Water	Myro slava	Khry styna	Wilks' Λ	Parti-al Λ	F to en-	p-level	Tole-rancy

	(10)	(10)	(15)	(15)			ter		
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	0,28	0,839	0,497

Table 9.5. Discriminant Function Analysis Summary. Non-electrolytic variables currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Intact rats (10)	Daily Water (10)	Myroslava (15)	Khrystyna (15)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
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	0,06	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	0,00	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	0,40	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	0,28	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	0,97	0,423	0,260
Urea Plasma,	7,42 1	9,46 1,27	7,65 1,03	9,05 1,22	0,024	0,939	0,48	0,702	0,313

mM/L	0	+1,19	+0,13	+0,95					
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	0,43	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	0,65	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	0,38	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	0,57	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	0,51	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	0,48	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	0,04	0,988	0,393
Malonic dialdehyde 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	0,17	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	0,55	0,654	0,128

The dividing information contained in 24 variables is condensed in 3 canonical discriminant roots (Table 9.6). 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 9.6) 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 9.6. 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
Malonic dialdehyde 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 9.7 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.

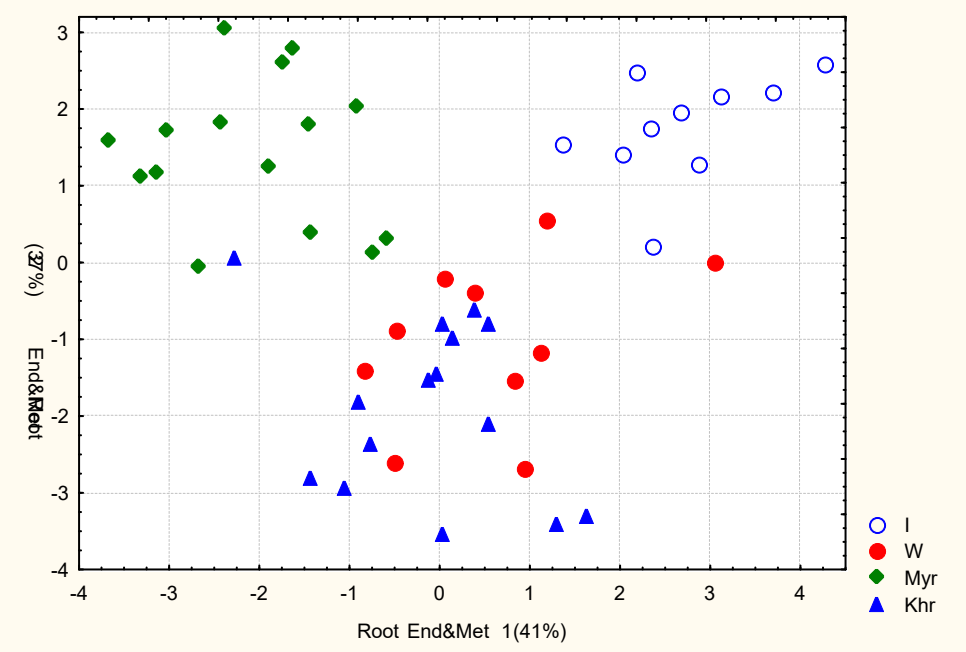
The localization of rats loaded with **Myroslava** water in the extreme left zone of the first root axis (Fig. 9.1 above) reflects their maximally reduced levels of erythrocyte superoxide dismutase activity and plasma corticosterone in combination with maximally elevated levels of uric acid and glucose, 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 9.7. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots			**Myro-slava**	**Khry-styna**	**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
Malonic dialdehyde 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
Malonic dialdehyde 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.



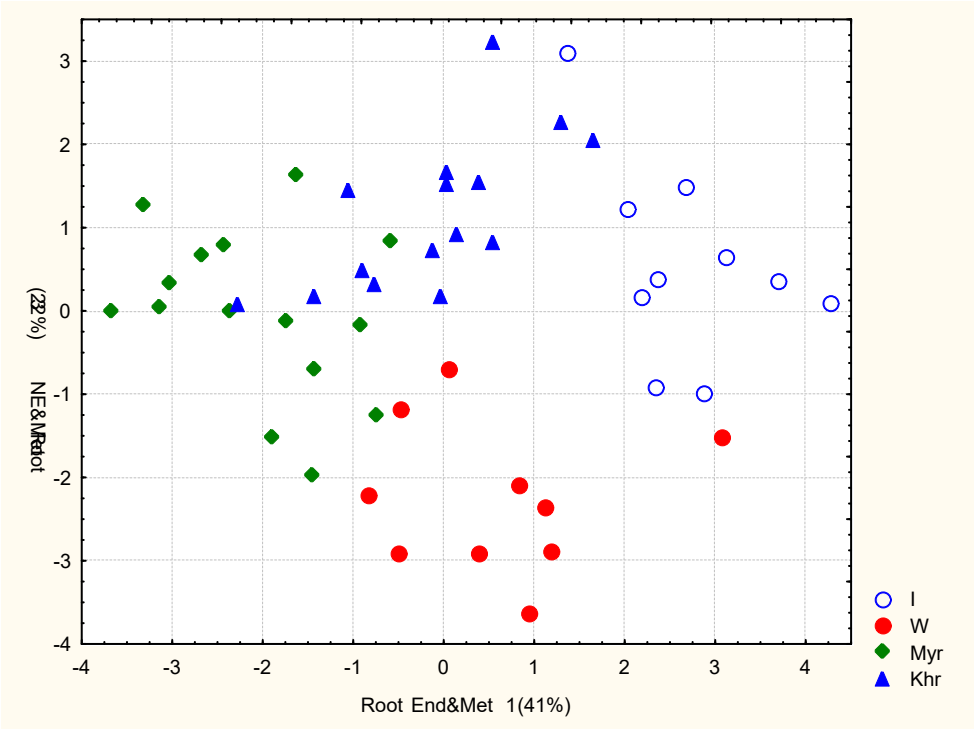


Fig. 9.1. Individual values of the first and second (above) and the first and third (below) roots of the endocrine and metabolic parameters in intact rats (o) and loaded with Daily water (W) and mineral waters Myroslava (Myr) and Khrystyna (Khr)

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. 9.1 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.

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 9.8).

Table 9.8. 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 9.9) enables the retrospective identification of the intact rats without mistake, and the latter with a single error (Table 9.10).

Table 9.9. 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 9.10. Classification Matrix

Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent 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 (Hrytsan II et al., 2019), 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.

9.2. Immunotropic effects

On the second stage, the immune parameters of all four groups were subjected to discriminant analysis. The program included 15 parameters in the model: 3 parameters of **thymocytogram**, 4 parameters of **splenocytogram**, 7 parameters of **leukocytogram and phagocytosis**, and also parameter of **immunocytogram** of blood (Tables 9.11-9.12).

Table 9.11. Discriminant Function Analysis Summary
Step 15, N of Variables currently in the model: 15; Grouping: 4 groups
Wilks' Lambda: 0,1528; approx. $F_{(46)}$=1,88; p=0,005

Variables currently in the model	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myroslava (15)	Khrystyna (15)	Intact rats (10)	Wilks' Λ	Partial Λ	F-remove (3,3)	p-level	Tolerancy
Microbial Count Neutrophils, Bac/Phag	8,2 0,95 -0,21	7,3 0,84 -0,70	7,5 0,87 -0,60	8,6 1 0	0,280	0,545	8,91	10^{-4}	0,119
Monocytes Blood, %	4,20 0,88 -0,20	4,87 1,01 +0,02	5,33 1,11 +0,18	4,80 1 0	0,195	0,785	2,93	0,049	0,106
Eosinophiles Blood, %	3,80 0,83 -0,27	3,33 0,72 -0,42	3,93 0,86 -0,22	4,60 1 0	0,184	0,829	2,20	0,107	0,735
Phagocytic Index Neutrophils, %	71,9 1,03 +0,56	68,9 0,99 -0,13	69,2 1,00 -0,07	69,5 1 0	0,192	0,796	2,73	0,060	0,313
Entropy Leukocytogram	0,557 0,94 -0,66	0,592 0,99 -0,07	0,552 0,93 -0,76	0,596 1 0	0,197	0,777	3,07	0,042	0,725
Macrophages Spleen, %	9,1 1,15 +0,75	7,9 1,00 +0,02	8,3 1,05 +0,27	7,9 1 0	0,203	0,751	3,54	0,026	0,507
Plasmocytes Thymus, %	2,44 1,36 +0,82	2,00 1,11 +0,25	1,93 1,07 +0,17	1,80 1 0	0,193	0,791	2,82	0,055	0,549
Leukocytes Blood, 10^9/L	12,55 0,99 -0,02	10,51 0,83 -0,36	11,53 0,91 -0,19	12,68 1 0	0,165	0,927	0,85	0,479	0,669
Eosinophiles Spleen, %	1,40 0,93 -0,09	1,73 1,16 +0,22	1,13 0,76 -0,34	1,50 1 0	0,169	0,903	1,15	0,343	0,747
NK Lymphocytes Blood, %	14,8 0,95 -0,30	16,3 1,04 +0,23	16,4 1,05 +0,26	15,6 1 0	0,179	0,853	1,83	0,161	0,099
Phagocytic Index Monocytes %	2,75 0,95 -0,21	2,83 0,98 -0,10	2,83 0,98 -0, 10	2,90 1 0	0,162	0,941	0,67	0,579	0,603
Spleen Mass Index, mg/100g Body Mass	294 0,94 -0,18	268 0,86 -0,44	309 0,99 -0,03	312 1 0	0,190	0,806	2,57	0,071	0,470
Lymphoblastes Spleen, %	3,80 0,97 -0,08	4,00 1,03 +0,08	4,40 1,13 +0,42	3,90 1 0	0,182	0,838	2,06	0,125	0,419
Lymphocytes Thymus, %	69,3 0,99 -0,43	68,2 0,97 -0,88	69,5 0,99 -0,33	70,3 1 0	0,188	0,813	2,45	0,081	0,417
Endotheliocytes Thymus, %	3,00 1,15 +0,41	2,47 0,95 -0,14	2,53 0,97 -0,07	2,60 1 0	0,172	0,887	1,37	0,269	0,507

Table 9.12. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Microbial Count Neutrophils, Bac/Phag	2,64	0,060	0,853	2,64	0,060
Monocytes Blood, %	4,43	0,008	0,658	3,49	0,004
Eosinophils Blood, %	2,63	0,062	0,558	3,23	0,002
Phagocytic Index Neutrophils, %	2,10	0,114	0,487	2,97	0,001
Entropy Leukocytogram	1,79	0,163	0,431	2,76	0,001
Macrophages Spleen, %	1,87	0,150	0,380	2,64	0,001
Plasmocytes Thymus, %	1,87	0,150	0,333	2,57	0,001
Leukocytes Blood, 10^9/L	1,24	0,309	0,304	2,41	0,001
Eosinophiles Spleen, %	1,07	0,372	0,280	2,26	0,002
NK Lymphocytes Blood, %	1,07	0,372	0,258	2,14	0,002
Phagocytic Index Monocytes, %	1,38	0,266	0,231	2,08	0,003
Spleen Mass Index, mg/100g Body Mass	1,09	0,367	0,211	2,00	0,003
Lymphoblastes Spleen, %	1,12	0,354	0,192	1,94	0,004
Lymphocytes Thymus, %	1,28	0,299	0,172	1,90	0,005
Endotheliocytes Thymus, %	1,37	0,269	0,153	1,88	0,005

To complete the picture, we present immune parameters not included in the model (Tables 9.13-9.16).

Table 9.13. Immune Variables of Thymus currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myroslava (15)	Khrystyna (15)	Intact rats (10)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Thymus Mass Index, mg/100g Body Mass	32,4 1,14 +0,34	27,0 0,95 -0,13	27,6 0,97 -0,08	28,5 1 0	0,149	0,976	0,25	0,860	0,677
Lymphoblastes Thymus, %	7,22 0,98 -0,21	6,93 0,94 -0,55	6,93 0,94 -0,55	7,40 1 0	0,148	0,968	0,34	0,796	0,801
Reticulocytes Thymus, %	4,44 0,95 -0,15	5,13 1,09 +0,25	4,53 0,96 -0,10	4,70 1 0	0,141	0,920	0,90	0,454	0,573
Epitheliocytes Thymus, %	8,78 1,00 -0,01	9,80 1,11 +0,50	9,53 1,08 +0,37	8,80 1 0	0,147	0,964	0,38	0,767	0,357
Macrophages Thymus, %	3,00 1,11 +0,22	3,47 1,28 +0,57	3,00 1,11 +0,22	2,70 1 0	0,143	0,936	0,71	0,555	0,632
Hassal's corpuscles Thymus, %	1,83 1,08 +0,25	2,00 1,18 +0,56	2,03 1,20 +0,62	1,70 1 0	0,144	0,941	0,65	0,588	0,578
Entropy Thymocytogram	0,560 1,04 +0,61	0,568 1,05 +0,85	0,551 1,02 +0,35	0,538 1 0	0,151	0,987	0,13	0,941	0,031

Table 9.14. Immune Variables of Spleen currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myroslava (15)	Khrystyna (15)	Intact rats (10)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Lymphocytes Spleen, %	48,2 0,99 -0,18	48,8 1,00 +0,04	48,2 0,99 -0,18	48,7 1 0	0,146	0,954	0,50	0,685	0,647
Plasmocytes Spleen, %	2,00 0,80 -0,32	1,73 0,69 -0,49	1,80 0,72 -0,44	2,50 1 0	0,144	0,941	0,65	0,590	0,450
Reticulocytes Spleen, %	14,8 1,03 +0,26	14,7 1,03 +0,23	15,4 1,08 +0,58	14,3 1 0	0,148	0,968	0,35	0,792	0,568
Fibroblastes Spleen, %	7,90 0,96 -0,14	8,07 0,98 -0,06	7,87 0,96 -0,16	8,20 1 0	0,152	0,993	0,08	0,973	0,746
Microphages Spleen, %	12,8 0,98 -0,14	13,0 1,00 0,00	12,9 0,99 -0,09	13,0 1 0	0,151	0,986	0,15	0,930	0,621
Entropy Splenocytogram	0,750 1,00 -0,11	0,750 1,00 -0,11	0,749 0,99 -0,14	0,753 1 0	0,149	0,972	0,29	0,831	0,494

Table 9.15. Immune Variables of Blood currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myroslava (15)	Khrystyna (15)	Intact rats (10)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Blast Transformation T-Lymphocytes Blood, %	78,5 1,00 -0,04	73,4 0,93 -0,75	76,8 0,97 -0,28	78,9 1 0	0,150	0,980	0,21	0,891	0,623
T helper Lymphocytes Blood, %	30,5 0,97 -0,32	30,7 0,97 -0,27	30,6 0,97 -0,29	31,5 1 0	0,152	0,997	0,03	0,991	0,711
T cytolytic Lymphocytes Blood, %	15,8 0,99 -0,08	15,6 0,98 -0,17	16,7 1,05 +0,31	16,0 1 0	0,144	0,942	0,64	0,595	0,655
B Lymphocytes Blood, %	16,7 1,04 +0,24	16,2 1,01 +0,07	15,9 1,00 -0,02	16,0 1 0	0,148	0,970	0,32	0,813	0,647
Plasmocytes Blood, %	0,86 1,84 +0,85	0,78 1,66 +0,66	0,93 1,97 +0,98	0,47 1 0	0,142	0,931	0,77	0,521	0,450
0-Lymphocytes Blood, %	23,5 1,06 +0,21	22,1 0,99 -0,02	20,7 0,93 -0,24	22,2 1 0	0,141	0,925	0,83	0,486	0,532
Entropy Immunocytogram	0,887 1,02 +0,76	0,886 1,01 +0,65	0,881 1,01 +0,37	0,887 1 0	0,145	0,951	0,53	0,665	0,516

Table 9.16. Variables of Leukocytogram and Phagocytosis currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myroslava (15)	Khrystyna (15)	Intact rats (10)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Pan Lymphocytes Blood, %	61,1 1,01 +0,04	59,7 0,98 -0,10	59,1 0,97 -0,17	60,7 1 0	0,146	0,955	0,48	0,696	0,633
Basophiles Blood, %	0,30 1,00 0,00	0,40 1,33 +0,21	0,47 1,56 +0,35	0,30 1 0	0,144	0,940	0,66	0,582	0,626
Rod-shaped Neutrophils Blood, %	3,20 0,89 -0,37	3,20 0,89 -0,37	3,27 0,91 -0,31	3,60 1 0	0,152	0,993	0,08	0,972	0,697
Polymorphonuclear Neutrophils Blood, %	27,4 1,05 +0,21	28,3 1,09 +0,34	27,9 1,07 +0,28	26,0 1 0	0,145	0,950	0,54	0,657	0,709
Killing Index Neutrophils, %	51,9 1,02 +0,19	53,4 1,05 +0,42	55,9 1,10 +0,81	50,7 1 0	0,143	0,934	0,74	0,539	0,758
Microbial Count Monocytes, Bacteria/Phagocyte	3,8 0,76 -0,64	4,8 0,97 -0,08	4,9 0,98 -0,05	5,0 1 0	0,145	0,948	0,57	0,638	0,322

The dividing information contained in 15 variables is condensed in 3 canonical discriminant roots (Table 9.17). The first root contains 53,6% of discriminative opportunities (r*=0,774; Wilks' Λ=0,153; $\chi^2_{(45)}$=74; p=0,004), the second 34,2% (r*=0,698; Wilks' Λ=0,381; $\chi^2_{(28)}$=38; p=0,097), the third 12,2% (r*=0,506; Wilks' Λ=0,744; $\chi^2_{(13)}$=12; p=0,553).

The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients (Table 9.17) to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots (Figs. 9.2-9.3).

Table 9.17. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Microbial Count Neutrophils, Bac/Phag	2,425	-0,709	0,482	1,831	-0,535	0,364
Monocytes Blood, %	0,821	-1,821	-0,219	0,336	-0,746	-0,090
Eosinophiles Blood, %	0,453	-0,467	0,121	0,222	-0,228	0,059
Phagocytic Index Neutrophils, %	-1,017	-0,249	0,099	-0,260	-0,064	0,025
Entropy Leukocytogram	0,523	0,456	0,407	8,584	7,483	6,668
Macrophages Spleen, %	-0,715	-0,610	-0,111	-0,393	-0,335	-0,061
Plasmocytes Thymus, %	-0,744	-0,274	0,223	-0,972	-0,358	0,292
Leukocytes Blood, 10^9/L	0,343	-0,278	-0,084	0,071	-0,058	-0,017
Eosinophiles Spleen, %	-0,086	0,066	0,695	-0,101	0,078	0,814
NK Lymphocytes Blood, %	1,123	1,199	0,298	0,513	0,548	0,136
Phagocytic Index Monocytes, %	-0,328	-0,215	-0,201	-0,375	-0,246	-0,230
Spleen Mass Index, mg/100g Body Mass	0,664	0,257	-0,678	0,010	0,004	-0,010
Lymphoblastes Spleen, %	0,564	0,274	-0,788	0,407	0,198	-0,569
Lymphocytes Thymus, %	-0,615	-0,659	0,207	-0,248	-0,266	0,084
Endotheliocytes Thymus, %	-0,358	-0,519	0,253	-0,394	-0,571	0,278
		Constants		7,663	22,85	-12,10
		Eigenvalues		1,495	0,951	0,345
	Cumulative Proportions			0,536	0,878	1

Table 9.18. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots			Daily Water	Myroslava	Khrystyna	Intact rats
Root 1 (53,6%)	**R1**	**R2**	**R3**	**-1,59**	-0,42	+0,13	+2,02
Plasmocytes Thymus	**-0,201**	-0,118	0,097	**+0,82**	+0,25	+0,25	0
Macrophages Spleen	**-0,147**	-0,184	-0,064	**+0,75**	+0,02	+0,15	0
Phagocytic Index Neutrophils	**-0,131**	-0,244	0,126	**+0,56**	-0,13	-0,03	0
Endotheliocytes Thymus	**-0,080**	-0,180	0,082	**+0,41**	-0,14	-0,10	0
Entropy Immunocytogram				**+0,76**	+0,65	+0,37	0
Thymus Mass Index				**+0,34**	-0,13	-0,08	0
B Lymphocytes Blood				**+0,24**	+0,07	-0,02	0
Monocytes Blood	**0,061**	0,086	-0,213	**-0,20**	+0,02	+0,09	0
Phagocytic Index Monocytes	**0,044**	0,017	0,003	**-0,21**	-0,10	-0,01	0
Microbial Count Monocytes				**-0,64**	-0,08	-0,05	0
Root 2 (34,0%)	**R1**	**R2**	**R3**	-1,29	**+1,24**	+0,03	-0,63
Microbial Count Neutrophils	0,137	**-0,350**	0,285	-0,21	**-0,70**	-0,54	0
Lymphocytes Thymus	0,164	**-0,243**	-0,113	-0,43	**-0,88**	-0,30	0
Spleen Mass Index	0,119	**-0,195**	-0,240	-0,18	**-0,44**	0,00	0
Leukocytes Blood	0,041	**-0,187**	0,030	-0,02	**-0,36**	-0,15	0
Eosinophils Blood	0,138	**-0,153**	-0,026	-0,27	**-0,42**	-0,20	0
Blast Transformation T-Lym				-0,04	**-0,75**	-0,28	0
Plasmocytes Spleen				-0,32	**-0,49**	-0,44	0
NK Lymphocytes Blood	0,072	**0,242**	-0,244	-0,30	**+0,23**	+0,15	0
Epitheliocytes Thymus				-0,01	**+0,50**	+0,37	0
Macrophages Thymus				+0,22	**+0,57**	+0,22	0
Reticulocytes Thymus				-0,15	**+0,25**	-0,10	0
Entropy Thymocytogram				+0,61	**+0,85**	+0,35	0
Root 3 (12,4%)	**R1**	**R2**	**R3**	+0,30	+0,37	**-0,86**	+0,43
Entropy Leukocytogram	0,132	0,142	0,439	-0,66	-0,07	**-0,76**	0
Eosinophils Spleen	-0,011	0,146	0,422	-0,09	+0,22	**-0,33**	0
0-Lymphocytes Blood				+0,21	-0,02	**-0,24**	0
Lymphoblastes Spleen	0,021	0,056	-0,281	-0,08	+0,08	**+0,38**	0
Killing Index Neutrophils				+0,19	+0,42	**+0,81**	0
T cytolytic Lymphocytes				-0,08	-0,17	**+0,31**	0
Reticulocytes Spleen				+0,26	+0,23	**+0,58**	0

As we can see, along the axis of the first root (Figs 9.2-9.3) immune clusters of intact and control rats are localized at opposite poles. This reflects the stress **activation/suppression** of 11 parameters, which is leveled or minimized to approximately the same extent by both mineral waters (Table 9.18).

Differences between the immunotropic effects of both mineral waters are visualized along the axes of the second and third roots. In particular, the top position of the Myroslava water cluster along the axis of the second root reflects the maximum **suppression/activation** of the constellation of 12 parameters, which is predominant under the influence of Khrystyna water. On the other hand, the lowest position of the Khrystyna water cluster along the axis of the third root reflects the maximum for sampling **suppression/activation** of another constellation of 7 parameters.

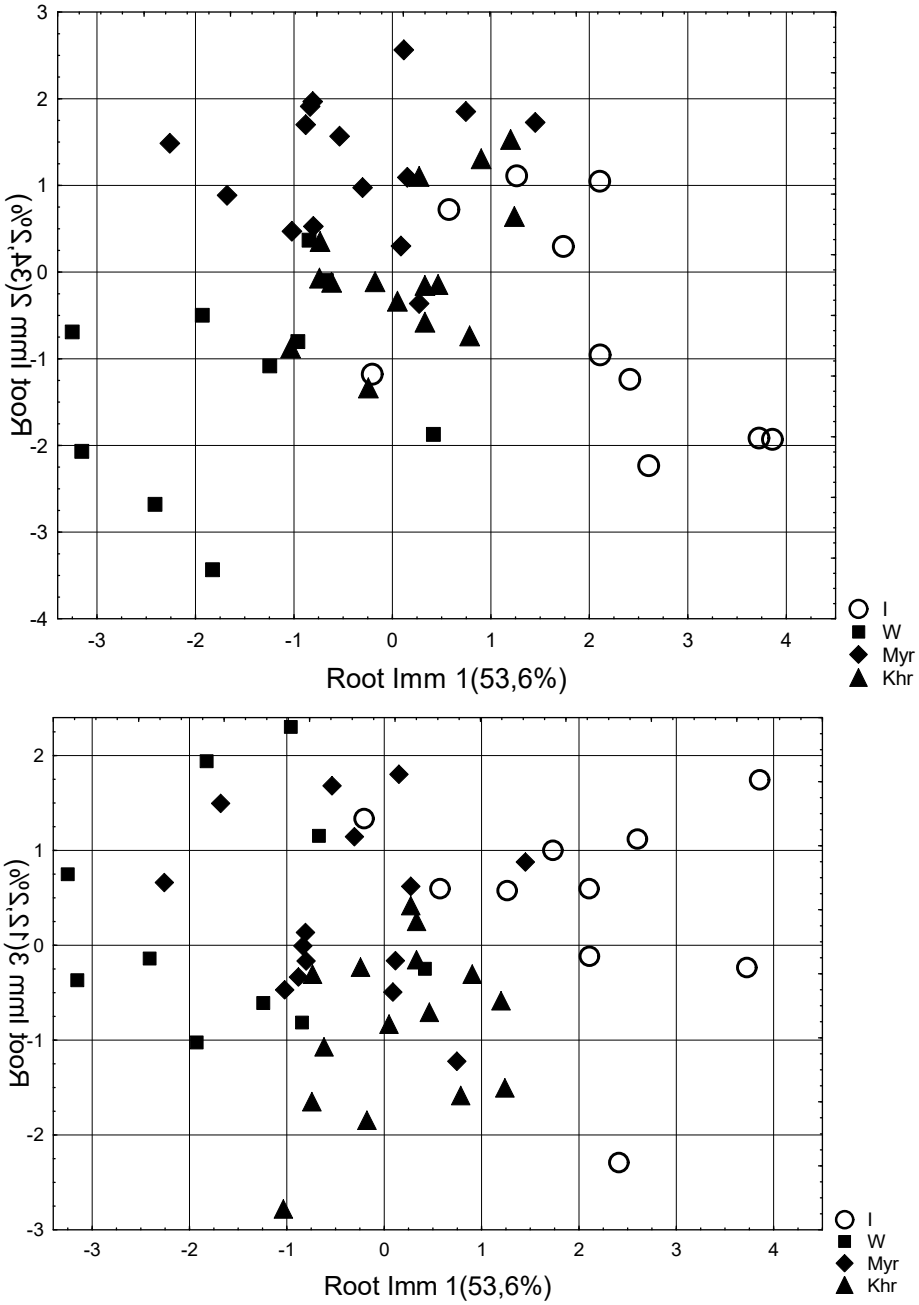


Fig. 9.2. Individual values of the first and second (above) and the first and third (below) roots of the immune parameters in intact rats (o) and loaded with Daily water (W) and mineral waters Myroslava (Myr) and Khrystyna (Khr)

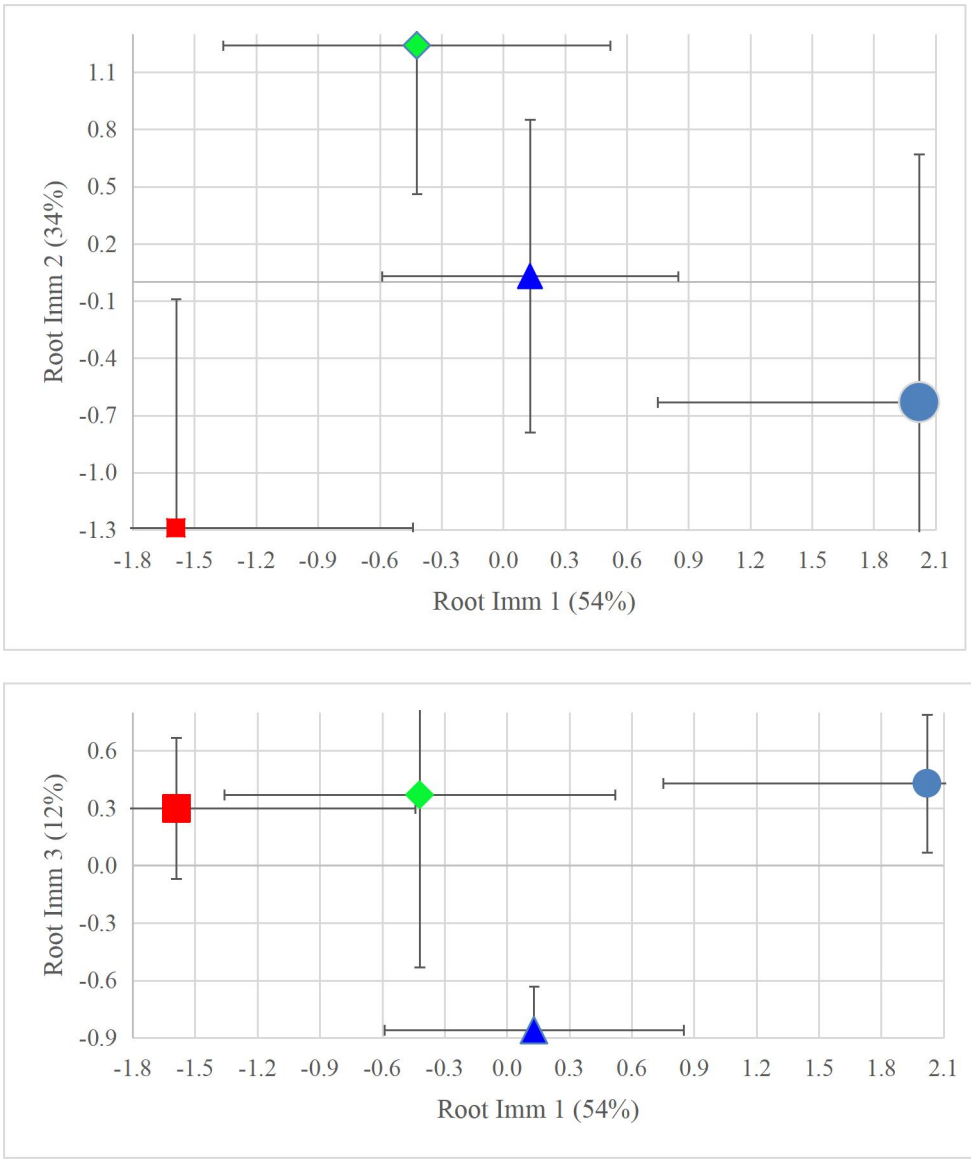


Fig. 9.3. Average values (Mean±SD) of the first and second (above) and the first and third (below) roots of the immune parameters in intact rats (o) and loaded with Daily water and mineral waters Myroslava and Khrystyna

However, judging by the distances of Mahalanobis (Table 9.19) and the accuracy of retrospective classification (Tables 9.20 and 9.21), the specificity of the immunomodulatory effects of mineral waters on the set of discriminant variables is not significant enough.

Table 9.19. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=15) and p-levels (under diagonal)

Groups	I (10)	DW (10)	Myr (15)	Khr (15)
Intact rats (I)	**0,0**	13,4	9,44	5,64
Daily Water (DW)	**3,12 ,003**	**0,0**	7,77	6,03
Water "Myroslava" (Myr)	**2,63 ,011**	**2,16 ,033**	**0,0**	3,29
Water "Khrystyna" (Khr)	**1,57 ,139**	**1,68 ,108**	**1,14 ,361**	**0,0**

Table 9.20. Coefficients and Constants for Classification Functions

Variables currently in the model	**Intact**	**Daily**	**Myro**	**Khry**

	rats	Water	slava	styna
Microbial Count Neutrophils, Bac/Phag	-5,543	-11,84	-11,03	-9,811
Monocytes Blood, %	-14,03	-14,73	-16,23	-15,03
Eosinophiles Blood, %	7,771	7,116	6,800	7,128
Phagocytic Index Neutrophils, %	15,53	16,51	16,05	15,95
Entropy Leukocytogram	94,33	57,53	86,99	74,49
Macrophages Spleen, %	9,139	10,79	9,474	9,738
Plasmocytes Thymus, %	46,53	50,23	48,21	47,75
Leukocytes Blood, 10^9/L	1,045	0,829	0,765	0,896
Eosinophils Spleen, %	26,64	26,84	26,98	25,83
NK Lymphocytes Blood, %	31,69	29,46	31,46	30,91
Phagocytic Index Monocytes, %	2,796	4,343	3,265	3,638
Spleen Mass Index, mg/100g Body Mass	-0,063	-0,098	-0,078	-0,066
Lymphoblastes Spleen, %	-15,83	-17,35	-16,42	-15,73
Lymphocytes Thymus, %	23,84	24,89	23,94	24,02
Endotheliocytes Thymus, %	23,92	25,68	23,79	23,93
Constants	-1714	-1755	-1688	-1696

Table 9.21. Classification Matrix
Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,20	DW p=,20	Myr p=,30	Khr p=,30
Intact rats (I)	70,0	**7**	1	2	
Daily Water (DW)	70,0	0	**7**	1	2
Myroslava (Myr)	80,0	0	0	**12**	3
Khrystyna (Khr)	86,7	0	0	2	**13**
Total	78,0	7	10	33	

Therefore, a different approach was used. It consists in creating 6 patterns of Z-scores of immune parameters (Table 9.18 and Fig. 9.4).

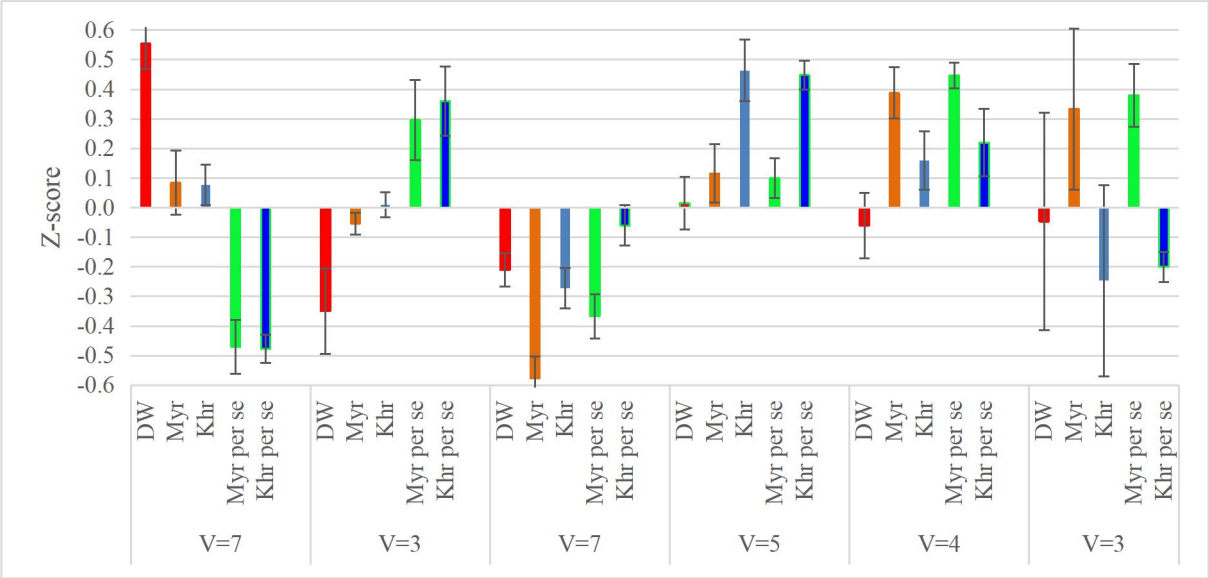


Fig. 9.4. Patterns (V - number of variables) of effects (Z±SD) of daily water and mineral waters and simulated partial effects of mineral waters

The first pattern shows how both mineral waters equally prevent the stress-induced increase in thymus mass and content in the thymocytogram of plasma cells and endothelial cells, in the splenocytogram macrophages, in the immunocytogram B-lymphocytes and its entropy, as well as the phagocytic index of blood neutrophils.

On the other hand (second pattern), they prevent a stress-induced decrease in blood cell counts and the activity and intensity of bacterial phagocytosis by monocytes.

The third pattern shows how Myroslava water significantly exacerbates the stress-induced decrease in lymphoblast of thymocytogram content, spleen mass and plasma cell of splenocytogram content, blood content of leukocytes in general and eosinophils in particular as well as the intensity of phagocytosis of bacteria by neutrophils and the transformation of T lymphocytes into blasts. On the other hand, Khrystyna water hardly potentiates the effect of stress on these parameters.

The fourth pattern demonstrates that stress-insensitive immune parameters (lymphoblast and reticulocyte content in splenocytogram, T cytolytic lymphocytes content in immunocytogram, and neutrophil killing index) increase (the content of 0-lymphocytes decreases) under the influence of mineral waters, and Khrystyna water is much more active than Myroslava water.

In contrast, Myroslava water is much more active than Khrystyna water in increasing the level in thymocytogram of epitheliocytes, macrophages and reticulocytes, as well as NK lymphocytes in the blood.

In addition, on the entropy of the leukocytogram and thymocytogram, as well as the content of eosinophils in the splenocytogram Khrystyna water has the opposite effect.

Calculating the algebraic difference between Z-scores of immune parameters in control and experimental groups allows us to estimate the partial immunotropic effects of mineral waters (Figs. 9.4 and 9.5).

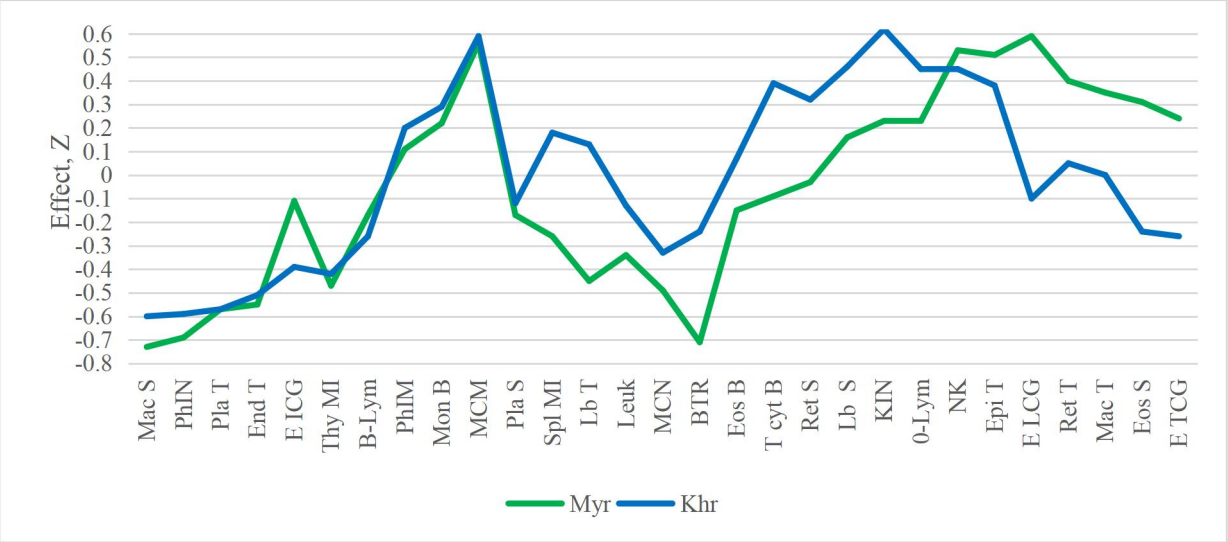


Fig. 9.5. Profiles of simulated immune Z-scores in rats after consumption of sulphate-chloride sodium-magnesium mineral waters Myroslava and Khrystyna

9.3. Integral assessment of specific effects of mineral waters

Thus, sulphate-chloride sodium-magnesium mineral waters Myroslava and Khrystyna have both common and specific modulating effects on the immune system of healthy female rats. The data obtained earlier on the same animals on the modulating neuroendocrine effects of these mineral waters give grounds to associate the identified immunotropic effects with them.

At the final stage, an integrated assessment of the specific effects of both mineral waters was performed.

Among the registered parameters, 7 neuroendocrine, 9 metabolic and 15 immune parameters (Tables 9.22 and 9.23) were identified by the method of discriminant analysis (forward stepwise program), according to which the intact, control and two main groups of animals differ significantly from each other.

