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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

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Abstract

Background. Earlier in experiments on rats, it was shown that drinking mineral water, regardless of their mineralization and chemical composition, have similar (nonspecific) course effects on a number of parameters of metabolism and neuroendocrine-immune complex. The aim of this study is to identify such parameters in patients of Truskavets' spa who received complex drinking balneotherapy. **Materials and Methods**. The object of clinical-physiological observation were 34 men aged 23-70 years, who underwent rehabilitation treatment of chronic cholecystitis and pyelonephritis in remission. The examination was performed twice, before and after a 7-10-day course of balneotherapy. All patients received bioactive water Naftussya, therewith, 11 men additionally drank sulfate-chloride sodium-magnesium water "Khrystyna" (5 g/L), and the other 11 men - water "Myroslava" with a similar chemical composition, but twice the mineralization. The object of the study were the parameters of the electroencephalogram, heart rate variability (HRV), hormones, metabolism, immunity, microbiota and cholekinetics. **Results**. The complex balneotherapy by interval use of Naftussya water with sulfate-chloride sodium-magnesium mineral waters causes similar significant changes in the constellation of 10 EEGs, 10 metabolic, 10 microbiota and 4 immune parameters as well as total power and entropy of HRV, calcitonin and testosterone plasma levels and cholecystokinetics activity index. **Conclusion**. Balneofactors of Truskavets' spa causes non-specific modulating effects on parameters of neuroendocrine

regulation, metabolism, immunity, microbiota and cholekinetics in patients with chronic pyelonephritis and cholecystitis.

Keywords: drinking mineral waters, Truskavets' spa, EEG, HRV, endocrine, immune, metabolic parameters, non-specific changes.

INRODUCTION

Earlier in experiments on rats, it was shown that drinking mineral water, regardless of their mineralization and chemical composition, have similar (nonspecific) course effects on a number of parameters of metabolism and neuroendocrine-immune complex [1,10,11,23,36]. The aim of this study is to identify such parameters in patients of Truskavets resort who received complex drinking balneotherapy.

MATERIALS AND METHODS

The object of clinical-physiological observation were 34 men aged 23-70 years, who underwent rehabilitation treatment in the Truskavets' spa 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. Patients received bioactive water Naftussya (3 ml/kg one hour before meals three times a day) and in half an hour additionally drank sulfate-chloride sodium-magnesium water "Khrystyna" (5 g/L) or "Myroslava" (10 g/L) [11] in the same dose.

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) [6]. Urinary syndrome was assessed by quantitative and quantitative-qualitative [27] levels of bacteriuria, erythrocyturia and leukocyturia.

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 [6] with the use of analyzers "Reflotron" (BRD) and "Pointe-180" (USA) with corresponding sets of reagents, and flamming photometer "СФ-47".

We determined also content in plasma major hormones of adaptation: Cortisol, Testosterone, Calcitonin and Triiodothyronine (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.). Paying tribute to tradition, we calculated Kerdö's Vegetative Index [33].

In basal conditions we estimated the state of the autonomous regulation by the method heart rate variability (HRV) [2,4,9,34], 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) bands. Time Domain Methods: HR, SDNN, RMSSD, pNN_{50}. Calculated the Shannon's CE entropy (h) of the relative spectral powers (SP) of the HRV bands by the formula Popovych IL [8]:

$$hHRV=- [SPHF \bullet \log_2 SPHF+SPLF \bullet \log_2 SPLF+SPVLF \bullet \log_2 SPVLF+SPULF \bullet \log_2 SPULF]/\log_2 4$$

Simultaneosly with HRV we 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, % = Σ [200•(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 [8]:

$$hEEG = - [PSD\alpha \bullet \log_2 PSD\alpha + PSD\beta \bullet \log_2 PSD\beta + PSD\theta \bullet \log_2 PSD\theta + PSD\delta \bullet \log_2 PSD\delta]/\log_2 4$$

Immune status evaluated as described in the manual [18]. 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 the capillary blood we counted up Leukocytogram and calculated the Entropy (h) of Leukocytogram (LCG) as well as Immunocytogram (ICG) using IL Popovych’s formulas [8]:

$$hLCG = - [L \bullet \log_2 L + M \bullet \log_2 M + E \bullet \log_2 E + SNN \bullet \log_2 SNN + StubN \bullet \log_2 StubN]/\log_2 5$$

$$hICG = - [CD4 \bullet \log_2 CD4 + CD8 \bullet \log_2 CD8 + CD22 \bullet \log_2 CD22 + CD56 \bullet \log_2 CD56]/\log_2 4$$

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [5] with moderately modification by MM Kovbasnyuk [15,31]. 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. 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 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. To quantify cholekinetics activity index, the area between the cholecystovolumogram and the basal line was calculated [19-21].

