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NEUROENDOCRINE-IMMUNE AND METABOLIC ACCOMPANIMENTS OF CHOLECYSTOKINETIC EFFECTS OF BALNEOTHERAPY ON SPA TRUSKAVETS'

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SUMMARY

Objective. Ability balneofactors spa Truskavets' (Ukraine), including bioactive water Naftussya, affect Gall-bladder motility has long been known. We set a goal to identify changes related neuroendocrine-immune complex and metabolism accompanying cholecystokinetic effect of balneotherapy on spa. **Results.** In observation of 22 men with chronic cholecystitis in combination with pyelonephritis, found that 10-12-day course of balneotherapy (drinking of bioactive water Naftussya, application of ozokerite, mineral baths) reduces Gall-bladder volume fasting by 16% ($p< 0,001$) and increases its contractile response to 40% solution of xylitol by 44% ($p<0,001$). This is accompanied by noticeable modulation of neuroendocrine-immune complex. In particular, decreases as the absolute and relative power spectral density (PSD) θ- and α-rhythms ($p<0,05÷0,01$) and increased relative PSD β-rhythm ($p<0,05÷0,02$) ongoing EEG. However, reduced heart rate variability indices: (VLF+LF)/HF by 34% ($p<0,05$) and LF/HF by 32% ($p>0,05$), by increasing PSD HF greater extent than LF. The basal levels of plasma cortisol decreased by 20% ($p<0,01$), testosterone by 15% ($p=0,01$), whereas increased levels of triiodothyronine (by 4%, $p<0,05$) and especially of calcitonin activity (92%, $p<0,001$)), calculated by urinary excretion of phosphates and calcium, which increases according by 103% ($p<0,001$) and 76% ($p<0,01$). It notes that higher urinary excretion of oxalates by 52% ($p<0,001$), creatinine by 32% ($p<0,02$), uric acid by 29% ($p<0,001$), urea by 21% ($p<0,01$), magnesium by 19% ($p=0,05$), with no significant changes in urinary excretion of chloride, potassium and sodium. This reduces the plasma level of creatinine by 14% ($p<0,001$) and urea by 11% ($p<0,01$). With respect to the parameters neutrophil phagocytic function stated increasing of index killing them Staphylococcus aureus by 19% ($p<0,001$) and Escherichia coli by 18% ($p<0,01$), coupled with a decrease in the number of microbes absorbed one phagocyte by 4% ($p>0,2$) and 8% ($p<0,05$) respectively. Regarding immunity parameters revealed significant increase in blood $CD16^+$ lymphocytes only (+17%, $p<0,01$) in the absence of changes in levels of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells and $CD19^+$ B lymphocytes. Do not change significantly either serum Igg G, M, A, or circulating immune complexes. Finally, stated a slight but significant increase electrokinetic index cell nuclei of buccal epithelium, indicating the "rejuvenation" of the body. **Conclusion**: balneotherapy on spa Truskavets' be significantly cholecystokinetic effect, combined with the activation excretory and depurative kidney functions and neutrophil bactericidal function against a background of lower levels of neuroendocrine markers of stress.

Keywords: Truskavets', balneotherapy, cholekinetic, diuresis, neuroendocrine-immune complex.

INTRODUCTION

Ability balneofactors spa Truskavets', including bioactive water Naftussya, affect Gall-bladder motility through the autonomic nervous gastroentero-pancreatic neuroendocrine system, has long been known [5,8,13]. At one time it was felt that it choleretic and cholecystokinetic effects play a key role in the mechanism sanogenesis chronic cholecystitis [8,13,14,32,33]. But according to modern conception IL Popovyc [4,22,24,25,26], essence therapeutic and prophylactic effect bioactive water Naftussya is activation adaptative and protective systems of the body by modulation state of neuroendocrine-immune complex [23] present in the water autochthonous microbes and organic matter oil (in Greek: naphta) and microbial origin. Under this concept, microbial-xenobiotic in nature and streslimiting adaptogenic essentially, **worship** for the inhabitants (unfortunately, for a significant proportion of doctors also) diuretic, cholekinetic and anti-inflammatory effects of balneotherapy is only in relation to **derivatives or concomitant major** streslimiting and immunomodulatory effects. Hence the hypothesis that the mechanism sanogenesis chronic cholecystitis and pyelonephritis (most common in patients nosology Truskavets resort) is not limited to banal "***washout of biliary and urinary tract pus and bacteria increased flow of bile and urine***" [cit. by: 22] and it involved **bactericidal** factors. In turn, both cholekinetic and bactericidity are objects neuroendocrine regulatory influences [13,17,18,25].