Table 9.22. Discriminant Function Analysis Summary

Step 31, N of Variables currently in the model: 31; Grouping: 4 groups

Wilks' Lambda: 0,00387; approx. $F_{(93)}$=2,83; p=0,0001

Variables currently in the model	Groups (n)				Parameters of Wilks' Statistics				
	Khrystyna (15)	Myroslava (15)	Daily Water (10)	Intact rats (10)	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
Calcium Plasma, mM/L	2,51 0,75 -0,83	2,91 0,87 -0,43	2,08 0,62 -1,24	3,35 1 0	0,004	0,910	0,52	0,672	0,361
Superoxide Dismutase Erythrocytes, un/mL	57,7 0,99 -0,03	49,9 0,86 -0,75	58,2 1,00 +0,02	58,0 1 0	0,005	0,814	1,22	0,335	0,263
Microbial Count	7,6	7,3	8,2	8,6	0,005	0,822	1,16	0,357	0,070

Neutrophils, Bacteria/Phagocyte	0,88 -0,54	0,84 -0,70	0,95 -0,21	1 0					
Sodium Excretion, µM/24h•100 g Body Mass	271 2,01 +1,62	167 1,24 +0,39	76 0,56 -0,70	135 1 0	0,005	0,782	1,49	0,255	0,057
Monocytes Blood, %	5,07 1,06 +0,09	4,87 1,01 +0,02	4,20 0,88 -0,20	4,80 1 0	0,006	0,655	2,81	0,073	0,053
Eosinophiles Blood, %	4,00 0,87 -0,20	3,33 0,72 -0,42	3,80 0,83 -0,27	4,60 1 0	0,007	0,550	4,37	0,020	0,267
Potassium Plasma, mM/L	3,33 0,79 -1,27	3,42 0,81 -1,15	3,54 0,84 -0,98	4,23 1 0	0,006	0,647	2,91	0,067	0,344
$(Cap/Pp)^{0.5}$ as Parathyroid Activity	1,75 0,68 -0,70	1,91 0,75 -0,56	1,58 0,62 -0,84	2,56 1 0	0,008	0,478	5,82	0,007	0,181
Testosterone Plasma, nM/L	4,50 1,15 +0,53	4,97 1,27 +0,98	6,04 1,54 +1,97	3,93 1 0	0,007	0,518	4,96	0,013	0,193
NK Lymphocytes Blood, %	16,1 1,03 +0,15	16,3 1,04 +0,23	14,8 0,95 -0,30	15,6 1 0	0,006	0,698	2,30	0,116	0,043
Malondialdehyde Urine, µM/L	96 1,04 +0,09	88 0,95 -0,10	75 0,81 -0,40	92 1 0	0,007	0,528	4,77	0,015	0,127
Leukocytes Blood, 10^9/L	11,76 0,93 -0,15	10,51 0,83 -0,36	12,55 0,99 -0,02	12,68 1 0	0,004	0,920	0,47	0,710	0,510
Spleen Mass Index, mg/100g Body Mass	312 1,00 0,00	268 0,86 -0,44	294 0,94 -0,18	312 1 0	0,004	0,902	0,58	0,635	0,365
Amylase Activity Urine, g/h•L	204 1,01 +0,02	204 1,01 0,04	217 1,07 +0,26	202 1 0	0,009	0,437	6,86	0,003	0,092
Katalase Activity Plasma, µM/h•L	128 1,24 +0,88	122 1,18 +0,67	148 1,43 +1,58	103 1 0	0,007	0,556	4,25	0,022	0,219
Chloride Excretion, µM/24h•100 g Body Mass	244 1,69 +1,02	195 1,35 +0,51	107 0,74 -0,38	144 1 0	0,007	0,552	4,34	0,020	0,062
Triiodothyronine Plasma, nM/L	2,38 1,11 +0,42	2,31 1,08 +0,30	2,11 0,99 -0,05	2,14 1 0	0,006	0,677	2,55	0,092	0,045
Corticosterone Plasma, nM/L	460 0,96 -0,17	365 0,76 -0,92	383 0,80 -0,78	482 1 0	0,006	0,684	2,46	0,100	0,332
Glucose Plasma, mM/L	5,22 1,05 +0,25	5,55 1,12 +0,55	5,49 1,11 +0,49	4,95 1 0	0,006	0,641	2,98	0,063	0,265
Phagocytic Index Monocytes %	2,89 1,00 -0,01	2,83 0,98 -0,10	2,75 0,95 -0,21	2,90 1 0	0,006	0,687	2,43	0,103	0,269
Sodium Erythrocytes, mM/L	24,2 1,10 +0,51	21,8 0,99 -0,04	22,6 1,03 +0,13	22,0 1 0	0,006	0,600	3,56	0,038	0,116
Amylase Activity Plasma, g/h•L	163 1,07 +0,46	155 1,02 +0,14	154 1,02 +0,10	152 1 0	0,005	0,717	2,10	0,140	0,266
Macrophages	8,1	7,9	9,1	7,9	0,005	0,759	1,70	0,208	0,247

Spleen, %	1,03 +0,15	1,00 +0,02	1,15 +0,75	1 0					
Phagocytic Index Neutrophils, %	69,4 1,00 -0,03	68,9 0,99 -0,13	71,9 1,03 +0,56	69,5 1 0	0,007	0,533	4,67	0,016	0,092
Reticular Zone of Adrenal Cortex, µM	42 0,98 -0,12	44 1,04 +0,20	40 0,95 -0,29	43 1 0	0,006	0,609	3,42	0,043	0,219
Entropy Leukocytogram	0,551 0,93 -0,76	0,592 0,99 -0,07	0,557 0,94 -0,66	0,596 1 0	0,006	0,622	3,24	0,050	0,295
Plasmocytes Thymus, %	2,00 1,11 +0,25	2,00 1,11 +0,25	2,44 1,36 +0,82	1,80 1 0	0,006	0,690	2,40	0,106	0,300
Eosinophiles Spleen, %	1,14 0,76 -0,33	1,73 1,16 +0,22	1,40 0,93 -0,09	1,50 1 0	0,006	0,684	2,46	0,100	0,352
Glomerular Zone of Adrenal Cortex, µM	185 0,96 -0,18	182 0,94 -0,25	207 1,07 +0,29	193 1 0	0,006	0,651	2,87	0,069	0,298
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,42 0,99 -0,02	1,37 0,95 -0,08	2,34 1,63 +1,09	1,44 1 0	0,005	0,781	1,49	0,254	0,172
Magnesium Urine, mM/L	2,89 1,13 +0,18	2,49 0,97 -0,04	2,34 0,91 -0,12	2,56 1 0	0,005	0,818	1,19	0,346	0,118

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 9.23. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Calcium Plasma, mM/L	4,49	0,008	0,773	4,49	0,008
Superoxide Dismutase Erythrocytes, un/mL	4,18	0,011	0,605	4,29	0,001
Microbial Count Neutrophils, Bac/Phag	3,38	0,027	0,492	4,03	10^{-4}
Sodium Excretion, µM/24h•100 g Body Mass	3,88	0,015	0,387	4,10	10^{-4}
Monocytes Blood, %	3,07	0,038	0,317	4,00	10^{-5}
Eosinophiles Blood, %	2,49	0,074	0,268	3,83	10^{-5}
Potassium Plasma, mM/L	2,04	0,124	0,233	3,63	10^{-5}
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	2,68	0,060	0,193	3,62	10^{-5}
Testosterone Plasma, nM/L	2,07	0,121	0,166	3,51	10^{-5}
NK Lymphocytes Blood, %	2,21	0,103	0,141	3,46	10^{-6}
Malondialdehyde Urine, µM/L	2,37	0,087	0,118	3,46	10^{-6}
Leukocytes Blood, 10^9/L	1,69	0,186	0,103	3,36	10^{-6}
Spleen Mass Index, mg/100g Body Mass	1,70	0,185	0,089	3,28	10^{-6}
Amylase Activity Urine, g/h•L	1,79	0,168	0,077	3,23	10^{-6}
Katalase Activity Plasma, µM/h•L	1,25	0,307	0,069	3,12	10^{-5}
Chloride Excretion, µM/24h•100 g Body Mass	1,74	0,179	0,059	3,09	10^{-5}
Triiodothyronine Plasma, nM/L	1,57	0,217	0,051	3,04	10^{-5}
Corticosterone Plasma, nM/L	1,55	0,224	0,044	3,00	10^{-5}
Glucose Plasma, mM/L	1,31	0,292	0,038	2,93	10^{-5}
Phagocytic Index Monocytes, %	1,38	0,270	0,033	2,89	10^{-5}
Sodium Erythrocytes, mM/L	1,63	0,207	0,028	2,88	10^{-5}
Amylase Activity Plasma, g/h•L	1,59	0,217	0,024	2,87	10^{-5}
Macrophages Spleen, %	1,11	0,363	0,021	2,80	10^{-5}
Phagocytic Index Neutrophils, %	1,27	0,308	0,018	2,76	10^{-5}
Reticular Zone of Adrenal Cortex, µM	1,61	0,216	0,015	2,77	10^{-5}
Entropy Leukocytogram	1,43	0,262	0,012	2,76	10^{-4}

Plasmocytes Thymus, %	1,38	0,279	0,010	2,74	10^{-4}
Eosinophiles Spleen, %	1,36	0,285	0,008	2,73	10^{-4}
Glomerular Zone of Adrenal Cortex, μM	2,76	0,072	0,006	2,92	10^{-4}
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,09	0,381	0,005	2,87	10^{-4}
Magnesium Urine, mM/L	1,19	0,346	0,004	2,83	10^{-4}

The dividing information contained in 31 variables is condensed in 3 canonical discriminant roots (Tables 9.24 and 9.25). The first root contains 53,0% of discriminative opportunities (r^*=0,950; Wilks' Λ=0,0039; $\chi^2_{(93)}$=175; $p<10^{-6}$), the second 28,9% (r^*=0,914; Wilks' Λ=0,0397; $\chi^2_{(60)}$=100; p=0,0006), the third 18,1% (r^*=0,871; Wilks' Λ=0,2406; $\chi^2_{(29)}$=45; p=0,030).

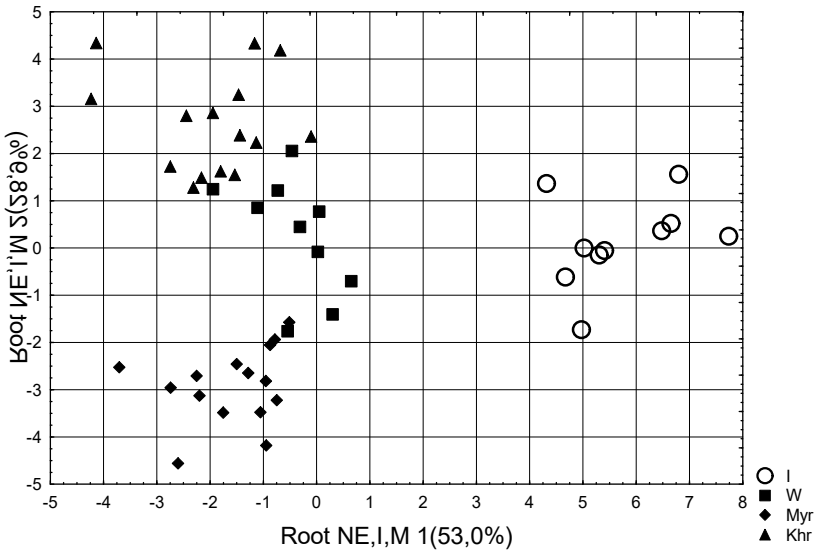
The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients (Table 9.24) to the individual values of discriminant variables together with the constants enables the visualization of each rat in the information space of the roots (Fig. 9.6).

Table 9.24. 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, mM/L	0,485	-0,198	-0,058	0,593	-0,243	-0,071
Superoxide Dismutase Erythrocytes, un/mL	-0,012	0,701	-0,625	-0,0013	0,079	-0,070
Microbial Count Neutrophils, Bac/Phag	1,398	0,690	0,702	1,055	0,521	0,530
Sodium Excretion, μM/24h•100 g	1,779	-0,952	0,524	0,010	-0,0055	0,0031
Monocytes Blood, %	2,493	-0,441	-1,006	1,021	-0,180	-0,412
Eosinophiles Blood, %	1,251	0,526	-0,241	0,612	0,257	-0,118
Potassium Plasma, mM/L	1,027	0,285	-0,079	1,344	0,373	-0,104
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	1,245	-1,222	0,551	1,812	-1,779	0,802
Testosterone Plasma, nM/L	1,136	-0,912	-0,913	0,549	-0,441	-0,442
NK Lymphocytes Blood, %	-1,105	1,289	2,461	-0,505	0,589	1,125
Malondialdehyde Urine, μM/L	0,765	1,886	0,528	0,023	0,058	0,016
Leukocytes Blood, 10^9/L	0,114	0,377	-0,188	0,024	0,078	-0,039
Spleen Mass Index, mg/100g Body Mass	0,181	0,436	0,327	0,0026	0,0063	0,0047
Amylase Activity Urine, g/h•L	-0,408	-2,591	0,686	-0,010	-0,065	0,017
Katalase Activity Plasma, μM/h•L	-0,670	-1,366	-0,280	-14,41	-29,38	-6,018
Chloride Excretion, μM/24h•100 g	-2,664	1,002	0,090	-0,018	0,0069	0,0006
Triiodothyronine Plasma, nM/L	-2,288	1,660	0,354	-5,598	4,062	0,866
Corticosterone Plasma, nM/L	-0,402	0,905	0,398	-0,0024	0,0055	0,0024
Glucose Plasma, mM/L	-1,140	-0,337	0,335	-1,375	-0,407	0,405
Phagocytic Index Monocytes, %	-0,764	0,094	-0,908	-0,874	0,108	-1,038
Sodium Erythrocytes, mM/L	-0,591	1,534	1,238	-0,123	0,320	0,258
Amylase Activity Plasma, g/h•L	-0,523	0,715	0,714	-0,015	0,021	0,021
Macrophages Spleen, %	-0,904	0,143	-0,541	-0,497	0,079	-0,298
Phagocytic Index Neutrophils, %	-1,157	2,080	-0,598	-0,296	0,533	-0,153
Reticular Zone of Adrenal Cortex, μM	-0,355	-1,414	-0,007	-0,033	-0,132	-0,001
Entropy Leukocytogram	0,754	-0,931	0,244	12,37	-15,26	3,994
Plasmocytes Thymus, %	-0,769	0,683	-0,384	-1,005	0,892	-0,503
Eosinophiles Spleen, %	0,112	-0,958	0,397	0,131	-1,121	0,465
Glomerular Zone of Adrenal Cortex, μM	0,273	1,032	-0,530	0,008	0,029	-0,015
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,076	0,519	-0,008	1,128	0,544	-0,008
Magnesium Urine, mM/L	1,208	0,111	-0,534	0,717	0,066	-0,317
		Constants		26,74	-54,47	-16,66
		Eigenvalues		9,26	5,06	3,16
	Cumulative Proportions			0,530	0,819	1

Table 9.25. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables Z-scores

	Correlations Variables-Roots			Khrystyna	Myroslava	Daily Water	Intact rats
Root 1 (53,0%)	**R1**	**R2**	**R3**	-1,96	-1,59	-0,41	+5,73
(Cap/Pp)^{0,5} as Parathyroid Act	**0,148**	-0,039	0,127	-0,70	-0,56	-0,84	**0**
Calcium Plasma	**0,112**	-0,084	0,212	-0,83	-0,43	-1,24	**0**
Potassium Plasma	**0,149**	-0,011	-0,003	-1,27	-1,15	-0,98	**0**
Microbial Count Neutrophils	**0,118**	0,040	-0,105	-0,54	-0,70	-0,21	**0**
Eosinophils Blood	**0,061**	0,057	0,006	-0,20	-0,42	-0,27	**0**
Glucose Plasma	**-0,070**	-0,074	-0,058	+0,25	+0,55	+0,49	**0**
Katalase Activity Plasma	**-0,068**	0,024	-0,120	+0,88	+0,67	+1,58	**0**
Amylase Activity Plasma	**-0,029**	0,038	0,027	+0,46	+0,14	+0,10	**0**
Root 2 (28,9%)	**R1**	**R2**	**R3**	+2,64	**-2,92**	+0,26	+0,15
Corticosterone Plasma	0,054	**0,104**	0,068	-0,17	**-0,92**	-0,78	0
SOD Erythrocytes	0,054	**0,166**	-0,082	-0,03	**-0,75**	+0,02	0
Sodium Erythrocytes	-0,030	**0,087**	0,021	+0,51	**-0,04**	+0,13	0
Spleen Mass Index	0,041	**0,110**	0,006	0,00	**-0,44**	-0,18	0
Leukocytes Blood	0,042	**0,044**	-0,056	-0,15	**-0,36**	-0,02	0
Eosinophils Spleen	0,015	**-0,126**	0,002	-0,33	**+0,22**	-0,09	0
Entropy Leukocytogram	0,060	**-0,114**	0,061	-0,76	**-0,07**	-0,66	0
Root 3 (18,1%)	**R1**	**R2**	**R3**	+1,06	+0,78	**-3,39**	+0,63
Testosterone Plasma	-0,059	-0,033	**-0,165**	+0,53	+0,98	**+1,97**	0
(Ku/Nau)^{0,5} as MC Activity	-0,004	0,021	**-0,228**	-0,02	-0,08	**+1,09**	0
Glomerular ZAC	0,028	0,025	**-0,136**	-0,18	-0,25	**+0,29**	0
Phagocytic Index Neutrophils	0,007	0,021	**-0,164**	-0,03	-0,13	**+0,56**	0
Plasmocytes Thymus	-0,034	-0,011	**-0,144**	+0,25	+0,25	**+0,82**	0
Macrophages Spleen	-0,024	0,045	**-0,127**	+0,15	+0,02	**+0,75**	0
Amylase Activity Urine	-0,008	-0,000	**-0,068**	+0,02	+0,04	**+0,26**	0
Triiodothyronine Plasma	-0,063	0,021	**0,117**	+0,42	+0,30	**-0,05**	0
Reticular ZAC	-0,000	-0,045	**0,052**	-0,12	+0,20	**-0,29**	0
Sodium Excretion	-0,062	0,095	**0,183**	+1,62	+0,39	**-0,70**	0
Chloride Excretion	-0,064	0,049	**0,163**	+1,02	+0,51	**-0,38**	0
Malondialdehyde Urine	0,004	0,037	**0,115**	+0,09	-0,10	**-0,40**	0
Magnesium Urine	-0,010	0,040	**0,048**	+0,18	-0,04	**-0,12**	0
NK Lymphocytes Blood	-0,036	-0,002	**0,151**	+0,15	+0,23	**-0,30**	0
Monocytes Blood	-0,014	0,029	**0,084**	+0,09	+0,02	**-0,20**	0
Phagocytic Index Monocytes	0,011	-0,001	**0,026**	-0,01	-0,10	**-0,21**	0



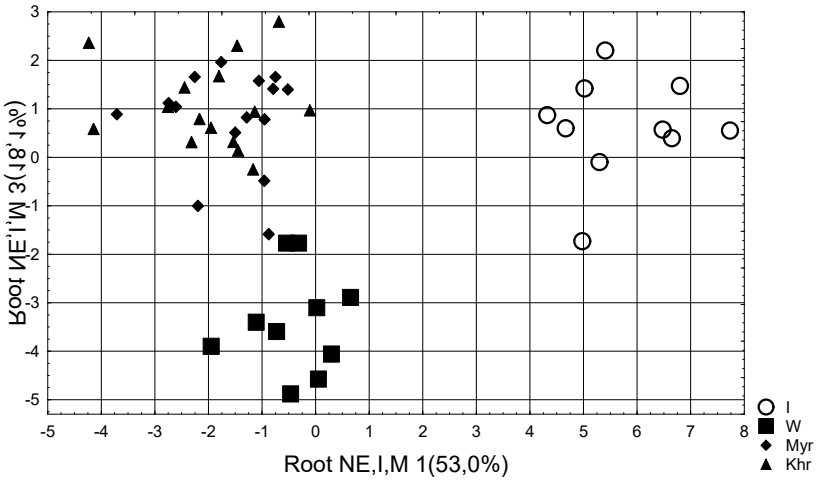


Fig. 9.6. Individual values of the first and second (above) and the first and third (below) roots of the endocrine and metabolic parameters in intact rats (o) and loaded with Daily water (W) and mineral waters Myroslava (Myr) and Khrystyna (Khr)

Pseudo-staining visualizes a combination of **hormonal**, **immune** and **metabolic** parameters in the structure of each root (Table 9.25), consistent with previously identified neuroendocrine-immune and neuroendocrine-metabolic linkages (Popovych IL, 2008; 2011).

As you can see (Fig. 9.6 above), along the axis of the first root of the rat, both control and both main groups, significantly distant from intact animals, while their projections on the axis are closely mixed.

This disposition reflects a decrease in parathyroid activity and plasma calcium and potassium levels, as well as eosinophils in the blood and the intensity of bacterial phagocytosis by neutrophils on the one hand, while increased plasma glucose levels and catalase and amylase activity on the other. The described changes are nonspecific and are caused, apparently, by adversarial stress.

Instead, the groups subjected to water loading are quite clearly delineated along the axis of the second root. The lowest position of Myroslava loaded rats showed the maximum decrease in plasma corticosterone, sodium and SOD in erythrocytes, leukocytes in blood and spleen mass in combination with the maximum content in the splenocytogram of eosinophils and maximum entropy of leukocytogram. At the opposite pole of the axis are animals loaded with Khrystyna water, and the rats of the control group occupy an intermediate position.

Obviously, this illustrates the specificity of the modulating effects of mineral waters with different mineralization.

Additional delimitation of rats of the control group occurs along the axis of the third root. Their lowest localization reflects elevated or maximal for sampling testosterone levels, mineralocorticoid activity, adrenal glomerular thickness, amylasuria, phagocytic index of blood neutrophils, as well as the content of plasma cells in the thymus and macrophages in the spleen. In contrast, this cluster is characterized by low or minimal sampling levels of triiodothyronine, adrenal reticular thickness, urinary excretion of sodium and chloride, urinary concentrations of magnesium and malonic dialdehyde, as well as phagocytic index of blood monocytes and the content of monocytes and natural killers.

Both mineral waters equally prevent changes in these parameters, which is a manifestation of their non-specific stress limiting effect.

In general, in the information field of the three roots, all four groups of animals are quite different from each other, as documented by the distances of Mahalanobis (Table 9.26).

Table 9.26. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=31) and p-levels (under diagonal)

Groups	I (10)	DW (10)	Myr (15)	Khr (15)
Intact rats (I)	**0,0**	54	63	65
Daily Water (DW)	**3,03 ,011**	**0,0**	29	28
Water Myroslava (Myr)	**4,25 ,002**	**1,95 ,080**	**0,0**	31
Water Khrystyna (Khr)	**4,41 ,001**	**1,88 ,092**	**2,61 ,023**	**0,0**

The application of the classifying functions (Table 9.27) enables the retrospective identification of all rats without mistake (Table 9.28).

Table 9.27. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact rats	Daily Water	Myro-slava	Khrys-tyna
Calcium Plasma, mM/L	-29,94	-33,33	-33,55	-35,14
Superoxide Dismutase Erythrocytes, un/mL	3,424	3,723	3,182	3,599
Microbial Count Neutrophils, Bac/Phag	62,39	53,83	53,14	55,80
Sodium Excretion, µM/24h•100 g BM	-0,818	-0,895	-0,877	-0,910
Monocytes Blood, %	-71,75	-76,39	-78,74	-80,23
Eosinophils Blood, %	2,272	-0,987	-3,021	-1,847
Potassium Plasma, mM/L	17,16	9,361	6,150	7,709
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	-124,1	-138,6	-131,8	-142,1
Testosterone Plasma, nM/L	-46,69	-48,34	-49,44	-52,21
NK Lymphocytes Blood, %	123,9	122,5	125,9	129,7
Malonic dialdehyde Urine, µM/L	2,913	2,710	2,567	2,883
Leukocytes Blood, 10^9/L	7,374	7,395	6,955	7,370
Spleen Mass Index, mg/100g Body Mass	0,582	0,548	0,544	0,579
Amylase Activity Urine, g/h•L	-3,227	-3,240	-2,952	-3,302
Katalase Activity Plasma, µM/h•L	-1238	-1129	-1043	-1203
Chloride Excretion, µM/24h•100 g BM	1,260	1,370	1,372	1,417
Triiodothyronine Plasma, nM/L	578,3	609,7	607,0	631,9
Corticosterone Plasma, nM/L	0,494	0,500	0,495	0,527
Glucose Plasma, mM/L	50,91	57,69	62,29	60,65
Phagocytic Index Monocytes, %	18,45	28,00	24,36	24,98
Sodium Erythrocytes, mM/L	31,46	31,22	31,42	33,32
Amylase Activity Plasma, g/h•L	2,534	2,546	2,584	2,710
Macrophages Spleen, %	12,83	17,09	16,19	16,72
Phagocytic Index Neutrophils, %	52,39	54,89	52,91	55,93
Reticular Zone of Adrenal Cortex, µM	-6,299	-6,106	-5,649	-6,372
Entropy Leukocytogram	-674,7	-768,5	-717,9	-806,0
Plasmocytes Thymus, %	113,4	121,7	118,0	123,1
Eosinophiles Spleen, %	-18,41	-21,20	-15,86	-22,00
Glomerular Zone of Adrenal Cortex, µM	1,349	1,365	1,203	1,356
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	-4,639	-11,47	-14,57	-11,96
Magnesium Urine, mM/L	-39,64	-42,77	-45,15	-45,13
Constants	-3953	-4046	-3974	-4291

Table 9.28. Classification Matrix

Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,20	DW p=,20	Myr p=,30	Khr p=,30
Intact rats (I)	100	**10**	0	0	0
Daily Water (DW)	100	0	**10**	0	0
Water Myroslava (Myr)	100	0	0	**15**	0
Water Khrystyna (Khr)	100	0	0	0	**15**
Total	100	10	10	15	15

Another approach to identifying the specificity of the effects is to create patterns of Z-scores parameters, both included in the discriminant model and extramodel, but carrying recognizable information. Calculating the algebraic difference between Z-scores parameters in control and experimental groups allows us to estimate the partial effects of mineral waters (Fig. 9.7).

The first pattern shows how both mineral waters equally prevent the stress-induced increase in thickness of the glomerular zone of the adrenal cortex and mineralocorticoid activity, glycemia and amylasuria, thymus mass and content in the thymocytogram of endothelial cells, in the splenocytogram macrophages as well as the phagocytic index of blood neutrophils.

Significantly higher stress-induced four parameters (testosterone, plasma catalase, thymocytogram plasma cells and immunocytogram entropy) under the influence of mineral waters are reduced to the upper zone of normal.

On the other hand (third pattern), they prevent a stress-induced decrease in thickness of the reticular zone of the adrenal cortex, triiodethyroninemia, parathyroid activity, calciumemia, urinary excretion of sodium and chloride, urinary concentration of malonic dialdehyde, as well as blood monocytes count, the activity and intensity of bacterial phagocytosis by monocytes.

The following three patterns reflect the differences in the effects of mineral waters. Myroslava water deepens chronic stress-induced decrease in corticosterone, SOD, lymphoblast of thymocytogram content, spleen mass and plasma cell of splenocytogram content, blood content of leukocytes in general and eosinophils in particular as well as the intensity of phagocytosis of bacteria by neutrophils and the transformation of T lymphocytes into blasts. On the other hand, Khrystyna water does not affect this constellation of parameters in general.

The next pattern demonstrates that stress-insensitive parameters (amylasemia, natrihistia, magnesiumuria, lymphoblast and reticulocyte content in splenocytogram, T cytolytic lymphocytes content in immunocytogram, and neutrophil killing index) increase under the influence of Khrystyna water while Myroslava water is inefficient for these parameters.

In contrast, Myroslava water, unlike Khrystyna water, initiates increase in the entropy of leukocytogram and thymocytogram, level in thymocytogram of epitheliocytes, macrophages and reticulocytes, as well as eosinophils in the splenocytogram, NK lymphocytes in the blood.

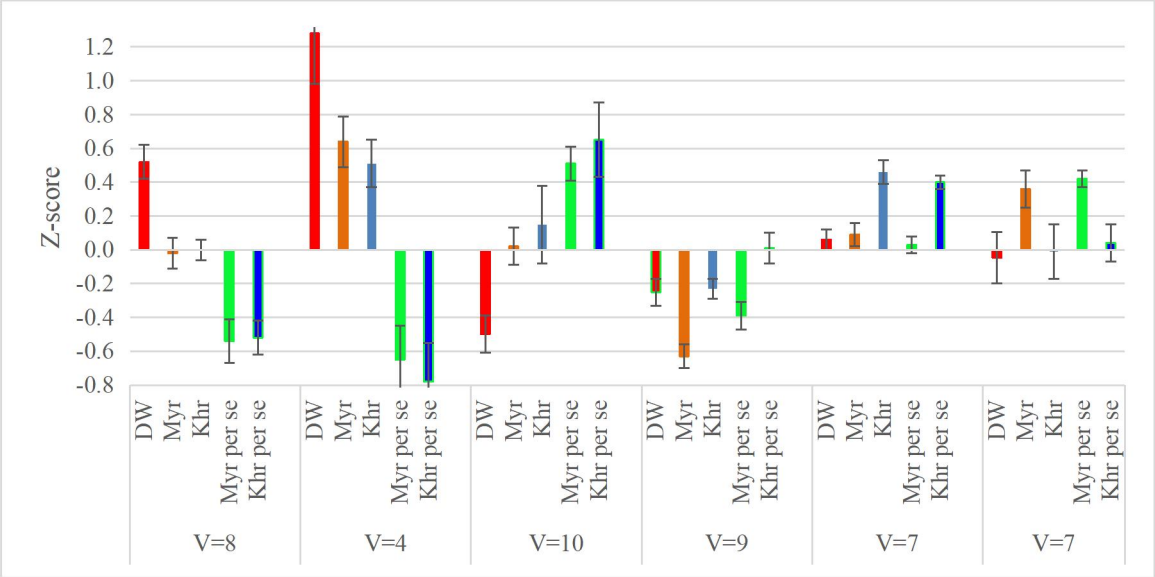


Fig. 9.7. Patterns (V - number of variables) of effects of daily water and mineral waters and simulated partial effects of mineral waters

Thus, the newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa has both similar and specific effects on the neuroendocrine-immune complex and metabolism at healthy old female rats with weekly use. This provides a basis for preclinical studies.

CHAPTER 10

GENERAL NON-SPECIFIC EFFECTS OF BALNEOFACTORS OF TRUSKAVETS' SPA ON PARAMETERS OF NEUROENDOCRINE REGULATION, METABOLISM, IMMUNITY AND MICROBIOTA IN PATIENTS WITH CHRONIC PYELONEPHRITIS AND CHOLECYSTITIS

Earlier in an experiment on rats, we showed that the newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa have neuroendocrine and metabolic effects significantly different from daily water. Adhering to the principle "Ex experimento ad clinic", we continued research in this direction with the participation of patients of the resort.

The object of clinical-physiological observation were 34 men aged 23-70 years, who underwent rehabilitation treatment in the Truskavets' resort of chronic pyelonephritis and cholecystitis in remission with of neuroendocrine-immune complex dysfunction. The examination was performed twice, before and after a 7-10-day course of balneotherapy. All patients received bioactive water Naftussya (3 ml/kg one hour before meals three times a day), however, 11 men in half an hour additionally drank water Khrystyna, and the other 11 men - water Myroslava in the same dose.

Tests in patients are carried out in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

The day before, daily urine was collected, in which was determined the concentration of electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (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). Urine lithogenicity index (Lith) was also calculated by the Tiselius' HS (1978) formula modifed by Flyunt et al., (2017):

$$Lith = (Uric\ acid \bullet Calcium/Magnesium \bullet Creatinine)^{0,25}.$$

The same metabolic parameters were determined in plasma as well as glucose (glucose-oxidase method), triglycerides (by a certain meta-periodate method), total cholesterol (by a direct method after the classic reaction by Zlatkis-Zack) and content of him in composition of α-lipoproteins (by the enzyme method after precipitation of notα-lipoproteins); prae-β-lipoproteins (expected by the level of triglycerides); β-lipoproteins (expected by a difference between a total cholesterol and cholesterol in composition α-and prae-β-lipoproteins).

The analysis carried out according to instructions with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) and corresponding sets of reagents.

According to the parameters of Ca and phosphates exchange, parathyroid activity was evaluated by coefficient $(Cap \bullet Pu/Cau \bullet Pp)^{0,25}$, based on its classical effects and recommendations by Popovych IL (2000) as well as evaluated sympatho-vagal balance by coefficient $(Cap/Kp)^{0,5}$ (Fajda et al., 2016).

We determined content in plasma major hormones of adaptation: Cortisol, Testosterone, Aldosterone, Triiodothyronine as well as Calcitonin (by the ELISA with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Алкор Био", XEMA Co., Ltd and DRG International Inc.).

In basal conditions we estimated the state of the autonomous regulation by the method heart rate variability (HRV) (Heart Rate Variability, 1996; Berntson et al., 1997; Baevskiy et al., 2001; Shaffer, Ginsberg, 2017;), using a hardware-programmatic complex "CardioLab+HRV" (KhAI Medica, Kharkiv, Ukraine). The following parameters were subject to analysis. Frequency Domain Methods: HF (0,4÷0,15 Hz), LF (0,15÷0,04 Hz), VLF (0,04÷0,015 Hz), ULF (0,015÷0,003 Hz) components. Time Domain Methods: HR, SDNN, RMSSD, pNN_{50}. Calculated as well as Kerdö's Vegetative Index (Kerdö , 1964; Fajda et al., 2015) and the Shannon's (1948) entropy (h) of the relative spectral powers (SP) of the HRV bands by the Popovych's IL formula (Ruzhylo et al., 2015):

$$hHRV = -(SPHF \bullet \log_2 SPHF + SPLF \bullet \log_2 SPLF + SPVLF \bullet \log_2 SPVLF + SPULF \bullet \log_2 SPULF)/\log_2 4$$

Simultaneosly recorded EEG a hardware-software complex "NeuroCom Standard" (KhAI MEDICA, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref tassels on the ears. The duration of the epoch was 25 sec. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz) as well as absolute (μV^2/Hz) and relative (%) power spectrum density (PSD) of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device. In addition, calculated Laterality Index (LI) for PSD each Rhythm using formula:

$$LI, \% = \Sigma\ (200 \bullet (Right - Left)/(Right + Left))/8.$$

We calculated also for each locus EEG Shannon's CE entropy (h) of normalized PSD using Popovych's IL formula (Ruzhylo et al., 2015):

$$hEEG = -(PSD\alpha \bullet \log_2 PSD\alpha + PSD\beta \bullet \log_2 PSD\beta + PSD\theta \bullet \log_2 PSD\theta + PSD\delta \bullet \log_2 PSD\delta)/\log_2 4$$

Immune status evaluated on a set of I and II levels recommended by the WHO as described in the manual (Lapovets', Lutsyk, 2004). For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD56 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Immunoglobulins classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by polyethylene glycol precipitation method) as well as C-reactive protein (by the ELISA with the use of analyzer "RT-2100C"), Interleukins 1β and 6 (ELISA, analyzer "Stat Fax 303", USA, reagents from "Vector-Best", RF).

In portion of capillary blood we counted up Leukocytogram (LCG) (Eosinophils, Rod-shaped and Segmentonucleary Neutrophils, Lymphocytes and Monocytes) and calculated its Strain Index by IL Popovych (Popovych, 2000; Popadynets' et al., 2019):

Strain Index-1 = $((Eo/3,5\text{-}1)^2 + (RSN/3,5\text{-}1)^2 + (Mon/5,5\text{-}1)^2 + (Leu/6\text{-}1)^2)/4$

Strain Index-2 = $((Eo/2,75\text{-}1)^2 + (RSN/4,25\text{-}1)^2 + (Mon/6\text{-}1)^2 + (Leu/5\text{-}1)^2)/4$

We calculated also the Entropy (h) of Leukocytogram (LCG) as well as Immunocytogram (ICG) using IL Popovych's formulas (2007):

$hLCG = -(L \bullet \log_2 L + M \bullet \log_2 M + E \bullet \log_2 E + SNN \bullet \log_2 SNN + RSN \bullet \log_2 StubN)/\log_2 5$

$hICG = -(CD4 \bullet \log_2 CD4 + CD8 \bullet \log_2 CD8 + CD22 \bullet \log_2 CD22 + CD56 \bullet \log_2 CD56)/\log_2 4$

Parameters of phagocytic function of neutrophils estimated as described by Douglas and Quie (1981) with moderately modification by Kovbasnyuk (Kul'chyns'kyi et al., 2016). The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". Take into account the following parameters of Phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index PhI), intensity (number of microbes absorbed one phagocytes - Microbial Count MC or Right's Index) and completeness (percentage of dead microbes - Killing Index KI). On the basis of the recorded partial parameters of Phagocytosis, taking into account the Neutrophils (N) content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula:

$BCCN\ (10^9\ Bact/L) = N\ (10^9/L) \bullet PhI\ (\%) \bullet MC\ (Bact/Phag) \bullet KI\ (\%) \bullet 10^{-4}$

On the tone and motility of gall-bladder judged by its volume on an empty stomach in the morning and after 5, 15 and 30 min after ingestion cholekinetic (50 ml of 40% solution of xylitol). The method echoscopy (echocamera "Radmir") applicated (Marfian et al., 2015; 2016). To quantify cholekinetics, the area between the cholecystovolumogram and the basal line was calculated.

The condition of microbiota is evaluated on the results of sowing of feces and urine.

Normal (reference) values of variables are taken from the database of the Truskavetsian Scientific School of Balneology.

For statistical analysis used the software package "Statistica 64".

According to the algorithm of Truskavetsian Scientific School, at the preparatory stage of data analysis the registered parameters were normalized, which allowed their correct comparison. Further, profiles of normalized parameters of the neuroendocrine-immune complex, microbiota and metabolism were created, the levels of which differ significantly before and after balneotherapy, as well as several parameters which according to the following discriminant analysis were still recognizable, despite the insignificant value of criterion t (Fig. 10.1).

Fig. 10.1. Profiles of normalized parameters (Z±SE) of neuroendocrine-immune complex, microbiota and metabolism before and after balneotherapy

At the next stage, the profiles were transformed into 9 patterns (Fig. 10.2).

The first pattern reflects the parameters (the Killing index vs both types of bacteria by neutrophils, the content in the feces of normal E. coli, Bifidobacter and Lactobacillus, Creatinine excretion and concentration of Phosphate in the urine, Entropy of HRV and plasma Calcitonin level) that were significantly reduced before balneotherapy and increased under its influence, but only to the lower zone of the normal range.

The next two patterns contain only one parameter, reflecting the complete normalization of attenuated Cholekinetics and the shift of the Phosphaturia level from the lower zone of the norm to the upper, respectively.

The fourth pattern shows a small but statistically significant increase in perfectly normal levels of HRV Total Power, EEG Entropy at the T5 locus, as well as PSD theta rhythm at the F7 locus as well as beta rhythm at the T4 and Fp2 loci.

The following two patterns illustrate how balneotherapy causes a significant increase in initially normal serum IgG level, Chloriduria and Calciuria, as well as a further increase in initially elevated levels of Diuresis and Urea and Uric acid excretion.

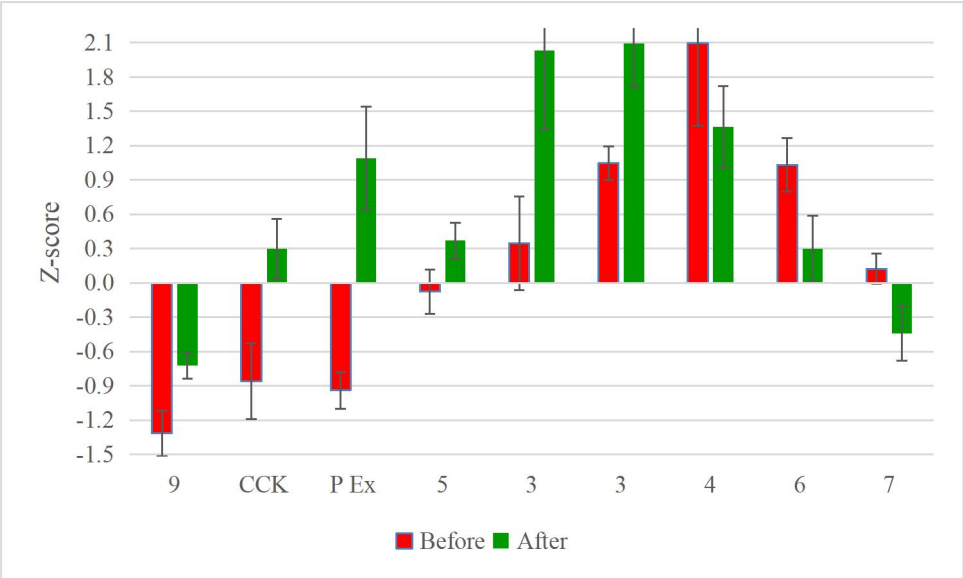


Fig. 10.2. Patterns of normalized parameters (Z±SD) of neuroendocrine-immune complex, microbiota and metabolism before and after balneotherapy. The number of pattern components is specified

In contrast to the described patterns of enhancing effects of balneotherapy, the last three patterns reflect its reducing effects. In particular, the first pattern shows a decrease (but not to the area of normal) in the severity of Bacteriuria, assessed both in lgCFU/mL and in points, the content in the feces of E. coli strain with impaired enzymatic activity, as well as Popovych's Leukocytary Strain Index-2 (but not Index-1) as a marker of dysadaptosis. In contrast, moderately elevated markers of dysbacteriosis (fecal content of hemolyzing strain E. coli and Klebsiela & Proteus) and pyelonephritis (Leukocyturia, assessed in both lgLeu/mL and in points), as well as plasma Creatinine and Testosterone levels are completely normalized. Finally, initially normal urinary Sodium concentrations as well as EEG Entropy at the Fp2 locus and PSD theta rhythm at the Fp2 and T4 loci are slightly but statistically significantly reduced. The decrease in the indices of Lateralization of beta, alpha and theta rhythms reflects the left-hand shift of their symmetry.

Another approach to quantifying balneoeffects is to calculate the direct differences between the final and initial parameters of each patient (Fig. 10.3).

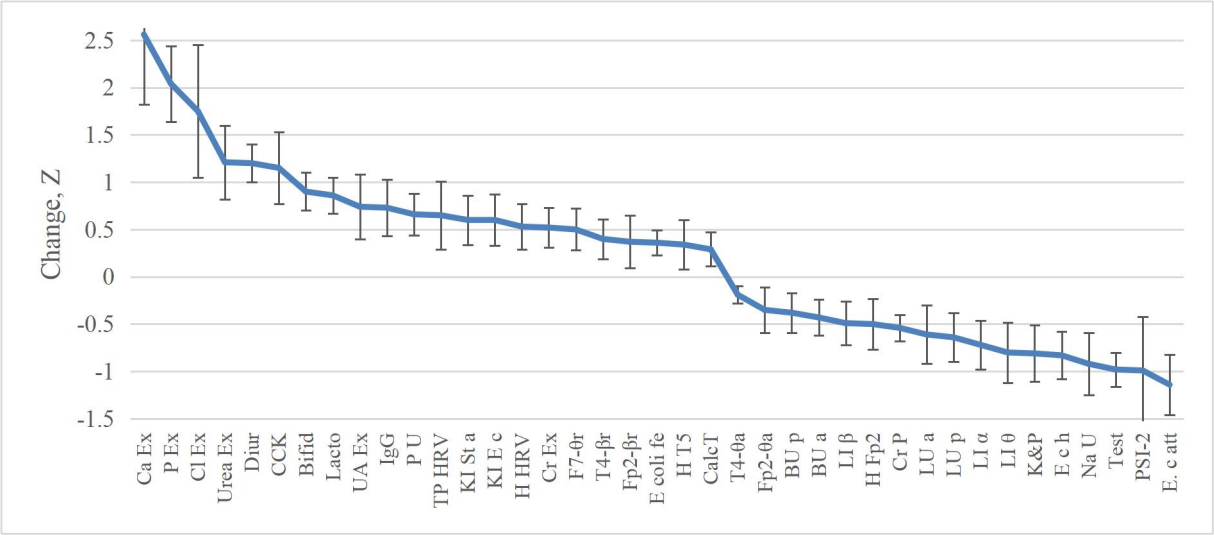


Fig. 10.3. Nonspecific effects (Z±SE) of balneotherapy on the parameters of neuroendocrine-immune complex, microbiota and metabolism

It seems that the enhancing effects of balneotherapy are more numerous and tangible than reducing. However, they are all physiologically favorable.

However, according to the results of discriminant analysis (method forward stepwise), only 22 parameters were included in the model: 9 **neuro-endocrine**, 3 **immune**, 4 **microbiota**, 5 **metabolic**, and **Cholecystokinetic Index** (Tables 10.1 and 10.2).