The condition of microbiota is evaluated on the results of sowing of feces.

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".

RESULTS AND DISCUSSION

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. 1).

Fig. 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. 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.

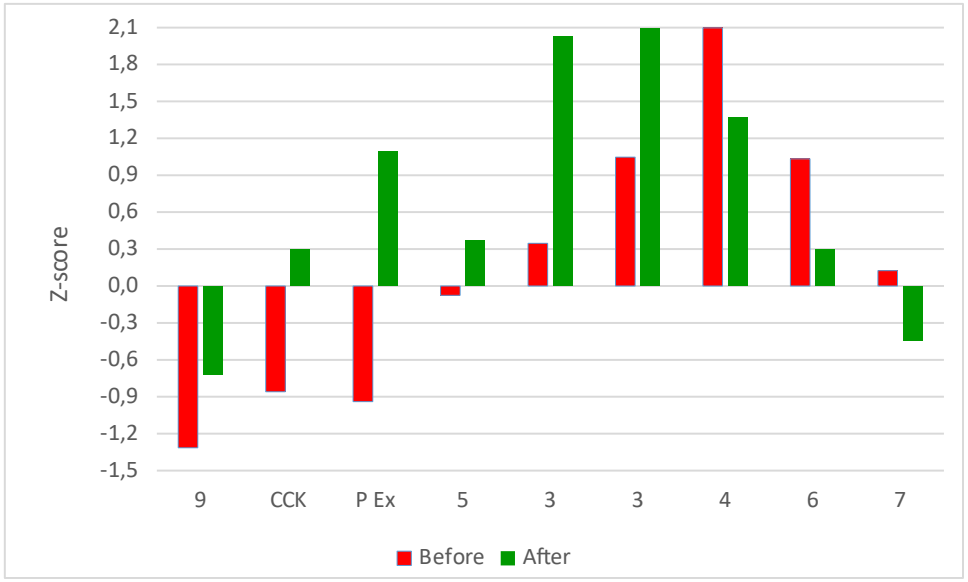


Fig. 2. Patterns of normalized parameters (Z±SD) of neuroendocrine-immune complex, microbiota and metabolism before and after balneotherapy. The number of pattern components is specified

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 Calciumuria, as well as a further increase in initially elevated levels of Diuresis and Urea and Uric acid excretion.

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 [8].

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. 3).