MATERIAL AND METHODS

The object of observation were 22 men aged 24-70 (mean 49,1±2,5) years, who arrived in Truskavets (Ukraine) for the treatment of chronic cholecystitis combined with pyelonephritis in remission. The main subject of study was Gall-bladder motility evaluated by echoscopy (echokamera company "Radmir") by Gall-bladder volume morning on an empty stomach and after 5, 15 and 30 minutes after drinking cholekinetic (50 ml of 40% solution of xylitol).

In addition, background electroencephalogram was recorded in 16 monopolar leads (hardware-software complex "NeuroCom Standard", company "KhAI-MEDICA", Kharkiv) and electrocardiogram in II standard lead to estimate parameters of heart rate variability (hardware-software complex "CardioLab+HRV" (company "KhAI-MEDICA", Kharkiv) [1,28,29].

On the cubital vein blood sample taken for testing in its plasma main adaptive hormones: cortisol, testosterone and triiodothyronine (by ELISA using the analyzer "Tecan", Oesterreich and kits for company "Alkor Bio", St. Petersburg, Russia).

In the same portions of plasma were tested for creatinine, urea and uric acid. These nitrogen metabolites and oxalates, phosphates, chloride, calcium, magnesium, potassium and sodium were determined in urine collected during the day. Used unified methods [cit. by: 11].

About phagocytic function of neutrophils judged by activity (percentage of neutrophils, which are found in bacteria), intensity (number of microbes, absorbed by one phagocyte) and completeness (percentage of dead germs - index of Killing) phagocytosis museum cultures Staphylococcus aureus (ATCC N 25 423 F49) and Escherichia coli (O55 K59) [9].

To assess the immune status conducted phenotyping $CD3^+$-, $CD4^+$-, $CD8^+$-, $CD16^+$- and $CD19^+$-lymphocytes blood (by indirect Immunofluorescent binding reaction monoclonal antibodies [20] company "Sorbent", Moscow reg.) and were tested serum immunoglobulins classes G, A, M (method of one-dimensional radial immunodiffusion in agar gel) and circulating immune complexes (by precipitation with polyethylene glycol) [cit. by: 19].

Finally, we tested the electrokinetic index of cell nuclei of buccal epithelium (by microelectrophoresis device "Biotest", Kharkiv University), which is considered a marker of biological age [31].

After examination, patients received standard balneotherapy [27] (drinking water Naftussya 3 ml/kg three times a day, ozokerite applications on the right upper quadrant, t^0 45^{0}C, lasting 20-30 minutes, every other day; mineral baths, concentration of Cl^--$SO_4^{2-}$$Na^+$-$Mg^{2+}$ salt 20-30 g/l, t^0 36-37^{0}C, duration 8-10 minutes, every other day) and 10-12 days retest was conducted.

Results processed by direct difference with calculation of average and standard error, as well as by discriminant analysis [15] using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Back in 1968, EZ Polyak [21] by serial Röntgenographs showed that in healthy subjects after administration of 20 ml classic cholekinetic egg yolk Gall-bladder volume after 90 minutes reduced to 34% original. A similar cholecystokinetic effect has 40% solution of xylitol: 33,3±2,7% [16]. W Hopman et al. [12] in applying the method of dynamic ultrasonography confirmed that the normal 70 minutes after a standard breakfast of mixed food Gall-bladder volume is reduced to 30-40% original. So cholecystokinetic effects of egg yolk, 40% solution of xylitol and mixed meal almost identical.

AYa Bul'ba [8] demonstrated that postprandial Gall-bladder volume at the reference 90th minute can be provided by its volume at the 30th minute. In particular, normal volumes are respectively 32-37% and 60-70%, during hyperkinesia 12-20% and 44-52%, during hypokinesia 55-75% 69-76% original. This gave us reason to reduce the duration of registration cholecystovolumogram to 30 min (Table 1).