Table 10.1. Summary of the analysis of discriminant functions in relation to the parameters of neuro-endocrine-immune complex and metabolism

Step 22, N of vars in model: 22; Grouping: 2 grps; Wilks' Λ: 0,1777; approx. $F_{(22)}$=9,5; $p<10^{-6}$

Variables currently in the model	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (34)	**After therapy (34)**	***Effect of therapy (34)***	Wilks' Λ	Partial Λ	F-remove (1,45)	p-level	Tolerancy	*Norm (30)*
Phosphate Excretion, mM/24h	18,2 1,2	33,3 3,3	*+15,1* *3,0*	0,183	0,969	1,43	0,238	0,480	25,2 0,294
Testosterone, nM/L	18,5 1,6	13,1 1,5	*-5,4* *1,0*	0,179	0,991	0,416	0,522	0,523	14,8 0,400
Laterality θ, %	-4 7	-30 7	*-26* *10*	0,186	0,955	2,11	0,154	0,296	-3 ***32***
Lactobacillus feces, lgCFU/g	5,92 0,25	7,17 0,18	*+1,25* *0,27*	0,206	0,861	7,29	0,010	0,021	8,10 0,015
Entropy T5	0,744 0,033	0,800 0,027	*+0,056* *0,043*	0,219	0,813	10,4	0,002	0,355	0,778 0,211
Ig G Serum, g/L	14,10 0,70	16,01 0,63	*+1,91* *0,80*	0,179	0,994	0,265	0,609	0,688	12,75 0,206
E. coli attenuated feces, %	63,5 4,8	43,6 4,5	*-19,9* *5,5*	0,178	1,000	0,017	0,898	0,057	17,4 1,000
Cholecystokinetic Index, units	554 27	648 22	*+94* *31*	0,189	0,942	2,75	0,104	0,610	624 0,131
Entropy Fp2	0,817 0,024	0,747 0,032	*-0,072* *0,039*	0,224	0,791	11,9	0,001	0,297	0,799 0,180
F7-θ PSD, %	7,1 0,7	9,3 0,9	*+2,2* *0,9*	0,231	0,769	13,5	0,001	0,327	7,9 0,568
Laterality β, %	-3 5	-17 4	*-14* *6*	0,263	0,676	21,6	10^{-4}	0,280	-6 ***28***
T4-β PSD, %	29,0 2,5	35,6 3,3	*+6,6* *3,4*	0,199	0,894	5,31	0,026	0,369	27,9 0,591
Fp2-β PSD, %	29,9 2,8	35,5 3,5	*+5,5* *4,2*	0,201	0,882	6,01	0,018	0,361	27,2 0,570
Fp2-θ PSD,	29	19	*-11*	0,206	0,860	7,30	0,010	0,344	25

μV²/Hz	7	3	*7*						1,186
Sodium Urine, mM/L	119 5	98 6	*-21* *8*	0,195	0,911	4,40	0,042	0,440	110 0,211
Creatinine Plasma, μM/L	92,6 2,6	85,5 1,7	*-7,1* *1,8*	0,209	0,851	7,88	0,007	0,496	79,5 0,167
Killing Index vs Staph. aur., %	48,2 1,5	53,3 1,5	*+5,1* *2,2*	0,179	0,994	0,29	0,590	0,330	58,9 0,142
Diuresis, L/24 h	1,86 0,12	2,32 0,11	*+0,46* *0,08*	0,202	0,880	6,16	0,017	0,269 0921	1,40 0,274
Creatinine Excretion, mM/24h	6,72 0,52	8,43 0,71	*+1,71* *0,68*	0,195	0,912	4,36	0,043	0,397	11,0 0,300
Bacteriuria, points	0,34 0,04	0,25 0,04	*-0,09* *0,05*	0,190	0,935	3,14	0,083	0,565	0 ***0,24***
Killing Index vs E. coli, %	46,7 2,7	52,5 1,8	*+5,8* *2,7*	0,190	0,936	3,09	0,085	0,060	62,0 0,156
Bifidobacter feces, lgCFU/g	5,23 0,20	6,26 0,15	*+1,03* *0,23*	0,183	0,969	1,42	0,240	0,025	6,94 0,011

Notes. In each column, the first line is the average, the second – SE. In norm column - the average and Cv or ***SD***. The "Effect" and "Norm" columns are not the result of discriminant analysis.

Table 10.2. Summary of stepwise analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	**Λ**	F-value	p-level
Phosphates Excretion, mM/24 h	18,4	10^{-4}	0,782	18,4	10^{-4}
Testosterone, nM/L	9,47	0,003	0,683	15,1	10^{-5}
Laterality θ, %	8,42	0,005	0,603	14,0	10^{-6}
Lactobacillus feces, lgCFU/g	9,41	0,003	0,525	14,3	10^{-6}
Entropy T5	9,19	0,004	0,457	14,7	10^{-6}
Ig G Serum, g/L	5,18	0,026	0,421	14,0	10^{-6}
E. coli attenuated feces, %	3,34	0,072	0,399	12,9	10^{-6}
Cholecystokinetic Index, units	3,36	0,072	0,378	12,2	10^{-6}
Entropy Fp2	3,99	0,050	0,353	11,8	10^{-6}
F7-θ PSD, %	4,66	0,035	0,327	11,8	10^{-6}
Laterality β, %	4,42	0,040	0,303	11,7	10^{-6}
T4-β PSD, %	3,78	0,057	0,283	11,6	10^{-6}
Fp2-β PSD, %	2,50	0,119	0,271	11,2	10^{-6}
Fp2-θ PSD, μV²/Hz	3,05	0,086	0,256	11,0	10^{-6}
Sodium Urine, mM/L	3,23	0,078	0,241	10,9	10^{-6}
Creatinine Plasma, μM/L	2,53	0,118	0,230	10,7	10^{-6}
Killing Index vs Staph. aureus, %	2,97	0,091	0,217	10,6	10^{-6}
Diuresis, L/24 h	2,35	0,132	0,207	10,4	10^{-6}
Creatinine Excretion, mM/24 h	2,12	0,152	0,198	10,2	10^{-6}
Bacteriuria, points	1,93	0,171	0,190	10,0	10^{-6}
Killing Index vs E. coli, %	1,76	0,191	0,183	9,76	10^{-6}
Bifidobacter feces, lgCFU/g	1,42	0,240	0,178	9,47	10^{-6}

Other variables, despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 10.3).

Table 10.3. Variables currently not in the discriminant model

Variables	**Groups (n) and Means±SE**			**Parameters of Wilks' Statistics**					
	Before therapy (34)	**After therapy (34)**	***Effect of therapy (34)***	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy	*Norm (30)*
Calcitonin, ng/L	6,95 0,62	8,96 0,93	+2,01 1,22	0,174	0,982	0,81	0,372	0,482	13,95 0,493
Urea Excretion, mM/24 h	543 41	647 39	+103 33	0,178	1,000	0,00	0,993	0,190	458 0,186
Bacteriuria,	1,50	1,08	-0,42	0,175	0,983	0,74	0,394	0,073	0

lgCFU/mL	0,17	0,18	0,19						***0,98***
Leukocyturia, points	0,18 0,03	0,08 0,02	-0,10 0,04	0,177	0,993	0,29	0,594	0,483	0 ***0,15***
Leukocyturia, lgLeu/L	3,40 0,14	3,09 0,09	-0,30 0,16	0,176	0,991	0,40	0,531	0,267	3,00 0,070
E. coli hemolytica feces, %	24 7	3 1	-21 6	0,178	1,000	0,02	0,900	0,497	0 ***25***
Klebsiela&Proteus feces, %	15,5 3,2	6,6 1,7	-8,9 3,3	0,177	0,995	0,23	0,633	0,358	0 ***11***
Escherichia coli feces, lgCFU/g	8,25 0,05	8,39 0,04	+0,14 0,05	0,177	0,999	0,06	0,801	0,297	8,66 0,045
Popovych Strain Index-2	0,225 0,032	0,171 0,023	-0,054 0,032	0,177	0,999	0,06	0,800	0,762	0,072 0,762
Total Power HRV, msec^2	2042 215	2615 345	+611 343	0,177	0,994	0,25	0,619	0,686	2379 0,402
Entropy HRV	0,696 0,021	0,745 0,022	+0,049 0,022	0,176	0,992	0,36	0,553	0,486	0,806 0,114
Phosphates Urine, mM/L	10,5 0,7	14,0 1,1	+3,5 1,2	0,177	0,996	0,16	0,688	0,123	18,0 0,294
Calcium Excretion, mM/24 h	4,26 0,41	6,66 0,62	+2,40 0,69	0,177	0,996	0,17	0,679	0,421	4,38 0,214
Chloride Excretion, mM/24 h	186 13	237 19	+51 20	0,178	1,000	0,01	0,943	0,342	167,5 0,172
Laterality α, %	-1 6	-20 4	-19 7	0,175	0,986	0,60	0,441	0,184	-4 ***27***
T4-θ PSD, μV^2/Hz	34 7	18 3	-16 7	0,176	0,992	0,35	0,560	0,179	32 2,582
Uric acid Excretion, mM/24h	3,70 0,24	4,26 0,26	+0,56 0,25	0,178	1,000	0,01	0,933	0,204	3,00 0,250

Calculating the value of the discriminant root for each patient as the sum of the products of non-standardized (raw) coefficients on the individual values of discriminant variables together with the constant (Table 10.4) allows visualization of each patient in the information space of the root (Figs. 10.4 and 10.5).

Table 10.4. Standardized and raw coefficients and constant for discriminant variables

	Coefficients	
Variables	**Standardized**	**Raw**
Phosphates Excretion, mM/24 h	-0,280	-0,143
Testosterone, nM/L	0,146	0,082
Laterality θ, %	-0,429	-0,012
Lactobacillus feces, lgCFU/g	-2,835	-2,239
Entropy T5	-0,800	-5,098
Ig G Serum, g/L	-0,102	-0,069
E. coli attenuated feces, %	0,089	0,003
Cholecystokinetic Index, units	-0,339	-0,002
Entropy Fp2	0,924	6,297
F7-θ PSD, %	-0,926	-0,226
Laterality β, %	1,185	0,045
T4-β PSD, %	-0,590	-0,040
Fp2-β PSD, %	0,630	0,039
Fp2-θ PSD, μV^2/Hz	0,702	0,026
Sodium Urine, mM/L	0,496	0,016
Creatinine Plasma, μM/L	0,604	0,0478
Killing Index vs Staph. aureus, %	0,155	0,018
Diuresis, L/24 h	-0,738	-1,081
Creatinine Excretion, mM/24 h	0,520	0,473
Bacteriuria, points	0,375	1,491
Killing Index vs E. coli, %	1,138	0,085
Bifidobacter feces, lgCFU/g	1,212	1,172

	Constant	0,920
	Eigenvalue	4,63
Squared Mahalanobis Distance=18; $F_{(22)}$=9,5; $p<10^{-6}$		
Canonical R=0,907; Wilks' Λ=0,1777; $\chi^2_{(22)}$=95; $p<10^{-6}$		

Fig. 10.4 illustrates that patients in all three groups had, firstly, almost the same initial integral state of discriminant variables, secondly, it changed significantly under the influence of balneotherapy, and thirdly, the integral influence of Naftussya water itself and in combination with one or another mineral water, almost the same. In other words, the effects of balneofactors are nonspecific. Reformatted Fig. 10.5 focuses on unidirectional, albeit differently expressed, changes in the integral state in all patients without exception.

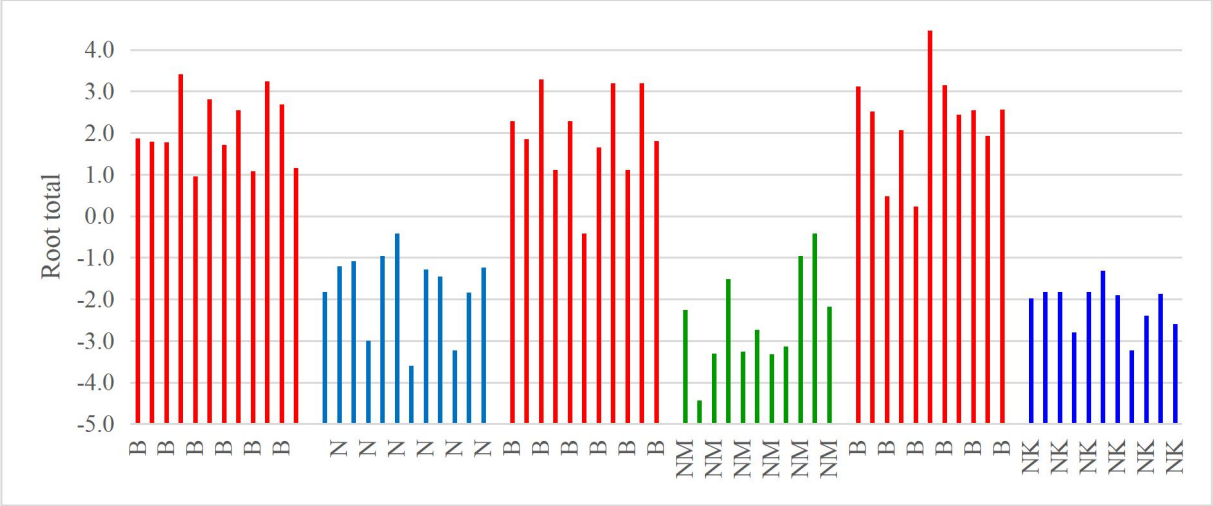


Fig. 10.4. Sectional values of the discriminant root before (B) and after course drinking of Naftussya only (N), Naftussya and Myroslava (NM), Naftussya and Khrystyna (NK) waters

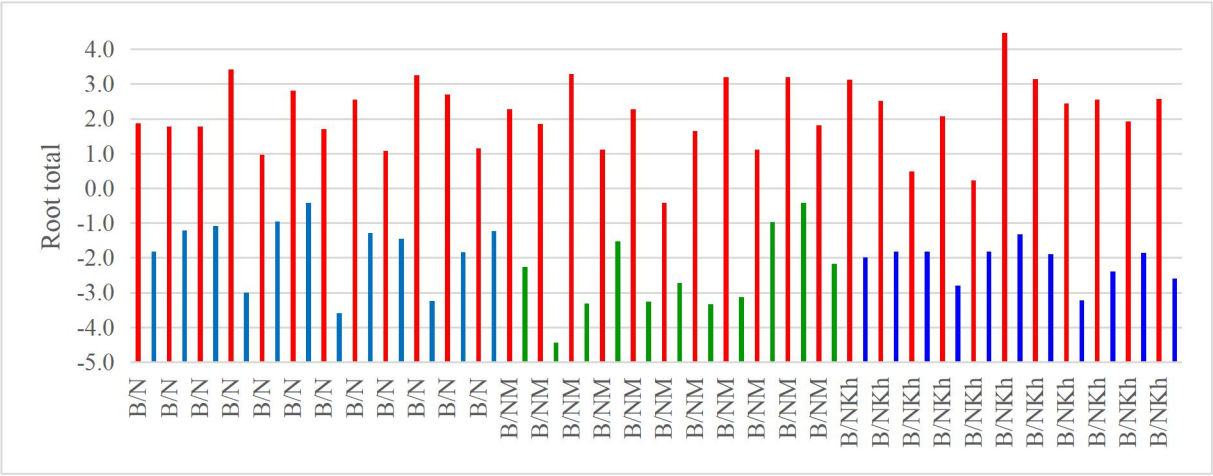


Fig. 10.5. Individual values of the discriminant root before (B) and after course drinking of Naftussya only (N), Naftussya and Myroslava (NM), Naftussya and Khrystyna (NKh) waters

Lower root levels after balneotherapy reflect its enhancing effect on 13 variables, information about which is reflected in the root in reverse, instead reducing effect on 9 variables that are directly related to the root (Table 10.5).

Table 10.5. Correlations between variables and root as well as Z-scores of variables

Variables	R	Before therapy (34)	After therapy (34)	*Effect of therapy (34)*
Phosphates Excretion	-0,245	-0,94±0,16	+1,09±0,45	*+2,04±0,40*
Bifidobacter feces	-0,234	-1,50±0,18	-0,60±0,13	*+0,90±0,20*
Lactobacillus feces	-0,232	-1,50±0,17	-0,64±0,13	*+0,86±0,19*
Diuresis	-0,159	+1,21±0,32	+2,41±0,29	*+1,20±0,20*

Cholecystokinetic Index, units	-0,156	-0,86±0,33	+0,30±0,26	*+1,15±0,38*
Killing Index vs Staph. aureus	-0,137	-1,28±0,17	-0,67±0,18	*+0,60±0,26*
Ig G Serum	-0,116	+0,51±0,27	+1,24±0,24	*+0,73±0,30*
Creatinine Excretion	-0,111	-1,30±0,16	-0,78±0,22	*+0,52±0,21*
Killing Index vs E. coli	-0,102	-1,58±0,28	-0,99±0,18	*+0,60±0,27*
F7-θ PSDr	-0,098	-0,11±0,16	+0,39±0,20	*+0,50±0,22*
T4-β PSDr	-0,083	+0,07±0,15	+0,47±0,20	*+0,40±0,21*
Fp2-β PSDr	-0,065	+0,17±0,18	+0,54±0,23	*+0,37±0,28*
Entropy T5	-0,066	-0,21±0,20	+0,13±0,16	*+0,34±0,26*
E. coli attenuated feces	0,173	+2,65±0,27	+1,51±0,26	*-1,14±0,32*
Sodium Urine	0,160	+0,39±0,21	-0,52±0,25	*-0,92±0,33*
Laterality θ	0,134	-0,04±0,21	-0,84±0,22	*-0,80±0,32*
Creatinine Plasma	0,133	+0,99±0,19	+0,45±0,13	*-0,54±0,14*
Testosterone	0,130	+0,84±0,33	-0,14±0,28	*-0,98±0,18*
Laterality β	0,100	+0,10±0,17	-0,39±0,19	*-0,49±0,23*
Entropy Fp2	0,090	+0,13±0,17	-0,36±0,23	*-0,50±0,27*
Bacteriuria, p	0,085	+1,42±0,18	+1,05±0,18	*-0,38±0,21*
Fp2-θ PSDa	0,070	+0,14±0,23	-0,20±0,10	*-0,35±0,24*

Note. The “Effect” column is not the result of discriminant analysis.

Visual impressions are documented by calculating the mean values of the discriminant root before and after balneotherapy for each group of patients (Fig. 10.6).

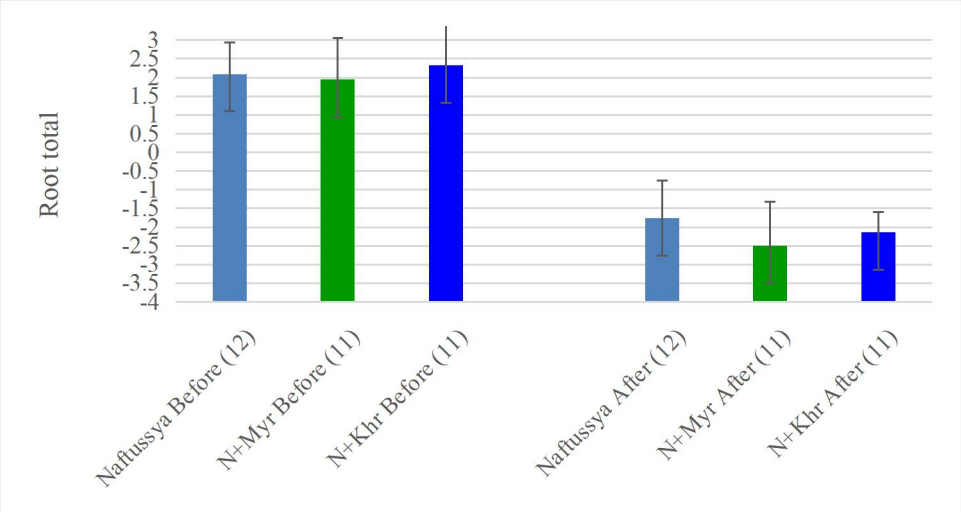


Fig. 10.6. Average values (Mean±SD) of the discriminant root before and after course drinking of Naftussya only, Naftussya and Myroslava (N+Myr), Naftussya and Khrystyna (N+Khr) waters

An additional criterion for a clear distinction between the integrated states of patients before and after balneotherapy is the 98,5% accuracy (only one error for 68 cases) of classification based on coefficients and constants for classification functions (Table 10.6).

Table 10.6. Coefficients and constants of classification functions

Clusters	Before therapy	After therapy
Variables	p=,500	p=,500
Phosphates Excretion, mM/24 h	-2,694	-3,299
Testosterone, nM/L	-9,193	-8,845
Laterality θ, %	-0,724	-0,775
Lactobacillus feces, lgCFU/g	-8,873	-18,37
Entropy T5	83,08	61,46
Ig G Serum, g/L	4,999	4,706
E. coli attenuated feces, %	5,058	5,072
Cholecystokinetic Index, units	0,133	0,123

Entropy Fp2	60,12	86,82
F7-θ PSD, %	0,726	-0,231
Laterality β, %	0,610	0,800
T4-β PSD, %	0,908	0,740
Fp2-β PSD, %	-0,399	-0,236
Fp2-θ PSD, $\mu V^2/Hz$	0,370	0,480
Sodium Urine, mM/L	0,836	0,903
Creatinine Plasma, μM/L	1,078	1,280
Killing Index vs Staph. aureus, %	0,879	0,955
Diuresis, L/24 h	16,16	11,57
Creatinine Excretion, mM/24 h	-3,183	-1,177
Bacteriuria, points	15,11	21,43
Killing Index vs E. coli, %	7,152	7,513
Bifidobacter feces, lgCFU/g	69,24	74,21
Constants	-737,7	-733,8

We consider it necessary to emphasize that both activating and reducing effects of balneofactors are physiologically favorable, because they are aimed, as a rule, at normalizing the deviations of body parameters from normal. Non-specificity and normalization are the main attributes of the adaptogenic effect (Balanovs'kyi et al., 1993; Popovych, 2011; Popovych et al., 2020; Gozhenko et al., 2021).

Earlier, both in the experiment and in the clinical-physiological observations of the Truskavetsian Scientific School of Balneology, close links were found between the parameters of the central and autonomic nervous and endocrine systems, on the one hand, and immunity and metabolism - on the other (Popovych, 2008; 2011; Popovych et al., 2013; 2014; 2020; Kozyavkina et al., 2015; Gozhenko et al., 2019; 2021), which are based on the concepts of neuro-endocrine-immune complex (Popovych, 2009) and functional-metabolic continuum (Gozhenko, 2016).

Therefore, it is logical to analyze such relationships in this sample. Following the accepted algorithm, a matrix of correlations was first created between changes in neuroendocrine parameters as factor traits, on the one hand, and immunity, microbiota and metabolism parameters as result traits, on the other hand (Table 10.7).

Table 10.7. Matrix of correlations between changes in neuro-endocrine and immune-microbiota-metabolic parameters

N=34	CT	Test	H HRV	LIB	LIT	H Fp2	Fp2Br	Fp2Ta	F7Tr	T4Br	H T5
Lg BU	0,28	-0,18	-0,04	0,08	-0,04	0,28	0,09	-0,16	0,05	0,07	0,27
Lg LU	0,22	-0,16	-0,40	0,10	0,14	0,27	-0,13	0,02	-0,09	-0,05	0,07
Lg Bifidobacter	-0,27	0,05	0,34	0,11	0,34	-0,29	-0,08	0,09	-0,32	-0,46	-0,23
Lg Lactob acillus	-0,29	0,04	0,41	0,11	0,32	-0,33	-0,10	0,12	-0,32	-0,47	-0,28
E. c oli att en,%	0,29	-0,10	-0,47	0,07	0,07	0,16	-0,08	-0,10	-0,14	0,01	-0,03
E. c oli hem ol,%	0,34	-0,21	-0,10	0,18	0,15	0,20	-0,13	0,12	-0,00	0,05	0,09
Klebs &P roteus ,%	0,20	0,13	-0,44	-0,01	-0,14	0,17	0,11	-0,17	0,01	0,23	-0,02
Lg E. coli feces	-0,23	-0,16	0,57	0,03	0,07	-0,06	-0,01	0,27	0,20	-0,08	0,03
Killing I vs St. aur	-0,25	0,17	0,14	-0,18	-0,04	-0,19	0,07	0,04	-0,16	0,01	-0,20
Killing I vs E. coli	-0,40	0,13	0,33	-0,15	-0,10	-0,18	0,14	0,04	0,08	0,11	-0,06
IgG	0,01	-0,27	0,01	0,11	0,07	-0,12	-0,20	0,12	-0,06	-0,09	-0,23
Cr P	-0,13	-0,06	0,01	-0,35	-0,07	0,03	0,00	-0,02	-0,02	0,02	0,06
Diuresis	-0,00	0,01	0,10	-0,26	-0,42	-0,03	0,27	-0,29	0,04	0,35	-0,13
Na U	-0,44	0,14	0,10	-0,40	0,06	-0,38	-0,12	-0,03	-0,32	-0,16	-0,25
Cr Exc	-0,25	-0,24	0,11	-0,48	-0,35	0,02	0,19	-0,14	0,01	0,16	-0,01
UA Exc	0,17	-0,11	-0,06	-0,12	-0,29	0,04	0,13	-0,16	0,03	0,18	0,08
Ca Exc	0,08	-0,13	-0,04	-0,03	-0,06	0,24	0,26	-0,06	0,21	0,32	0,25
P Exc	0,08	-0,36	0,06	0,08	0,09	0,07	-0,01	-0,12	-0,09	0,02	-0,05
Cl Exc	-0,29	0,10	0,01	-0,39	-0,11	-0,12	0,01	0,05	-0,06	-0,00	-0,06
Cholekinetics	0,29	-0,30	0,02	0,12	-0,17	0,13	0,33	-0,27	0,05	0,27	0,06
Urea Exc	-0,10	-0,01	-0,21	-0,40	-0,28	0,12	0,21	-0,10	0,06	0,24	0,01

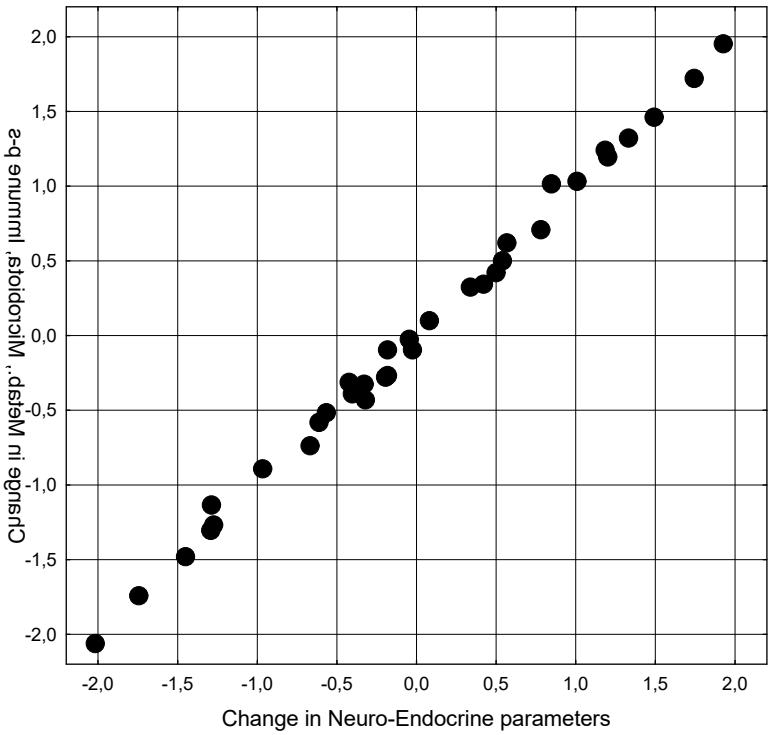
Note. According to the formula: $|r| \geq \{exp(2t/(n-1,5)^{0,5}) - 1\}/\{exp(2t/(n-1,5)^{0,5}) + 1\}$,

for a sample of 34 observations critical value of correlation coefficient module at p<0,05 (t>2,04) is 0,34, at p<0,02 (t>2,46) is 0,40, at p<0,01 (t>2,75) is 0,44, at p<0,001 (t>3,64) is 0,55. Technical limitations of the program allowed to use only 32 parameters in the canonical analysis (n=34-2).

As a result of the analysis, two pairs of canonical roots were identified. The factor structure of the neuro-endocrine root of the first pair is represented by changes in plasma levels of Testosterone and Calcitonin, PSD theta and beta rhythms and their Entropy and Lateralization. Changes in these regulatory parameters determine changes in the constellation of parameters of microbiota, immunity, metabolism and cholekinetics by 99,6% (Table 10.8 and Fig. 10.7).

Table 10.8. Factor structure of first pair of Neuro-Endocrine and Microbiota-Immune- Metabolic Roots of change

Neuro-Endocrine Variables	R 1
F7-θ PSD, %	-0,634
Testosterone, nM/L	-0,328
Entropy Fp2	-0,310
Entropy T5	-0,305
Fp2-β PSD, %	-0,180
Fp2-θ PSD, $\mu V^2/Hz$	-0,177
T4-β PSD, %	-0,151
Calcitonin, ng/L	0,404
Laterality θ, %	0,364
Laterality β, %	0,168
Microbiota-Immune-Metabolic Variables	**R 1**
E. coli hemolytica feces, %	0,318
E. coli attenuated feces, %	0,286
Phosphates Excretion, mM/24 h	0,284
Ig G Serum, g/L	0,233
Cholecystokinetic Index, units	0,233
Leukocyturia, lgLeu/L	0,205
Bacteriuria, lgCFU/mL	0,126
Uric acid Excretion, mM/24h	0,098
Lactobacillus feces, lgCFU/g	0,041
Bifidobacter feces, lgCFU/g	0,037
Killing Index vs E. coli, %	-0,288
Escherichia coli feces, lgCFU/g	-0,166
Chloride Excretion, mM/24 h	-0,138
Urea Excretion, mM/24 h	-0,136
Creatinine Excretion, mM/24 h	-0,074
Killing Index vs Staph. aureus, %	-0,069



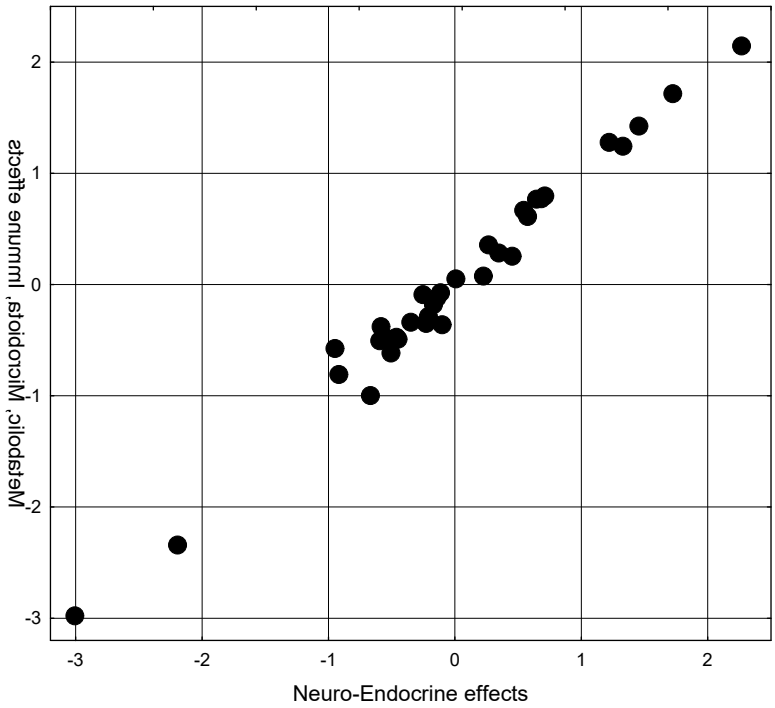
$R=0,998$; $R^2=0,996$; $\chi^2_{(231)}=330$; $p<10^{-4}$; Λ Prime$<10^{-6}$

Fig. 10.7. Scatterplot of canonical correlation between change in Neuro-Endocrine (X-line) and Metabolic, Microbiota, Immune (Y-line) parameters. First pair of Roots

The factor structure of the second pair of roots to some extent differs both in the composition of variables and factor loads (Table 10.9). However, the degree of neuroendocrine determination of cholekinetics, metabolism and microbiota remains the same (Fig. 10.8).

Table 10.9. Factor structure of second pair of Neuro-Endocrine and Metabolic-Microbiota Roots of change

Neuro-Endocrine Variables	R 2
Entropy Fp2	0,520
F7-θ PSD, %	0,347
Fp2-θ PSD, μV²/Hz	0,254
Entropy HRV	0,238
Fp2-β PSD, %	0,224
Laterality β, %	0,211
Laterality θ, %	0,208
Entropy T5	0,193
Calcitonin, ng/L	0,174
T4-β PSD, %	0,158
Testosterone, nM/L	-0,451
Metabolic&Microbiota Variables	**R 2**
Cholecystokinetic Index, units	0,382
Escherichia coli feces, lgCFU/g	0,349
E. coli hemolytica feces, %	0,261
Calcium Excretion, mM/24 h	0,242
Phosphates Excretion, mM/24 h	0,185
Bacteriuria, lgCFU/mL	0,175
Diuresis, L/24 h	0,118
Sodium Urine, mM/L	-0,479
Chloride Excretion, mM/24 h	-0,202
Creatinine Plasma, μM/L	-0,107
Klebsiela&Proteus feces, %	-0,049



R=0,991; R2=0,982; χ2(200)=240; p=0,027; Λ Prime<10-6
Fig. 10.8. Scatterplot of canonical correlation between change in Neuro-Endocrine (X-line) and Metabolic, Microbiota, Immune (Y-line) parameters. Second pair of Roots

Based on the above data, a picture of the neuro-endocrine mechanism of physiologically favorable modulating effect of balneofactors of Truskavets' spa on immunity, microbiota, metabolism and cholekinetics of patients with chronic pyelonephritis and cholecystitis is created. This mechanism will be discussed in more detail in the next chapter.

CHAPTER 11

COMPARATIVE STUDY OF THE EFFECTS ON THE METABOLISM AND NEUROENDOCRINE-IMMUNE COMPLEX OF DRINKING MONOTHERAPY WITH NAFTUSSYA WATER AND THERAPY SUPPLEMENTED WITH MYROSLAVA AND KHRYSTYNA MINERAL WATERS

Following the accepted algorithm, in this study, two research groups were combined to determine the effects common to both sulfate-magnesium mineral waters.

11.1. Metabolism, HRV, hormones, immunity

In order to identify those indicators for which the condition of patients on admission to treatment and after monotherapy or complex balneotherapy differ significantly, a discriminant analysis of registered indicators was conducted. The program included in the discriminant model 27 variables, including 15 **metabolic**, 4 **autonomous**, 3 **endocrine** and 5 **immune** (Tables 11.1 and 11.2).

Table 11.1. Summary of the analysis of discriminant functions in relation to the parameters of metabolism and neuro-endocrine-immune complex

Step 27, N of vars in model: 27; Grouping: 3 grps; Wilks' Λ: 0,022; approx. $F_{(57)}$=7,9; $p<10^{-6}$

Variables currently in the model	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (34)	After Naftussya (12)	After Salt Waters and N (22)	Wilks' Λ	Partial Λ	F-remove (2,4)	p-level	Tolerancy	Norm Cv (30)
Phosphates Excretion, mM/24 h	18,2 1,2	16,8 1,8	42,4 3,8	0,050	0,428	25,3	10^{-6}	0,233	25,2 0,294
Calcitonin, ng/L	6,95 0,62	6,16 1,11	10,48 1,21	0,029	0,747	6,42	0,004	0,427	13,95 0,493
Creatinine Plasma, μM/L	92,6 2,6	81,9 2,8	87,4 2,0	0,033	0,654	10,1	10^{-3}	0,426	79,5 0,167
Testosterone, nM/L	18,5 1,6	9,0 1,0	15,3 2,1	0,036	0,602	12,6	10^{-4}	0,359	14,8 0,400
Sodium Plasma, mM/L	141,5 1,5	146,7 2,1	142,3 2,0	0,031	0,699	8,19	0,001	0,260	145,0 0,034
Phosphate Plasma, mM/L	1,04 0,03	1,13 0,06	0,91 0,04	0,028	0,785	5,20	0,010	0,274	1,20 0,167
Magnesium Urine, mM/L	2,40 0,11	2,14 0,23	2,22 0,13	0,027	0,816	4,28	0,021	0,095	2,93 0,256
Chloride Excretion, mM/24 h	186 13	197 15	259 27	0,023	0,936	1,30	0,284	0,025	167,5 0,172
Interleukin-6, ng/L	4,45 0,36	3,67 0,56	4,58 0,33	0,026	0,843	3,54	0,039	0,240	4,25 0,324
LD Cholesterol Plasma, mM/L	3,54 0,18	3,43 0,32	3,25 0,21	0,032	0,670	9,35	10^{-3}	0,237	3,44 0,192
Sodium Urine, mM/L	119 5	114 8	89 7	0,031	0,689	8,57	0,001	0,010	110 0,211
Microbian Count for St. aur., B/Ph	62,8 1,2	66,0 2,0	60,2 2,3	0,024	0,883	2,52	0,094	0,624	61,6 0,160
Glucose Plasma, mM/L	4,77 0,17	4,68 0,33	4,59 0,18	0,027	0,807	4,55	0,017	0,532	4,70 0,160
Chloride Urine mM/L	102 3	127 14	96 10	0,026	0,840	3,63	0,036	0,027	120 0,172
Sodium Excretion, mM/24 h	225 18	179 11	238 19	0,029	0,743	6,56	0,004	0,014	154 0,211
$(Ca/K)^{0,5}$ as Symp-Vagal balance	0,728 0,012	0,729 0,014	0,708 0,010	0,023	0,928	1,48	0,240	0,194	0,710 0,104
VLF HRV PS, $msec^2$	969 99	869 141	1238 168	0,025	0,860	3,09	0,057	0,319	1250 0,572
HF HRV PS, $msec^2$	354 75	407 262	541 100	0,024	0,900	2,12	0,134	0,206	350 0,713
Magnesium Excretion, mM/24 h	4,40 0,29	3,43 0,36	5,98 0,43	0,031	0,703	8,04	0,001	0,035	4,10 0,256

Lithogenicity Urine	0,86 0,03	0,83 0,03	0,95 0,03	0,028	0,770	5,67	0,007	0,443	0,73 0,300
Killing Index vs Staph. aur., %	48,2 1,5	45,2 1,9	57,7 1,4	0,026	0,833	3,81	0,031	0,375	58,9 0,142
$CD3^+$ active T-Lymphocytes, %	28,3 0,8	31,3 0,9	26,1 1,1	0,026	0,817	4,27	0,021	0,470	30,0 0,167
Interleukin-1, ng/L	4,94 0,19	4,36 0,37	5,17 0,30	0,022	0,964	0,72	0,495	0,613	4,51 0,173
Potassium Urine, mM/L	39,5 3,2	41,5 3,6	30,5 1,7	0,026	0,827	3,99	0,027	0,022	46,4 0,269
Aldosterone, pM/L	225 5	236 10	229 4	0,025	0,861	3,06	0,058	0,023	238 0,187
ULF HRV PS, $msec^2$	73 15	139 56	110 34	0,024	0,908	1,92	0,161	0,331	122 0,892
HD Cholesterol Plasma, mM/L	1,35 0,08	1,41 0,14	1,31 0,08	0,023	0,949	1,02	0,370	0,458	1,34 0,300

Note. In each column, the first line is the average, the second – SE or Cv.

Table 11.2. Summary of step-by-step analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Phosphates Excretion, mM/24 h	33,0	10^{-6}	0,496	33,0	10^{-6}
Calcitonin, ng/L	7,42	0,001	0,403	18,4	10^{-6}
Creatinine Plasma, µM/L	5,95	0,004	0,279	13,8	10^{-6}
Testosterone, nM/L	5,86	0,005	0,234	13,0	10^{-6}
Sodium Plasma, mM/L	5,68	0,005	0,197	12,5	10^{-6}
Phosphate Plasma, mM/L	5,55	0,006	0,166	12,3	10^{-6}
Magnesium Urine, mM/L	5,20	0,008	0,140	12,1	10^{-6}
Chloride Excretion, mM/24 h	4,11	0,022	0,123	11,7	10^{-6}
Interleukin-6, ng/L	3,38	0,041	0,110	11,3	10^{-6}
LD Cholesterol Plasma, mM/L	3,83	0,028	0,096	11,1	10^{-6}
Sodium Urine, mM/L	2,83	0,068	0,087	10,8	10^{-6}
Microbian Count for Staph. aur., Bac/Ph	2,49	0,092	0,080	10,4	10^{-6}
Glucose Plasma, mM/L	2,44	0,097	0,073	10,1	10^{-6}
Chloride Urine mM/L	2,51	0,092	0,066	9,81	10^{-6}
Sodium Excretion, mM/24 h	2,05	0,140	0,061	9,51	10^{-6}
$(Ca/K)^{0,5}$ Plasma as Symp/Vagal balance	1,75	0,185	0,057	9,18	10^{-6}
VLF HRV PS, $msec^2$	1,40	0,255	0,054	8,81	10^{-6}
HF HRV PS, $msec^2$	4,41	0,018	0,045	9,13	10^{-6}
Magnesium Excretion, mM/24 h	2,16	0,127	0,042	8,99	10^{-6}
$(UA•Ca)/(Cr•Mg)^{0,25}$Lithogenicity Urine	3,04	0,058	0,037	9,06	10^{-6}
Killing Index vs Staph. aur., %	2,17	0,126	0,033	8,96	10^{-6}
$CD3^+$ active T-Lymphocytes, %	2,33	0,109	0,030	8,92	10^{-6}
Interleukin-1, ng/L	1,10	0,341	0,029	8,61	10^{-6}
Potassium Urine, mM/L	1,17	0,321	0,027	8,34	10^{-6}
Aldosterone, pM/L	1,54	0,226	0,025	8,18	10^{-6}
ULF HRV PS, $msec^2$	1,95	0,155	0,023	8,12	10^{-6}
HD Cholesterol Plasma, mM/L	1,02	0,370	0,022	7,87	10^{-6}

A number of variables, primarily **cholecystokinetic activity** to the standard stimulus, despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 11.3).

Table 11.3. Metabolic and neuroendocrine-immune complex parameters not included in the model

Variables	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (34)	After Naftussya (12)	After Salt Waters and N (22)	Wilks Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv (30)
Cholecystokinetic Activity, units	553 22	584 24	675 28	0,021	0,982	0,34	0,715	0,572	624 0,131
Calcium Urine, mM/L	2,34 0,18	2,40 0,82	3,04 0,26	0,022	0,995	0,10	0,910	0,354	3,13 0,214
Phosphates Urine, mM/L	10,8 0,7	10,5 1,1	15,8 1,3	0,021	0,975	0,47	0,629	0,071	18,0 0,294
Potassium Plasma, mM/L	4,21 0,10	4,25 0,16	4,43 0,10	0,021	0,990	0,19	0,831	0,457	4,55 0,104
Uric Acid Urine, mM/L	2,33 0,23	1,93 0,10	1,79 0,12	0,021	0,979	0,39	0,681	0,344	2,14 0,250
VLF HRV PS, %	50,8 3,0	51,1 6,5	44,4 2,5	0,021	0,980	0,37	0,691	0,346	53,9 0,277
Triiodothyronine, nM/L	1,97 0,13	1,93 0,30	1,78 0,13	0,022	0,996	0,07	0,932	0,142	2,20 0,227
Chloride Plasma, mM/L	100,8 1,0	105,3 1,2	101,3 1,6	0,022	0,999	0,02	0,980	0,044	101,5 0,032
$CD4^+$ T-helper Lymphocytes, %	28,0 1,3	34,8 2,1	26,7 0,9	0,022	0,999	0,02	0,980	0,044	39,5 0,082
$CD8^+$ T-cytolytic Lymphocytes, %	22,6 0,8	24,3 1,5	21,5 1,0	0,021	0,974	0,49	0,613	0,057	23,5 0,138
VLD Cholesterol Plasma, mM/L	0,57 0,05	0,48 0,08	0,64 0,08	0,022	0,999	0,02	0,980	0,474	0,54 0,612
LF HRV PS, $msec^2$	717 101	691 213	1604 158	0,021	0,965	0,67	0,519	0,189	625 0,482
Calcium Plasma, mM/L	2,20 0,04	2,23 0,04	2,20 0,04	0,022	0,997	0,06	0,943	0,169	2,30 0,065
Urea Plasma, mM/L	5,60 0,17	6,17 0,19	6,04 0,26	0,021	0,984	0,31	0,737	0,442	5,00 0,330
Creatinine Urine, mM/L	3,9 0,3	5,2 0,6	3,1 0,3	0,021	0,969	0,60	0,554	0,246	7,9 0,300
Cortisol, nM/L	373 26	441 30	419 41	0,021	0,993	0,13	0,875	0,716	405 0,524
Parathyroid activity, units	1,81 0,06	1,73 0,07	1,90 0,04	0,022	0,999	0,37	0,519	0,189	1,82 0,230
Blood Pressure systolic, mmHg	141,2 2,7	141,9 5,3	141,4 3,5	0,021	0,978	0,42	0,663	0,493	124,5 0,076
Blood Pressure diastolic, mmHg	84,6 1,7	85,7 1,7	86,0 8,2	0,021	0,987	0,25	0,780	0,476	79,0 0,054
Kerdoe Vegetative Index, units	-18,8 3,4	-23,1 5,1	-19,5 3,8	0,021	0,969	0,13	0,663	0,442	-23,5 20,1

The identifying information contained in the 27 discriminant variables is condensed into two roots. The major root contains 80% of discriminatory opportunities ($r^*=0,958$; Wilks' $\Lambda=0,022$; $\chi^2_{(56)}=197$; $p<10^{-6}$), while minor root - 20% only ($r^*=0,857$; Wilks' $\Lambda=0,265$; $\chi^2_{(27)}=68$; $p<10^{-4}$).