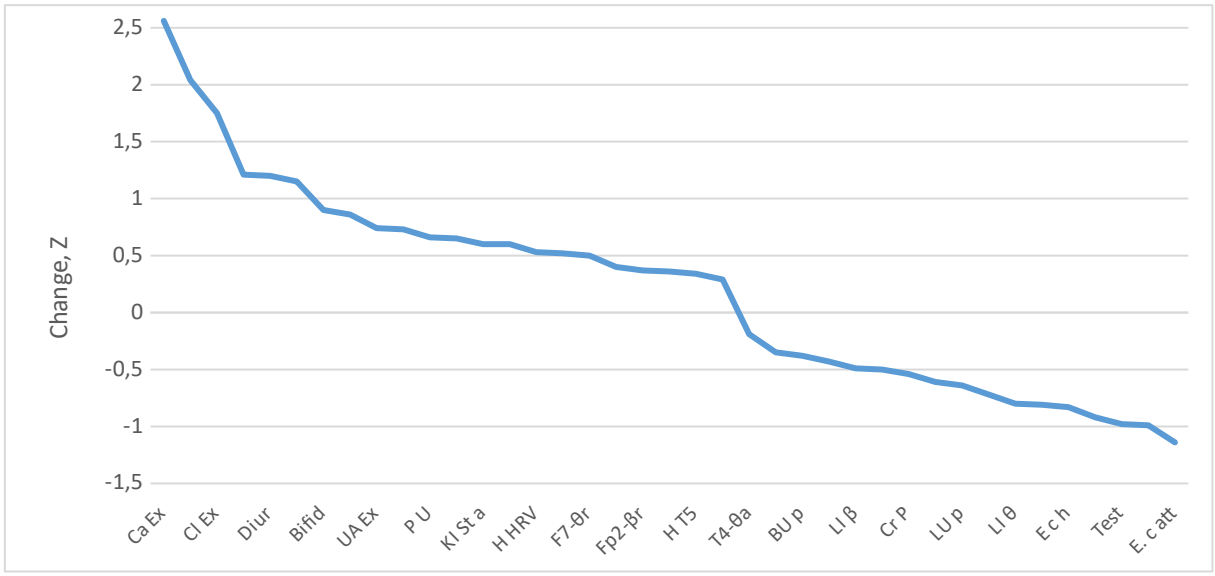


Fig. 3. Nonspecific effects (Z±SE) of balneotherapy on the parameters of neuroendocrine-immune complex, microbiota and metabolism

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 [13] (method forward stepwise), only 22 parameters were included in the model: 9 **neuro-endocrine**, 3 **immune**, 4 **microbiota**, 5 **metabolic**, and **Cholecystokinetic Index** (Tables 1 and 2).

Table 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, $\mu V^2/Hz$	29 7	19 3	*-11* *7*	0,206	0,860	7,30	0,010	0,344	25 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 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, $\mu V^2/Hz$	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 3).

Table 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, lgCFU/mL	1,50 0,17	1,08 0,18	-0,42 0,19	0,175	0,983	0,74	0,394	0,073	0 ***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	10,5	14,0	+3,5	0,177	0,996	0,16	0,688	0,123	18,0

Urine, mM/L	0,7	1,1	1,2						0,294
Calcium Excre-tion, 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 Excre-tion, 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, μV²/Hz	34 7	18 3	-16 7	0,176	0,992	0,35	0,560	0,179	32 2,582
Uric acid Exc-retion, 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 4) allows visualization of each patient in the information space of the root (Figs. 4 and 5).

Table 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, μV²/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; $p<10^{-6}$		

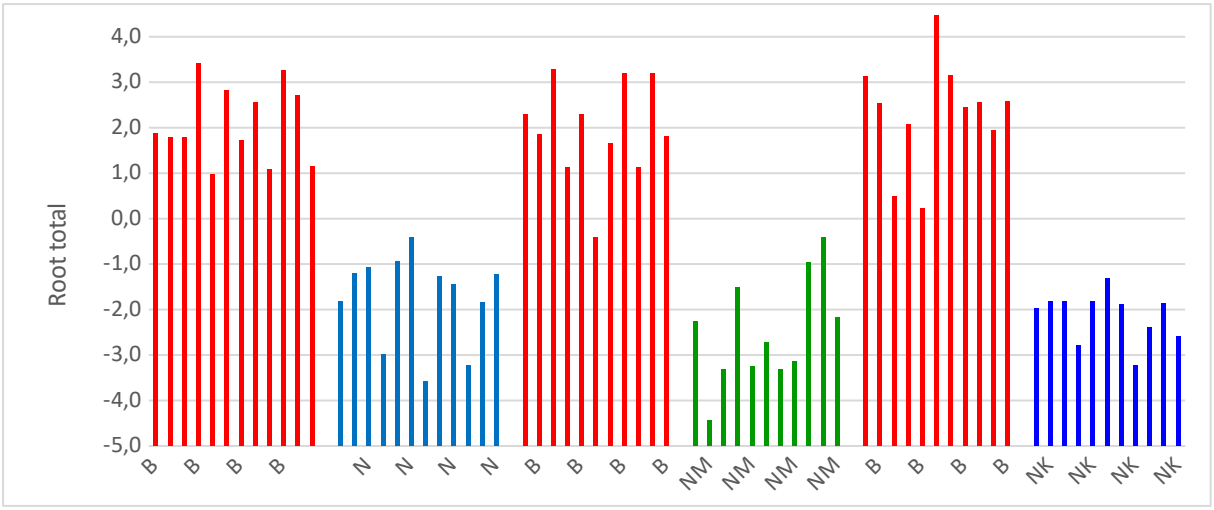


Fig. 4. Sectional values of the discriminant root before (B) and after course drinking of Naftussya only (N), Naftussya and "Myroslava" (NM), Naftussya and "Khrystyna" (NK) waters