Based on averages, Gall-bladder motility at admission to treatment was in the lower zone norm. On the effect of balneotherapy tried both direct performance differences and changes in % from the original. It can be seen that the course of balneotherapy, first, reduces the amount of Gall-bladder volume fasting, and secondly, it increases the contraction in response to cholekinetic to the upper zone norm, even to the level hyperkinesia.

For the purpose of integrated assessment cholecystokinetic reaction calculated area between the basal level (100%) and cholecystovolumogram (Fig. 1). Revealed that cholecystokinetic reaction increases by an average from 493%•min to 707%•min, ie by 44%.

Table 1. Parameters of Cholecystovolumegrams

Gall-bladder Volumes	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
Initial, ml	50,7±3,3	42,8±2,4	-7,9±1,3	-16	6,08	<0,001
5 min Postprandial, ml	49,4±3,3	40,9±2,3	-8,5±1,2	-17	7,08	<0,001
15 min Postprandial, ml	41,7±3,2	32,0±2,0	-9,8±1,3	-23	7,54	<0,001
30 min Postprandial, ml	35,5±3,0	23,0±1,5	-12,5±1,7	-31	7,35	<0,001
5 min Postprandial, %	97,2±0,4	95,5±0,4	-1,8±0,4	-2	4,50	<0,001
15 min Postprandial, %	81,3±1,5	74,2±1,3	-7,1±1,2	-9	5,92	<0,001
30 min Postprandial, %	68,3±2,0	53,2±0,8	-15,2±1,7	-22	8,94	<0,001

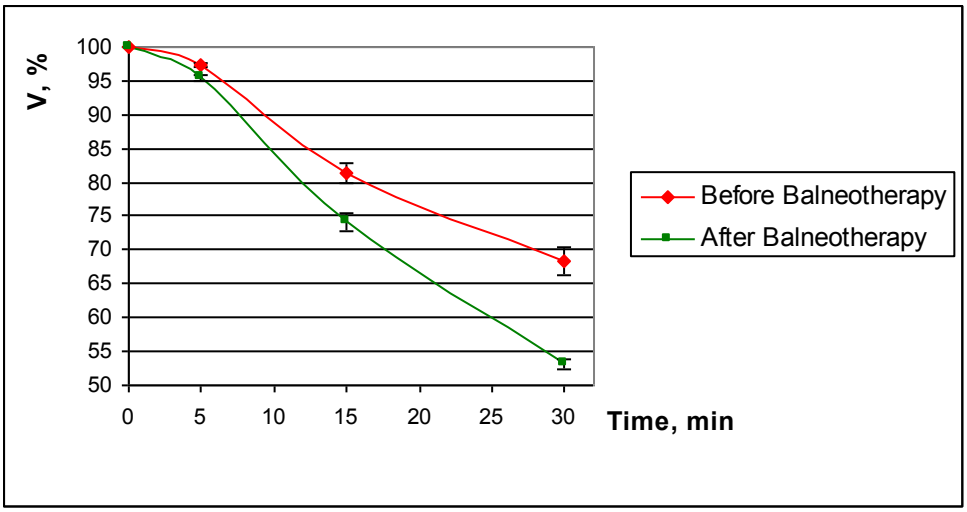

Fig. 1. Cholecystovolumegrams before and after Balneotherapy on spa Truskavets'

Consequently, the course balneotherapy increases lowered Gall-bladder fasting tone and increases its postprandial contractile response.

How varies with bioelectrical brain activity? We **first** found that the most notable changes in the absolute power spectral density (PSD) stated regarding θ-rhythm in the left prefrontal locus (Table 2). Much smaller but statistically significant reduction PSD stated in relation θ-rhythm in two other loci, and α-rhythm in the right loci.

In contrast to the clearly negative dynamics of **absolute** PSD, pattern **relative** PSD (Table 3) dual: increased β-rhythm and decreased θ- and α-rhythm, and only in the left-hand loci.