Calculating the values of discriminant roots for each patient based on coefficients and constants (Table 11.4) allows visualization of each patient in the information space of roots (Fig. 11.1).

Table 11.4. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Phosphates Excretion, mM/24 h	-1,619	0,244	-0,1389	0,0210
Calcitonin, ng/L	-0,802	0,038	-0,1817	0,0085
Creatinine Plasma, µM/L	0,857	-0,435	0,0678	-0,0344
Testosterone, nM/L	0,389	-1,150	0,0448	-0,1325
Sodium Plasma, mM/L	-0,434	1,159	-0,0493	0,1318
Phosphate Plasma, mM/L	-0,0586	1,031	-0,3130	5,5092
Magnesium Urine, mM/L	-1,217	-0,886	-1,8362	-1,3358
Chloride Excretion, mM/24 h	0,666	-1,712	0,0071	-0,0182
Interleukin-6, ng/L	-0,161	-0,926	-0,0841	-0,4839
LD Cholesterol Plasma, mM/L	1,059	0,701	1,0159	0,6727
Sodium Urine, mM/L	5,833	-0,777	0,1913	-0,0255
Microbian Count for Staph. aur., Bac/Ph	0,4101	0,2130	0,0491	0,0255
Glucose Plasma, mM/L	-0,502	0,423	-0,5240	0,4415
Chloride Urine mM/L	-1,351	2,433	-0,0379	0,0683
Sodium Excretion, mM/24 h	-4,335	1,370	-0,0463	0,0146
$(Ca/K)^{0,5}$ Plasma as Symp/Vagal balance	-0,566	0,327	-9,7651	5,6385
VLF HRV PS, $msec^2$	-0,440	0,596	-0,0007	0,0009
HF HRV PS, $msec^2$	0,596	-0,468	0,0011	-0,0008
Magnesium Excretion, mM/24 h	2,973	0,751	1,7012	0,4299
$(UA•Ca)/(Cr•Mg)^{0,25}$Lithogenicity Urine	-0,714	-0,262	-5,0313	-1,8465
Killing Index vs Staph. aur., %	-0,624	-0,346	-0,0821	-0,0455
$CD3^+$ active T-Lymphocytes, %	0,325	0,632	0,0700	0,1363
Interleukin-1, ng/L	-0,191	0,187	-0,1547	0,1510
Potassium Urine, mM/L	-2,663	-1,391	-0,1784	-0,0932
Aldosterone, pM/L	2,345	1,221	0,0876	0,0456
ULF HRV PS, $msec^2$	-0,3119	-0,5054	-0,0023	-0,0037
HD Cholesterol Plasma, mM/L	0,344	0,057	0,7764	0,1275
	Constants		-10,23	-37,42
	Eigenvalues		11,26	2,77
Cumulative Proportion			0,802	1

Following the accepted algorithm, Table 11.5 collects the Z-scores of discriminant variables together with those that are not included in the model, but still reflect the specifics of the water used.

Table 11.5. Correlations between immune variables and roots, centroids of clusters and Z-scores of clusters

Variables	Correlations Variables-Roots		After Salt Waters and N (22)	After Naftussya (12)	Before therapy (44)
Root 1(80 %)	**Root 1**	**Root 2**	**-4,73**	+1,87	+2,40
Phosphates Excretion	**-0,299**	-0,063	**+2,31**	-1,14	-0,94
Magnesium Excretion	**-0,146**	-0,141	**+1,79**	-0,64	+0,29
Chloride Excretion	**-0,106**	0,012	**+3,16**	+1,02	+0,62
$(UA•Ca)/(Cr•Mg)^{0,25}$Lithogenicity Urine	**-0,094**	-0,066	**+0,98**	+0,43	+0,59
HF HRV PS	**-0,045**	0,016	**+0,82**	-0,03	-0,04
Cholecystokinetic Activity			**+0,62**	-0,30	-0,86
Killing Index vs Staph. aureus	**-0,190**	-0,111	**-0,15**	-1,64	-1,28
Potassium Plasma			**-0,25**	-0,64	-0,72
Calcium Urine			**-0,13**	-1,09	-1,18
Phosphates Urine			**-0,42**	-1,36	-1,41
Triiodothyronine			**-0,46**	-0,55	-0,85
VLF HRV PS^2	**-0,065**	-0,043	**-0,01**	-0,47	-0,36
Calcitonin	**-0,119**	-0,054	**-0,51**	-1,14	-1,02
Phosphate Plasma	**0,108**	0,122	**-1,43**	-0,36	-0,82
Sodium Urine	**0,135**	-0,022	**-0,90**	+0,17	+0,39

Uric Acid Urine			**-0,65**	-0,24	+0,30
Potassium Urine	**0,089**	0,041	**-1,27**	-0,40	-0,56
LD Cholesterol Plasma	**0,036**	-0,019	**-0,28**	-0,11	+0,16
Glucose Plasma	**0,025**	-0,018	**-0,15**	-0,02	+0,09
(Ca/K)^{0,5} Plasma as Symp/Vagal balance	**0,051**	0,009	**-0,05**	+0,23	+0,24
Root 2(20 %)	**Root 1**	**Root 2**	-0,14	**+3,39**	-1,11
Testosterone	0,019	**-0,241**	+0,24	**-0,82**	+0,84
Magnesium Urine	0,027	**-0,082**	-0,95	**-1,05**	-0,71
Interleukin-6	-0,022	**-0,093**	+0,24	**-0,42**	+0,14
Interleukin-1	-0,040	**-0,110**	+0,78	**-0,24**	+0,50
VLD Cholesterol Plasma			+0,09	**-0,21**	+0,32
Parathyroid activity			+0,19	**-0,22**	-0,03
Creatinine Plasma	0,032	**-0,185**	+0,60	**+0,18**	+0,99
Urea Plasma			+0,63	**+0,45**	+0,83
Sodium Excretion	-0,035	**-0,111**	+2,58	**+0,78**	+2,17
Chloride Urine	0,047	**0,164**	-1,15	**+0,38**	-0,85
Creatinine Urine			-2,02	**-1,11**	-1,69
Chloride Plasma			-0,07	**+1,00**	-0,26
CD3^{+} active T-Lymphocytes	0,088	**0,151**	-0,78	**+0,25**	-0,33
Sodium Plasma	0,005	**0,130**	-0,55	**+0,33**	-0,71
Calcium Plasma			-0,64	**-0,44**	-0,66
Microbian Count for Staph. aureus	0,056	**0,092**	-0,14	**+0,44**	+0,12
HD Cholesterol Plasma	0,017	0,031	-0,08	**+0,21**	+0,04
ULF HRV PS	-0,023	0,103	-0,11	**+0,16**	-0,45
Aldosterone	-0,012	0,091	-0,19	**-0,05**	-0,30

The localization in the extreme left zone of the axis of the first root of the cluster of patients who received two mineral waters shows a significant increase relative to baseline levels of parameters that are **negatively** associated with the root, and a significant decrease in **positively** correlated with the root parameters. In contrast, in patients receiving **Naftussya** water only, these parameters remained unchanged or changed to a much lesser extent.

On the other hand, such patients are characterized by a significant **decrease**/**increase** in another number of parameters associated with the second root **negatively**/**positively**, while in combination balneotherapy their changes are insignificant or much less pronounced.

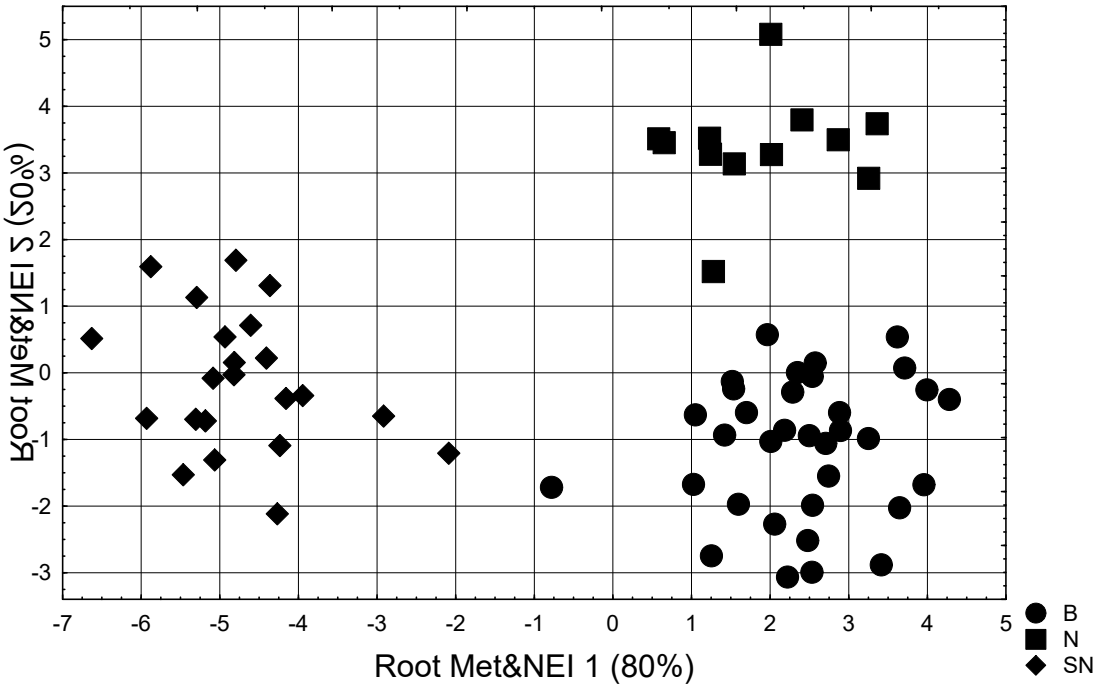


Fig. 11.1. Scattering of individual values of the first and second discriminant roots of patients before (circles) and after the course of drinking only water Naftussya (squares) and in combination with water Myroslava or Khrystyna (rhombuses)

Fig. 11.2 illustrates that the integrated initial state of all three groups of patients was almost the same as the effect on the discriminant variables of both sulfate-chloride sodium-magnesium mineral waters.

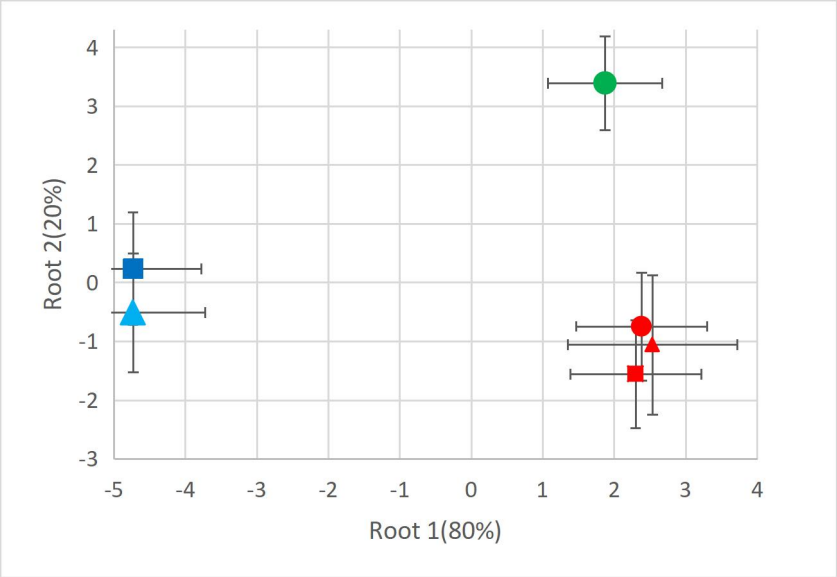


Fig. 11.2. Mean values (M±SD) of the first and second discriminant roots of patients before (red fill) and after the course of drinking only water Naftussya (circle) and in combination with water Myroslava (triangle) or Khrystyna (square)

The visual impression of a clear demarcation of the three clusters in the information field of the two roots is documented by calculating the distances of Mahalanobis (Table 11.6).

Table 11.6. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=15,4) and p-levels (below the diagonal)

Clusters	Before therapy	After Naftussya	After SW& N
Before therapy	0	21	52
After Naftussya	3,8 10^{-4}	0	56
After SW&N	14,5 10^{-6}	9,1 10^{-6}	0

Selected discriminant variables were used to identify the affiliation of a patient to a particular cluster. This goal of discriminant analysis is realized with the help of classification functions (Table 11.7).

Table 11.7. Coefficients and constants of classification functions

Clusters	Before therapy	After Naftussya	After Salt W&N
Variables	p=,500	p=,176	p=,324
Phosphates Excretion, mM/24 h	-0,742	-0,574	0,270
Calcitonin, ng/L	-2,613	-2,478	-1,308
Creatinine Plasma, µM/L	-0,164	-0,355	-0,681
Testosterone, nM/L	-2,692	-3,312	-3,141
Sodium Plasma, mM/L	5,563	6,182	6,043
Phosphate Plasma, mM/L	471,4	496,4	479,0
Magnesium Urine, mM/L	-41,67	-46,71	-29,86
Chloride Excretion, mM/24 h	-0,108	-0,194	-0,176
Interleukin-6, ng/L	-30,56	-32,69	-30,43

LD Cholesterol Plasma, mM/L	70,60	73,08	64,00
Sodium Urine, mM/L	7,063	6,846	5,673
Microbian Count for Staph. aur., Bac/Ph	3,221	3,310	2,895
Glucose Plasma, mM/L	12,50	14,76	16,66
Chloride Urine mM/L	0,682	1,009	1,019
Sodium Excretion, mM/24 h	-0,511	-0,420	-0,166
$(Ca/K)^{0,5}$ Plasma as Symp/Vagal balance	149,1	179,7	224,2
VLF HRV PS, $msec^2$	0,091	0,095	0,097
HF HRV PS, $msec^2$	-0,088	-0,092	-0,097
Magnesium Excretion, mM/24 h	34,30	35,33	22,57
$(UA•Ca)/(Cr•Mg)^{0,25}$Lithogenicity Urine	40,54	34,90	74,66
Killing Index vs Staph. aureus, %	-0,166	-0,328	0,376
$CD3^+$ active T-Lymphocytes, %	10,05	10,62	9,678
Interleukin-1, ng/L	7,842	8,604	9,093
Potassium Urine, mM/L	-19,99	-20,32	-18,81
Aldosterone, pM/L	10,02	10,18	9,436
ULF HRV PS, $msec^2$	-0,512	-0,528	-0,500
HD Cholesterol Plasma, mM/L	26,96	27,12	21,55
Constants	-2151	-2319	-2122

The use of classification functions allows unmistakable retrospective identification of all clusters (Table 11.8).

Table 11.8. Classification matrix
Rows: observed classifications; columns: projected classifications

	Percent Correct	**Before therapy**	**After Naftussya**	**After Salt W&N**
Groups		p=,500	p=,176	p=,324
Before therapy	100	**34**	0	0
After Naftussya	100	0	**12**	0
After Salt W&N	100	0	0	**22**
Total	100	34	12	22

Thus, we have shown that complex balneotherapy by interval use of sulfate-chloride sodium-magnesium mineral water with Naftussya water causes significant changes in the constellation of neuroendocrine, metabolic and immune parameters, which are different from the effects of Naftussya water monotherapy.

In the conditions of the resort, it was organizationally (but also ethically) impossible to offer patients to use only newly created mineral waters. However, the calculation of algebraic differences between the mean Z-scores of the parameters in both groups of patients still allows us to assess the independent effects of sulfate-chloride sodium-magnesium mineral waters.

This approach suggests that sulfate-chloride sodium-magnesium mineral waters have their own (per se) more or less pronounced effect on the constellation of parameters of the neuro-endocrine-immune complex and metabolism, regardless of their initial levels (Fig. 11.3).

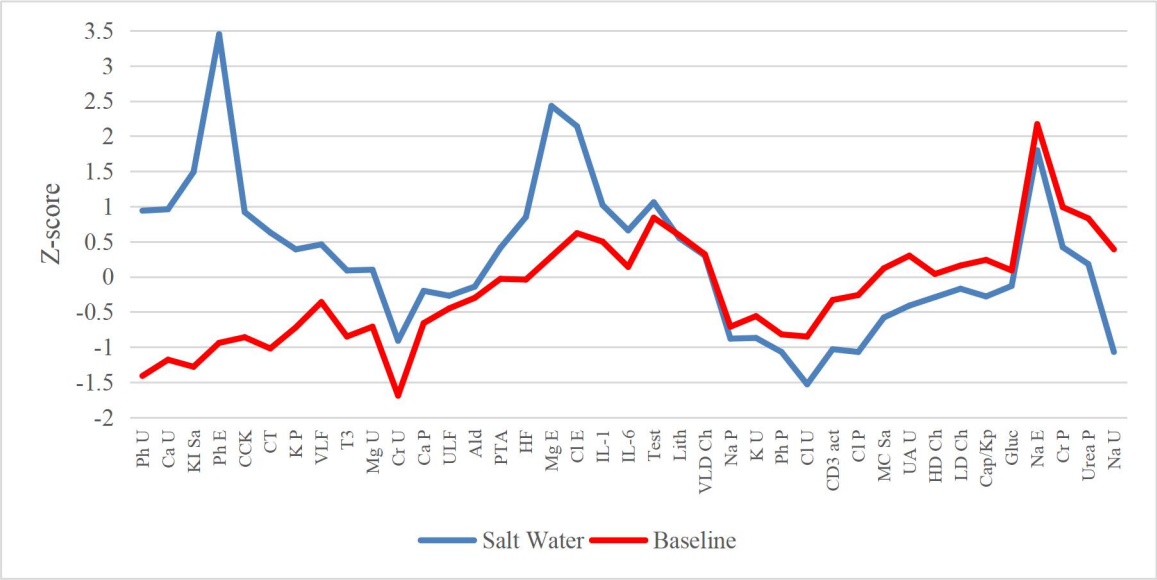


Fig. 11.3. Profiles of real Z-scores of initial discriminant variables and their simulated Z-scores after consumption of sulphate-chloride sodium-magnesium mineral waters

In particular, initially reduced neuroendocrine (VLF and ULF bands HRV, calcitonin, triiodothyronine, to a lesser extent aldosterone and parathyroid activity) and metabolic (urine concentrations of phosphate, calcium, magnesium and creatinine, phosphaturia, plasma potassium and calcium, cholecystokinetic activity) variables as well as the completion of phagocytosis of Staphylococcus aureus increase, as a rule, to the zone of norm. On the other hand, initially increased urinary excretion and concentration of sodium and plasma creatinine and urea levels are reduced. Such effects are consistent with the ancient concept of the ambivalent-balancing nature of the effects of balneal factors on the body (Balanovs'kyi et al., 1993).

However, there is an increase in initially normal levels of vagal tone, parathyroid activity, excretion of magnesium and chloride and interleukins 1 and 6 plasma, as well as a decrease in initially normal levels of Ca/K marker sympathetic-vagal balance, concentration of uric acid in urine as well as glucose and cholesterol in plasma as well as the intensity of Staphylococcus aureus phagocytosis. The latter pattern is formed by initially reduced plasma levels of sodium, phosphate and chloride, chloride and potassium of urine, as well as active T-lymphocytes of blood, which continue to decline. Such effects do not fit into this concept, but are consistent with the known data on the diversity of responses of the neuroendocrine-immune complex and metabolism to balneal factors (Popovych, 2011; Chebanenko et al., 2013; Kozyavkina et al., 2015; Popovych et al., 2020; Gozhenko et al., 2021).

11.2. Electroencephalogram

Given the significant number of registered EEG parameters (n=168), their analysis is assigned a separate section.

Following the accepted algorithm, the method of discriminant analysis revealed 30 EEG parameters, according to which the conditions of patients before and after the two balneotherapy regimens differ significantly. Characteristic were 4 parameters of **beta**-rhythm, 6 parameters of **alpha**- and **theta**-rhythm and 8 parameters of **delta**-rhythm, as well as the entropy of PSD in 6 loci (Tables 11.9 and 11.10).

Table 11.9. Summary of the analysis of discriminant functions in relation to the parameters of EEG
Step 30, N of vars in model: 30; Grouping: 3 grps; Wilks' Λ: 0,057; approx. $F_{(61)}$=3,8; $p<10^{-6}$

Variables currently in the model	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	After Naftus-sya (12)	After Salt Waters and N (22)	Before thera-py (34)	Wil ks' Λ	Par-tial Λ	F-re-move (2,36)	p-level	Tole-rancy	Norm Cv/σ (122)
Laterality β, %	-33 10	-4 4	-3 5	0,086	0,666	9,03	0,001	0,179	-6 ***28***
F4-β PSD, μV²/Hz	68 11	92 12	86 9	0,061	0,938	1,19	0,317	0,101	73 0,612
T4-β PSD, %	33,6 4,7	37,3 4,6	29,0 2,4	0,071	0,798	4,56	0,017	0,111	27,9 0,591
Fp2-β PSD, μV²/Hz	50 7	74 10	74 8	0,061	0,936	1,23	0,305	0,232	61 0,629
Laterality α,	-23	-18	-1	0,066	0,858	2,97	0,064	0,096	-4

%	9	5	6						***27***
T4-α PSD, %	23,0 3,2	32,6 3,9	28,0 2,9	0,058	0,987	0,23	0,794	0,069	29,2 0,628
F8-α PSD, μV²/Hz	37 13	23 2	37 4	0,063	0,909	1,80	0,179	0,267	40 0,957
F4-α PSD, %	22,0 3,8	31,5 3,1	31,4 3,4	0,120	0,475	19,9	10^{-6}	0,031	32,7 0,564
P3-α PSD, %	37,7 5,5	49,5 3,8	42,1 3,6	0,077	0,737	6,44	0,004	0,032	40,8 0,480
C3-α PSD, %	30,1 4,8	38,9 3,4	35,5 3,2	0,071	0,803	4,43	0,019	0,057	35,3 0,510
Laterality θ, %	-24 10	-35 10	-4 7	0,119	0,478	19,7	10^{-5}	0,036	-3 ***32***
T4-θ PSD, μV²/Hz	22 4	19 3	34 7	0,079	0,724	6,85	0,003	0,032	32 2,582
F7-θ PSD, %	9,8 1,0	8,8 1,3	7,1 0,7	0,127	0,450	22,0	10^{-6}	0,055	7,9 0,568
T4-θ PSD, %	9,1 0,8	6,4 0,7	9,5 1,2	0,101	0,565	13,86	10^{-4}	0,016	8,7 0,539
Fp2-θ PSD, %	8,9 0,8	6,7 1,3	9,7 1,5	0,095	0,600	12,0	10^{-4}	0,028	8,3 0,588
Fp2-θ PSD, μV²/Hz	18 3	20 4	29 7	0,083	0,690	8,08	0,001	0,033	25 1,186
Deviation δ, Hz	0,71 0,10	0,57 0,04	0,73 0,05	0,067	0,846	3,27	0,050	0,490	0,66 0,405
T6-δ PSD, μV²/Hz	53 11	125 60	97 23	0,124	0,458	21,3	10^{-6}	0,006	74 1,108
T5-δ PSD, μV²/Hz	85 21	110 53	179 40	0,078	0,735	6,49	0,004	0,084	89 1,404
F7-δ PSD, μV²/Hz	84 26	93 42	163 45	0,095	0,602	11,9	10^{-4}	0,016	80 1,750
F8-δ PSD, %	50,2 8,8	28,3 7,3	38,8 4,7	0,074	0,770	5,37	0,009	0,183	38,3 0,700
C4-δ PSD, %	34,8 6,5	22,9 4,0	28,6 3,5	0,066	0,865	2,81	0,073	0,107	29,9 0,617
O2-δ PSD, μV²/Hz	104 19	219 104	148 35	0,062	0,913	1,72	0,193	0,086	95 0,968
P3-δ PSD, %	27,5 4,9	19,8 3,4	27,3 3,3	0,082	0,695	7,89	0,001	0,036	26,5 0,672
Entropy F7	0,851 0,024	0,724 0,054	0,704 0,039	0,060	0,956	0,83	0,446	0,122	0,751 0,282
Entropy Fp2	0,797 0,036	0,705 0,048	0,817 0,024	0,202	0,282	45,9	10^{-6}	0,021	0,799 0,180
Entropy T4	0,843 0,029	0,736 0,030	0,819 0,022	0,131	0,434	23,5	10^{-6}	0,029	0,790 0,215
Entropy O2	0,798 0,027	0,669 0,037	0,769 0,028	0,082	0,698	7,77	0,002	0,106	0,727 0,242
Entropy T6	0,834 0,026	0,710 0,046	0,790 0,031	0,069	0,826	3,79	0,032	0,108	0,761 0,249
Entropy P3	0,851 0,032	0,771 0,025	0,797 0,024	0,068	0,834	3,57	0,038	0,098	0,804 0,155

Note. In each column, the first line is the average, the second – SE for variables and Cv or ***SD*** for Norm.

Table 11.10. Summary of stepwize analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Laterality β, %	6,28	0,003	0,84	6,28	0,003
Laterality θ, %	4,02	0,023	0,74	5,09	0,001
Entropy F7	2,83	0,067	0,68	4,41	10^{-3}
Entropy Fp2	3,31	0,043	0,62	4,23	10^{-3}
T4-α PSD, %	2,00	0,144	0,58	3,83	10^{-3}
Entropy T4	2,37	0,102	0,54	3,65	10^{-3}
T6-δ PSD, μV²/Hz	2,87	0,065	0,49	3,62	10^{-4}
T4-θ PSD, μV²/Hz	1,96	0,150	0,46	3,46	10^{-4}
F8-α PSD, μV²/Hz	2,25	0,115	0,42	3,38	10^{-4}
F4-α PSD, %	1,72	0,188	0,40	3,25	10^{-4}
P3-α PSD, %	3,02	0,057	0,36	3,32	10^{-4}
T5-δ PSD, μV²/Hz	3,22	0,048	0,32	3,43	10^{-5}
F7-θ PSD, %	2,08	0,135	0,30	3,38	10^{-5}
Entropy O2	2,78	0,071	0,27	3,43	10^{-5}
T4-θ PSD, %	2,26	0,115	0,25	3,43	10^{-5}
F7-δ PSD, μV²/Hz	5,41	0,007	0,20	3,80	10^{-6}
F4-β PSD, μV²/Hz	3,21	0,049	0,18	3,91	10^{-6}
Fp2-θ PSD, %	1,63	0,207	0,17	3,82	10^{-6}
Laterality α, %	2,22	0,120	0,15	3,83	10^{-6}
Fp2-θ PSD, μV²/Hz	2,13	0,131	0,14	3,82	10^{-6}
Deviation δ, Hz	2,06	0,139	0,13	3,82	10^{-6}
F8-δ PSD, %	1,97	0,152	0,12	3,80	10^{-6}
C4-δ PSD, %	1,77	0,183	0,11	3,77	10^{-6}
O2-δ PSD, μV²/Hz	2,61	0,085	0,10	3,85	10^{-6}
P3-δ PSD, %	1,34	0,272	0,09	3,78	10^{-6}
Entropy T6	2,29	0,114	0,08	3,83	10^{-6}
Entropy P3	1,13	0,334	0,08	3,74	10^{-6}
T4-β PSD, %	1,05	0,359	0,07	3,65	10^{-6}
C3-α PSD, %	3,86	0,030	0,06	3,89	10^{-6}
Fp2-β PSD, μV²/Hz	1,23	0,305	0,06	3,83	10^{-6}

A number of variables despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 11.11).

Table 11.11. EEGs parameters not included in the model

	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
Variables currently in the model	**After Naftussya (12)**	**After Salt Waters and N (22)**	**Before therapy (34)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv/σ (122)
F8-β PSD, %	23,9 4,9	39,0 5,1	29,9 3,5	0,057	0,995	0,09	0,912	0,067	28,7 0,702
F8-θ PSD, μV²/Hz	23 5	11 2	22 5	0,056	0,985	0,26	0,772	0,252	19 1,791
O2-θ PSD, %	7,2 0,8	5,1 0,6	6,1 0,7	0,057	0,996	0,07	0,928	0,255	6,0 0,603
Entropy T5	0,835 0,028	0,770 0,041	0,744 0,033	0,057	0,998	0,03	0,969	0,170	0,778 0,211

The identifying information contained in the 30 discriminant variables is condensed into two roots. The major root contains 90% of discriminatory opportunities (r^*=0,944; Wilks' Λ=0,057; $\chi^2_{(60)}$=145; $p<10^{-6}$), while minor root 10% only (r^*=0,689; Wilks' Λ=0,526; $\chi^2_{(29)}$=32; p=0,299).

Calculating the values of discriminant roots for each patient by the raw coefficients and the constant (Table 11.12) allows visualization of each patient in the information space of roots.

Table 11.12. Standardized and raw coefficients and constants for discriminant EEG variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Laterality β, %	1,374	-0,613	0,0550	-0,0245
Laterality θ, %	-3,999	0,492	-0,1119	0,0138
Entropy F7	-0,591	-0,314	-3,1466	-1,6701
Entropy Fp2	6,220	-0,378	42,538	-2,5875
T4-α PSD, %	-0,174	-0,578	-0,0122	-0,0404
Entropy T4	-4,676	0,530	-40,737	4,6211
T6-δ PSD, $\mu V^2/Hz$	10,024	0,449	0,0252	0,0011
T4-θ PSD, $\mu V^2/Hz$	-3,112	0,440	-0,1137	0,0161
F8-α PSD, $\mu V^2/Hz$	0,083	0,840	0,0034	0,0350
F4-α PSD, %	4,266	1,032	0,2782	0,0673
P3-α PSD, %	-3,000	-0,717	-0,1691	-0,0404
T5-δ PSD, $\mu V^2/Hz$	-1,756	0,903	-0,0022	0,0011
F7-θ PSD, %	-3,351	-0,172	-0,8149	-0,0419
Entropy O2	1,788	0,087	13,073	0,6355
T4-θ PSD, %	5,478	-1,528	1,1257	-0,3139
F7-δ PSD, $\mu V^2/Hz$	-5,196	-1,160	-0,0036	-0,0008
F4-β PSD, $\mu V^2/Hz$	-0,261	-1,081	-0,0057	-0,0237
Fp2-θ PSD, %	-4,001	0,581	-0,6332	0,0920
Laterality α, %	1,280	-0,107	0,0480	-0,0040
Fp2-θ PSD, $\mu V^2/Hz$	3,187	0,695	0,1170	0,0255
Deviation δ, Hz	-0,575	0,198	-2,3259	0,8004
F8-δ PSD, %	-1,147	0,423	-0,0429	0,0159
C4-δ PSD, %	1,024	-0,824	0,0565	-0,0454
O2-δ PSD, $\mu V^2/Hz$	0,121	-1,455	0,0001	-0,0017
P3-δ PSD, %	-3,103	0,037	-0,1946	0,0023
Entropy T6	1,313	-0,371	8,3683	-2,3617
Entropy P3	-1,378	0,050	-12,129	0,4444
T4-β PSD, %	-1,370	-0,549	-0,0913	-0,0366
C3-α PSD, %	-1,903	-0,704	-0,1222	-0,0452
Fp2-β PSD, $\mu V^2/Hz$	0,487	0,366	0,0126	0,0095
	Constants		7,567	5,967
	Eigenvalues		8,22	0,90
Cumulative Proportion			0,901	1

The localization of the cluster of patients who received only **Naftussya** water in the extreme left zone of the first root axis (Fig. 11.4) reflects the maximum decrease in the initial parameters that are **positively** related to the root, as well as the maximum increase **inversely** correlated parameters (Table 11.13). Recall that a negative value of the Laterality Index indicates a left shift of symmetry. In contrast, in patients receiving **complex balneotherapy**, these EEG parameters deviated from the initial to a much lesser extent or remained unchanged.

On the other hand, these patients are characterized by reduced or minimal for sample EEG parameters that correlate positively with the second root, and correspondingly increased or maximum for sample EEG parameters that correlate **negatively** with it, which is visualized by localization of the cluster in the lower root axis.

Table11.13. Correlations between EEGs variables and roots, centroids of clusters and Z-scores of variables

Variables	Correlations Variables-Roots		After Naftussya (12)	After Salt Waters and N (22)	Before therapy (34)
Root 1 (90%)	**Root 1**	**Root 2**	**-5,77**	+0,06	+2,00
Laterality β	**0,149**	-0,112	**-0,95**	+0,08	+0,10
Laterality α	**0,098**	0,144	**-0,71**	-0,52	+0,12
F4-α PSDr	**0,076**	-0,065	**-0,58**	-0,06	-0,07
Fp2-β PSDa	**0,075**	-0,061	**-0,26**	+0,35	+0,35
Fp2-θ PSDa	**0,048**	0,079	**-0,23**	-0,17	+0,14
T5-δ PSDa	**0,047**	0,032	**-0,04**	+0,17	+0,72

F7-δ PSDa	**0,025**	-0,145	**+0,03**	+0,09	+0,59
Entropy F7	**-0,096**	0,039	**+0,47**	-0,13	-0,22
Entropy T5			**+0,35**	-0,05	-0,20
F7-θ PSDr	**-0,077**	-0,082	**+0,51**	+0,29	-0,11
Root 2 (10%)	**Root 1**	**Root 2**	+0,61	**-1,34**	+0,65
Laterality θ	0,063	**0,240**	-0,66	**-0,98**	-0,04
Entropy Fp2	0,012	**0,256**	-0,02	**-0,66**	+0,13
T4-θ PSDr	0,006	**0,215**	+0,08	**-0,49**	+0,17
F8-α PSDa	-0,007	**0,201**	-0,07	**-0,44**	-0,08
Entropy O2	-0,031	**0,277**	+0,40	**-0,32**	+0,24
Entropy T4	-0,032	**0,272**	+0,31	**-0,32**	+0,17
Deviation δ	0,007	**0,218**	+0,17	**-0,34**	+0,26
Entropy T6	-0,039	**0,206**	+0,39	**-0,27**	+0,15
F8-δ PSDr	-0,056	**0,180**	+0,45	**-0,37**	+0,02
P3-δ PSDr	-0,005	**0,166**	+0,06	**-0,38**	+0,04
Fp2-θ PSDr	0,012	**0,157**	+0,13	**-0,34**	+0,29
T4-θ PSDa	0,049	**0,155**	-0,12	**-0,16**	+0,03
C4-δ PSDr	-0,045	**0,145**	+0,27	**-0,38**	-0,07
O2-θ PSDr			+0,32	**-0,25**	+0,03
F8-θ PSDa			+0,11	**-0,24**	+0,08
Entropy P3	-0,061	**0,127**	+0,38	**-0,27**	-0,06
O2-δ PSDa	0,028	**-0,167**	+0,09	**+1,35**	+0,58
T4-β PSDr	-0,034	**-0,164**	+0,35	**+0,58**	+0,07
T6-δ PSDa	0,040	**-0,123**	-0,25	**+0,62**	+0,28
F8-β PSDr			-0,24	**+0,51**	+0,06
P3-α PSDr	0,034	**-0,171**	-0,15	**+0,45**	+0,07
F4-β PSDa	0,050	**-0,085**	-0,11	**+0,43**	+0,30
T4-α PSDr	0,046	**-0,147**	-0,34	**+0,19**	-0,07
C3-α PSDr	0,045	**-0,111**	-0,29	**+0,20**	+0,01

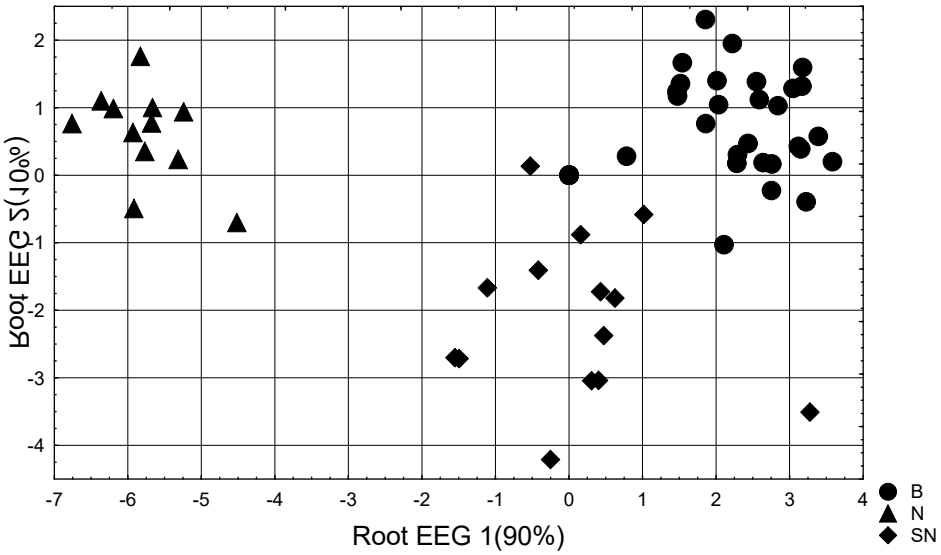


Fig. 11.4. Scattering of individual values of the first and second EEG discriminant roots of patients before (circles) and after the course of drinking only water Naftussya (triangles) and in combination with water Myroslava or Khrystyna (rhombuses)

Fig. 11.5 illustrates that the integrated initial state of all three groups of patients was almost the same as the effect on the discriminant EEG variables of both sulfate-chloride sodium-magnesium mineral waters.

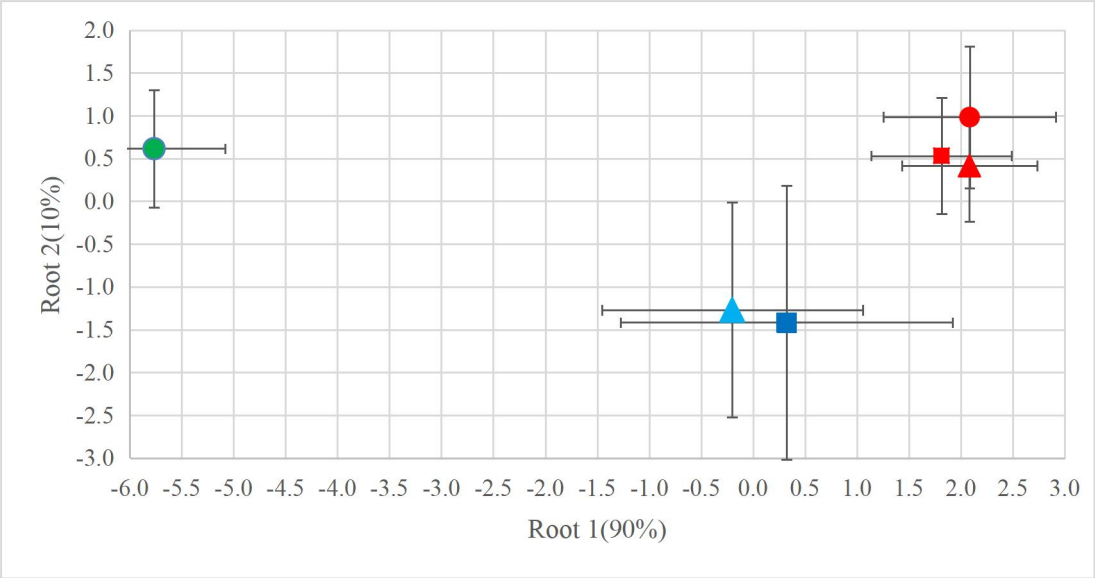


Fig. 11.5. Mean values (M±SD) of the first and second discriminant EEG roots of patients before (red fill) and after the course of drinking only water Naftussya (circle) and in combination with water Myroslava (triangle) or Khrystyna (square)

The visual impression of a clear demarcation of the three clusters in the information field of the two roots is documented by calculating the distances of Mahalanobis (Table 11.14).

Table 11.14. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=30,3) and p-levels (below the diagonal)

Clusters	Before therapy	After Naftussya	After SW& N
Before therapy	0	60	7,7
After Naftussya	**9,9** 10^{-6}	0	38
After SW&N	**1,91** 0,033	**5,4** 10^{-5}	0

Selected discriminant variables were used to identify the affiliation of a patient to a particular cluster. This goal of discriminant analysis is realized with the help of classification functions (Table 11.15).

Table 11.15. Coefficients and constants of classification functions

Clusters	Before therapy	After Naftussya	After Salt W&N
Variables	p=,500	p=,176	p=,324
Laterality β, %	-0,914	-1,340	-0,972
Laterality θ, %	-0,046	0,822	0,143
Entropy F7	233,3	257,8	242,8
Entropy Fp2	-83,74	-413,79	-160,9
T4-α PSD, %	2,086	2,182	2,190
Entropy T4	-353,5	-37,51	-283,9
T6-δ PSD, $\mu V^2/Hz$	-0,226	-0,422	-0,277
T4-θ PSD, $\mu V^2/Hz$	-0,583	0,299	-0,395
F8-α PSD, $\mu V^2/Hz$	0,032	0,004	-0,045
F4-α PSD, %	-0,515	-2,678	-1,188
P3-α PSD, %	6,436	7,750	6,844
T5-δ PSD, $\mu V^2/Hz$	0,037	0,055	0,040
F7-θ PSD, %	-4,860	1,466	-3,200
Entropy O2	386,6	285,1	360,0
T4-θ PSD, %	15,31	6,583	13,76
F7-δ PSD, $\mu V^2/Hz$	0,054	0,081	0,062

F4-β PSD, μV²/Hz	0,976	1,021	1,035
Fp2-θ PSD, %	2,844	7,755	3,886
Laterality α, %	1,108	0,735	1,023
Fp2-θ PSD, μV²/Hz	-2,362	-3,270	-2,639
Deviation δ, Hz	11,68	29,70	14,59
F8-δ PSD, %	0,296	0,628	0,347
C4-δ PSD, %	2,239	1,802	2,220
O2-δ PSD, μV²/Hz	0,069	0,068	0,072
P3-δ PSD, %	6,638	8,148	7,010
Entropy T6	-135,2	-200,0	-146,7
Entropy P3	757,5	851,7	780,1
T4-β PSD, %	5,517	6,227	5,766
C3-α PSD, %	5,831	6,781	6,158
Fp2-β PSD, μV²/Hz	-0,295	-0,392	-0,338
Constants	-838,8	-913,5	-864,5

The accuracy of the classification is 91,2% (Table 11.16).