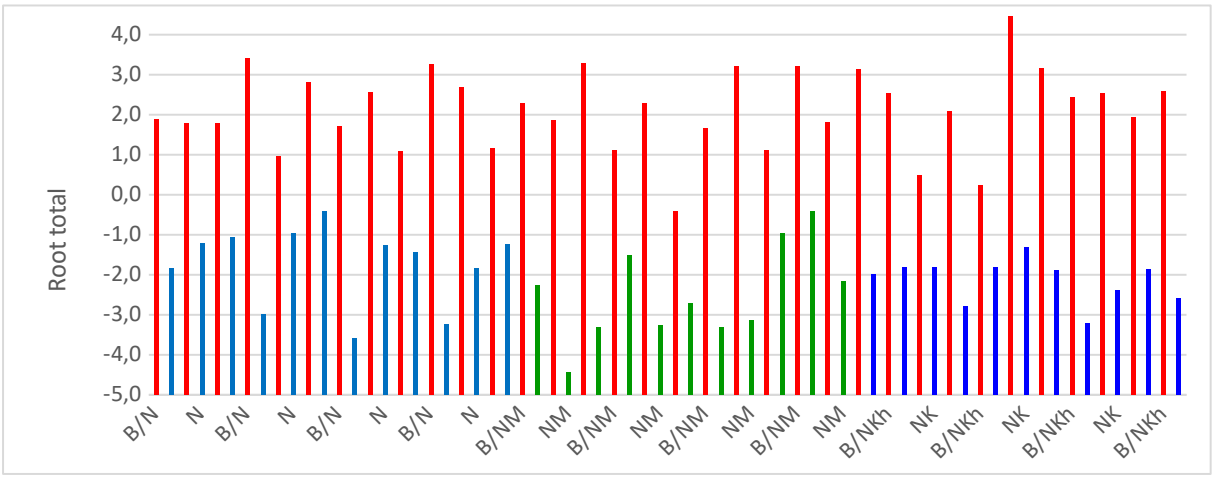


Fig. 5. Individual values of the discriminant root before (B) and after course drinking of Naftussya only (N), Naftussya and "Myroslava" (NM), Naftussya and "Khrystyna" (NKh) waters

Fig. 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. 5 focuses on unidirectional, albeit differently expressed, changes in the integral state in all patients without exception.

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 5).

Table 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. 7.6).

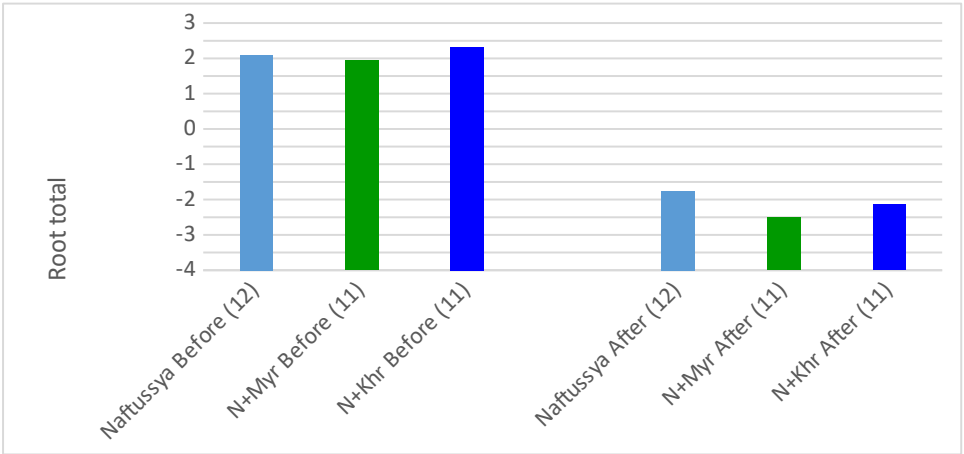


Fig. 6. Average values (Mean±SD) of the discriminant root before and after course drinking of Naftussya only, Naftussya and "Myroslava" (N+Myr), Naftussya and "Khrystyna" (N+Khr) waters

An additional criterion for a clear distinction between the integrated states of patients before and after balneotherapy is the 98,5% accuracy (the only error for 68 cases) of classification based on coefficients and constants for classification functions (Table 6).