Earlier on this cohort of patients, we found the relationships between the parameters of EEG and heart rate variability (HRV) [28]. It is therefore quite expected to reduce them centralization index (VLF + LF)/HF from 141% to 93% rule (6,87±0,81) and sympathetic-vagal balance index LF/HF from 200% to 136% rule (1,96±0,13), by increasing PSD HF more than LF (Table 4).

Vagotonic shift of tone autonomic regulation combined with a reduction in plasma cortisol from 171% to 137% of normal (405±23 nM/l) and testosterone from 120% to 102% rule (25,2±1,2 nM/l). Instead triiodothyronine level increased slightly in the normal range (1,90±0,08 nM/l) (Table 5).

Mineralocorticoid activity (MCA), evaluated the ratio of content in urine potassium and sodium, showed only a tendency to increase. Instead calcitonin activity (CTA), measured by the ratio of content in urine calcium and phosphates, increased drastical from 91% to 177% rule (10,5±0,5 units).

Table 2. Accompanied changes in EEG Rhythms absolute Power Spectrum Density

EEG Rhythms PSD, $\mu V^2/Hz$	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
Fp1-θ	34±10	10±3	-24±10	-71	2,40	<0,02
C3-θ	61±15	35±8	-26±12	-43	2,17	<0,05
F8-θ	16±3	10±3	-6,1±2,8	-38	2,18	<0,05
F8-α	42±7	28±6	-14±5	-33	2,60	<0,02
Fp1-α	101±15	72±12	-29±9	-29	3,22	<0,01
Fp2-α	103±16	77±13	-26±9	-25	2,89	<0,01
T5-β	76±13	54±9	-19±7	-25	2,76	<0,01
P4-α	319±59	248±32	-71±34	-22	2,09	<0,05
F4-α	135±18	116±17	-19±8	-14	2,38	<0,05

Table 3. Accompanied changes in EEG Rhythms relative Power Spectrum Density

EEG Rhythms PSD, %	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
T6-β	28,9±4,2	40,1±5,4	+11,2±5,7	+51	1,98	>0,05
T4-β	28,6±3,0	39,1±4,3	+10,5±4,4	+48	2,39	<0,02
F8-β	29,5±4,4	43,2±4,9	+13,7±5,7	+41	2,40	<0,02
C4-β	22,9±1,9	31,0±3,0	+5,6±2,5	+35	2,24	<0,05
P4-β	20,6±2,5	25,1±3,2	4,5±2,3	+36	1,98	>0,05
T4-θ	10,7±1,7	6,2±0,9	-4,5±2,0	-37	2,25	<0,05
T6-θ	8,0±1,1	5,6±1,0	-2,4±1,1	-30	2,18	<0,05
C4-θ	12,3±1,6	8,9±1,0	-3,4±1,6	-28	2,13	<0,05
P4-α	56,6±3,7	48,9±3,1	-7,7±3,3	-19	2,33	<0,05

Table 4. Accompanied changes in Heart Rate Variability Power Spectrum Density and Indices

HRV PSD, $msec^2$	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
VLF	1192±288	1288±153	+96±214	+8	0,45	>0,5
LF	791±177	988±169	+197±153	+25	1,29	>0,10
HF	552±173	717±174	+165±190	+30	0,87	>0,2
Indices, units						
(VLF+LF)/HF	9,68±1,23	6,41±0,96	-3,27±1,52	-34	2,15	<0,05
LF/HF	3,92±0,83	2,66±0,46	-1,26±0,70	-32	1,80	>0,05

Table 5. Accompanied changes in Endocrine parameters

Hormone level or activity	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
Cortisol, nM/l	693±68	556±53	-137±47	-20	2,91	<0,01
Testosterone, nM/l	30,3±2,0	25,8±1,8	-4,5±1,7	-15	2,64	=0,01
MCA=$(Ku/Nau)^{0,5}$, units	0,58±0,03	0,63±0,03	+0,05±0,05	+8	1,00	>0,2
Triiodthyronin, nM/l	1,93±0,03	2,01±0,03	+0,07±0,03	+4	2,33	<0,05
CTA=$(Pu•Cau)^{0,5}$, units	9,6±0,7	18,4±1,6	+8,8±1,9	+92	4,63	<0,001

It is well known that calcitonin prevents stress ulceration of the stomach, so we treat it together with aldosterone, triiodothyronine and vagal tone, as streslimiting factor, as opposed stresrealisating factors cortisol and sympathetic tone [1,2,3,10,17,30]. Thus, the cholecystokinetic effect of balneotherapy combined with its streslimiting effect, which is expected in line with the concept IL Popovych [25].