Table 11.16. Classification matrix
Rows: observed classifications; columns: projected classifications

	Percent Correct	**Before therapy**	**After Naftussya**	**After Salt W&N**
Groups		p=,500	p=,176	p=,324
Before therapy	82,4	**28**	0	6
After Naftussya	100	0	**12**	0
After Salt W&N	100	0	0	**22**
Total	91,2	28	12	28

Thus, we have shown that complex balneotherapy by interval use of sulfate-chloride sodium-magnesium mineral water with Naftussya water causes significant changes in the constellation of EEG parameters, which are different from the effects of Naftussya water monotherapy.

Using the algebraic approach, we modeled the neurotropic effects of mineral waters themselves.

Three patterns of neurotropic effects of mineral waters emerge (Fig. 11.6). The first pattern (12 parameters) reflects a more or less pronounced activation of neurons that generate delta, alpha and beta rhythms, as well as a right-hand shift of symmetry of beta-rhythm. In contrast, the antipode pattern (17 parameters) reflects the inhibition of neurons that generate delta, alpha, and theta rhythms and the left-hand shift of theta-rhythm symmetry, as well as the decrease in EEG entropy. The intermediate position in the profiles is occupied by 5 parameters, the changes of which are insignificant.

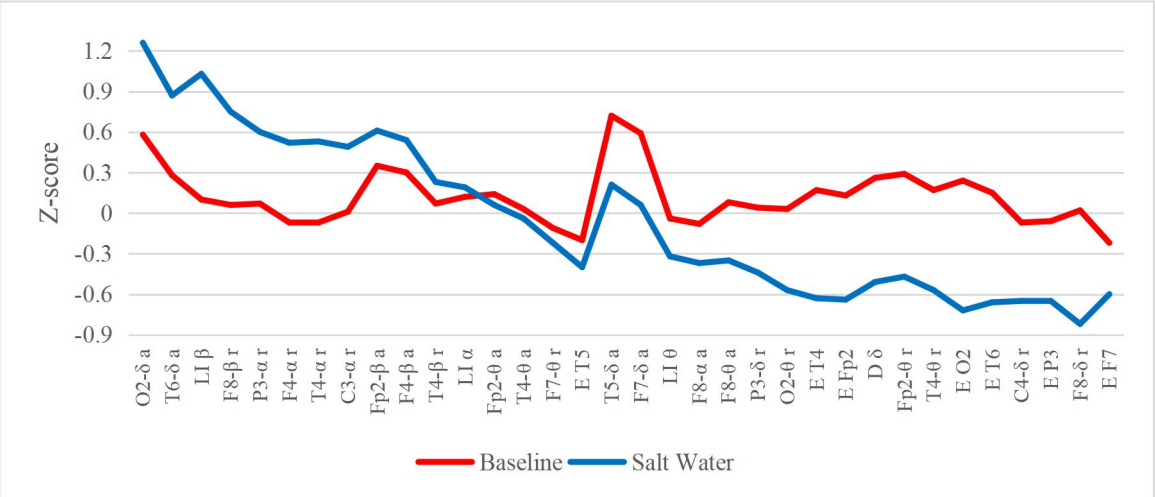


Fig. 11.6. Profiles of real Z-scores of initial discriminant EEGs variables and their simulated Z-scores after consumption of sulphate-chloride sodium-magnesium mineral waters

11.3. Neuroendocrine-immune complex and metabolism

At the final stage, the object of discriminant analysis was 168 EEG parameters, 17 HRV parameters, 5 hormonal, 33 immune and 34 metabolic. The method of discriminant analysis revealed 35 parameters, according to which the conditions of patients before and after the two balneotherapy regimens differ significantly. Characteristic were 14 **EEGs**, 2 **HRVs**, 2 **endocrine**, 4 **immune** and 13 **metabolic** parameters (Tables 11.17 and 11.18).

Table 11.17. Summary of the analysis of discriminant functions

Step 35, N of vars in model: 35; Grouping: 3 grps; Wilks' Λ: 0,0072; approx. $F_{(71)}$=9,5; $p<10^{-6}$

Variables currently in the model	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (34)	After Naftussya (12)	After SW and N (22)	Wilks' Λ	Partial Λ	F-remove (2,31)	p-level	Tolerancy	Norm Cv
Phosphates Excretion, mM/24 h	18,2 1,2	16,8 1,8	42,4 3,8	0,013	0,538	13,3	10^{-4}	0,187	25,2 0,294
Calcitonin, ng/L	6,95 0,62	6,16 1,11	10,48 1,21	0,009	0,778	4,41	0,021	0,404	13,95 0,493
Killing Index vs Staph. aur., %	48,2 1,5	45,2 1,9	57,7 1,4	0,011	0,631	9,04	0,001	0,286	58,9 0,142
Testosterone, nM/L	18,5 1,6	9,0 1,0	15,3 2,1	0,013	0,568	11,8	10^{-4}	0,286	14,8 0,400
Magnesium Urine, mM/L	2,40 0,11	2,14 0,23	2,22 0,13	0,011	0,655	8,16	0,001	0,055	2,93 0,256
Creatinine Plasma, μM/L	92,6 2,6	81,9 2,8	87,4 2,0	0,011	0,657	8,10	0,001	0,352	79,5 0,167
Laterality β, %	-3 5	-33 10	-4 4	0,008	0,925	1,25	0,301	0,286	-6 ***28***
Sodium Plasma, mM/L	141,5 1,5	146,7 2,1	142,3 2,0	0,012	0,613	9,79	0,001	0,254	145,0 0,034
LD Cholesterol Plasma, mM/L	3,54 0,18	3,43 0,32	3,25 0,21	0,010	0,729	5,77	0,007	0,269	3,44 0,192
Interleukin-6, ng/L	4,45 0,36	3,67 0,56	4,58 0,33	0,011	0,673	7,52	0,002	0,146	4,25 0,324
F7-θ PSD, %	7,1 0,7	9,8 1,0	8,8 1,3	0,010	0,748	5,23	0,011	0,128	7,9 0,568
VLF HRV PS, $msec^2$	969 99	869 141	1238 168	0,010	0,730	5,72	0,008	0,259	1250 0,572
Entropy Fp2	0,817 0,024	0,797 0,036	0,705 0,048	0,008	0,948	0,84	0,440	0,208	0,799 0,180
Sodium Urine, mM/L	119 5	114 8	89 7	0,009	0,759	4,93	0,014	0,017	110 0,211
Chloride Urine mM/L	102 3	127 14	96 10	0,009	0,814	3,54	0,041	0,023	120 0,172
Phosphate Plasma, mM/L	1,04 0,03	1,13 0,06	0,91 0,04	0,007	0,997	0,05	0,949	0,306	1,20 0,167
$CD3^+$ active T-Lymphocytes, %	28,3 0,8	31,3 0,9	26,1 1,1	0,009	0,824	3,32	0,050	0,508	30,0 0,167
Lithogenicity Urine	0,86 0,03	0,83 0,03	0,95 0,03	0,010	0,750	5,17	0,012	0,358	0,73 0,300
T4-θ PSD, $\mu V^2/Hz$	34 7	22 4	19 3	0,009	0,819	3,42	0,045	0,210	32 2,582
Laterality θ, %	-4 7	-24 10	-35 10	0,011	0,670	7,65	0,002	0,096	-3 ***32***
C3-α PSD, %	35,5 3,2	30,1 4,8	38,9 3,4	0,012	0,588	10,9	10^{-3}	0,089	35,3 0,510
P3-α PSD, %	42,1 3,6	37,7 5,5	49,5 3,8	0,010	0,731	5,71	0,008	0,089	40,8 0,480
Sodium Excretion, mM/24 h	225 18	179 11	238 19	0,009	0,821	3,39	0,047	0,011	154 0,211

Magnesium Excretion, mM/24 h	4,40 0,29	3,43 0,36	5,98 0,43	0,012	0,613	9,78	0,001	0,022	4,10 0,256
Chloride Excretion, mM/24 h	186 13	197 15	259 27	0,008	0,874	2,23	0,124	0,020	167,5 0,172
HF HRV PS, msec^2	354 75	407 262	541 100	0,008	0,854	2,65	0,087	0,266	350 0,713
Glucose Plasma, mM/L	4,77 0,17	4,68 0,33	4,59 0,18	0,008	0,883	2,06	0,145	0,441	4,70 0,160
F8-δ PSD, %	38,8 4,7	50,2 8,8	28,3 7,3	0,007	0,987	0,21	0,816	0,316	38,3 0,700
Fp2-β PSD, μV^2/Hz	74 8	50 7	74 10	0,009	0,793	4,05	0,027	0,130	61 0,629
T5-δ PSD, μV^2/Hz	179 40	85 21	110 53	0,008	0,916	1,42	0,258	0,252	89 1,404
F4-β PSD, μV^2/Hz	86 9	68 11	92 12	0,008	0,923	1,29	0,290	0,106	73 0,612
Entropy T6	0,790 0,031	0,834 0,026	0,710 0,046	0,009	0,801	3,86	0,032	0,126	0,761 0,249
Microbian Count for St. aur., B/Ph	62,8 1,2	66,0 2,0	60,2 2,3	0,009	0,847	2,81	0,076	0,478	61,6 0,160
Laterality α, %	-1 6	-23 9	-18 5	0,008	0,854	2,64	0,087	0,129	-4 ***27***
Entropy F7	0,704 0,039	0,851 0,024	0,724 0,054	0,008	0,911	1,52	0,235	0,127	0,751 0,282

Note. In each column, the first line is the average, the second – SE for variables and Cv or ***SD*** for Norm.

Table 11.18. Summary of step-by-step analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	**Λ**	F-value	p-level
Phosphates Excretion, mM/24 h	33,0	10^{-6}	0,496	33,0	10^{-6}
Calcitonin, ng/L	7,42	0,001	0,403	18,4	10^{-6}
Killing Index vs Staph. aureus, %	6,14	0,004	0,337	15,2	10^{-6}
Testosterone, nM/L	5,86	0,005	0,283	13,6	10^{-6}
Magnesium Urine, mM/L	5,57	0,006	0,240	12,7	10^{-6}
Creatinine Plasma,μM/L	6,31	0,003	0,198	12,5	10^{-6}
Laterality β, %	6,32	0,003	0,163	12,4	10^{-6}
Sodium Plasma, mM/L	4,61	0,014	0,141	12,1	10^{-6}
LD Cholesterol Plasma, mM/L	3,61	0,033	0,125	11,6	10^{-6}
Interleukin-6, ng/L	4,05	0,023	0,109	11,4	10^{-6}
F7-θ PSD, %	4,09	0,022	0,095	11,2	10^{-6}
VLF HRV PS, msec^2	2,99	0,058	0,085	10,9	10^{-6}
Entropy Fp2	3,84	0,028	0,075	10,8	10^{-6}
Sodium Urine, mM/L	3,43	0,040	0,066	10,7	10^{-6}
Chloride Urine mM/L	2,49	0,094	0,060	10,5	10^{-6}
Phosphate Plasma, mM/L	2,81	0,069	0,054	10,3	10^{-6}
CD3$^+$ active T-Lymphocytes, %	1,76	0,183	0,050	9,96	10^{-6}
Lithogenicity Urine	2,51	0,092	0,046	9,82	10^{-6}
T4-θ PSD, μV^2/Hz	2,33	0,109	0,042	9,66	10^{-6}
Laterality θ, %	4,06	0,024	0,035	9,94	10^{-6}
C3-α PSD, %	2,03	0,144	0,032	9,76	10^{-6}
P3-α PSD, %	2,36	0,106	0,029	9,70	10^{-6}
Sodium Excretion, mM/24 h	1,58	0,218	0,027	9,46	10^{-6}
Magnesium Excretion, mM/24 h	4,18	0,022	0,023	9,86	10^{-6}
Chloride Excretion, mM/24 h	2,95	0,063	0,020	10,0	10^{-6}
HF HRV PS, msec^2	2,62	0,085	0,018	10,1	10^{-6}
Glucose Plasma, mM/L	2,97	0,063	0,015	10,3	10^{-6}
F8-δ PSD, %	1,73	0,191	0,014	10,1	10^{-6}
Fp2-β PSD, μV^2/Hz	1,62	0,211	0,013	9,98	10^{-6}

T5-δ PSD, μV²/Hz	1,48	0,241	0,012	9,82	10^{-6}
F4-β PSD, μV²/Hz	1,78	0,184	0,011	9,75	10^{-6}
Entropy T6	1,73	0,192	0,010	9,69	10^{-6}
Microbian Count for St. aureus, B/Ph	1,60	0,218	0,009	9,59	10^{-6}
Laterality α, %	2,02	0,149	0,008	9,64	10^{-6}
Entropy F7	1,52	0,235	0,007	9,55	10^{-6}

A number of variables despite their recognizable properties, were outside the discriminant model, apparently due to duplication and/or redundancy of information (Table 11.19).

Table 11.19. Variables not included in the model

Variables	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (34)	After Naftussya (12)	After Salt Waters and N (22)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv/σ
Deviation δ, Hz	0,73 0,05	0,71 0,10	0,57 0,04	0,007	0,979	0,33	0,725	0,364	0,66 0,405
Fp2-θ PSD, %	9,7 1,5	8,9 0,8	6,7 1,3	0,007	0,994	0,09	0,911	0,173	8,3 0,588
Fp2-θ PSD, μV²/Hz	29 7	18 3	20 4	0,007	0,999	0,02	0,983	0,150	25 1,186
F4-α PSD, %	31,4 3,4	22,0 3,8	31,5 3,1	0,007	0,959	0,65	0,530	0,100	32,7 0,564
F7-δ PSD, μV²/Hz	163 45	84 26	93 42	0,007	0,992	0,12	0,887	0,153	80 1,750
F8-α PSD, μV²/Hz	37 4	37 13	23 2	0,007	0,955	0,71	0,499	0,222	40 0,957
T4-β PSD, %	29,0 2,4	33,6 4,7	37,3 4,6	0,007	0,974	0,40	0,676	0,256	27,9 0,591
T4-α PSD, %	28,0 2,9	23,0 3,2	32,6 3,9	0,007	0,997	0,04	0,963	0,113	29,2 0,628
Entropy T4	0,819 0,022	0,843 0,029	0,736 0,030	0,007	0,960	0,62	0,545	0,326	0,790 0,215
T4-θ PSD, %	9,5 1,2	9,1 0,8	6,4 0,7	0,007	0,996	0,06	0,943	0,171	8,7 0,539
C4-δ PSD, %	28,6 3,5	34,8 6,5	22,9 4,0	0,007	0,994	0,10	0,908	0,221	29,9 0,617
T6-δ PSD, μV²/Hz	97 23	53 11	125 60	0,007	0,994	0,09	0,910	0,075	74 1,080
Entropy P3	0,797 0,024	0,851 0,032	0,771 0,025	0,007	0,975	0,38	0,687	0,261	0,804 0,155
P3-δ PSD, %	27,3 3,3	27,5 4,9	19,8 3,4	0,007	0,966	0,53	0,595	0,121	26,5 0,672
Entropy O2	0,769 0,028	0,798 0,027	0,669 0,037	0,007	0,981	0,30	0,746	0,163	0,727 0,242
O2-δ PSD, μV²/Hz	148 35	104 19	219 104	0,007	0,942	0,93	0,407	0,200	95 0,968
Potassium Urine, mM/L	39,5 3,2	41,5 3,6	30,5 1,7	0,007	0,957	0,67	0,519	0,386	46,4 0,269
Cholecystokinetic Activity, units	553 22	584 24	675 28	0,007	0,973	0,42	0,661	0,437	624 0,131
$(Ca/K)^{0,5}$ as S/V balance	0,728 0,012	0,729 0,014	0,708 0,010	0,007	0,972	0,43	0,654	0,410	0,710 0,104
Interleukin-1, ng/L	4,94 0,19	4,36 0,37	5,17 0,30	0,007	0,987	0,19	0,825	0,428	4,51 0,173
Aldosterone, pM/L	225 5	236 10	229 4	0,007	0,982	0,27	0,764	0,516	238 0,187
ULF HRV PS, msec²	73 15	139 56	110 34	0,007	0,967	0,52	0,602	0,276	122 0,892

The identifying information contained in the 35 discriminant variables is condensed into two roots. The major root contains 71% of discriminatory opportunities (r^*=0,971; Wilks' Λ=0,0072; $\chi^2_{(70)}$=237; $p<10^{-6}$), while minor root 29% only (r^*=0,934; Wilks' Λ=0,127; $\chi^2_{(34)}$=99; $p<10^{-6}$).

Calculating the values of discriminant roots for each patient by coefficients and constants given in Table 11.20 allows visualization of each patient in the information space of roots (Fig. 11.7).

Table 11.20. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Phosphates Excretion, mM/24 h	1,447	-0,749	0,124	-0,064
Calcitonin, ng/L	0,760	-0,067	0,172	-0,015
Killing Index vs Staph. aureus, %	1,123	-0,334	0,148	-0,044
Testosterone, nM/L	-0,771	-1,043	-0,089	-0,120
Magnesium Urine, mM/L	1,093	-2,423	1,649	-3,655
Creatinine Plasma,μM/L	-0,989	-0,241	-0,078	-0,019
Laterality β, %	-0,305	-0,445	-0,012	-0,018
Sodium Plasma, mM/L	0,135	1,313	0,015	0,149
LD Cholesterol Plasma, mM/L	-0,894	0,543	-0,857	0,521
Interleukin-6, ng/L	-0,368	-1,556	-0,192	-0,813
F7-θ PSD, %	0,512	1,407	0,124	0,342
VLF HRV PS, msec^2	0,936	0,496	0,0015	0,0008
Entropy Fp2	-0,111	-0,520	-0,762	-3,557
Sodium Urine, mM/L	-3,648	-1,215	-0,120	-0,040
Chloride Urine mM/L	0,173	3,030	0,005	0,085
Phosphate Plasma, mM/L	0,052	0,099	0,279	0,527
CD3$^+$ active T-Lymphocytes, %	-0,603	0,072	-0,130	0,016
Lithogenicity Urine	0,673	-0,558	4,746	-3,934
T4-θ PSD, μV^2/Hz	-0,476	-0,863	-0,017	-0,032
Laterality θ, %	-0,389	1,942	-0,011	0,054
C3-α PSD, %	-2,167	-0,498	-0,139	-0,032
P3-α PSD, %	1,787	-0,101	0,101	-0,006
Sodium Excretion, mM/24 h	3,994	1,081	0,043	0,012
Magnesium Excretion, mM/24 h	-3,408	2,714	-1,950	1,553
Chloride Excretion, mM/24 h	0,528	-2,613	0,0056	-0,0278
HF HRV PS, msec^2	-0,758	-0,079	-0,0014	-0,0001
Glucose Plasma, mM/L	0,514	0,136	0,537	0,142
F8-δ PSD, %	-0,192	0,087	-0,007	0,003
Fp2-β PSD, μV^2/Hz	-0,996	-0,870	-0,026	-0,022
T5-δ PSD, μV^2/Hz	-0,532	-0,275	-0,0007	-0,0003
F4-β PSD, μV^2/Hz	0,857	0,181	0,0188	0,0040
Entropy T6	-0,298	-1,309	-1,901	-8,342
Microbian Count for St. aureus, Bac/Phag	-0,126	0,592	-0,015	0,071
Laterality α, %	0,159	-1,125	0,006	-0,042
Entropy F7	-0,085	0,892	-0,450	4,748
	Constants		4,70	-8,79
	Eigenvalues		16,7	6,86
Cumulative Proportion			0,708	1

Following the accepted algorithm, Table 5 collects the Z-scores of discriminant variables together with those that are not included in the model, but still reflect the specifics of the water used.

Pseudo-staining visualizes a combination of **neuro, endocrine**, **metabolic** and **immune** parameters in the structure of each root (Table 11.21), consistent with previously identified neuroendocrine-immune and neuroendocrine-metabolic linkages.

Table 11.21. Correlations between variables and roots, centroids of clusters and Z-scores of clusters

Variables	Correlations Variables-Roots		Before therapy (34)	After Naftus-sya (12)	After Salt Waters and N (22)
Root 1 (70,8 %)	**Root 1**	**Root 2**	-3,27	-1,11	**+5,66**
Calcitonin	**0,094**	-0,055	-1,02	-1,14	**-0,51**
Triiodothyronine			-0,85	-0,55	**-0,46**
VLF HRV PS	**0,050**	-0,039	-0,36	-0,47	**-0,01**
P3-α PSDr	**0,035**	-0,048	+0,07	**-0,15**	**+0,45**
HF HRV PS	**0,038**	0,002	-0,04	-0,03	**+0,82**
Cholecystokinetic Activity			-0,86	-0,30	**+0,62**
Killing Index vs Staph. aureus	**0,148**	-0,104	-1,28	-1,64	**-0,15**
Phosphates Excretion	**0,240**	-0,093	-0,94	-1,14	**+2,31**
Magnesium Excretion	**0,111**	-0,115	+0,29	-0,64	**+1,79**
Chloride Excretion	**0,087**	-0,011	+0,62	+1,02	**+3,16**
(UA•Ca)/(Cr•Mg)^{0,25}Lithogenicity Urine	**0,072**	-0,058	+0,59	+0,43	**+0,98**
Potassium Plasma			-0,72	-0,64	**-0,25**
Calcium Urine			-1,18	-1,09	**-0,13**
Phosphates Urine			-1,41	-1,36	**-0,42**
Laterality θ	**-0,063**	-0,052	-0,04	-0,66	**-0,98**
Entropy Fp2	**-0,060**	0,004	+0,13	-0,02	**-0,66**
T4-θ PSDa	**-0,042**	-0,043	+0,03	-0,12	**-0,16**
(Ca/K)^{0,5} Plasma as Symp/Vagal balance			+0,24	+0,23	**-0,05**
Phosphate Plasma	**-0,081**	0,096	-0,82	-0,36	**-1,43**
Potassium Urine			-0,56	-0,40	**-1,27**
Sodium Urine	**-0,111**	0,010	+0,39	+0,17	**-0,90**
Uric Acid Urine			+0,30	-0,24	**-0,65**
LD Cholesterol Plasma	**-0,031**	-0,006	+0,16	-0,11	**-0,28**
Glucose Plasma	**-0,021**	-0,007	+0,09	-0,02	**-0,15**
Root 2 (29,2 %)	**Root 1**	**Root 2**	-1,47	**+5,49**	-0,72
Laterality β	0,007	**-0,167**	+0,10	**-0,95**	+0,08
Laterality α	-0,045	**-0,096**	+0,12	**-0,71**	-0,52
Fp2-β PSDa	0,004	**-0,085**	+0,35	**-0,26**	+0,35
F4-β PSDa	0,013	**-0,060**	+0,30	**-0,11**	+0,43
C3-α PSDr	0,020	**-0,055**	+0,01	**-0,29**	+0,20
T5-δ PSDa	-0,013	**-0,049**	+0,72	**-0,04**	+0,17
Testosterone	-0,029	**-0,148**	+0,84	**-0,82**	+0,24
Parathyroid activity			-0,03	**-0,22**	+0,19
Interleukin-6	0,013	**-0,062**	+0,14	**-0,42**	+0,24
Interleukin-1			+0,50	**-0,24**	+0,78
VLD Cholesterol Plasma			+0,32	**-0,21**	+0,09
Creatinine Plasma	-0,037	**-0,111**	+0,99	**+0,18**	+0,60
Urea Plasma			+0,83	**+0,45**	+0,63
Sodium Excretion	0,022	**-0,076**	+2,17	**+0,78**	+2,58
Magnesium Urine	-0,027	**-0,047**	-0,71	**-1,05**	-0,95
Entropy F7	0,003	**0,106**	-0,22	**+0,47**	-0,13
F7-θ PSDr	0,029	**0,078**	-0,11	**+0,51**	+0,29
F8-δ PSDr	-0,034	**0,072**	+0,02	**+0,45**	-0,37
Entropy T6	-0,042	**0,055**	+0,15	**+0,39**	-0,27
ULF HRV PS			-0,45	**+0,16**	-0,11
Aldosterone			-0,30	**-0,05**	-0,19
CD3$^+$ active T-Lymphocytes	-0,063	**0,110**	-0,33	**+0,25**	-0,78
Microbian Count for Staph. aureus	-0,040	**0,068**	+0,12	**+0,44**	-0,14
Chloride Urine	-0,029	**0,111**	-0,85	**+0,38**	-1,15
Chloride Plasma			-0,26	**+1,00**	-0,07
Sodium Plasma	0,003	**0,083**	-0,71	**+0,33**	-0,55
Calcium Plasma			-0,66	**-0,44**	-0,64
Creatinine Urine			-1,69	**-1,11**	-2,02

The localization in the extreme right zone of the axis of the first root of the cluster of patients who received two mineral waters shows a significant increase of initially reduced or minimum for sampling parameters that are **positively** associated with the root, and reduction of initially normal or even deeper fall of initially reduced parameters correlating with the root **negatively**. Instead, in patients receiving **Naftussya** water only, these parameters remained unchanged or changed to a much lesser extent.

On the other hand, such patients are characterized by a significant **decrease**/**increase** in another number of parameters associated with the second root **negatively**/**positively**, while in combination balneotherapy their changes are insignificant or much less pronounced.

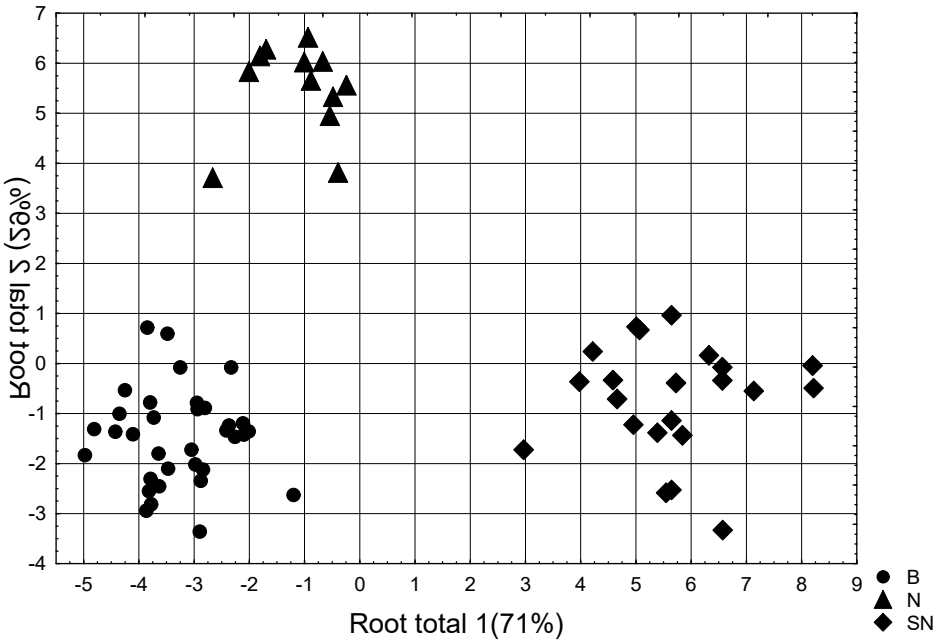


Fig. 11.7. Scattering of individual values of the first and second discriminant roots of patients before (circles) and after the course of drinking only water Naftussya (triangles) and in combination with water Myroslava or Khrystyna (rhombuses)

Fig. 11.8 illustrates that the integrated initial state of all three groups of patients was almost the same as the effect on the discriminant variables of both sulfate-chloride sodium-magnesium mineral waters.

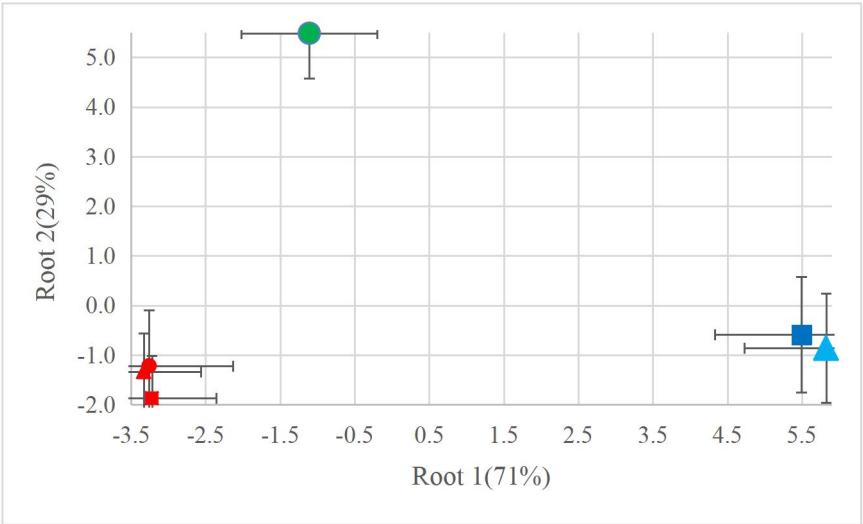


Fig. 11.8. Mean values (M±SD) of the first and second discriminant roots of patients before (red fill) and after the course of drinking only water Naftussya (circle) and in combination with water Myroslava (triangle) or Khrystyna (square)

The visual impression of a clear demarcation of the three clusters in the information field of the two roots is documented by calculating the distances of Mahalanobis (Table 11.22).

Table 11.22. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=35.3) and p-levels (below the diagonal)

Clusters	Before therapy	After Naftussya	After SW&N
Before therapy	0	53	80
After Naftussya	6,4 10^{-5}	0	84
After SW&N	14,6 10^{-6}	8,9 10^{-6}	0

Selected discriminant variables were used to identify the affiliation of a patient to a particular cluster. This goal of discriminant analysis is realized with the help of classification functions (Table 11.23).

Table 11.23. Coefficients and constants of classification functions

Clusters	Before therapy	After Naftussya	After Salt W&N
Variables	p=,500	p=,176	p=,324
Phosphates Excretion, mM/24 h	-0,132	-0,310	0,929
Calcitonin, ng/L	-0,193	0,073	1,332
Killing Index vs Staph. aureus, %	1,681	1,695	2,969
Testosterone, nM/L	-1,552	-2,580	-2,434
Magnesium Urine, mM/L	-17,24	-39,09	-5,234
Creatinine Plasma, µM/L	0,956	0,654	0,243
Laterality β, %	0,346	0,195	0,223
Sodium Plasma, mM/L	6,044	7,115	6,292
LD Cholesterol Plasma, mM/L	27,72	29,49	20,46
Interleukin-6, ng/L	-15,60	-21,67	-17,92
F7-θ PSD, %	-8,705	-6,057	-7,339
VLF HRV PS, $msec^2$	0,036	0,045	0,050
Entropy Fp2	161,3	134,9	151,9
Sodium Urine, mM/L	0,716	0,181	-0,382
Chloride Urine mM/L	1,289	1,891	1,395
Phosphate Plasma, mM/L	159,5	163,7	162,3
$CD3^+$ active T-Lymphocytes, %	4,920	4,748	3,772
Lithogenicity Urine	135,3	118,2	174,7
T4-θ PSD, $µV^2/Hz$	0,758	0,501	0,579
Laterality θ, %	-0,953	-0,599	-1,010
C3-α PSD, %	2,162	1,639	0,896
P3-α PSD, %	-2,219	-2,041	-1,324
Sodium Excretion, mM/24 h	-0,041	0,132	0,349
Magnesium Excretion, mM/24 h	26,65	33,23	10,39
Chloride Excretion, mM/24 h	-0,398	-0,579	-0,368
HF HRV PS, $msec^2$	-0,049	-0,052	-0,061
Glucose Plasma, mM/L	22,68	24,82	27,57
F8-δ PSD, %	0,186	0,193	0,124
Fp2-β PSD, $µV^2/Hz$	0,506	0,295	0,260
T5-δ PSD, $µV^2/Hz$	0,041	0,037	0,034
F4-β PSD, $µV^2/Hz$	-0,399	-0,331	-0,228
Entropy T6	1,772	-60,33	-21,40
Microbian Count for St. aureus, Bac/Phag	0,501	0,962	0,420
Laterality α, %	1,040	0,759	1,062
Entropy F7	233,8	265,8	233,3
Costants	-1041	-1102	-1015

The calculation of algebraic differences between the mean Z-scores of the parameters in both groups of patients still allows us to assess the partial effects of sulfate-chloride sodium-magnesium mineral waters.

This approach suggests that sulfate-chloride sodium-magnesium mineral waters have their own (per se) more or less pronounced effect on the constellation of parameters of the neuro-endocrine-immune complex and metabolism, regardless of their initial levels (Fig. 11.9).

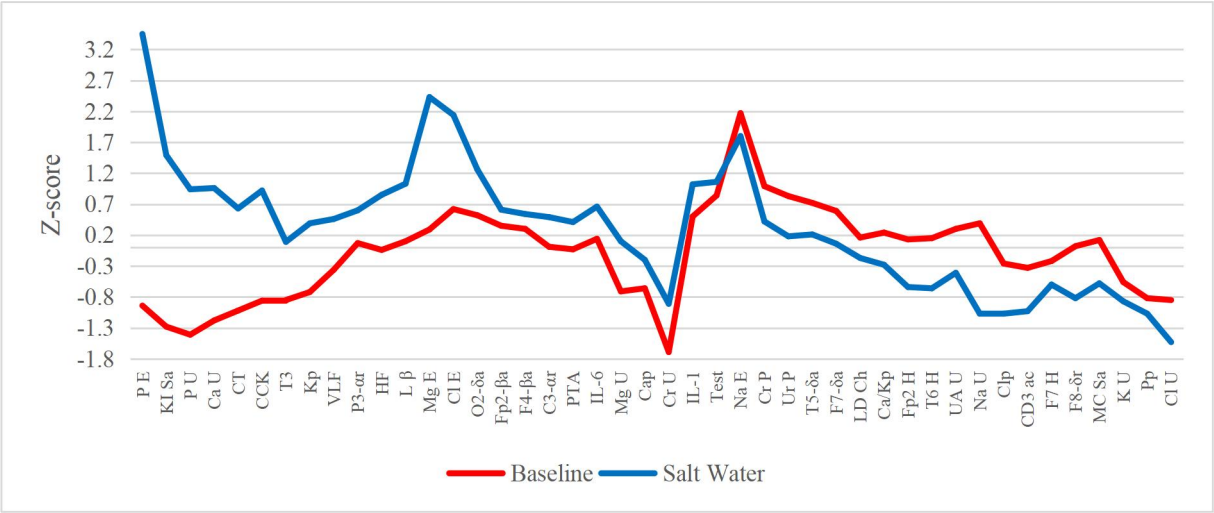


Fig. 11.9. Profiles of real Z-scores of initial EEG, HRV, endocrine, immune and metabolic variables and their simulated Z-scores after consumption of sulphate-chloride sodium-magnesium mineral waters

In particular, initially reduced neuroendocrine (VLF band HRV, calcitonin, triiodothyronine) and metabolic (urine concentrations of phosphate, calcium, magnesium and creatinine, phosphaturia, plasma potassium and calcium, cholecystokinetics activity) variables as well as the completion of phagocytosis of Staphylococcus aureus increase, as a rule, to the zone of norm. On the other hand, initially increased urinary excretion and concentration of sodium and plasma creatinine and urea levels are reduced. Such effects are consistent with the ancient concept of the ambivalent-balancing nature of the effects of balneal factors on the body.

However, there are an increase in initially normal levels of PSD of alpha-rhythm in loci P3 and C3, delta-rhythm in locus O2, laterality and PSD of beta rhythm in loci Fp2 and F4, vagal tone, plasma testosterone, parathyroid activity, excretion of magnesium and chloride, plasma interleukins 6 and 1, as well as a decrease in initially normal levels of EEG entropy in loci Fp2 and T6, Ca/K marker of sympathetic-vagal balance, concentration of uric acid in urine as well as LD cholesterol in plasma as well as the intensity of Staphylococcus aureus phagocytosis. The latter pattern is formed by initially reduced EEG entropy in locus F7, PSD of delta rhythm in locus F8, plasma levels of phosphate and chloride, chloride and potassium of urine, as well as active T-lymphocytes of blood, which continue to decline. Such effects do not fit into this concept, but are consistent with the known data on the diversity of responses of the neuroendocrine-immune complex and metabolism to balneal factors.

CHAPTER 12

SPECIFIC BALNEOEFFECTS OF MYROSLAV AND CHRISTINA MINERAL WATERS

Thus, we found effects on the neuroendocrine-immune complex and metabolism that are common to the mineral waters of Myroslava and Khrystyna, but different from the effects of Naftussya water. The purpose of this chapter is to find the specific effects of these waters.

12.1. Screening of parameters whose changes are different under the influence of two balneotherapy regimens

The effects of balneotherapy were assessed by direct differences between the final and initial Z-scores of the recorded parameters. Screening revealed specific changes in 37 parameters grouped into 5 patterns (Figs 12.1 and 12.2).

The first pattern combines 11 parameters that decrease under the influence of Myroslava water, while increase under the influence of Khrystyna water. In particular, it is power spectral density (PSD) of beta rhythm in loci Fp1, F4, F8, C4, T6 and O2 as well as LF band of HRV; diastolic but not systolic blood pressure; plasma Aldosterone, Potassium excretion and relative level blood Monocytes.

For the other 7 parameters of the second pattern (PSD T5-β and F4-α; plasma Sodium and Chloride; relative level blood T active Lymphocytes as well as both variants Popovych's leukocytary Strain Index) Myroslava water acts similarly, while Khrystyna water is ineffective. Both mineral waters have a enhancing effect on 8 parameters, while Myroslava is inferior to Khrystyna. In particular, it is Diuresis and urinary excretion of Creatinine, Uric acid and Magnesium; serum IgG level, completeness phagocytosis E. coli by blood Neutrophils; content in the feces of E. coli as well as Cholecystokinetics index. It should be noted that the content in the feces of E. coli strains with hemolytic and weakened enzymatic ability, as well as Klebsiela&Proteus is reduced to the same extent. Myroslava water has a upregulating effect on 9 parameters of the fourth pattern, while Khrystyna water has a downregulating effect. In particular, it is PSD of delta rhythm in loci Fp1, F4, C3 and C4 as well as VLF band of HRV and Kerdoe's Vegetative Index; plasma Cortisol and serum IgM levels. Finally, Sodium excretion and Leukocyturia are reduced under the influence of both waters, but to a greater extent under the influence of Khrystyna.

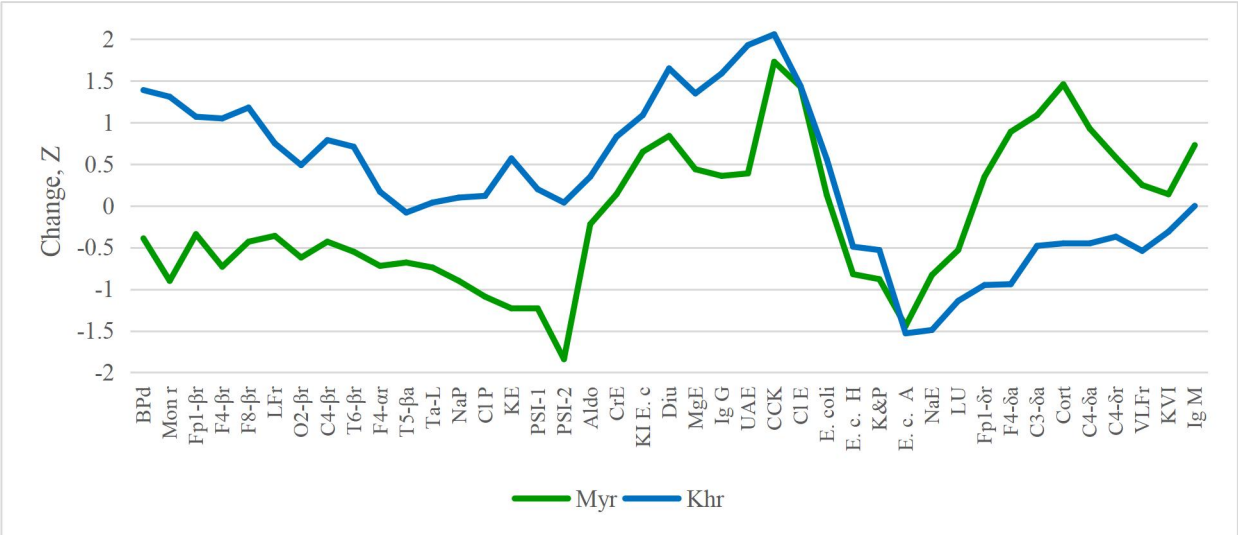


Fig. 12.1. Profiles of specific effects of Myroslava and Khrystyna mineral waters

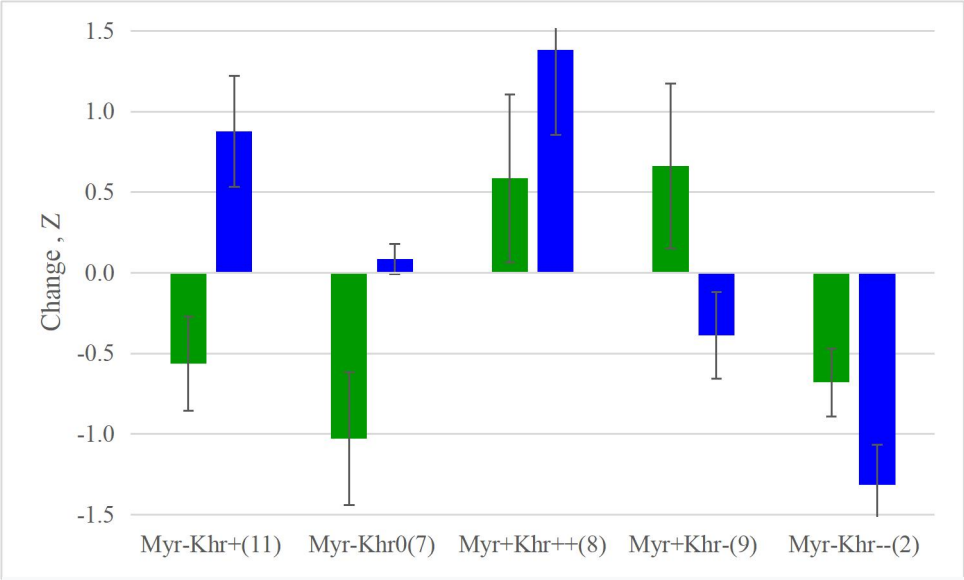


Fig. 12.2. Patterns of specific balneoeffects (Mean±SD) of mineral waters. In parentheses is the number of parameters

However, based on the discriminant analysis, only 12 parameters were included in the model (Tables 12.1 and 12.2), while the rest were outside the model (Tables 12.3-12.5), apparently, as carriers of redundant/duplicate information.