Table 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 [3,8,26,28].

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 [14-17,22,24,26,28-32,35], which are based on the concepts of neuro-endocrine-immune complex [8,24-26,28] and functional-metabolic continuum [7].

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 7).

Table 7. Matrix of correlations between changes in neuro-endocrine and immune-microbiota-metabolic parameters

N=34	CT	Test	HHR	LIB	LIT	HFp2	Fp2B	Fp2Ta	F7fl	T4E	HT5
LgBU	0,28	-0,1	-0,0	0,0	-0,0	0,27	0,08	-0,1	0,04	0,06	0,26
LgLU	0,2	-0,1	-0,4	0,0	0,1	0,26	-0,1	0,02	-0,0	-0,0	0,06
LgBifidobacter	-0,2	0,0	0,3	0,1	0,3	-0,2	-0,0	0,09	-0,3	-0,4	-0,2
LgLactobacillus	-0,2	0,0	0,4	0,1	0,3	-0,3	-0,1	0,11	-0,3	-0,4	-0,2
E. oliatep%	0,29	-0,1	-0,4	0,0	0,0	0,15	-0,0	-0,1	-0,1	0,0	-0,0
E. olihep%	0,34	-0,2	-0,0	0,1	0,1	0,20	-0,1	0,11	-0,0	0,04	0,09
Kleb&Proteus%	0,20	0,1	-0,4	-0,0	-0,1	0,17	0,11	-0,1	0,00	0,23	-0,0
Lg E. coliices	-0,2	-0,1	0,57	0,0	0,0	-0,0	-0,0	0,26	0,20	-0,0	0,03
Killingvs St. a	-0,2	0,17	0,1	-0,1	-0,0	-0,1	0,07	0,03	-0,1	0,00	-0,1
Killingvs E. co	-0,4	0,1	0,3	-0,1	-0,1	-0,1	0,14	0,03	0,07	0,11	-0,0
lgG	0,00	-0,2	0,0	0,1	0,06	-0,1	-0,2	0,12	-0,0	-0,0	-0,2
CrP	-0,1	-0,0	0,0	-0,3	-0,0	0,03	0,00	-0,0	-0,0	0,02	0,05
Diuresis	-0,0	0,00	0,1	-0,2	-0,4	-0,0	0,26	-0,2	0,04	0,34	-0,1
NaU	-0,4	0,14	0,09	-0,3	0,06	-0,3	-0,1	-0,0	-0,3	-0,1	-0,2
Cr Exc	-0,2	-0,2	0,1	-0,4	-0,3	0,0	0,18	-0,1	0,00	0,16	-0,0
UA Exc	0,17	-0,1	-0,0	-0,1	-0,2	0,04	0,13	-0,1	0,03	0,17	0,08
Ca Exc	0,07	-0,1	-0,0	-0,0	-0,0	0,23	0,26	-0,0	0,2	0,32	0,24
P Exc	0,07	-0,3	0,06	0,0	0,08	0,07	-0,0	-0,1	-0,0	0,02	-0,0
Cl Exc	-0,2	0,09	0,0	-0,3	-0,1	-0,1	0,0	0,04	-0,0	-0,0	-0,0
Cholekinetics	0,29	-0,2	0,02	0,1	-0,1	0,13	0,33	-0,2	0,05	0,26	0,05
UreaExc	-0,1	-0,0	-0,2	-0,3	-0,2	0,11	0,21	-0,0	0,06	0,23	0,00

Note. According to the formula:

$|r| \geq \{exp[2t/(n-1,5)^{0,5}] - 1\}/\{exp[2t/(n-1,5)^{0,5}] + 1\}$,

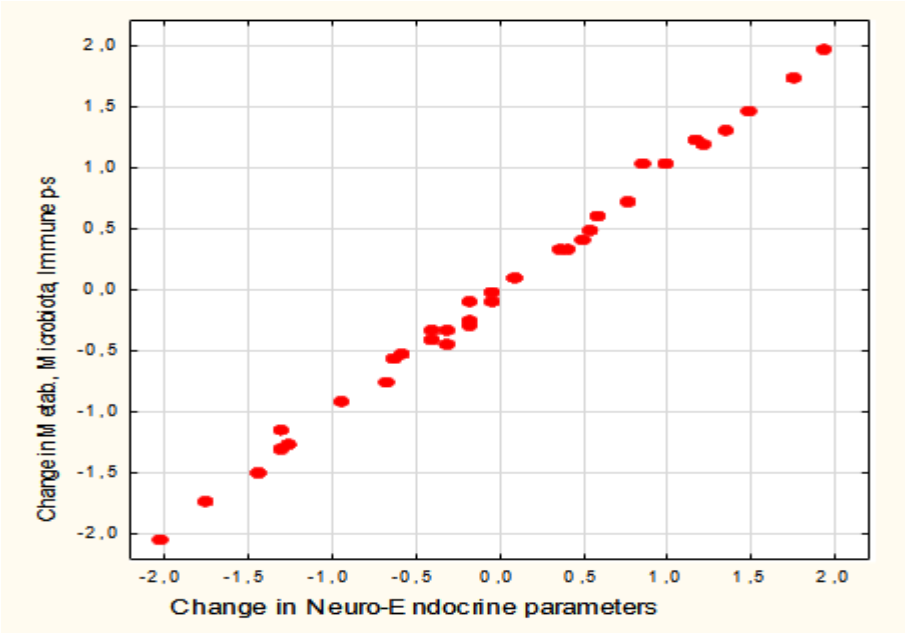
for a sample of 34 observations critical value of correlation coefficient module at $p<0,05$ ($t>2,04$) is 0,340, at $p<0,02$ ($t>2,46$) is 0,400, at $p<0,01$ ($t>2,75$) is 0,441, at $p<0,001$ ($t>3,64$) is 0,554.

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 8 and Fig. 7).

Table 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



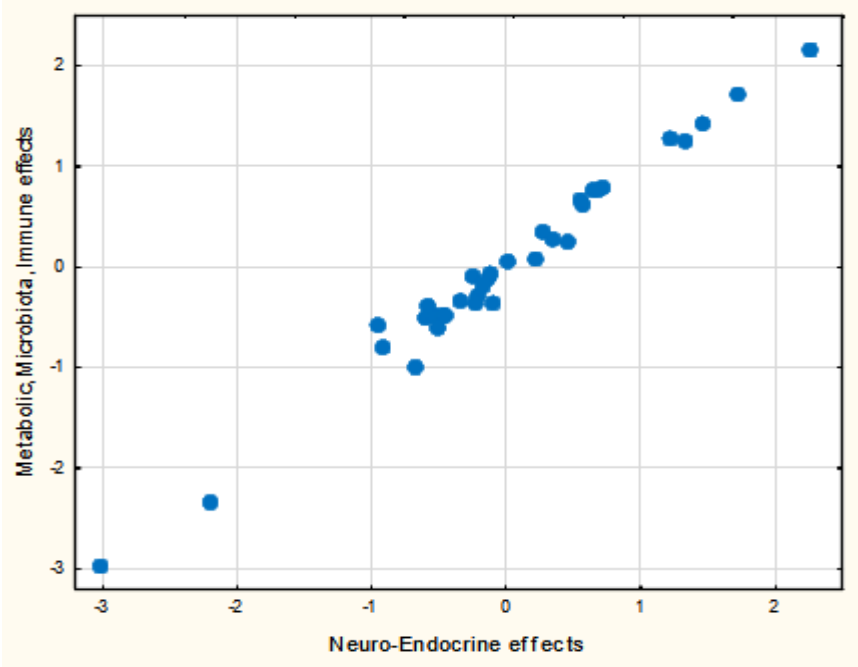
R=0,998; R^2=0,996; $\chi^2_{(231)}$=330; p<10^{-4}; Λ Prime<10^{-6}

Fig. 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 9). However, the degree of neuroendocrine determination of cholekinetics, metabolism and microbiota remains the same (Fig. 8).

Table 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; R^2=0,982; $\chi^2_{(200)}$=240; p=0,027; Λ Prime<10^{-6}

Fig. 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 article.

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ACCORDANCE TO ETHICS STANDARDS

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.

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