Also quite expected increase daily urine output (Table 6) accompanied by increased excretion of phosphates (mainly $H_2PO_4^-$ because urine pH about 5), calcium, magnesium, oxalates and nitrogen metabolites: creatinine, uric acid and urea ("slag"). Instead, levels creatinine and urea in plasma decreases, which together shows for well-known depurative effect of balneotherapy. However, we have not confirmed previous data [6,14,32,33] an increase in the excretion of chloride, potassium and sodium.

Table 6. Accompanied changes in Diurese and Excretion Electrolytes and Nitrous metabolites

Diurese and Excretion with Urina in 24 h	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
Diurese, l	2,06±0,11	2,63±0,07	+0,57±0,10	+28	5,70	<0,001
Phosphates, mM	20,9±1,4	42,5±4,0	+21,6±4,2	+103	5,14	<0,001
Calcium, mM	4,70±0,52	8,27±0,78	+3,57±1,09	+76	3,28	<0,01
Oxalates, μM	145±14	220±12	+75±12	+52	6,25	<0,001
Creatinin, mM	6,41±0,60	8,43±0,94	+2,02±0,82	+32	2,46	<0,02
Uric acid, mM	3,73±0,32	4,80±0,32	+1,07±0,28	+29	3,82	<0,001
Urea, mM	600±40	726±38	+126±43	+21	2,93	<0,01
Magnesium, mM	5,03±0,29	6,00±0,43	+0,97±0,48	+19	2,02	=0,05
Chloride, mM	208±15	220±19	+12±22	+6	0,55	>0,5
Potassium, mM	85±10	85±8	0±14	0	0,03	-
Sodium, mM	257±22	229±19	-28±27	-11	1,04	>0,2
Plasma level						
Creatinin, μM/l	101±3	88±2	-13,2±1,4	-14	9,4	<0,001
Urea, mM/l	6,92±0,27	6,27±0,26	-0,65±0,20	-9	3,10	<0,01
Uric acid, μM/l	354±23	349±20	-5±16	-1	0,31	>0,5

Analysis parameters of the phagocytic function of neutrophils indicates (Table 7) that in respect of Staphylococcus aureus initially normal phagocytosis activity (98,3±0,2%) and intensity (61,6±1,0 Microbs/Phagocyte) remain unchanged. Instead reduced killing index completely normalized, increasing from 82% to 98% rule (58,9±0,8%).

For the purpose of integrated assessment phagocytic function of neutrophils counted the number of microbes, which are able to kill neutrophils contained in 1 liter of blood (bactericidal index) by the formula [27]:

Bactericidity = Leukocytes•Neutrophiles•Phagocytose Index•Microbian Number•Killing Index/10^4

Table 7. Accompanied changes in parameters of Phagocytary Function of Neutrophiles

Parameters of Phagocytose	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
Leukocytes blood level, 10^9/l	6,16±0,28	6,09±0,33	-0,07±0,30	-1	0,23	-
Neutrophiles content, %	52,3±1,1	53,3±1,5	+1,0±1,5	+2	0,67	>0,5
Against Staphylococ. aureus						
Phagocytose Index, %	98,2±0,3	98,1±0,3	-0,1±0,2	0	0,50	>0,5
Microbial Count, Micr/Phagoc	61,5±1,2	59,1±2,6	-2,4±2,4	-4	1,00	>0,2
Killing Index, %	48,4±1,1	57,6±1,5	+9,2±1,8	+19	5,11	<0,001
Bactericidity, 10^9 Bacterias/l	92±5	106±8	+14±8	+15	1,75	>0,05
Against Escherichia coli						
Phagocytose Index, %	98,6±0,2	98,3±0,4	-0,3±0,3	0	1,00	>0,2
Microb Count, Micr/Phagocyte	62,9±1,0	57,7±2,0	-5,2±2,2	-8	2,36	<0,05
Killing Index, %	45,6±1,3	53,8±1,8	+8,2±2,5	+18	3,28	<0,01
Bactericidity, 10^9 Bacterias/l	85±5	94±6	+9±6	+11	1,50	>0,10

It turned out that balneotherapy enhances bactericidal index regarding Staphylococcus aureus from 78% to 90% of normal (118±7 10^9 Bacterias/l). These data confirming previous observations in for children [24] and adults [17] as well as in experiments on rats [4].