Table 12.1. Summary of the analysis of discriminant functions in relation to the changes in neuroendocrine, metabolic and immune parameters

Step 12, N of vars in model: 12; Grouping: 2 grps; Wilks' Λ: 0,078; appr. $F_{(13)}$=8,8; p=0,0014

Variables currently in the model	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (22)	Effect by Myroslava (11)	Effect by Khrystyna (11)	Wilks' Λ	Partial Λ	F-remove (1,9)	p-level	Tolerancy	Norm Cv
F8-β PSD, %	31,0 +0,11	-8,6 -0,43	+23,7 +1,18	0,296	0,265	25,01	0,001	0,116	28,7 0,702
Monocytes, %	6,8 +0,77	-0,9 -0,90	+1,3 +1,31	0,213	0,369	15,40	0,003	0,096	6,0 0166
Uric acid Excretion, mM/24 h	3,90 +1,19	+0,29 +0,39	+1,45 +1,93	0,259	0,303	20,75	0,001	0,164	3,00 0,250
Corticosterone, nM/L	363 -0,06	+163 +1,46	-51 -0,45	0,159	0,492	9,30	0,014	0,180	370 0,303
Sodium Excretion, mM/24 h	276 +3,74	-27 -0,83	-48 -1,49	0,146	0,538	7,72	0,021	0,283	154 0,211
Escherichia coli feces, lgCFU/g	8,33 -0,86	+0,05 +0,13	+0,22 +0,56	0,087	0,905	0,94	0,357	0,388	8,66 0,045
C3-δ PSD, $\mu V^2/Hz$	87 -0,26	+90 +1,09	-0,40 -0,48	0,083	0,950	0,48	0,507	0,243	108 0,769
Creatinine Excretion, mM/24 h	6,84 -1,26	+0,47 +0,14	+2,73 +0,86	0,144	0,543	7,56	0,022	0,273	11,0 0,300
Killing Index vs E. coli, %	48,3 -1,41	+6,3 +0,65	+10,6 +1,09	0,101	0,776	2,59	0,142	0,274	62,0 0,156
VLF HRV, %	46,8 -0,51	+3,4 +0,25	-8,2 -0,54	0,141	0,558	7,13	0,026	0,184	53,9 0,277
Ig G Serum, g/L	13,49 +0,28	+0,95 +0,36	+4,18 +1,59	0,113	0,697	3,92	0,079	0,318	12,75 0,206
T5-β PSD, $\mu V^2/Hz$	94 +0,19	-53 -0,68	-6 -0,08	0,098	0,802	2,22	0,170	0,132	79 0,995

Notes. In the first column, the top row is the average actual value, the bottom row is the average Z-value. The second and third columns show the average direct differences between the final and initial values. In the last column, the top row is the average reference value, the bottom row is Cv or ***SD***.

Table 12.2. Summary of step-by-step analysis of discriminant variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	**Λ**	F-value	p-level
F8-β PSD, %	5,54	0,029	0,783	5,54	0,029
Monocytes, %	8,23	0,010	0,547	7,88	0,003
Uric acid Excretion, mM/24 h	5,69	0,028	0,415	8,45	0,001
Corticosterone, nM/L	3,21	0,091	0,349	7,92	0,001
Sodium Excretion, mM/24 h	4,97	0,040	0,266	8,81	0,0004
Escherichia coli feces, lgCFU/g	3,11	0,098	0,221	8,83	0,0003
C3-δ PSD, μV²/Hz	1,76	0,205	0,196	8,20	0,001
Creatinine Excretion, mM/24 h	1,53	0,239	0,175	7,64	0,001
Killing Index vs E. coli, %	1,96	0,187	0,151	7,51	0,001
VLF HRV, %	1,86	0,200	0,129	7,43	0,001
Ig G Serum, g/L	3,19	0,104	0,098	8,39	0,001
T5-β PSD, μV²/Hz	2,22	0,170	0,078	8,82	0,001

Table 12.3. Neuro-Endocrine Variables currently not in the model

Variables	**Groups (n) and Means±SE**			**Parameters of Wilks' Statistics**					
	Before therapy (22)	**Effect of Myroslava (11)**	**Effect of Khrystyna (11)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv/**σ**
LF HRV, %	33,5 +0,92	-3,3 -0,26	+6,9 +0,75	0,076	0,969	0,25	0,629	0,055	26,1 0,312
Fp1-β PSD, %	30,7 +0,19	-4,7 -0,34	+14,8 +1,07	0,078	0,997	0,02	0,886	0,297	28,1 0,492
Fp1-δ PSD, %	25,0 -0,04	+6,8 +0,35	-18,5 -0,95	0,078	0,995	0,04	0,847	0,258	25,9 0,748
F4-β PSD, %	28,5 +0,30	-9,8 -0,73	+14,0 +1,05	0,078	1,000	0,00	0,960	0,162	24,5 0,544
F4-α PSD, %	38,4 +0,31	-13,4 -0,72	+3,2 +0,17	0,071	0,910	0,79	0,399	0,308	32,7 0,564
F4-δ PSD, μV²/Hz	101 -0,12	+103 +0,89	-109 -0,94	0,078	1,000	0,00	0,970	0,146	115 1,009
C4-δ PSD, %	20,2 -0,52	+10,7 +0,58	-6,9 -0,37	0,078	0,998	0,02	0,900	0,104	29,9 0,617
C4-δ PSD, μV²/Hz	82 -0,35	+87 +0,93	-42 -0,45	0,075	0,954	0,39	0,551	0,114	114 0,816
T6-β PSD, %	32,0 +0,10	-10,6 -0,55	+13,9 +0,71	0,078	0,997	0,03	0,872	0,078	30,1 0,646
C4-β PSD, %	26,2 -0,01	-5,6 -0,43	+10,3 +0,79	0,077	0,980	0,16	0,698	0,275	26,3 0,493
O2-β PSD, %	24,5 +0,07	-9,5 -0,62	+7,5 +0,49	0,077	0,977	0,18	0,679	0,379	23,4 0,652
Aldosterone, pM/L	227 -0,25	-10 -0,22	+15 +0,35	0,078	0,996	0,03	0,863	0,439	238 0,187
Kerdoe Vegetative Index, units	-18 +0,28	+3 +0,14	-6 -0,31	0,078	0,998	0,02	0,900	0,104	-23 ***20***
Blood Pressure Diastolic, mmHg	83,7 -0,18	-1,8 -0,39	+6,3 +1,38	0,072	0,918	0,71	0,423	0,371	84,5 0,054

Table 12.4. Metabolic Variables currently not in the model

Variables	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (22)	Effect of Myroslava (11)	Effect of Khrystyna (11)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv/σ
Diuresis, L/24 h	2,19 +2,07	+0,32 +0,84	+0,63 +1,65	0,076	0,969	0,25	0,628	0,214	1,40 0,274
Potassium Excretion, mM/24 h	88 +1,31	-21 -1,23	+10 +0,57	0,074	0,947	0,45	0,522	0,423	65 0,269
Magnesium Excretion, mM/24 h	5,04 +0,89	+0,46 +0,44	+1,42 +1,35	0,078	0,998	0,02	0,900	0,104	4,10 0,256
Chloride Excretion, mM/24 h	217 +1.73	+41 +1.43	+41 +1.44	0,078	1,000	0,00	0,960	0,162	167,5 0,172
Chloride Plasma, mM/L	102,9 +0,42	-3,6 -1,09	+0,4 +0,12	0,071	0,910	0,79	0,399	0,308	101,5 0,032
Sodium Plasma, mM/L	144,3 -0,15	-4,5 -0,90	+0,5 +0,10	0,072	0,918	0,71	0,423	0,371	145,0 0,034
Cholecystokinetic Index, units	519 -1,28	+142 +1,73	+168 +2,06	0,078	1,000	0,00	0,960	0,162	624 0,131

Table 12.5. Immune and Microbiota Variables currently not in the model

Variables	Groups (n) and Means±SE			Parameters of Wilks' Statistics					
	Before therapy (22)	Effect of Myroslava (11)	Effect of Khrystyna (11)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy	Norm Cv/σ
$CD3^+$ active T-Lymphocytes, %	27,9 -0,43	-3,7 -0,74	+0,2 +0,04	0,075	0,952	0,40	0,544	0,627	30,0 0,167
Ig M Serum, g/L	1,35 +0,71	+0,20 +0,73	0,00 0,00	0,076	0,969	0,25	0,628	0,214	1,15 0,239
Popovych Strain Index-1	0,162 +1,18	-0,068 -1,23	+0,011 +0,20	0,074	0,947	0,45	0,522	0,423	0,097 0,559
Popovych Strain Index-2	0,206 +2,43	-0,101 -1,84	+0,002 +0,04	0,074	0,947	0,45	0,522	0,423	0,072 0,762
E. coli hemolytica feces, %	20 0,82	-20 -0,82	-12 -0,49	0,074	0,945	0,46	0,515	0,382	0 ***25***
E. coli attenuated feces, %	58,1 +2,34	-25,0 -1,44	-26,5 -1,53	0,078	0,998	0,02	0,900	0,104	17,4 1,000
Klebsiela&Proteus feces, %	9,7 +0,88	-9,6 -0,88	-5,9 -0,53	0,071	0,904	0,85	0,384	0,140	*0* ***11***
Leukocyturia, lgLeu/L	3,29 +0,58	-0,26 -0,53	-0,57 -1,14	0,078	0,995	0,04	0,847	0,258	3,00 0,070

Calculation of individual values of the discriminant root by raw coefficients and constant (Table 12.6) allows to visualize the balneoeffects of mineral waters for each patient (Fig. 12.3).

Table 12.6. Standardized and raw coefficients and constants for discriminant variables

Coefficients	Standardized	Raw
Variables	Root 1	Root 1
F8-β PSD, %	-2,618	-0,128
Monocytes, %	-2,677	-1,091
Uric acid Excretion, mM/24 h	-2,151	-1,643
Corticosterone, nM/L	-1,751	-4,038
Sodium Excretion, mM/24 h	-1,331	-0,012
Escherichia coli feces, lgCFU/g	0,515	1,845
C3-δ PSD, $\mu V^2/Hz$	-0,474	-0,004
Creatinine Excretion, mM/24 h	1,347	0,361
Killing Index vs E. coli, %	-0,941	-0,072
VLF HRV, %	1,616	0,129
Ig G Serum, g/L	-1,018	-0,189

T5-β PSD, μV²/Hz	1,273	0,031
	Constant	3,992
	Eigenvalue	11,75
Squared Mahalanobis Distance=43; F=8,8; p=0,0014		
Canonical R=0,960; Wilks' Λ=0,0784; $\chi^2_{(12)}$=37; p=0,0004		

Opposite root values of patients receiving different mineral waters reflect (Fig 12.3 and Table 12.7), first, an increase under the influence of Myroslava water versus decrease under the influence of Khrystyna water in initially normal Cortisol levels and electrical activity of delta-rhythm-generating neurons projected at locus C3 (probably the left hippocampus (Romodanov, 1993)) and moderately reduced relative power of VLF components of HRV. As noted, Shaffer & Ginsberg (2017), there is uncertainty regarding the physiological mechanisms responsible for activity within the VLF (0,04÷0,0033 Hz) band. The heart's intrinsic nervous system appears to contribute to the VLF rhythm and the sympathetic nervous system influences the amplitude and frequency of its oscillations. VLF power may also be generated by physical activity, thermoregulatory, renin–angiotensin, and endothelial influences on the heart. Vagal activity may contribute to VLF power since parasympathetic blockade almost completely abolishes it. In contrast, sympathetic blockade does not affect VLF power. The VLF rhythm appears to be generated by the stimulation of afferent sensory neurons in the heart. This, in turn, activates various levels of the feedback and feed-forward loops in the heart's intrinsic cardiac nervous system, as well as between the heart, the extrinsic cardiac ganglia, and spinal column. This experimental evidence suggests that the heart intrinsically generates the VLF rhythm and efferent sympathetic nervous system activity due to physical activity and stress responses modulates its amplitude and frequency.

Second, Myroslava water reduces increased Sodium excretion to a lesser extent than Khrystyna water.

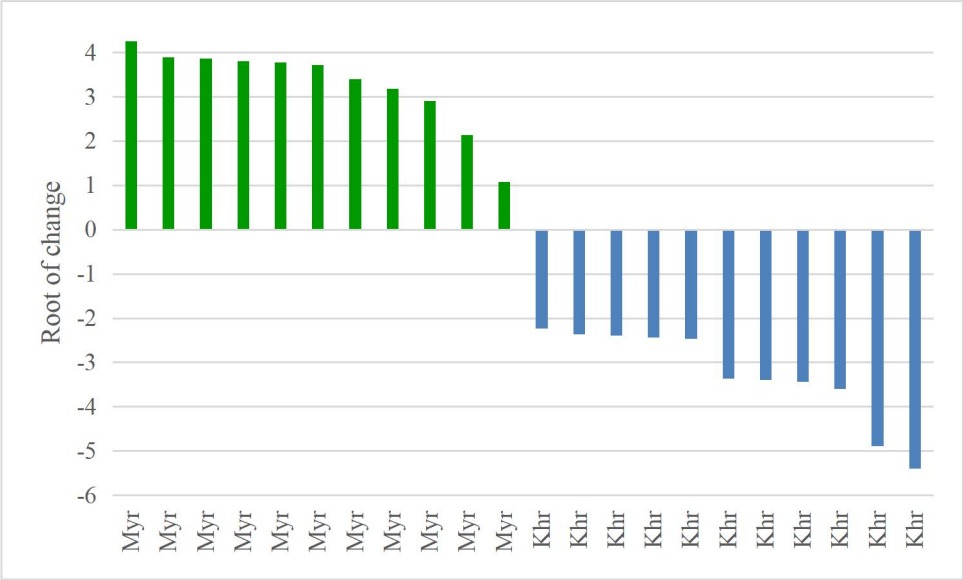


Fig. 12.3. Individual values of the discriminant root of balneoeffects

Third, Myroslava water inhibits the initially normal activity of beta-rhythm-generating neurons projected at the F8 and T5 loci, whereas Khrystyna water is excitatory or neutral, respectively. It is more likely that the locus F8 record the activity of right anterior cingulate cortex and the locus T5 projected left caudal anterior cingulate cortex. These cortical structures affect the activity of the vagal and sympathetic nuclei (Winkelmann et al., 2017; Yoo et al., 2018). In the same opposite way, mineral waters affect the increased relative level of blood monocytes.

Table 12.7. Correlations between variables and root and Z-scores of changes in variables

Variables	R	Effect of Myroslava	Effect of Khrystyna
VLF HRVr	0,142	+0,25	-0,54
Kerdoe Vegetative Index		+0,14	-0,31
C3-δ PSDa	0,114	+1,09	-0,48
C4-δ PSDa		+0,93	-0,45
F4-δ PSDa		+0,89	-0,94
C4-δ PSDr		+0,58	-0,37
Fp1-δ PSDr		+0,35	-0,95

Corticosterone	0,015	+1,46	-0,45
Ig M Serum		+0,73	0,00
Sodium Excretion	0,031	-0,83	-1,49
Leukocyturia, lg L/mL		-0,53	-1,14
LF HRVr		-0,36	+0,75
F8-β PSDr	-0,153	-0,43	+1,18
Fp1-β PSDr		-0,34	+1,07
F4-β PSDr		-0,73	+1,05
O2-β PSDr		-0,62	+0,49
C4-β PSDr		-0,43	+0,79
T6-β PSDr		-0,55	+0,71
F4-α PSDr		-0,72	+0,17
T5-β PSDa	-0,112	-0,68	-0,08
Aldosterone		-0,22	+0,35
Blood Pressure Diastolic		-0,39	+1,39
Monocytes	-0,137	-1,80	+2,62
$CD3^+$ active T-Lymphoc		-0,74	+0,04
Popovych' Strain Index-1		-1,23	+0,20
Popovych' Strain Index-2		-1,84	+0,04
E. coli hemolytica feces		-0,82	-0,49
Klebsiela&Proteus feces		-0,88	-0,53
Potassium Excretion		-1,23	+0,57
Sodium Plasma		-0,90	+0,10
Chloride Plasma		-1,09	+0,12
Uric acid Excretion	-0,135	+0,39	+1,93
Diuresis		+0,84	+1,65
Magnesium Excretion		+0,44	+1,35
Creatinine Excretion	-0,093	+0,14	+0,83
Ig G Serum	-0,092	+0,36	+1,59
Killing Index vs E. coli	-0,050	+0,65	+1,09
Cholecystokinetic Index		+1,73	+2,06
Escherichia coli feces	-0,078	+0,13	+0,56

Fourth, Khrystyna water significantly increases the initially reduced bactericidal activity of neutrophils against E. coli and its content in feces, as well as Creatinine excretion. In addition, there is an increase in normal IgG levels and further increase in Hyperuricosuria. On the other hand, Myroslava water affects these parameters to a much lesser extent.

The visual impression of a very clear delineation of the effects of both mineral waters is documented by the calculation of Mahalanobis Distance (Table 12.6), as well as 100% accuracy of classification based on the coefficients and constants given in Table 12.8.

Table 12.8. Coefficients and constants of classification functions

Clusters	Effect of Myroslava	Effect of Khrystyna
Variables	p=,500	p=,500
F8-β PSD, %	0,120	0,957
Monocytes, %	0,365	7,497
Uric acid Excretion, mM/24 h	2,013	12,76
Corticosterone, nM/L	2,181	28,58
Sodium Excretion, mM/24 h	-0,002	0,079
Escherichia coli feces, lgCFU/g	-5,650	-17,71
C3-δ PSD, $\mu V^2/Hz$	0,004	0,031
Creatinine Excretion, mM/24 h	-0,430	-2,793
Killing Index vs E. coli, %	0,116	0,587
VLF HRV, %	-0,191	-1,037
Ig G Serum, g/L	0,354	1,588
T5-β PSD, $\mu V^2/Hz$	-0,087	-0,291
Constants	-2,75	-28,8

It should be noted that the direction of balneal effects is not fully consistent with the previously proposed concept about ambivalence-equilibratory character of influence of curative water Naftussya on organism of human. Indeed, along with the increase in decreased and decrease in increased parameters, other variants of effects were found, usually physiologically favorable, except for the increase in diastolic pressure under the influence of Khrystyna water, due, apparently, its high content of sodium chloride.

12.2. Canonical analysis of the neuro-endocrine mechanism of specific balneoeffects

In previous studies of the Truskavetsian Scientific School of Balneology, in patients of the spa found significant relationships between EEG and HRV parameters (Popovych et al., 2013; 2014), EEG and HRV, on the one hand, and leukocytogram (Kul'chyns'kyi et al., 2017), phagocytosis (Kul'chyns'kyi et al., 2016) and immunocytogram (Kul'chyns'kyi et al., 2017a) – on the other, and between changes in these constellations under the influence of balneotherapy (Kul'chyns'kyi et al., 2017b; Popovych et al., 2017; 2018). In addition, we found close neuroendocrine-metabolic and neuroendocrine-immune relationships in healthy rats, both intact and exposed to water-salt loads.

These findings provide grounds for the hypothesis of the neuroendocrine mechanism of specific effects of mineral waters on the parameters of immunity, microbiota, metabolism, cholekinetics and diastolic pressure.

At the first stage, the correlations between individual changes in hormone levels, on the one hand, and immunity parameters, on the other, were screened. Given the significant number of registered parameters of Immunity (n=25), for further analysis were purposefully selected only those which are object to the **significant** modulating effect of Hormones (Table 12.9).

Table 12.9. Matrix of correlations between changes in hormone levels and immune parameters

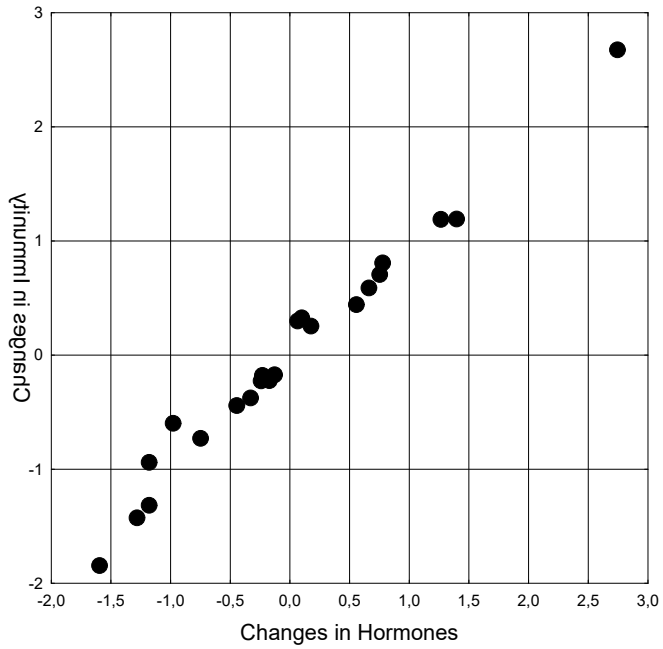
	Correlations N=22					
Variable	PTA	Ald	T3	Cor	CT	Test
PhI A	0,11	0,20	-0,43	-0,21	0,08	0,15
KI A	-0,15	-0,22	-0,01	-0,25	-0,62	0,07
PhI E	0,02	-0,01	-0,44	-0,37	0,05	0,08
MC E	0,01	-0,02	-0,20	-0,04	0,57	-0,19
KI E	-0,26	-0,04	0,24	0,02	-0,73	0,02
H LCG	0,41	-0,36	-0,43	-0,50	0,17	0,11
BC A	0,20	-0,18	-0,14	-0,74	-0,18	0,09
BC E	0,07	-0,15	-0,23	-0,63	-0,17	0,07
Leuk	0,29	-0,34	-0,44	-0,88	-0,03	0,18
Mon	0,18	0,00	-0,09	-0,59	-0,15	0,05
Lymph	-0,20	-0,26	-0,13	0,49	0,18	-0,04
CD4	-0,10	-0,21	-0,02	0,38	0,25	0,01
CD22	-0,32	-0,20	0,46	0,23	-0,15	0,17
CIC	-0,23	-0,04	0,10	-0,08	-0,49	0,10
IgG	0,46	0,07	-0,09	-0,15	-0,13	-0,26
IgA	0,20	-0,01	-0,03	-0,14	-0,33	-0,14
IgM	0,14	-0,02	-0,34	-0,04	0,37	-0,10
O-Lym	0,33	0,26	-0,43	-0,30	0,09	-0,12
IL-1	-0,23	-0,09	0,13	0,34	0,13	0,33
IL-6	-0,42	-0,21	-0,05	0,43	0,33	-0,02

In the second stage, the analysis of the canonical correlation between endocrine and immune sets of variables was performed. The maximum factor load on the endocrine root is given by changes in cortisol, which confirms its reputation as a major immunomodulator, while the aldosterone load is minimal (Table 12.10.).

Due to technical/mathematical limitations of the program, only 20 parameters can be used in the analysis, ie 2 less than the number of patients. Therefore, only 14 variables that are subject to **suppressive** or **enhancing** hormonal regulation fit into the root structure of immune changes. Judging by the coefficient of determination, hormonal reactions cause immunomodulation by 98,9% (Fig. 12.4).

Table 12.10. Factor structure of endocrine and immune canonical roots of changes

Endocrine Variables	R
Cortisol, nM/L	-0,750
Triiodothyronine, nM/L	-0,345
Aldosterone, pM/L	-0,075
Calcitonin, ng/L	0,451
Parathyroid Activity, units	0,277
Testosterone, nM/L	0,151
Immune Variables	**R**
Bactericidity vs Staph. aureus, Bact/L	0,548
Entropy of Leukocytogram	0,508
Monocytes, %	0,475
Bactericidity vs E. coli, Bacteria/L	0,453
Microbial Count vs E. coli, Bact/Phag	0,430
Immunoglobulins G, g/L	0,111
0-Lymphocytes, %	0,386
Killing Index vs E. coli, %	-0,547
$CD22^+$ B-Lymphocytes, %	-0,359
Circulating Immune Complexes, units	-0,288
Killing Index vs Staph. aureus, %	-0,280
Interleukin-6, ng/L	-0,255
Interleukin-1, ng/L	-0,251
$CD4^+$ T-helper Lymphocytes, %	-0,227



R=0,994; R^2=0,989; $\chi^2_{(84)}$=128; p=0,0012; Λ Prime<10^{-5}

Fig. 12.4. Scatterplot of canonical correlation between changes in Endocrine (X-line) and Immune (Y-line) parameters

Autonomic-immune relationships were analyzed by a similar algorithm (Tables 12.11 and 12.12).

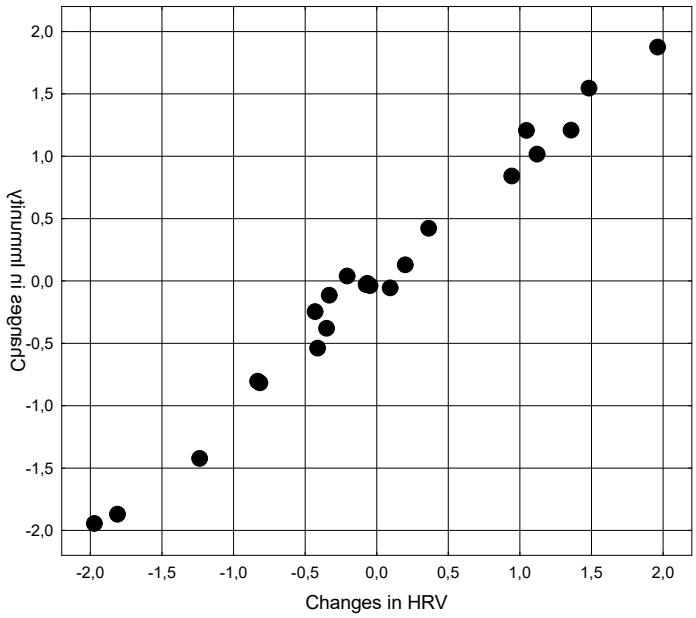
Table 12.11. Matrix of correlations between changes in HRV and immune parameters

Variable	Correlations N=22						
	RMSSD	ULF	VLF	LF	HF	hHRV	LFnu
KI A	0,05	0,14	0,07	-0,08	0,15	0,42	-0,30
MC A	-0,10	-0,13	0,06	0,04	-0,26	-0,52	0,48
PhI E	-0,37	0,00	-0,32	-0,47	-0,43	-0,08	0,04
MC E	-0,24	-0,54	-0,26	-0,27	-0,42	-0,54	0,21
KI E	0,47	0,46	0,39	0,25	0,56	0,72	-0,44
PMNN	-0,18	-0,01	-0,08	0,24	-0,16	0,15	0,54
CD4	0,25	0,01	0,23	-0,04	0,10	-0,35	-0,20
CD8	-0,21	-0,38	-0,32	-0,21	-0,12	0,12	-0,02
CD22	0,39	0,27	0,43	0,38	0,49	0,01	-0,10
CIC	0,50	0,16	0,34	0,02	0,46	0,25	-0,36
IgA	-0,29	0,50	0,04	-0,09	-0,28	0,35	0,22
IgM	-0,16	-0,60	-0,33	-0,29	-0,33	-0,41	0,10
CD56	0,06	0,43	0,20	0,28	0,06	0,13	0,17
O-Lym	-0,40	-0,18	-0,40	-0,29	-0,45	0,08	0,16
IL-1	0,39	0,49	0,50	0,41	0,35	-0,08	-0,08
IL-6	0,41	0,15	0,32	0,07	0,15	-0,25	-0,15

Table 12.12. Factor structure of HRV and immune canonical roots of changes

HRV Variables	**R**
LFnu, %	**-0,668**
Entropy of HRV	**0,781**
ULF band HRV, msec^2	**0,673**
HF band HRV, msec^2	**0,485**
VLF band HRV, msec^2	**0,436**
LF band HRV, msec^2	**0,166**
Immune Variables	**R**
Killing Index vs E. coli, %	**0,735**
Killing Index vs Staph. aureus, %	**0,396**
Circulating Immune Complexes, units	**0,368**
Immunoglobulins A, g/L	**0,264**
Interleukin-1, ng/L	**0,222**
CD56$^+$ NK-Lymphocytes, %	**0,184**
CD22$^+$ B-Lymphocytes, %	**0,085**
Microbial Count vs Staph. aur, Bac/Ph	**-0,503**
Microbial Count vs E. coli, Bacter/Phag	**-0,494**
Immunoglobulins M, g/L	**-0,415**
Polymorphonucleary Neutrophils, %	**-0,191**
CD8$^+$ T-cytolytic Lymphocytes, %	**-0,143**
Phagocytosis Index vs E. coli, %	**-0,083**
0-Lymphocytes, %	**-0,042**

The large factor load on the root of the autonomic response to balneotherapy by the HRV-marker of sympathetic tone (LFnu) confirms its important role in immunomodulation, along with the HRV-marker of vagal tone (HF). However, even more load is given by the ULF band (physiological interpretation is given above). Nevertheless, the maximum factor load is given by the entropy of HRV, the physiological essence of which is analyzed in detail in the just published monograph (Gozhenko et al., 2021). Judging by the coefficient of determination, autonomous reactions cause immunomodulation by 99,9% (Fig. 12.5).



R=0,9996; R^2=0,9993; $\chi^2_{(84)}$=145; p<10^{-4}; Λ Prime<10^{-6}

Fig. 12.5. Scatterplot of canonical correlation between changes in HRV (X-line) and Immune (Y-line) parameters

The CNS has a similar strength of immunomodulatory effect (Tables 12.13-12.14, Fig. 12.6).

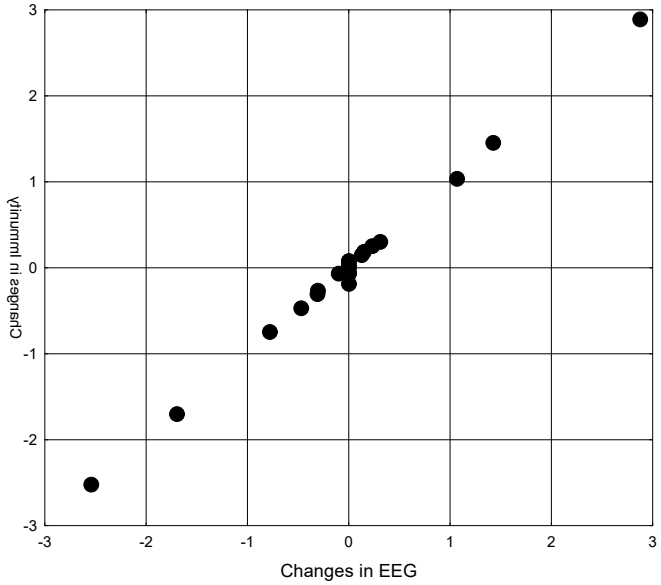
Table 12.13. Matrix of correlations between changes in EEG and immune parameters

Root Variable	Correlations, left set with right set									
	IL-6	CRP	IL-1	HLCG	PMNN	Eos	IgG	IgM	0-Lym	HICG
DD	0,55	0,55	0,26	-0,25	-0,30	0,04	-0,20	0,01	-0,30	0,18
Fp2 T	0,03	0,11	-0,42	0,41	-0,18	0,22	0,16	0,45	0,22	0,02
H F7	0,47	0,66	-0,02	-0,21	-0,02	-0,13	-0,16	0,13	-0,25	0,28
F7 T%	0,53	0,63	0,06	0,06	-0,12	0,01	-0,03	0,17	0,04	-0,08
F7 D	-0,23	-0,50	-0,13	0,23	-0,05	0,12	0,11	0,18	0,43	-0,47
F8 A	-0,09	0,10	-0,23	0,48	-0,41	0,42	0,22	0,19	0,15	0,12
T4 T	0,03	0,11	-0,53	0,39	-0,18	0,20	0,13	0,58	0,30	-0,06
H T6	0,47	0,62	-0,05	-0,16	-0,07	-0,03	-0,16	0,12	-0,20	0,24
H O2	0,60	0,64	0,06	-0,19	-0,18	0,04	-0,19	0,09	-0,16	0,13
O2 D	-0,59	-0,49	-0,30	0,41	0,05	0,10	0,32	0,07	0,23	-0,04

Table 12.14. Factor structure of EEG and Immune canonical roots of changes

EEG Variables	**R**
F8-α PSD, μV^2/Hz	**0,432**
O2-δ PSD, μV^2/Hz	**0,335**
Fp2-θ PSD, μV^2/Hz	**0,118**
Entropy F7	**0,105**
Entropy T6	**0,043**
F7-θ PSD, %	**0,004**
F7-δ PSD, μV^2/Hz	**-0,466**
Entropy O2	**-0,138**
Deviation δ, Hz	**-0,129**
T4-θ PSD, μV^2/Hz	**-0,039**
Immune Variables	**R**
Interleukin-6, ng/L	**-0,319**
Immunoglobulins M, g/L	**-0,264**
Interleukin-1, ng/L	**-0,074**
0-Lymphocytes, %	**-0,305**
Entropy of Immunocytogram	**0,597**

Immunoglobulins G, g/L	0,247
Entropy of Leukocytogram	0,212
Eosinophils, %	0,062
Polymorphonucleary Neutrophils, %	0,035
C-RP, mg/L	0,019



R=0,998; R^2=0,997; $\chi^2_{(100)}$=132; p=0,019; Λ Prime<10^{-5}

Fig. 12.6. Scatterplot of canonical correlation between changes in EEG (X-line) and Immune (Y-line) parameters

Because it is difficult for us to imagine how the CNS can directly perform immunomodulation, the mediating role of the endocrine and autonomic nervous systems seems more realistic. The results of canonical correlation analysis show a significant impact of the CNS on both the endocrine (Tables 12.15-12.16 and Fig. 12.7) and autonomic nervous (Tables 12.17-12.18 and Fig. 12.8) systems, the immunomodulatory effect of which is well known and documented in this study.

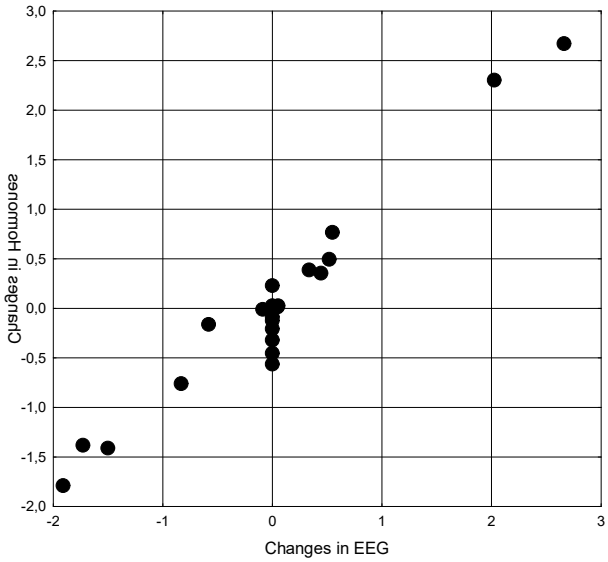
Table 12.15. Matrix of correlations between changes in EEG and Endocrine parameters

Root Variable	Correlations, left set with right set					
	PTA	AId	T3	Cor	CT	Test
DD	-0,73	0,00	0,07	0,38	0,24	0,04
Fp2 T	0,03	-0,31	-0,19	-0,16	-0,01	-0,29
F7 H	-0,55	0,01	0,25	0,40	0,34	-0,31
F7 T%	-0,40	-0,11	-0,10	0,28	0,34	-0,05
F7 D	0,44	-0,11	-0,33	-0,24	-0,32	0,13
F8 D%	0,77	-0,18	-0,18	-0,19	-0,08	-0,02
F8 A	0,20	-0,34	-0,39	-0,26	0,21	-0,05
T4 B%	-0,44	0,05	0,11	0,44	0,24	0,23
T4 T	0,08	-0,24	-0,20	-0,13	0,06	-0,33
T6 H	-0,60	0,09	0,29	0,35	0,14	-0,30
O2 H	-0,56	-0,06	0,23	0,47	-0,01	-0,34
O2 D	0,75	-0,21	-0,28	-0,57	-0,14	0,02

Table 12.16. Factor structure of EEG and Endocrine canonical roots of changes

EEG Variables	R
T4-β PSD, %	0,491
Entropy F7	0,476
Deviation δ, Hz	0,470
F7-θ PSD, %	0,413
Entropy T6	0,300

Entropy O2	**0,186**
F7-δ PSD, μV²/Hz	**-0,459**
O2-δ PSD, μV²/Hz	**-0,443**
F8-δ PSD, %	**-0,290**
Fp2-θ PSD, μV²/Hz	**-0,149**
T4-θ PSD, μV²/Hz	**-0,113**
Endocrine Variables	**R**
Calcitonin, ng/L	**0,861**
Cortisol, nM/L	**0,501**
Testosterone, nM/L	**0,210**
Triiodothyronine, nM/L	**0,022**
Parathyroid Activity, units	**-0,390**
Aldosterone, pM/L	**-0,073**



R=0,972; R^2=0,946; $\chi^2_{(66)}$=85; p=0,058; Λ Prime=0,0008

Fig. 12.7. Scatterplot of canonical correlation between changes in EEG (X-line) and Endocrine (Y-line) parameters

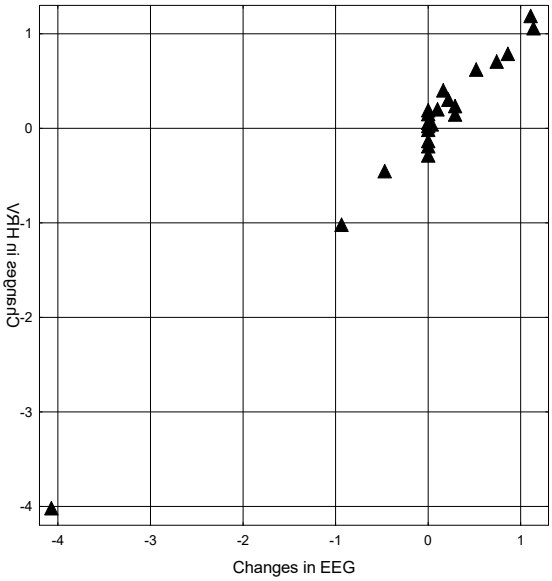
Table 12.17. Matrix of correlations between changes in EEG and HRV parameter

Root Variable	Correlations, left set with right set					
	RMSSD	ULF	VLF	LF	HHRV	LFnu
DD	0,29	0,16	0,33	0,04	-0,27	-0,08
Fp2 T	0,08	-0,58	-0,36	-0,38	0,08	-0,45
F7 H	0,13	-0,30	-0,10	-0,10	-0,38	0,05
F7 T%	0,25	-0,25	-0,10	-0,24	-0,14	-0,28
F7 D	0,01	0,09	0,10	0,02	0,39	-0,28
F8 A	-0,16	-0,13	-0,41	-0,27	0,15	-0,25
T4 B%	-0,00	0,47	0,25	0,03	-0,10	0,11
T4 T	0,03	-0,69	-0,41	-0,36	-0,04	-0,37
T6 H	0,20	-0,25	-0,05	-0,12	-0,29	-0,01
O2 H	0,21	0,07	0,04	-0,06	-0,01	-0,15
O2 D	-0,31	-0,19	-0,39	-0,14	0,19	-0,03

Table 12.18. Factor structure of EEG and HRV canonical roots of changes

EEG Variables	**R**
T4-θ PSD, μV²/Hz	**0,869**
Fp2-θ PSD, μV²/Hz	**0,806**
F7-θ PSD, %	**0,458**
F8-α PSD, μV²/Hz	**0,248**
Entropy T6	**0,357**

Entropy F7	**0,334**
Entropy O2	**0,126**
T4-β PSD, %	**-0,476**
HRV Variables	**R**
ULF band HRV, msec²	**-0,885**
VLF band HRV, msec²	**-0,412**
LF band HRV, msec²	**-0,425**
Entropy of HRV	**-0,341**
LFnu, %	**-0,223**



R=0,992; R^2=0,985; $\chi^2_{(77)}$=130; p=0,0001; Λ Prime=0,00001

Fig. 12.8. Scatterplot of canonical correlation between changes in EEG (X-line) and HRV (Y-line) parameters

To determine the neuro-endocrine mechanism of the detected effects of mineral waters, the discriminant parameters were divided into two sets. The first set consisted of neuroendocrine parameters as causal, and the second set - parameters of immunity, metabolism, microbiota, cholekinetics and diastolic pressure, which, apparently, are object to regulatory effects of the former (Table 12.19).

Table 12.19. Matrix of correlations between changes in Neuro-Endocrine and others parameters

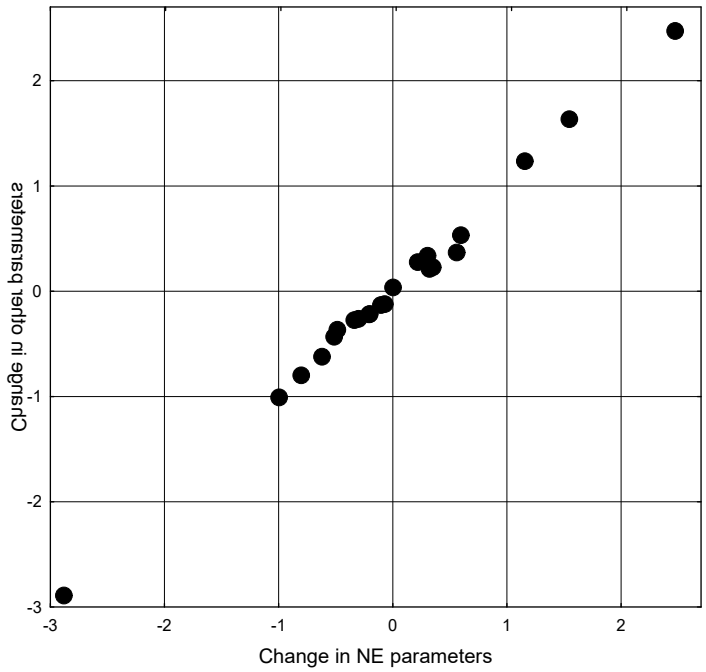
Vari-ables	KI EC	Mo n	Pop SI-1	CD 3Ta L	Ig G	Ig M	Cr Ex	UA Ex	Na Ex	CC K Ind	Mg Ex	BP d	lg LU	lg EC
LF r	**-0,42**	**0,43**	0,41	0,05	0,03	0,12	0,41	0,14	-0,34	0,40	-0,15	0,12	**0,43**	-0,34
VLF r	-0,02	-0,34	-0,34	-0,23	0,14	-0,03	-0,29	-0,08	0,35	**-0,46**	0,28	-0,07	-0,14	-0,15
T5-β a	-0,27	0,08	0,18	-0,07	-0,08	-0,22	0,14	0,36	-0,39	**0,63**	0,03	0,18	0,35	-0,31
C3-δ a	-0,20	-0,04	-0,17	-0,03	-0,07	0,36	-0,22	-0,29	0,25	-0,53	**-0,49**	-0,18	0,03	0,04
Cortisol	0,02	**-0,59**	-0,34	-0,23	-0,15	-0,04	0,01	-0,04	-0,20	0,35	-0,17	-0,16	-0,00	-0,06
F8-β r	0,12	-0,12	0,17	-0,06	-0,23	**-0,45**	0,11	0,26	**-0,44**	0,36	0,28	0,32	-0,04	0,01
Aldost	-0,04	0,00	0,03	-0,12	0,07	-0,02	0,35	0,03	-0,15	-0,04	-0,05	0,03	0,15	-0,09

The canonical correlation between the two sets of parameters was then analyzed. Two pairs of canonical roots are distinguished.

The factor structure of the first neuroendocrine root (Table 12.20) is formed by: LF component of HRV (reflects both sympathetic and vagal effects), VLF band and beta-rhythm-generating nerve structures (their physiological essence is described above), as well as cortisol and delta-rhythm-generating nerve structures projected at locus C3 (probably the left hippocampus). Changes in the mentioned neuro-endocrine factors determine the changes in the parameters of the subordinate set by 99,5% (Fig. 12.9).

Table 12.20. Factor load on first pair of canonical roots

Neuro-Endocrine Variables	R 1
LF, %	-0,823
T5 β, PSD, $\mu V^2/Hz$	-0,536
VLF, %	0,559
Cortisol	0,489
C3-δ PSD, $\mu V^2/Hz$	0,175
Other Variables	**R 1**
Monocytes, %	-0,643
Popovych's Strain Index-1	-0,469
Leukocyturia, lgLeu/L	-0,435
Creatinine Excretion, mM/24 h	-0,354
Cholecystokinetic Index, units	-0,298
Ig G Serum, g/L	-0,211
Uric acid Excretion, mM/24 h	-0,200
Blood Pressure Diastolic, mmHg	-0,126
Ig M Serum, g/L	-0,124
$CD3^+$ active T-Lymphocytes, %	-0,121
Killing Index vs E. coli, %	0,421
Escherichia coli feces, lgCFU/g	0,375
Sodium Excretion, mM/24 h	0,141



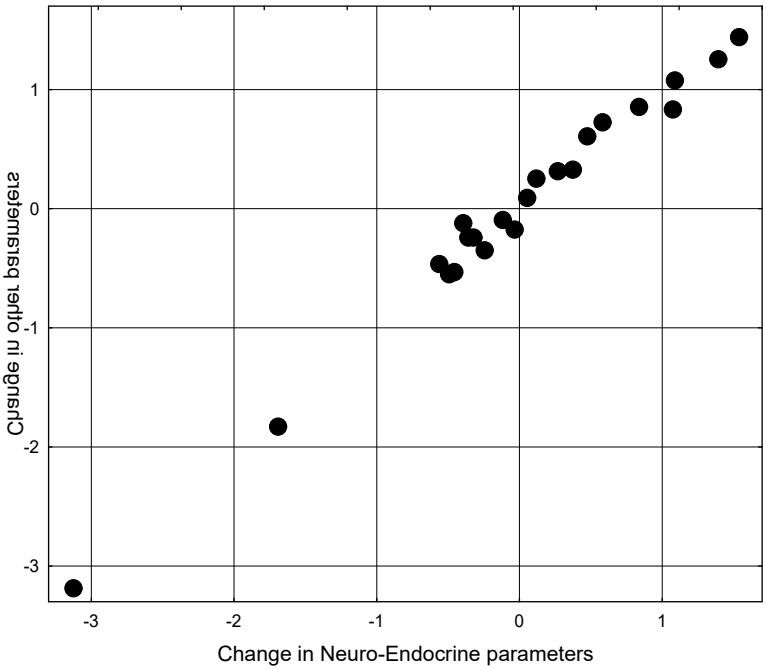
R=0,997; R^2=0,995; $\chi^2_{(91)}$=155; $p<10^{-4}$; Λ Prime$<10^{-6}$
Fig. 12.9. Scatterplot of canonical correlation between change in Neuro-Endocrine (X-line) and other (Y-line) parameters. First pair of Roots

The factor structure of the neuroendocrine root of the second pair (Table 12.21) is supplemented by two parameters (F8-β PSD and aldosterone). Their changes determine changes in the parameters of cholekinetics, metabolism, microbiota and immunity by 98,6% (Fig. 12.10).

Table 12.21. Factor load on second pair of canonical roots

Neuro-Endocrine Variables	R 2

F8-β PSD, %	0,710
T5 β, PSD, μV²/Hz	0,674
Cortisol	0,669
LF, %	0,307
Aldosterone	0,125
C3-δ PSD, μV²/Hz	-0,574
VLF, %	-0,189
Other Variables	R 2
Cholecystokinetic Index, units	0,615
Uric acid Excretion, mM/24 h	0,216
Leukocyturia, lgLeu/L	0,163
Blood Pressure Diastolic, mmHg	0,130
Creatinine Excretion, mM/24 h	0,103
Sodium Excretion, mM/24 h	-0,466
Monocytes, %	-0,330
Escherichia coli feces, lgCFU/g	-0,272
Ig M Serum, g/L	-0,238
Ig G Serum, g/L	-0,209
CD3⁺ active T-Lymphocytes, %	-0,197
Killing Index vs E. coli, %	-0,182



R=0,993; R^2=0,986; $\chi^2_{(72)}$=100; p=0,017; Λ Prime<10^{-4}

Fig. 12.10. Scatterplot of canonical correlation between change in Neuro-Endocrine (X-line) and other (Y-line) parameters. Second pair of Roots

Given the presence of chronic cholecystitis in the observed contingent, the mechanism of normalizing the effect of mineral waters on the reduced evacuation function of the gallbladder, estimated by the cholecystokinetic index, deserves a separate analysis. The correlations between the dynamics of the cholecystokinetic index and the discriminant variables were first screened. After that, a regression model was created by step-by-step exclusion of variables until the maximum value of Adjusted R^2 was reached (Table 12.22).

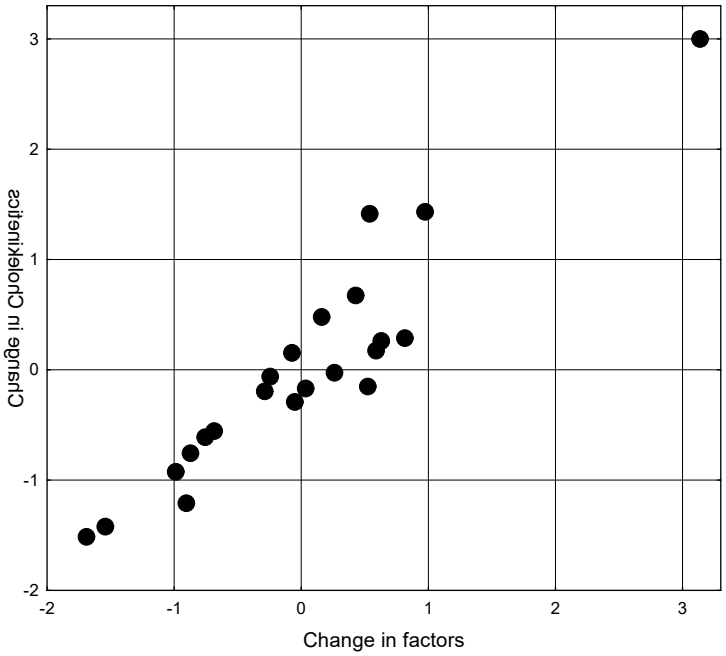
Table 12.22. Regression model for changes in cholecystokinetic index

R=0,939; R^2=0,882; Adjusted R^2=0,794; $F_{(9,1)}$=10,0; p=0,0003

N=22		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(12)}$	p-level
Variables	r		Intercpt	239	35,5	6,74	10^{-4}

Triiodothyronine, nM/L	**0,48**	-0,306	0,160	-80,13	42,06	-1,90	0,081
T4-β PSD, %	**0,42**	0,519	0,158	4,586	1,393	3,29	0,006
Entropy T4	**0,42**	0,564	0,172	746,4	227,0	3,29	0,006
Entropy O2	**0,40**	0,638	0,269	541,6	228,7	2,37	0,036
Entropy F7	**0,38**	-0,570	0,237	-381,8	158,8	-2,40	0,033
LD Cholesterol, mM/L	**0,32**	-0,254	0,174	-33,66	23,08	-1,46	0,170
Glucose Plasma, mM/L	**-0,55**	-0,272	0,146	-39,63	21,33	-1,86	0,088
Cl Urine, mM/L	**-0,55**	-0,653	0,170	-2,285	0,595	-3,84	0,002
Mg Urine, mM/L	**-0,33**	-0,230	0,124	-58,91	31,82	-1,85	0,089

It is stated that the dynamics of the cholecystokinetic index is positively associated with changes in plasma triiodothyronine levels, activity of beta-rhythm-generating nerve structures projected at the T4 locus (probably the right amygdala (Romodanov, 1993) and/or temporal gyrus of the cortex (Winkelmann et al., 2016)), EEG entropy at the T4, O2 and F7 loci, as well as low-density lipoprotein cholesterol. In contrast, changes in glycemia and urinary chloride and magnesium concentrations are negatively related to cholecystokinetic index dynamics. Taken together, changes in these factors due to mineral waters determine changes in cholekinetics by 88% (Fig. 12.11).



R=0,939; R^2=0,882; $\chi^2_{(9)}$=33; p=0,0001; Λ Prime=0,118
Fig. 12.11. Scatterplot of canonical correlation between change in Neuro-Endocrine and Metabolic (X-line) and Cholecystokinetics (Y-line) parameters

12.3. Neural determination of metabolic parameters

According to the results of screening the correlations between changes in EEG parameters, on the one hand, and the concentration of electrolytes and nitrogenous metabolites in the urine, on the other hand, a matrix was created, which includes only EEG parameters with significant links (Table 12.23).

Table 12.23. Matrix of correlations between changes in EEG and urine variables

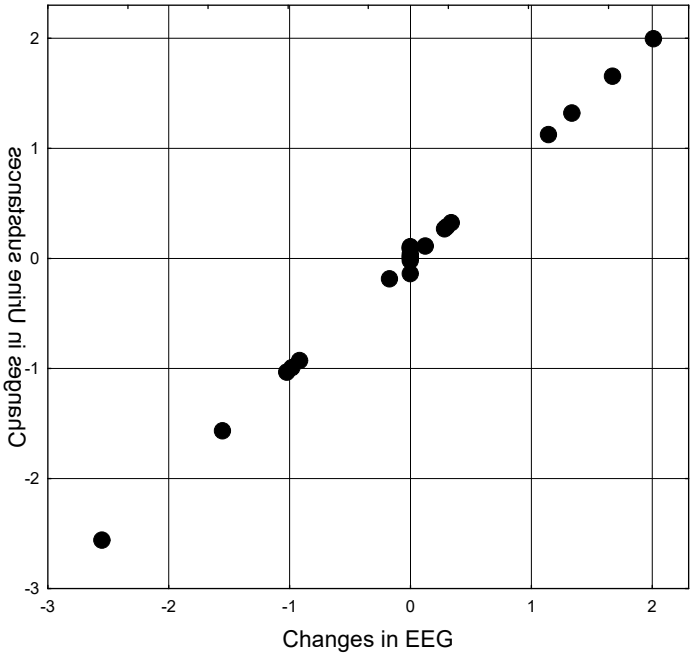
Variables	Nau	Clu	Cau	Mgu	Ku	Pu	Cru	Uru	Lith
F7-δ PSD, μV²/Hz	**0,69**	**0,50**	-0,15	-0,03	0,15	0,14	-0,07	-0,16	-0,08
T6-δ PSD, μV²/Hz	**0,58**	0,30	**-0,44**	-0,25	0,00	0,04	-0,06	-0,10	-0,28
F8-δ PSD, %	**0,58**	0,24	**-0,52**	-0,21	-0,02	0,17	-0,05	-0,12	**-0,45**
F7-δ PSD, %	0,41	0,10	**-0,67**	-0,39	0,02	0,08	-0,02	-0,11	**-0,50**
T5-δ PSD, μV²/Hz	0,29	-0,04	**-0,56**	-0,34	-0,18	-0,10	-0,01	0,01	-0,37
O2-δ PSD, μV²/Hz	0,24	-0,03	**-0,63**	-0,31	-0,20	0,04	0,01	0,03	**-0,45**
Deviation δ, Hz	**-0,49**	-0,16	**0,59**	0,12	-0,13	-0,11	0,01	0,11	**0,57**

Entropy F7	**-0,67**	-0,26	0,39	0,11	-0,26	0,02	0,02	0,08	0,30
Entropy T6	**-0,54**	-0,11	**0,47**	0,24	-0,11	0,01	0,02	0,05	0,34
Entropy Fp2	**-0,53**	-0,18	0,32	0,15	-0,17	0,12	0,05	0,04	0,16
Entropy O2	-0,31	0,07	**0,59**	0,32	-0,14	0,21	-0,03	-0,03	**0,44**
F8 β PSD, %	**-0,50**	-0,33	0,32	0,10	0,15	-0,26	0,04	0,10	0,30
F4-α PSD, %	-0,07	-0,09	0,37	**0,49**	0,08	0,12	0,11	0,08	0,13
Laterality β, %	**-0,47**	-0,35	-0,04	-0,21	-0,24	-0,14	-0,02	0,14	0,15

Next, the canonical correlation between the two sets of balneotherapy effects is analyzed. The program included in the canonical roots not all elements of the matrix (Table 2). Due to the pseudo-staining, the main mediating role in the effect of balneotherapy on the electrolytes of the urine parameters of **delta** rhythm and **entropy** is clearly visible. In this case, the delta-rhythm-generating neurons localized in different loci up-regulate changes in sodium, chloride and less potassium concentrations, while delta-rhythm frequency variability, beta-rhythm-generating neurons projected at the F8 locus, EEG entropy as well as right-hand shift of beta-rhythm symmetry (not included in the model), carry out down-regulation. In contrast, changes in urinary concentrations of uric acid and calcium (mostly) as well as magnesium and creatinine (less) are object to opposite regulatory effects of these nerve structures as well as of alpha-rhythm generating neurons projected at the F4 locus. Judging by the coefficient of determination, changes in the concentrations of electrolytes and nitric metabolites in the urine caused by balneotherapy are mediated by changes in neurodynamics by 99,8% (Fig. 1).

Table 12.24. Factor structure of EEG and urinary electrolytes roots changes

EEG Variables	**R**
F7-δ PSD, $\mu V^2/Hz$	**-0,523**
T6-δ PSD, $\mu V^2/Hz$	**-0,375**
F8-δ PSD, %	**-0,339**
F7-δ PSD, %	**-0,271**
T5-δ PSD, $\mu V^2/Hz$	**-0,079**
O2-δ PSD, $\mu V^2/Hz$	**-0,015**
Deviation δ, Hz	**0,335**
F8 β PSD, %	**0,217**
Entropy F7	**0,554**
Entropy Fp2	**0,437**
Entropy T6	**0,400**
Entropy O2	**0,267**
F4-α PSD, %	**0,069**
Electrolytes Urine	**R**
Na, mM/L	**-0,841**
K, mM/L	**-0.809**
Cl, mM/L	**-0,411**
Uric acid, µM/L	**0,180**
Ca, mM/L	**0,153**
Mg, mM/L	**0,034**
Creatinine, µM/L	**0,099**



R=0,999; R^2=0,998; $\chi^2_{(91)}$=125; p=0,010; Λ Prime<10^{-5}

Fig. 12.12. Scatterplot of canonical correlation between changes in EEG parameters (X-line) and Urine electrolytes&nitric metabolites concentration (Y-line)

Interestingly, the dynamics of lithogenicity of urine is unidirectional with the dynamics of only the concentration of calcium in it.

According to a similar algorithm, the relationships between changes in EEG parameters and the concentration of electrolytes and nitrogen metabolites in plasma were analyzed (Tables 3 and 4).

Table 12.25. Matrix of correlations between changes in EEG and Plasma variables

Variables	Mgp	Urp	Clp	Nap	Crp	Kp	Pp	Cap
T5-δ PSD, μV^2/Hz	0,90	0,16	0,39	0,39	0,15	-0,19	-0,38	-0,26
T6-δ PSD, μV^2/Hz	0,85	0,29	0,27	0,27	0,19	-0,36	-0,59	-0,08
O2-δ PSD, μV^2/Hz	0,77	0,38	0,19	0,19	0,04	-0,20	-0,50	-0,11
F7-δ PSD, μV^2/Hz	0,63	0,29	0,19	0,19	0,22	-0,38	-0,57	0,03
F7-δ PSD, %	0,81	0,12	0,26	0,26	0,26	-0,54	-0,42	-0,20
F8-δ PSD, %	0,76	0,11	0,19	0,19	0,14	-0,41	-0,56	-0,12
Deviation δ, Hz	-0,50	-0,43	-0,23	-0,23	-0,32	0,41	0,28	-0,04
F8 β PSD, %	-0,67	-0,08	-0,14	-0,14	0,01	0,29	0,72	0,23
Entropy F7	-0,71	-0,41	-0,20	-0,20	-0,31	0,37	0,30	-0,21
Entropy O2	-0,69	-0,38	-0,16	-0,16	-0,11	0,23	0,28	-0,10
Entropy T6	-0,74	-0,24	-0,16	-0,16	-0,19	0,32	0,28	-0,16
Entropy Fp2	-0,67	-0,36	-0,26	-0,26	-0,27	0,24	0,24	-0,16
F4-α PSD, %	-0,39	-0,04	-0,05	-0,05	-0,07	0,19	0,24	0,09
Laterality β, %	-0,20	-0,13	-0,16	-0,16	-0,37	0,37	0,03	-0,00

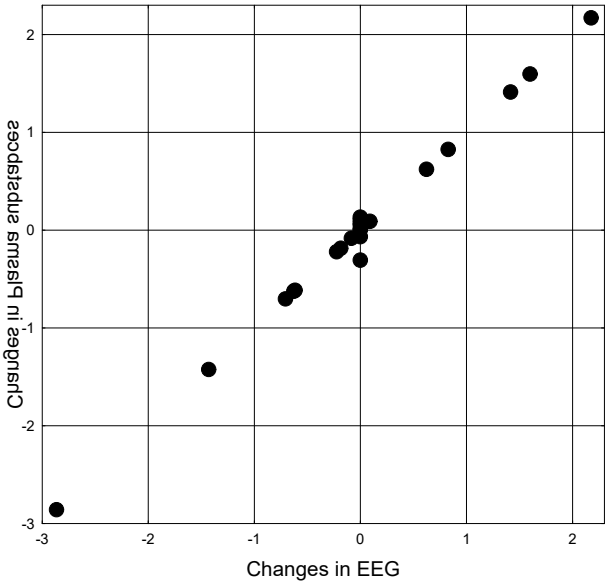
Table 12.26. Factor structure of EEG and Plasma variables roots changes

EEG Variables	R
T5-δ PSD, μV^2/Hz	0,714
O2-δ PSD, μV^2/Hz	0,691
T6-δ PSD, μV^2/Hz	0,614
F7-δ PSD, %	0,463
F8-δ PSD, %	0,460
F7-δ PSD, μV^2/Hz	0,408
F8 β PSD, %	-0,490
F4-α PSD, %	-0,249
Entropy O2	-0,524
Entropy Fp2	-0,464

Entropy F7	**-0,440**
Entropy T6	**-0,422**
Deviation δ, Hz	**-0,370**
Plasma Variables	**R**
Mg, mM/L	**0,698**
Uric acid, µM/L	**0,546**
Cl, mM/L	**0,536**
Na, mM/L	**0,536**
K, mM/L	**0,166**
Creatinine, µM/L	**0,030**
Phosphates, mM/L	**-0,555**
Ca, mM/L	**-0,417**

It was found that the delta-rhythm-generating neurons up-regulate changes in magnesium, uric acid, sodium, chloride and less potassium and creatinine plasma concentrations, while delta-rhythm frequency variability, beta-rhythm-generating neurons projected at the F8 locus, alpha-rhythm generating neurons projected at the F4 locus as well as EEG entropy carry out down-regulation. In contrast, changes in concentrations of phosphates and calcium are object to opposite regulatory effects of these nerve structures and entropy.

Judging by the coefficient of determination, changes in the concentrations of electrolytes and nitric metabolites in the urine caused by balneotherapy are mediated by changes in neurodynamics by 99,4% (Fig. 12.13).



R=0,997; R^2=0,994; $\chi^2_{(91)}$=155; p<10^{-4}; Λ Prime<10^{-6}

Fig. 12.13. Scatterplot of canonical correlation between changes in EEG parameters (X-line) and Plasma component concentration (Y-line)

CONCLUSION

Drinking mineral water, along with fresh water, is one of the environmental factors that affect the condition of the human body. Back in 1975, with the chemical analysis of over 300 mineral waters of the then USSR, organic matter was discovered in all of them without exception. It is shown that for water of one type, the presence of bitumen, naphthenic acids and phenols is typical, while for other types of humic, carboxylic acids and again phenols are characteristic (Tsarfis and Danilova, 1975). Despite this, it is still assumed that the physiological activity of drinking mineral waters is due to their electrolytes, the concentration of which is from 2 to 30 g/L, as well as the trace elements, while the role of organic substances is ignored, apparently because of their relatively insignificant concentration (5-40 mg/L). And only for Naftussya, Berezivs'ka, Kala-Alta, Kurgazak, Volzhanka, Serebryanyi klyuch, Munoc waters, which are not formally mineral, because they contain less than 1 g/L of electrolytes, organic substances are considered as active principles (Baladjayeva et al., 1975; Yessypenko, 1978, 1981; Yaremenko et al., 1989; Ivassivka, 1997; Khutoryansky et al., 2013; Shestopalov et al., 2013; Moiseev, 2017).

We adduce data by Dats'ko et al., (2008) about organic compounds (in mg/L) Naftussya water obtained by Solid Phase Extraction method and mass-spectroscopy by using as Sorbents Tenacle GC 60/80 and Polysorb-2. Paraffins 4,10 and 4,20; monoolefins 1,67 and 1,75; dienes and monocycloolefins 0,84 and 0,85; alkylbenzene 1,55 and 1,54; alkenylbenzene 0,47 and 0,46; esters of aromatic acids 1,32 and 1,33; alkyl phenols 1,14 and 1,14; polyaromatic hydrocarbons 0,077 and 0,059; oxygene-containing connections (acids) 1,12 and 1,14; sulfur-containing connections 0,30 and 0,31; alkylnaphthalenes 0,53 and 0,53; carboxylic acids 1,12 and 1,14; unidentified polyaromatic hydrocarbons 0,19 and 0,19; connections required subsquent identification 0,48 and 0,50 respectively. As such a complete analysis is extremely labor intensive and expensive, the organic component of water is usually judged by the gross organic carbon and nitrogen content.

The likelihood of **direct** effects of organic substances on immunocytes through their **aryl hydrocarbon** receptors was analyzed in Chapter 7. However, it is very likely to assume the realization of the immunotropic effects of organic matter through neuroendocrine structures. About it later, and now we will present other operating principle of medical water - autochthonous microflora which is very closely connected with organic substances: 1/3 of their weight is a product of microbic transformation of primary organic substances of aquifer (Dats'ko et al., 2008).

900 bacterial and yeast cultures were found in Naftussya water. Identified bacteria are classified as genera: Pseudomonas, Bacillus, Nocardia, Corinebacterium, Micrococcus, Brevibacterium. By type of food bacteria are ammonifying, denitrifying, iron bacteria, oligonitrophilic, sulfate-reducing, thionic acid bacteria and hydrocarbon oxidizing (Kvasnikov et al., 1978). However, only the last three groups are considered specific, which together with aquifer and filtration water are an attribute of the Naftussya "water-forming triad" (Ivassivka et al., 2010).

Based on the data of our previous studies and presented in this monograph, we hypothesize that the primary effect of mineral waters is the modulation of the structures of the autonomic and central nervous and endocrine systems, which, in turn, have regulatory modulating effects on the immune system, microbiota, metabolism, cholekinetics, blood pressure and, apparently, others, not yet registered body parameters.

To visualize our ideas about the possible ways of influence of the organic substances and autochthonous microflora of Naftussya water on the neuroendocrine-immune complex, we use the **wonderful** review and figures of Yoo and Mazmanian, (2017). Authors describe the current understanding of the anatomy and physiology of the gastrointestinal (GI) tract, focusing on the enteric nervous system (ENS) and the mucosal immune system. They highlight emerging literature that the ENS is essential for important aspects of microbe-induced immune responses in the gut. While most basic and applied research in neuroscience has focused on the brain, the proximity of the ENS to the immune system and its interface with the external environment suggest that ***novel paradigms for nervous system function await discovery***. The ENS senses and reacts to the dynamic ecosystem of the GI tract by translating chemical cues from the environment into neuronal impulses that propagate throughout the gut, and into other organs in the body including the central nervous system (CNS). Receptors on enteric neurons mediate important GI functions. Chemoreceptors respond to various chemical stimuli in the lumen, such as pH, osmolarity, and nutrients, including components of used mineral water, especially organic substances as agonists of **aryl hydrocarbon** receptors as well as **carboxylic acids** (acetic, myristic, oleic, palmitic, stearic) (Ivassivka, 1997; Shestopalov et al., 2013).

It is known that Short Chain Fatty Acids (SCFAs) are microbial metabolic products of dietary fibers, and the most studied SCFAs are butyrate, propionate, and acetate. These metabolites are sensed by the intestinal epithelium, but can also diffuse across the epithelium where they can be accessed by the enteric nervous and immune systems. G-protein coupled receptors (GPR) 41 and 43 are activated by acetate and propionate, whereas GPR109A is specific for butyrate (cit. by: Yoo and Mazmanian, 2017). In our case**, carboxylic acids** are microbial metabolic products of organic substances of aquifer (Ivassivka, 1997).

The GI tract is comprised of distinct cross-sectional compartments (Fig. 13.1). Extrinsic, sympathetic and parasympathetic nerve fibers enter the GI tract through the mesentery and can extend throughout all layers of

intestinal tissue. The myenteric and submucosal plexuses form the dense nerve network that innervates the entire length and depth of the GI tract. Various immune cells are resident to the muscularis, but are also highly abundant in the lamina propria, especially in Peyer's patches and lymphoid follicles. These immune cells are also in close proximity to neurons and glia. The epithelium shown here is made up of 5 different cell types. These include absorptive enterocytes, enteroendocrine cells (EECs), goblet cells, Paneth cells, and M-cells.

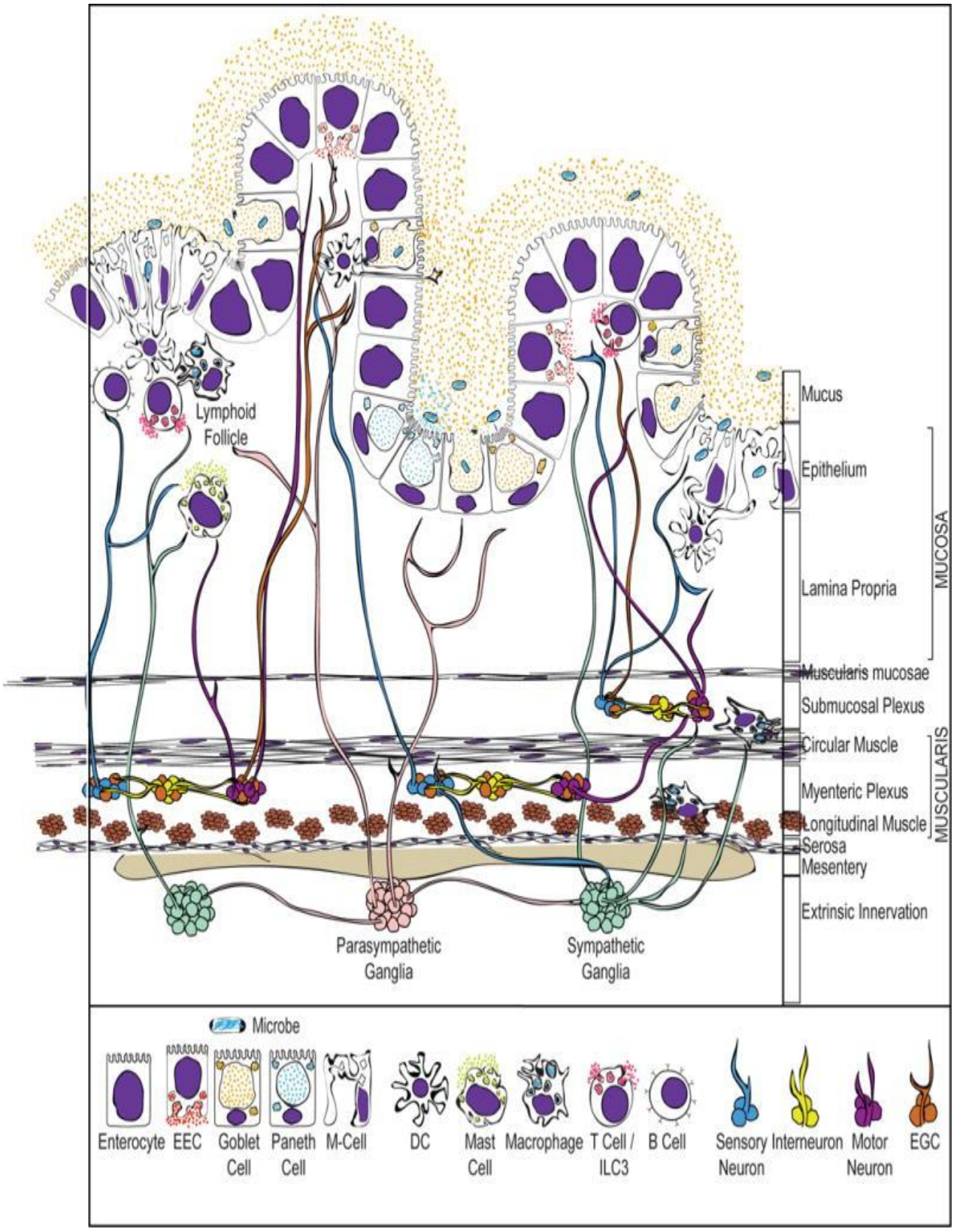


Fig. 13.1. Anatomy of the GI Tract (Yoo and Mazmanian, 2017)

Among the cells with which microbes interact (Fig. 13.2), we are most interested Paneth cells and enteroendocrine cells (EECs). Paneth cells reside mainly in ileal intestinal crypts and secrete potent antimicrobial products via release of granule contents. As such, antimicrobial peptides (AMPs) are secreted when the epithelium senses microbe associated molecular patterns. EECs produce a variety of modulatory, neuroendocrine molecules. These cells have been commonly referred to as the "taste" cells of the gut, as they are popularly known for their chemosensation and production of molecules that control aspects of digestive system functions, such as gastric and pancreatic secretion, cholekinetics etc. The influence of mineral waters on GEPES is analyzed by us in the review. It is important to add that EECs, in particular G-cells, are a source not only of regulatory polypeptides, but also of classical neurotransmitters and hormones: cathecholamines (Grube, 1982) and ACTH (Larsson, 1981).

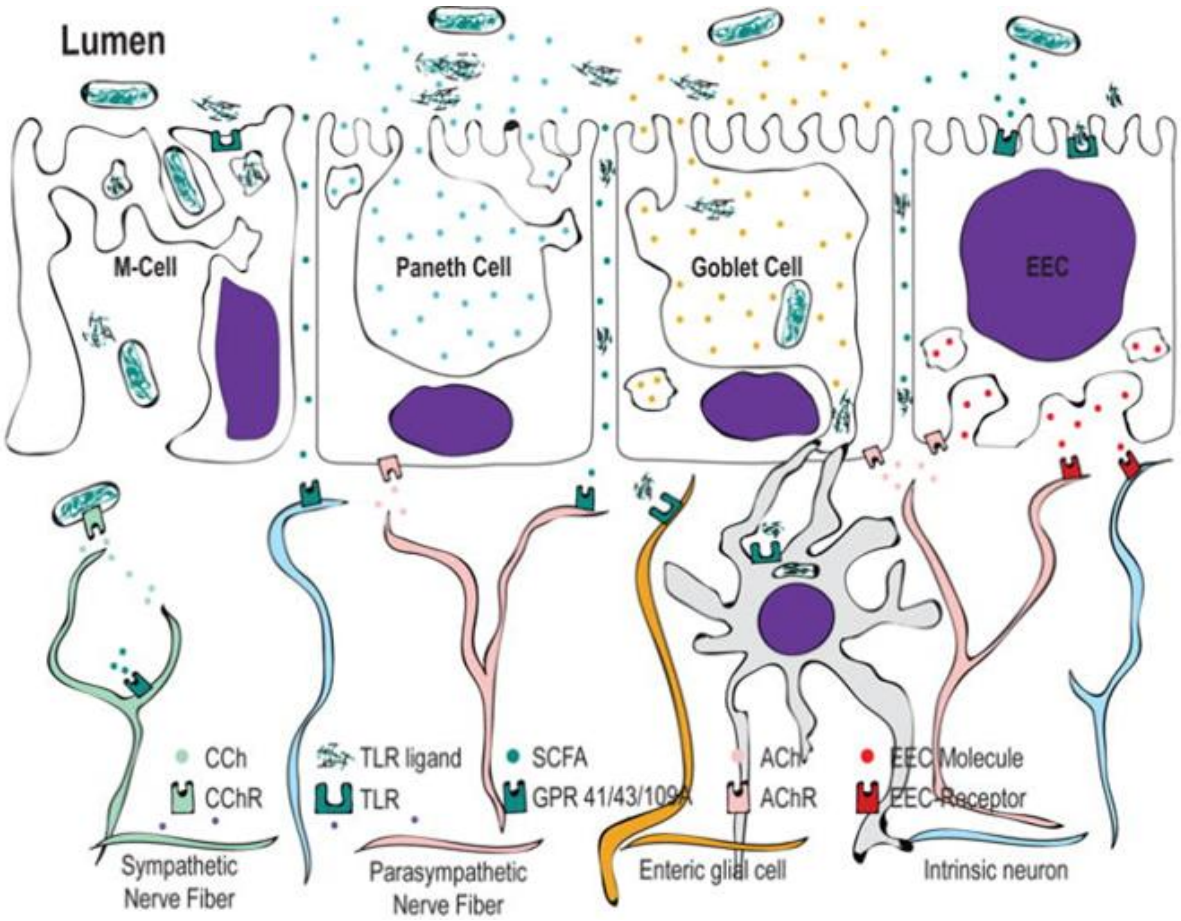


Fig. 13.2. Interactions at the Intestinal Epithelium (Yoo and Mazmanian, 2017)

The intestinal epithelium is where luminal constituents are actively or passively transported into the tissue. Extrinsic nerves and neurons are found near the epithelium, and thus the molecules that cross the epithelium and those that are secreted basolaterally can potentially have an effect on their activity. Microbes or microbial parts can cross the epithelium and affect other cell types through M-cells, immunoglobulin-mediated transcytosis, goblet cell-associated passages (GAPs), and by general leakiness of the epithelium. Dendritic cells (DCs) and macrophages can phagocytose microbial antigens and secrete cytokines that can have an effect on neurons as well. Parasympathetic fibers release acetylcholine (ACh) and induce secretion of intracellular stores of molecules. In goblet cells, ACh also increases rates of DC luminal sampling via GAPs. EECs can also release neuroendocrine molecules in response to TLR stimulation and SCFAs. These molecules released basolaterally can potentially regulate the activity of neurons. Enteric glial cells can also project towards the epithelium, potentially allowing microbes to impact their function. Enteric neurons can be activated by commensal and pathogenic bacteria, as well as short chain fatty acids that diffuse across the epithelium (Yoo and Mazmanian, 2017).

Extrinsic nerves, intrinsic neurons, and enteric glial cells are in close proximity to each other and to the immune cells in the GI tract **(**Fig. 13.3**).** Thus, the molecules that are produced by one cell can have an effect on another cell, given that the latter expresses a receptor to recognize the molecule. By these parameters, interactions between neurons/glia and immune cells are, in theory, abundant, and some of these putative interactions are presented here. Immune cells can be influenced by neurotransmitters and neuropeptides

produced by intrinsic neurons of the ENS (as well as those produced by extrinsic nerve fibers), and cytokines produced by immune cells can have a reciprocal effect on neurons (Yoo and Mazmanian, 2017).

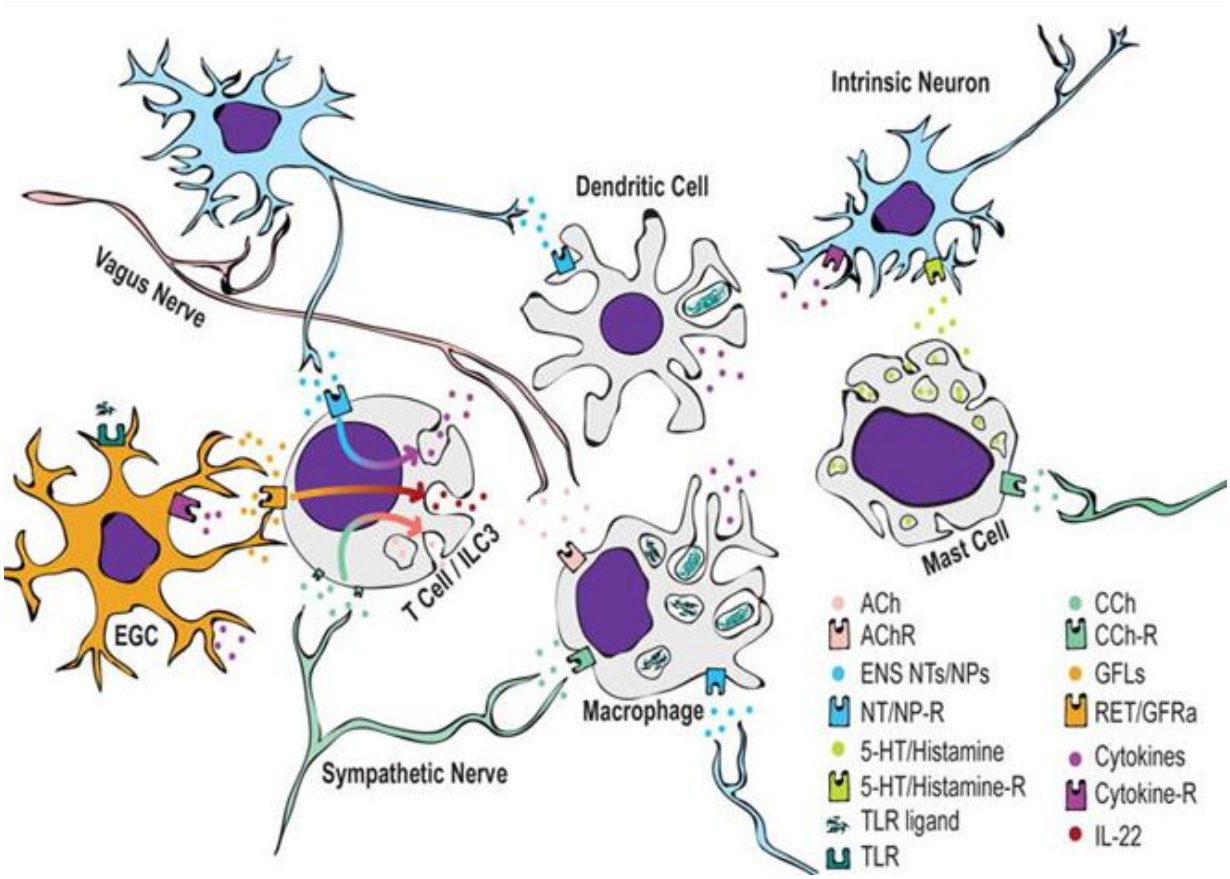


Fig. 13.3. Interactions between GI Immune Cells and the ENS (Yoo and Mazmanian, 2017)

The key effect of the action on macrophages of microbes (via TL and NL receptors) and organic matter (via Ah, GP41, GP43 and GP109A receptors) of drinking mineral water, in our opinion, is their release of pro-inflammatory cytokines. To visualize the following processes, we used a **wonderful** illustration by Pavlov and Tracey (2012) and Tracey (2009).

Cytokines through chemosensitive glomus cells localized along autonomic nerves (Goehler et al., 2000), activate the terminals of vagal afferents and through them the nucleus tractus solitarius (NTS), more precisely, its dorsal medial portion (dmNTS) (Tang and Dworkin, 2009). From dmNTS the common pathway in the integrative center diverges to the parasympathetic and sympathetic branches. On the parasympathetic branch dmNTS neurons are projected on the dorsal motor nucleus of the vagus nerve (DMN), nucleus ambiguus (NA) and area postrema (AP). Neuronal interconnections between the NTS, AP, DMN, NA, and higher forebrain regions (not shown) integrate afferent signalling and efferent vagus nerve-mediated immunoregulatory output. Efferent vagus nerve cholinergic output to the spleen, liver and gastrointestinal tract (blue) regulates immune activation and suppresses proinflammatory cytokine release (dotted red lines). This efferent cholinergic arm of the inflammatory reflex can be activated in the brain through muscarinic acetylcholine receptor (mAChR)-mediated mechanisms triggered by mAChR ligands and acetylcholinesterase (AChE) inhibitors, such as galantamine **(**Fig. 13.4**)** (Pavlov and Tracey, 2012).

We emphasize the fact that these same efferent vagal nuclei innervate the heart, so the heart rate variability simultaneously reflects the vagal effects on the heart and other organs, including the immune and digestive systems.

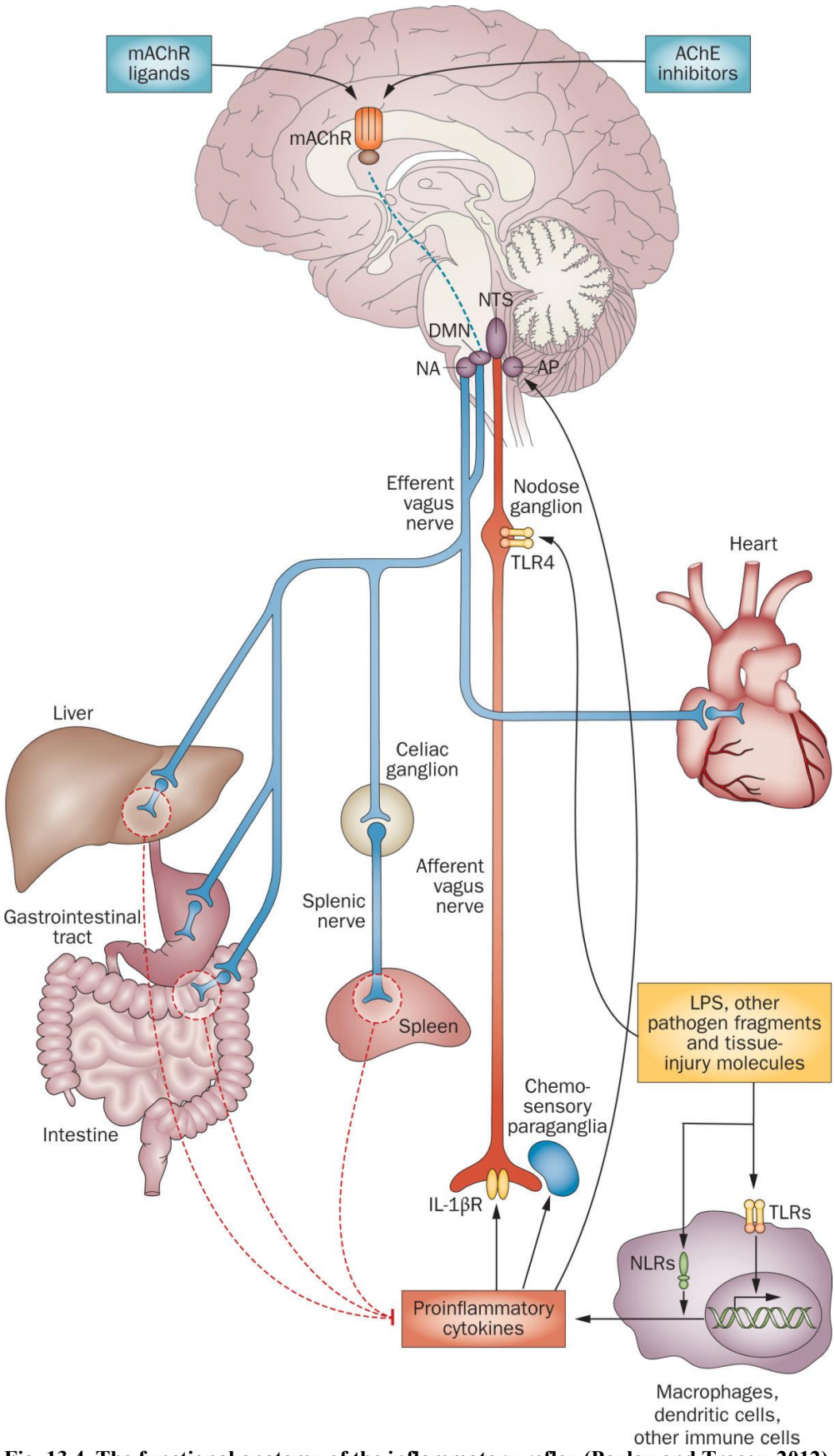


Fig. 13.4. The functional anatomy of the inflammatory reflex (Pavlov and Tracey, 2012)

On the other hand, the sympathetic branch is projected on the caudal ventro-lateral medulla, and then on the rostral ventro-lateral medulla (RVLM), the signal from the nuclei of which enters the ganglion coeliacum, and hence to the N-cholinoreceptors of splenic macrophages **(**Fig. 13.5**)**.