A somewhat different situation with respect bactericidal Escherichia coli, evaluated for the **first time**. A similar measure initial deficit of 77% of normal (110±6 10^9 Bacterias/l) was caused greater violation of completion of phagocytosis - 74% rule (62,0±0,9%), which was offset by an increase in the number of microbes absorbed up to 115% of normal (54,7±1,0 Microbs/Phagocyte), again at a normal phagocytic activity (98,3±0,1%). Under the influence of balneotherapy bactericidal increased to 85% of normal, while Killing index rose to 87% of normal, and microbial count decreases to 105% rule.

So, balneotherapy normalize neutrophil bactericidal regarding Staphylococcus aureus (perhaps, other gram-positive bacteria) more than regarding Escherichia coli (perhaps, other gram-negative bacteria). Unfortunately, the various strains of Escherichia coli is the most common pathogens of chronic cholecystitis and pyelonephritis.

Change parameters of humoral immunity (Table 8) had character tendencies only. In particular, the excess IgG further increased from 120% to 133% rule (11,5±0,4 g/l) and IgM from 115% to 123% rule (1,15±0,05 g/l). Given the ability of these antibodies opsonization showed a trend of dynamics probably plays a role in increasing neutrophil bactericidal. The level of circulating immune complexes continued to decline from 72% to 59% of normal (54±5 units), probably due to activation of macrophages absorption. However, 23% deficiency of IgA (norm 1,90±0,06 g/l) remained without solution.

Table 8. Accompanied changes in parameters of Immunity

Lymphocytes blood level	Before Balneotherapy (n=22)	After Balneotherapy (n=22)	change absolute	change in %	t	p
Total, 10^9/l	1,91±0,08	1,92±0,10	+0,01±0,05	0	0,20	-
$CD16^+$ NK, %	9,9±0,4	11,6±0,6	+1,7±0,6	+17	2,83	<0,01
$CD3^+CD4^+$ T, %	24,7±0,7	25,7±0,6	+1,0±0,8	+4	1,25	>0,10
$CD3^+CD8^+$ T, %	25,0±0,9	25,9±0,6	+0,9±1,0	+4	0,90	>0,10
$CD19^+$ B, %	23,6±0,5	24,4±0,4	+0,8±0,6	+3	1,33	>0,10
Serum level						
IgG, g/l	13,8±0,9	15,3±0,9	+1,5±1,2	+11	1,25	>0,10
IgM, g/l	1,32±0,06	1,42±0,07	+0,10±0,11	+8	0,91	>0,2
IgA, g/l	1,46±0,11	1,47±0,09	+0,01±0,14	+1	0,07	-
CIC, units	39±4	32±4	-7±6	-18	1,16	>0,10

Among the registered parameters of cellular immunity significant changes were found only in relation to the content of natural killer cells, which increased from 60% to 71% rule (16,4±0,8%). Instead, as a 15% deficit of T-helpers (normal 29,1±1,0%), and 9% surplus of B-lymphocytes (normal 21,7±0,8%) did not significantly respond to balneofactors. Initially normal level of T-killer cells (normal 24,8±0,5%) remained unchanged.

Finally, electrokinetic index buccal epithelium cell nuclei increased from 41,9±2,3% by 1,0±0,4% (t=2,43; p <0,05), which, according to the authors of the method [31] evidenced by the reduction of biological age from 48,9±1,8 years by 0,8±0,4 years (t=2,02; p=0,05), ie there is a "rejuvenation" of the body.

In order to identify those with registered data on set as the condition of patients before and after balneotherapy significantly different, conducted discriminant analysis (method forward stepwise [15]).

The program includes in model 14 parameters (variables) (Table 9). It is noteworthy that among the specific effects of balneotherapy in favor by the discriminant (distinctive) ability appeared to cholecystokinetic effect.

Calculation Unstandardized Canonical Scores for all patients by summation the products of personal values discriminant variables on Raw Coefficients for Canonical Variables plus Constant (Table 10) allows you to visualize the status of each patient before and after balneotherapy on spa Truskavets' (Fig. 2).

Table 9. Discriminant Function Analysis Summary

Step 14, N of variables in model: 14; Grouping: 2 groups. Wilks' Lambda: 0,061; approx. $F_{(14)}=32$; $p<10^{-6}$

Variables currently in model	Wilks' Λ	Partial Λ	F-rem (1,29)	p-level	Tole-rancy	F to enter	p-level	Λ	F-value	p-level
V30 min Postprand %	,114	,533	25,39	,00002	,303	50,81	10^{-6}	,453	50,8	10^{-6}
Creatininemia	,101	,603	19,07	,00015	,272	17,88	,0001	,315	44,6	10^{-6}
Oxaluria	,106	,574	21,54	,00007	,246	15,80	,0003	,226	45,7	10^{-6}
Killing Index vs St. aur.	,116	,526	26,09	,00002	,422	10,48	,002	,178	45,0	10^{-6}
Creatininuria	,078	,778	8,28	,00744	,387	5,45	,025	,156	41,2	10^{-6}
Electrokinetic Index	,087	,698	12,55	,00136	,374	3,69	,063	,142	37,4	10^{-6}
V15 min Postprand %	,090	,675	13,98	,00081	,244	5,74	,022	,122	37,0	10^{-6}
V 5 min Postprand %	,082	,740	10,20	,00337	,316	6,97	,012	,102	38,6	10^{-6}
Microb. Count vs E. coli	,068	,899	3,26	,08157	,876	3,69	,063	,092	37,3	10^{-6}
θ-Rhythm Laterality	,068	,895	3,39	,07600	,159	3,72	,062	,083	36,7	10^{-6}
Urea Plasma	,068	,903	3,12	,08797	,533	2,79	,104	,076	35,4	10^{-6}
Testosterone Plasma	,069	,887	3,71	,06410	,644	4,08	,052	,067	35,9	10^{-6}
δ-Rhythm Laterality	,065	,944	1,72	,19960	,169	1,62	,213	,064	33,9	10^{-6}
Diurese	,064	,958	1,29	,26605	,284	1,29	,266	,061	31,9	10^{-6}

Table 10. Standardized, Structural and Raw Coefficients and Means for Canonical Variables

Variables currently in model	Coefficients for Canonical Variables			Means for Canonical Variables	
	Standar-dized	Structural	Raw	Before Balneother	After Balneother
V30 min Postprandial, %	-1,280	**-0,280**	-0,181	68,3±2,0	53,2±0,8
Creatininemia, μM/l	-1,245	**-0,161**	-0,119	101±3	88±2
V15 min Postprandial, %	1,190	**-0,142**	0,184	81,3±1,5	74,2±1,3
V 5 min Postprandial, %	-0,937	**-0,137**	-0,548	97,2±0,4	95,5±0,4
Microbial Count vs E. coli	-0,350	**-0,085**	-0,047	62,9±1,0	57,7±2,0
θ-Rhythm Laterality, %	-0,838	**-0,085**	-0,021	+8±8	-26±11
Urea Plasma, mM/l	0,440	**-0,076**	0,368	6,92±0,27	6,27±0,26
Testosterone Plasma, nM/l	-0,433	**-0,056**	-0,058	30,3±2,0	25,8±1,8
δ-Rhythm Laterality, %	0,594	**-0,050**	0,013	+8±9	-15±13
Diurese, l/24 h	0,399	**0,175**	0,931	2,06±0,11	2,63±0,07
Killing Index vs Staph. aur., %	1,093	**0,165**	0,195	48,4±1,1	57,6±1,5
Oxaluria, μM/24 h	1,358	**0,150**	0,023	145±14	220±12
Creatininuria, mM/24 h	-0,782	**0,058**	-0,215	6,41±0,60	8,43±0,94
Electrokinetic Index, %	0,927	**0,011**	0,093	41,9±2,3	42,9±2,2
Squar. Mahalanobis Distance	**62**	**Constant**	**43,57**		
F	**32**		**Mean**	-3,83	+3,83
p	**10^{-6}**	Canonic R=0,97; Wilks'Λ=0,06; $\chi^2_{(14)}=98$; $p<10^{-6}$			