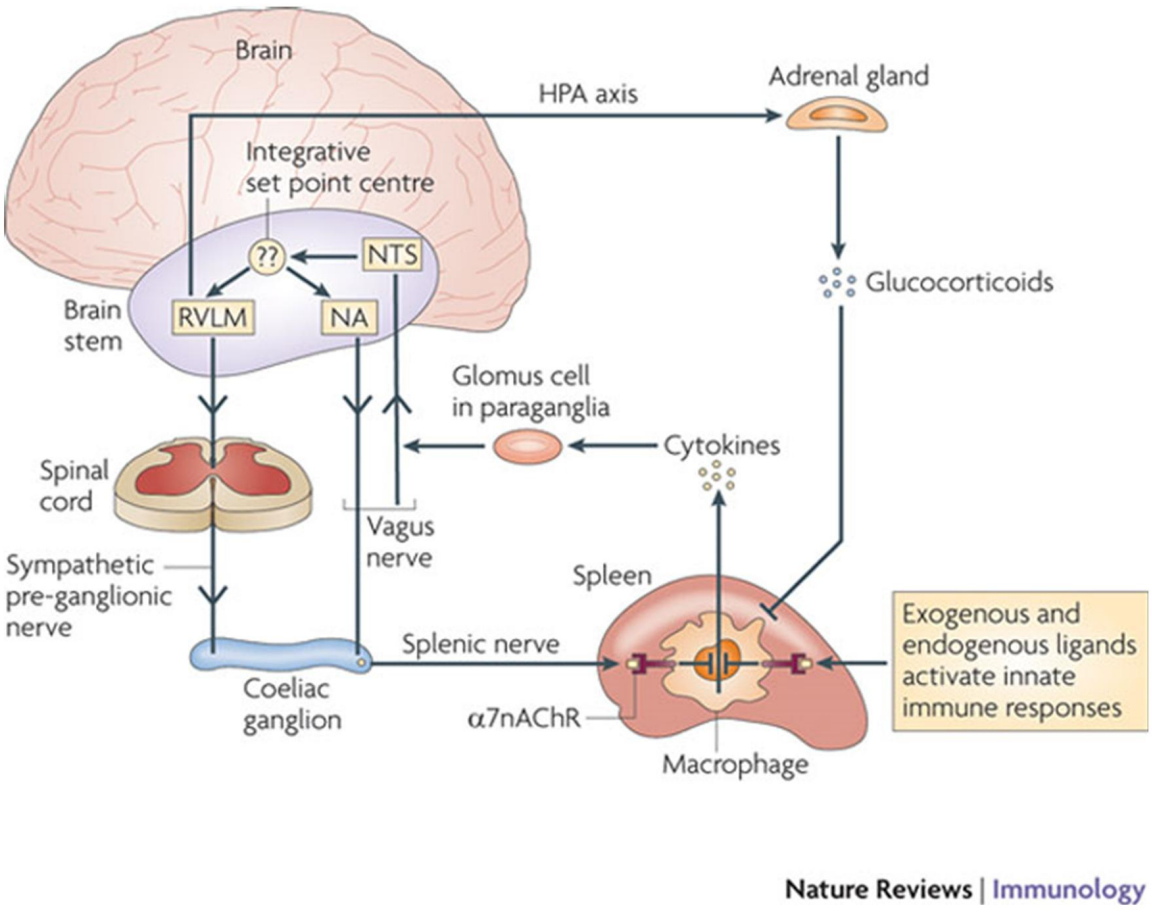


Fig. 13.5. The functional anatomy of the inflammatory reflex (Tracey, 2009)

However, RVLM impulses activate the adrenal cortex, increasing its release of glucocorticoids (Thayer and Sternberg, 2010). The scheme shows (Fig. 13.6) that the objects of regulatory influence of the parasympathetic and sympathetic divisions of the autonomic nervous system and the hypothalamic-pituitary-adrenocortical axis, more precisely their mediators acetylcholine, norepinephrine and glucocorticoids, are in addition to the spleen, lymph nodes, bone marrow, or more precisely their macrophages-monocytes, microphages-neutrophils, NK-, T- and B-lymphocytes, as well as epitheliocytes of the gastroduodenal mucosa as components of the classical stress triad Selye.

The mechanisms of inhibitory effect of the main anti-inflammatory factors are illustrated in Fig. 13.7 (Sternberg, 2006; Tracey, 2010). Acetylcholine through the α-7 subunit of the N-cholinoreceptor inhibits the biosynthesis of proinflammatory cytokines by at least three mechanisms: through activation of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 STAT3; increased levels of intracellular calcium followed by inhibition of mitogen-activated protein kinase MAPK; preventing the detachment of the nuclear factor inhibitor (IκB) from the p50 and 65 protein complex. All three mechanisms are ultimately reduced to inhibition of nuclear factor κB. Another anti-inflammatory factor, norepinephrine, exerts its effect through β_2-adrenoceptors of macrophages and dendritic cells, increasing the formation of c-AMP and thus activating protein kinase A, which in turn leads to suppression of proinflammatory cytokine production again through inhibition of nuclear factor κB.

Finally, the classical anti-inflammatory factors, glucocorticoids, exert their effect by binding to the cytosolic receptor, which displaces the HSP90 heat shock protein and allows dimerization of the receptor, its entry into the nucleus and binding of the glucocorticoid-glucocorticoid receptor to the DNA. This leads to the transcription and translation of proteins, including IκB. IκB, in turn, sequesters NF-κB, thereby preventing the activation of transcription of proinflammatory cytokines. However, the glucocorticoid-glucocorticoid receptor complex can interact with NF-κB directly, suppressing cytokine production.

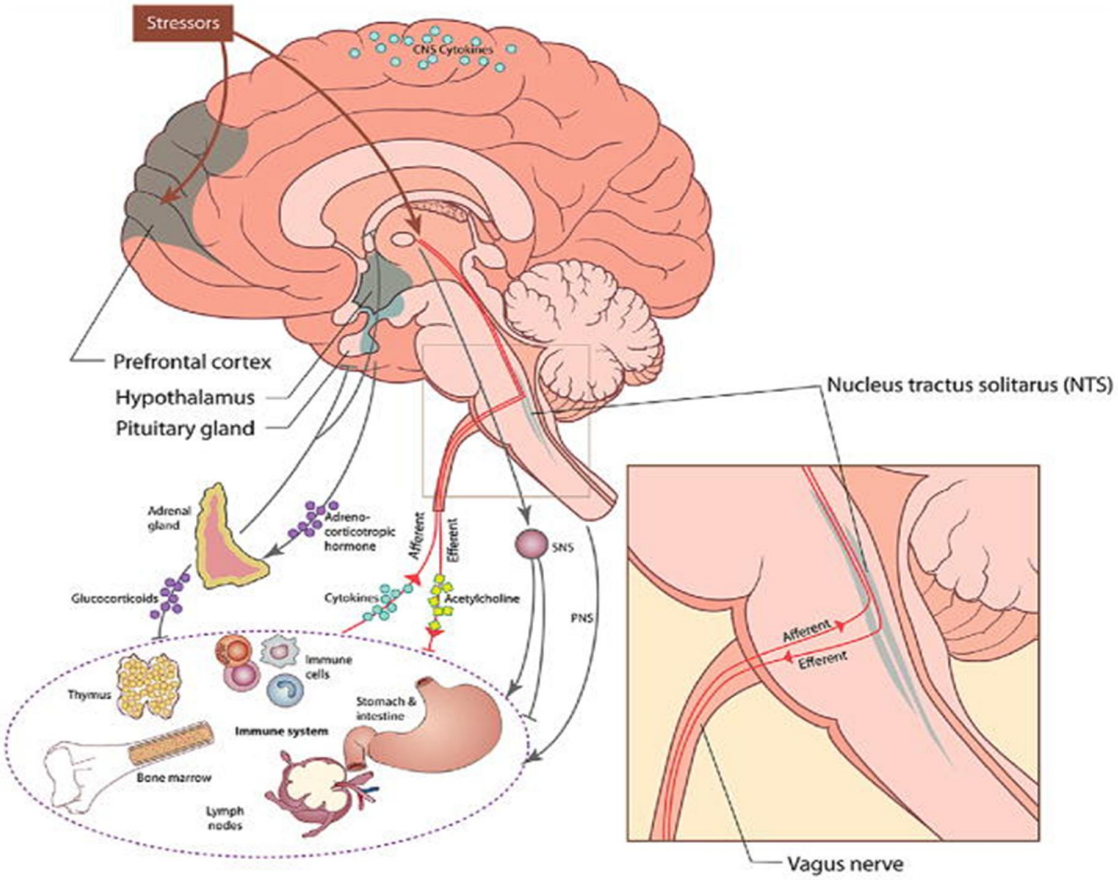


Fig. 13.6. Neuro-endocrine regulation of immunity (Thayer and Sternberg, 2010)

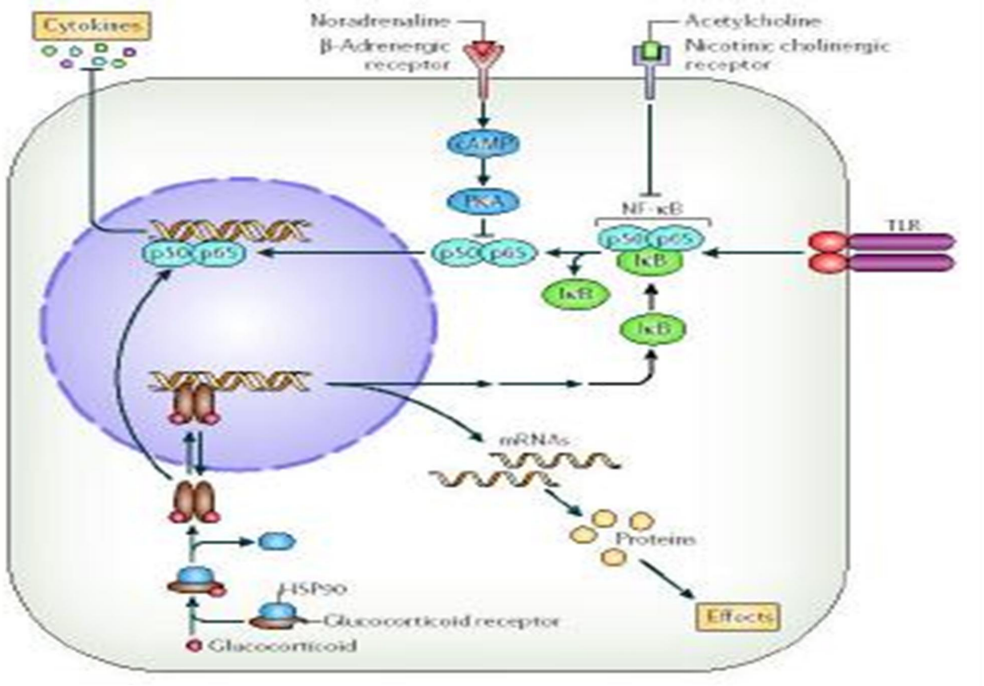


Fig. 13.7. Mechanisms of inhibitory effect of the main anti-inflammatory factors (Sternberg, 2006; Tracey, 2010)

Chang, Chavan and Pavlov, (2019) detail molecular mechanisms of cholinergic control of inflammation.

Cholinergic neurons in the brainstem dorsal motor nucleus of the vagus (DMN) and nucleus ambiguus (NA) provide axonal projections within preganglionic efferent vagus nerve fibers. These long fibers interact with short postganglionic neurons in proximity or within the innervated organs, including the heart, lungs, gastrointestinal

tract, liver, and pancreas. Acetylcholine (ACh) released from these neurons interacts with muscarinic acetylcholine receptors (mAChRs) on targeted cells and regulates several metabolic functions. ACh also regulates (inhibits) the release of pro-inflammatory cytokines and inflammation via alpha 7 nicotinic acetylcholine receptors (α7nAChRs) on immune cells (Fig. 13.8).

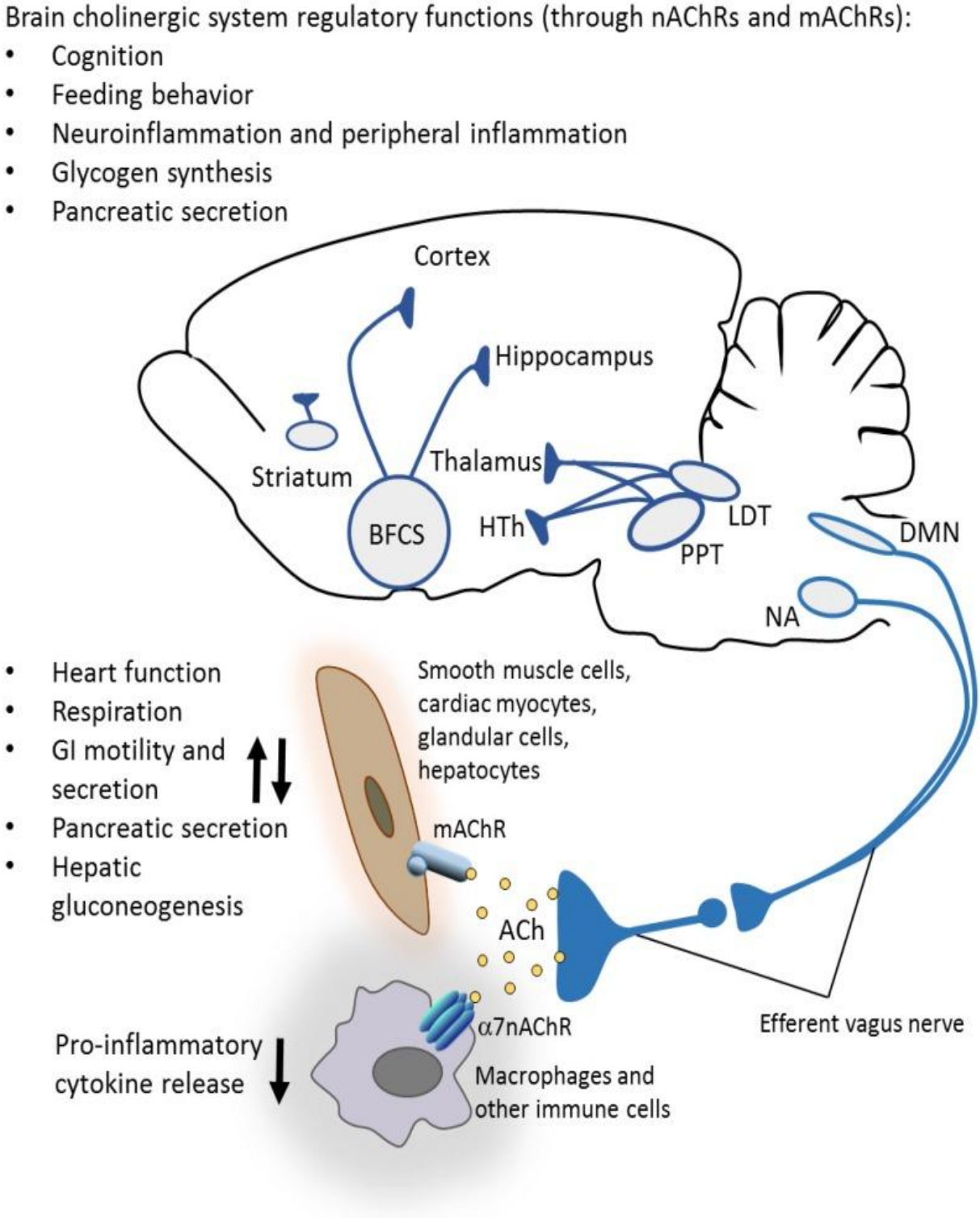


Fig. 13.8. Brain cholinergic system regulatory functions (Chang, Chavan and Pavlov, 2019)

Efferent vagus nerve activity is translated into catecholamine-mediated activation of T-cell-derived ACh release in the spleen and into direct ACh release from efferent vagus nerve endings in other organs. Inhibition of NF-κB nuclear translocation and activation of a JAK2-STAT3-mediated signaling cascade in macrophages and other immune cells are implicated in cholinergic α7nAChR-mediated control of pro-inflammatory cytokine production. ACh, acetylcholine; β_2AR, β_2 adrenergic receptor; JAK2, Janus kinase 2; α7nAChR, α 7 nicotinic

acetylcholine receptor; NA, noradrenaline; NF-κB, nuclear factor κB; STAT3, signal transducer and activator of transcription 3 (Fig. 13.9).

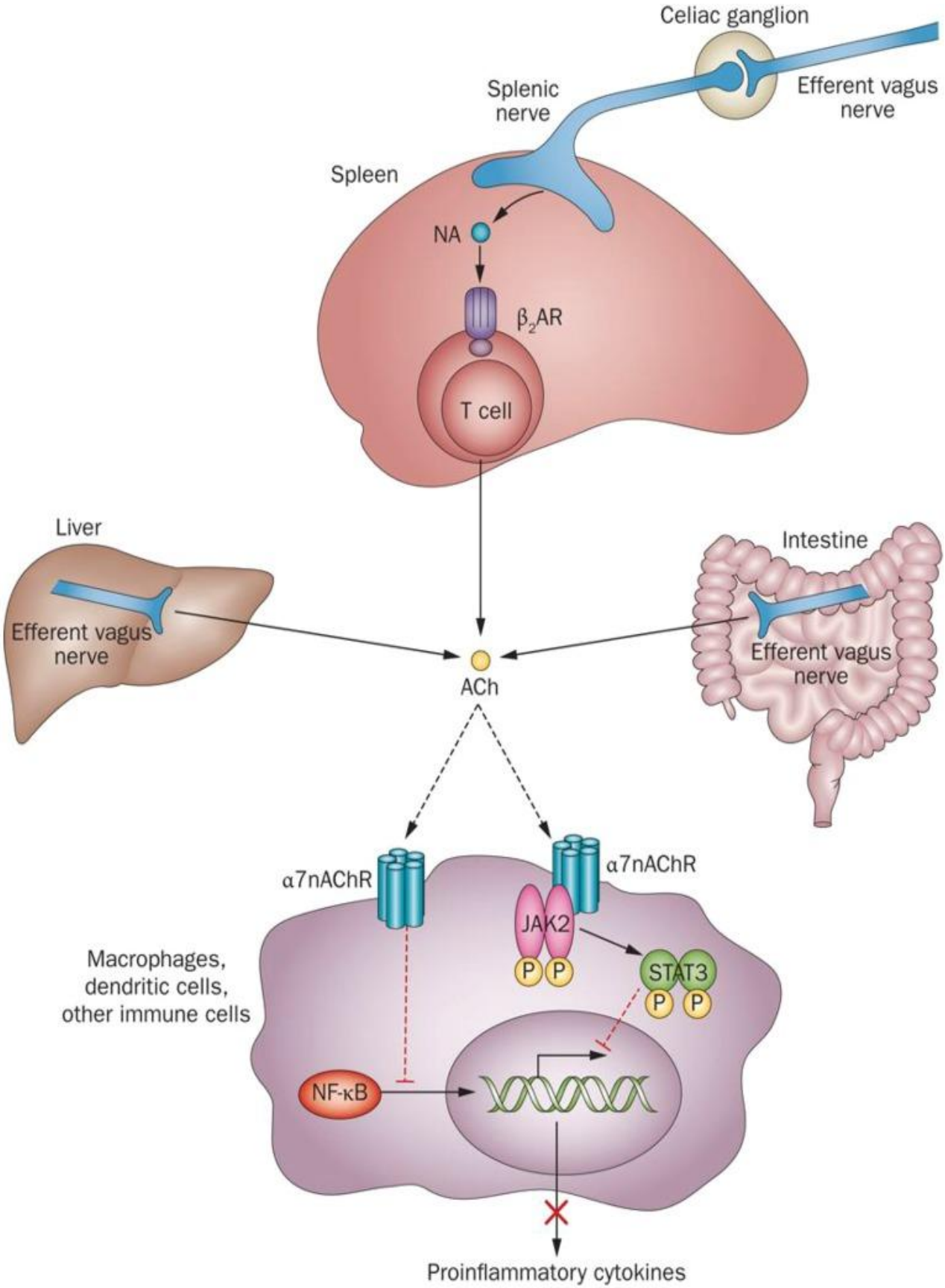


Fig. 13.9. Molecular mechanisms of cholinergic control of inflammation (Chang, Chavan and Pavlov, 2019)

Finally, we climb to the top - to the brain. According to the concept of "central autonomic network (CAN)" (Benarroch, 1993; Palma and Benarroch, 2014; Thayer and Lane, 2009) it include following cortical, subcortical, and medullary structures: the anterior cingulate, insular, orbitofrontal, and ventromedial cortices; the central nucleus of the amygdala; the paraventricular and related nuclei of the hypothalamus; the periaqueductal gray matter; the nucleus of the solitary tract; the nucleus ambiguous; the ventrolateral medulla; the ventromedial medulla and the medullary tegmental field (Fig. 13.10). The primary output of the CAN is mediated through the preganglionic sympathetic and parasympathetic neurons, which exert control over the heart via the stellate ganglia and the vagus nerve, respectively. The interplay of sympathetic and parasympathetic influences on sinoatrial node pacemaker activity generates the complex variability that characterizes the healthy heart rate rhythm, which is called HRV. A fundamental principle of the neural control of the heart is its hierarchical organization, with cortical structures providing inhibitory control over limbic and brainstem sympathoexcitatory, cardioacceleratory circuits. The prefrontal, cingulate, and insula cortices form an interconnected network with bi-directional communication with the amygdala. The amygdala is under tonic inhibitory control via prefrontal vagal pathways to intercalated cells in the amygdala. The activation of the central nucleus of the amygdala (CeA) inhibits the nucleus of the solitary tract (NTS) which in turn inhibits inhibitory caudal ventrolateral medullary (CVLM) inputs to the rostral ventrolateral medullary (RVLM) sympathoexcitatory neurons, and simultaneously inhibits vagal motor neurons in the nucleus ambiguus (NA) and the dorsal vagal motor nucleus (DVN). In addition, the CeA can directly activate the sympathoexcitatory neurons in the RVLM. Indeed, disruption of prefrontal activity leads to disinhibition of sympathoexcitatory circuits, with a resultant increase in heart rate and decrease in vagally-mediated HRV (Verberne et al., 1996; 1997; Thayer and Lane, 2009; Sakaki et al., 2016]. Conversely, let's add from ourselves, activation of prefrontal cortex leads to increase in vagal tone.

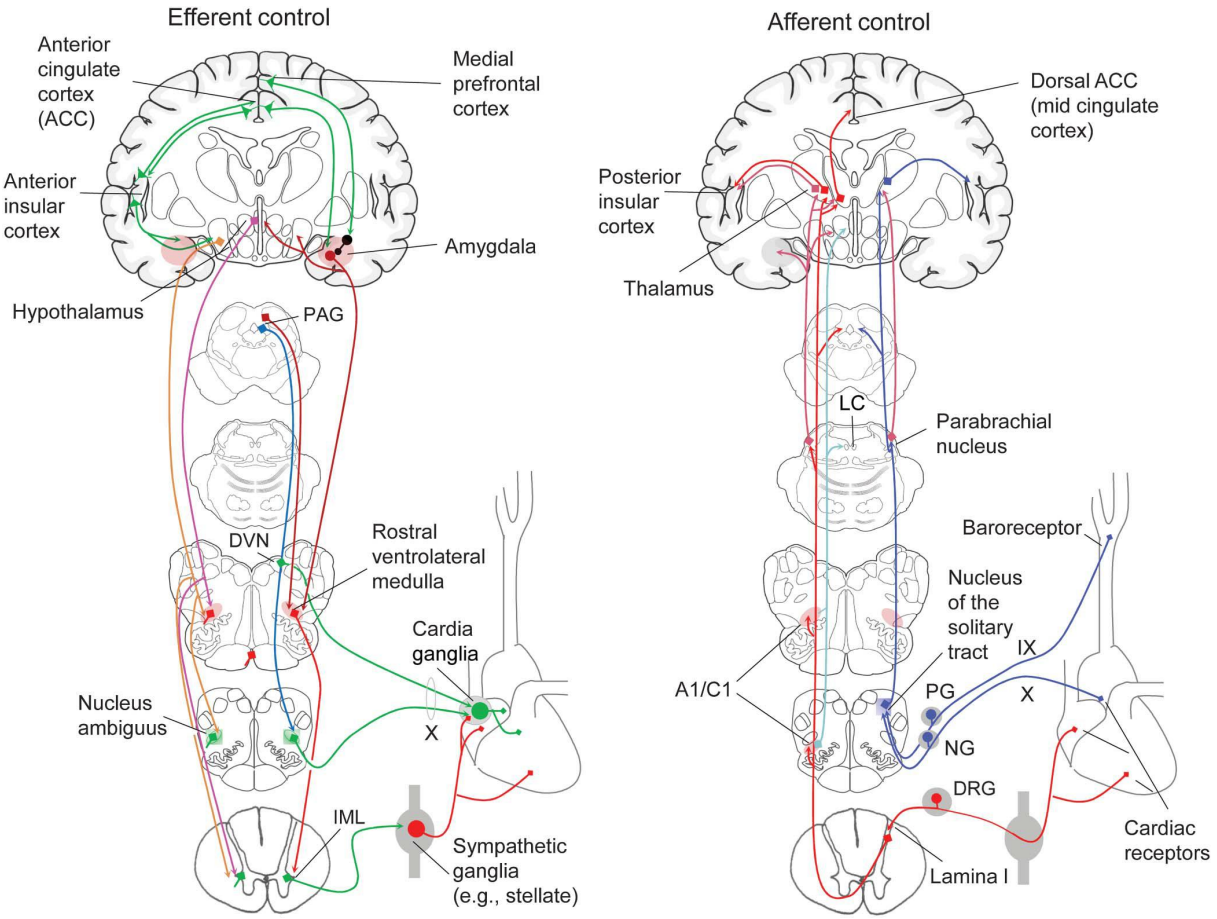


Fig. 13.10. Efferent and afferent control of cardiac function (Palma and Benarroch, 2014)

Mo, Huang, Peng, Ocak, Zhang and Zhang, (2019) presented their CAN visualization (Figs 13.11 and 13.12).

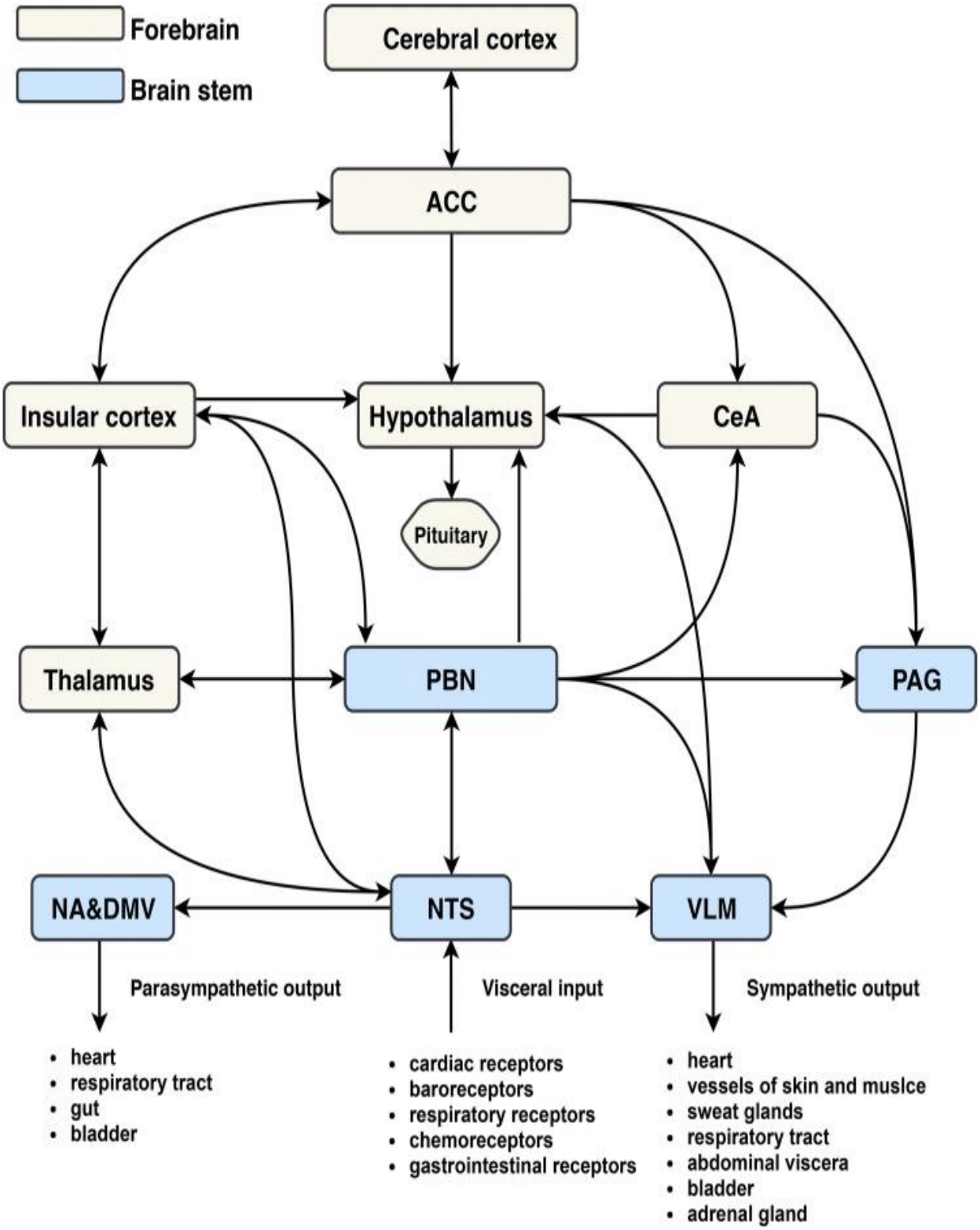


Fig. 13.11. Diagram of the central autonomic network

Visceral information is relayed through the NTS and PBN to forebrain areas such as the hypothalamus, amygdala, thalamus, and insular cortex. The insular cortex has dense reciprocal connections with the ACC, lateral hypothalamic area, NTS, and PBN. These regions are also reciprocally connected. Infarction of the insular cortex may result in the loss of overall autonomic modulation and a decline in parasympathetic tone and baroreflex sensitivity, as well as a shift towards sympathetic dominance. ACC, anterior cingulate cortex; CeA, central amygdala; PBN, parabrachial nucleus; PAG, periaqueductal gray; NA, nucleus ambiguus; DMV, dorsal motor nucleus of the vagus; NTS, nucleus of the solitary tract; VLM, ventrolateral medulla (Mo, Huang, Peng, Ocak, Zhang, Zhang, 2019).

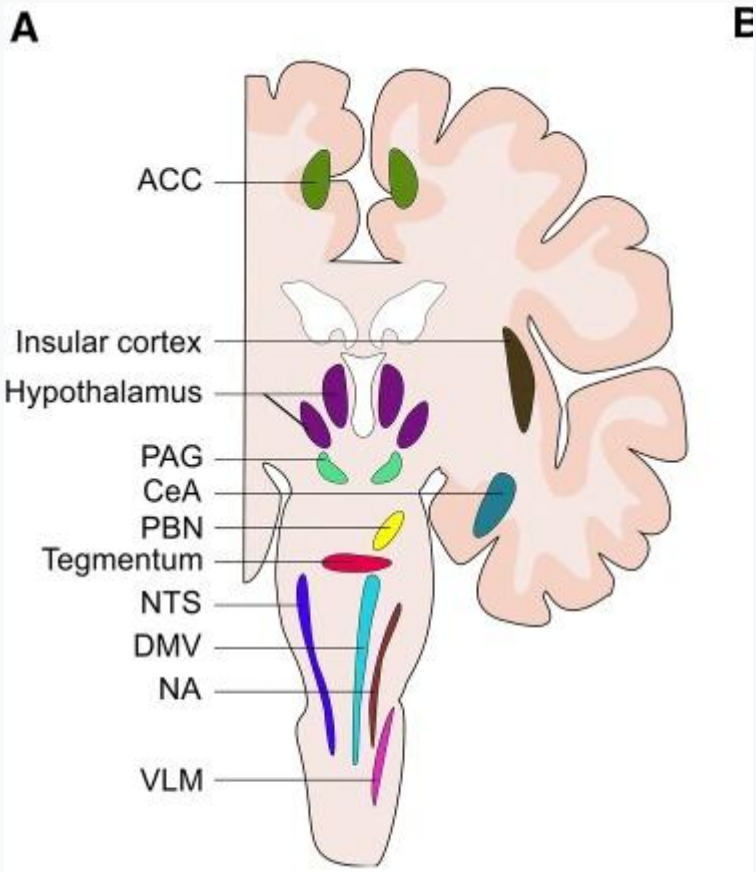


Fig. 13.12. Brain structures of the central autonomic network

ACC, anterior cingulate cortex; PAG, periaqueductal gray; CeA, central amygdala; PBN, parabrachial nucleus; NTS, nucleus of the solitary tract; DMV, dorsal motor nucleus of the vagus; NA, nucleus ambiguus; VLM, ventrolateral medulla (Mo, Huang, Peng, Ocak, Zhang, Zhang, 2019).

The apotheosis of the above we consider Tracy's, (2007) concept of "immunological homunculus".

According to the author, there is a structured, somatotopically organized neural network that controls the specific components of the immune response through the connection of the input and output. Such a theoretical organization is similar to the classic homunculus, which demonstrates that specific areas of the brain control the specific parts of the body, and in the future it will be possible to construct an "immunological homunculus". For example, one region of the brain can control cytokine responses in the liver, and the other - the activation of T cells in the spleen or lymph nodes. Certain centers can integrate information about the presentation of antigens, while others are about the process of maturation of dendritic cells. Separate neurological domains in the central nervous system may regulate the state of general readiness of the innate immunity to respond to pathogens or injury. The existence of neuroanatomical maps of cholinergic anti-inflammatory reflex is a significant step towards the identification of other domains in the immunological homunculus, which is crucial for maximizing body protection and maintaining health during immune responses.

However, in subsequent works of this laboratory (Chavan and Tracey, 2017; Chavan et al., 2017), this hypothesis was not developed. And only in 2018, the mention of him appeared in their review (Pavlov et al., 2018), but, unfortunately, without specification.

We draw attention to the fact that the author's scheme (Fig. 13.13) at the end of each signature is a question mark (?), that is, it is not a statement, but a hypothesis.

Under the influence of this hypothesis we carried out research for its verification (Kul'chyns'kyi et al., 2016; 2017; 2017a; 2017b; Popovych et al., 2017; 2018; Mel'nyk et al., 2019).

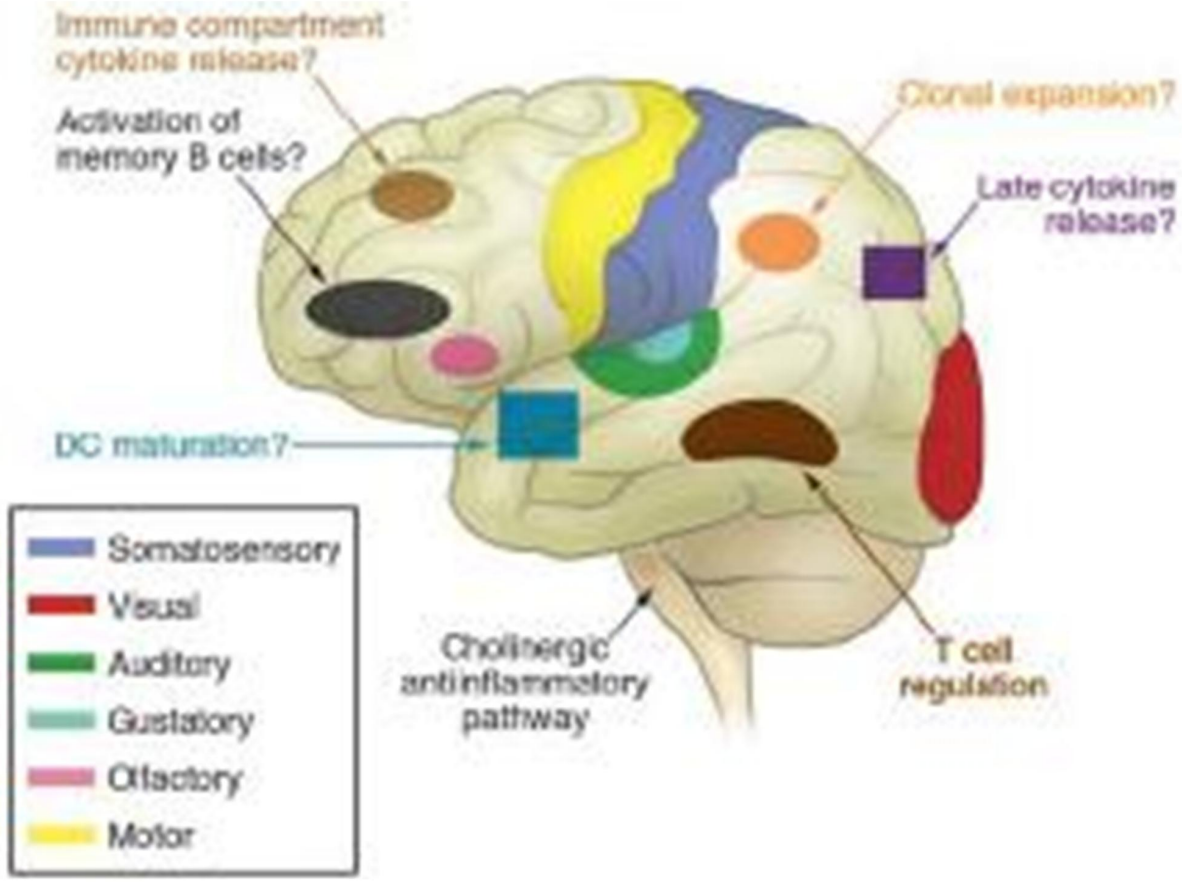


Fig. 13.13. Scheme of immunological homunculus (Tracey, 2007)

If you reformat the parameters of the EEG of persons, whose immune status is sensitive or resistant to chronic stress, within the framework of the Tracey's scheme of immunological homunculus, then they will be organically fit into it (Table 13.1).

Table 13.1. Comparative activity of the nerve structures responsible for various immune responses (Mel'nyk, Lukyanchenko, Gozhenko, Popovych, 2019)

Immune response and EEG Z-score	**Stress-sensitive**	**Stress-resistant**
Tracey's hypothesis		
Immune compartment cytokine release		
F4-δ PSD, μV²/Hz	+0,53±0,46	+1,83±0,77
F4-δ PSD, %	+0,52±0,37	+1,55±0,47
F4-β PSD, %	+0,20±0,24	-0,32±0,21
F3-δ PSD, %	+0,38±0,28	+1,44±0,35
F3-β PSD, %	+0,12±0,21	-0,53±0,12
Clonal expansion		
P4-δ PSD, %	+0,01±0,21	+1,25±0,44
P4-β PSD, %	+0,05±0,19	-0,46±0,13
P3-δ PSD, μV²/Hz	+0,09±0,26	+0,90±0,46
P3-β PSD, %	-0,11±0,16	-0,45±0,12
Dendritic cells maturation		
T3-δ PSD, %	+0,74±0,36	+1,53±0,46
T3-θ PSD, μV²/Hz	-0,16±0,22	+0,40±0,28
T3-β PSD, %	+0,06±0,26	-0,65±0,15
T3-β PSD, μV²/Hz	-0,12±0,19	-0,44±0,06
T4-δ PSD, %	+0,43±0,33	+1,11±0,32
T4-β PSD, %	+0,19±0,24	-0,24±0,14

T cells regulation		
T5-δ PSD, %	+0,38±0,25	+1,04±0,42
T5-β PSD, %	-0,11±0,17	-0,57±0,15
T5-β PSD, $\mu V^2/Hz$	-0,15±0,19	-0,48±0,06
T5 Entropy	+0,69±0,19	-0,06±0,37
T6-δ PSD, %	+0,28±0,23	+0,86±0,32
T6-β PSD, $\mu V^2/Hz$	+0,38±0,44	-0,51±0,09
Activation of memory B cells		
Fp2-δ PSD, %	+0,02±0,21	+0,54±0,26
Our hypothesis		
Activation of Phagocytosis?		
O1-δ PSD, $\mu V^2/Hz$	+0,06±0,25	+1,61±1,16
O2-δ PSD, %	+0,45±0,20	+1,37±0,44

Assuming Tracey, (2007), we can state the following.

First, the nerve structures responsible for one or another immune response are located in both hemispheres, but their activity is uneven.

Secondly, in the same locus there are different neurons, some of which generate immuno-protective δ-rhythm, while other generate immuno-suppressive β-rhythm.

Thirdly, the activity of the nervous structures is significantly different in persons whose immune status is different in response to chronic stress, but rather to stress-induced sympathetic displacement of sympathetic-vagal balance. In particular, the activity of the nerve structures that activate the release of the cytokines, as well as the clonal expansion, is more pronounced on the right, and the activation of memory B cells is carried out exclusively by right-sided structures. Instead, the structures responsible for maturation of dendritic cells, as well as the regulation of T cells, are characterized by left-sided lateralization. The activity of immunosuppressive structures is more pronounced on the left with respect to the effect on the release of cytokines and the maturation of dendritic cells, but is symmetrical in the structures responsible for the clonal expansion and regulation of T cells.

In addition, we want to supplement the concept with our own hypothesis about the existence in O1 and O2 loci of the nerve structures generating δ-rhythm that activate phagocytosis, or more precisely, prevent its depression caused by stress.

The above gives us reason to say that drinking mineral waters are an effective modulator of the neuro-endocrine-immune complex, through which their therapeutic effect is realized.

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MINERAL WATERS, METABOLISM, NEURO-ENDOCRINE-IMMUNE COMPLEX

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The monograph systematizes these writers and highlights the results of their own priority experimental and clinical-physiological studies of the impact of drinking mineral waters of Ukraine on neuroendocrine regulation, metabolism and immunity of healthy rats and patients in the process of rehabilitation of chronic pyelonephritis and cholecystitis in remission. In line with the concepts of functional-metabolic continuum and neuroendocrine-immune complex using the methods of factor, discriminant and canonical correlation analysis, it is demonstrated that mineral waters have both similar and specific physiologically favorable modulating effects on the parameters of the studied body systems.

For specialists in medical rehabilitation, endocrinologists, immunologists, biochemists, pathophysiologists.

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