It is seen (Fig. 2) that in **all** 22 patients Means for Canonical Root greater or lesser degree increases. This reflects, first, reduction negatively correlated with Root Variables: postprandial Gall-bladder Volume after 30, 15 and 5 min drinking of cholekinetic (cholecystokinetic effect); plasma levels of Creatinin and Urea (depurative effect) and increasing positively correlated with Root Variables: Diurese, Oxaluria and Creatininuria (excretory effect).

However, we **first** show that in addition to the long-known cholekinetic and diuretic effects of balneotherapy attribute should be considered in relation to its bactericidal effect of Staphylococcus aureus and the so-called "anti-aging" effect. Among the wide range of neurotropic effects were typical left-sided lateralization of θ-and δ-rhythms only.

Unexpected decline Testosterone plasma level and Microbial Count versus Escherichia coli we interpret as favorable changes because both initially raised parameters are reduced to normal, ie there are a normalizing endocrine and immune effects of balneotherapy.

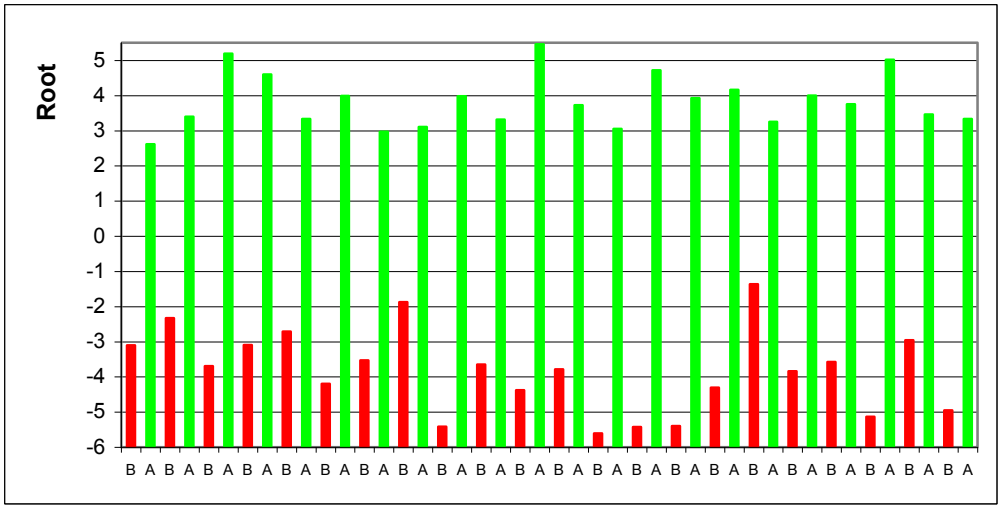

Fig. 2. Unstandardized Canonical Scores for all Patients before (B) and after (A) Balneotherapy on spa Truskavets'

Detected discriminant (and essentially attribute) variables allow by calculating Classification Functions (Table 11) accurately identify the patient before and after balneotherapy.

Table 11. Coefficients and Constants for Classification Functions

Variables currently in model	Before	After
V30 min Postprandial, %	5,58	4,19
Creatininemia, μM/l	6,17	5,25
Oxaluria, μM/24 h	-0,53	-0,35
Killing Index vs Staph. aur., %	-5,92	-4,42
Creatininuria, mM/24 h	13,31	11,67
Electrokinetic Index, %	-4,04	-3,32
V15 min Postprandial, %	-15,47	-14,06
V 5 min Postprandial, %	89,37	85,17
Microbial Count for E. coli	3,72	3,35
θ-Rhythm Laterality, %	0,40	0,23
Urea Plasma, mM/l	-5,92	-3,09
Testosterone Plasma, nM/l	4,70	4,25
δ-Rhythm Laterality, %	-0,59	-0,49
Diurese, l/24 h	-104,2	-97,0
Constant	-4049	-3715

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