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NEUROENDOCRINE-IMMUNE COMPLEX AS THE MIRROR OF THE STATE OF EXCHANGE OF NITROGENOUS METABOLITES AT RATS

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Abstract

Background. Earlier we found that even in intact rats, certain parameters of nitrogenous metabolism fluctuate in a fairly wide range, which further expands in cases of prolonged water loads. Based on this, we have been created three groups that are homogeneous in the parameters of nitrogenous metabolism. We have been shown that each cluster is accompanied by a specific constellation of immune parameters. In this study, an attempt will be made to supplement the immune accompaniment of each constellation of nitrogenous metabolites with parameters of the autonomic nervous and endocrine systems. **Material and methods**. Experiment was performed on 60 healthy female Wistar rats, both intact and loaded with different mineral waters. Immune status was assessed by thymocytogram, splenocytogram, blood leukocytogram and immunocytogram, as well as by phagocytosis parameters of blood neutrophils and monocytes. The state of autonomous regulation assessed by HRV. The plasma levels of the hormones of adaptation: corticosterone, triiodothyronine and testosterone (by the ELISA) were determined as well as mineralocorticoid, calcitonin and parathyroid activity calculated by their electrolyte markers. **Results**. 9 neuro-endocrine and 17 immune parameters were identified, the set of which three clusters of nitrogen metabolism clearly differ from each other. The overall accuracy of the classification is 93,3%. **Conclusion**. The variety of states of exchange of nitrogenous metabolites is accompanied by specific constellations of 26 parameters of neuro-endocrine-immune complex.

Key words: bilirubin, creatinine, urea, uric acid, immunity, HRV, hormones, rats.

INTRODUCTION

Earlier we found that even in intact rats, certain parameters of nitrogenous metabolism fluctuate in a fairly wide range, which further expands in cases of prolonged water loads [19]. Based on this, we have been created three groups that are homogeneous in the parameters of nitrogenous metabolism. The characteristic features of the members of the most numerous (48,3% animals) of the first cluster are moderately elevated plasma urea and upper borderline creatinineemia in combination with lower borderline uricemia. In members of the third cluster (36,7% rats), all seven parameters are in the range of normal. In contrast, rats of the second cluster (15,0% cases) were found to have significantly increased levels of urea and creatinine excretion, as well as uricemia. We have been shown that each cluster is accompanied by a specific constellation of immune parameters [11].

In this study, conducted in line with the concepts of functional-metabolic continuum [5] and neuroendocrine-immune complex [7], an attempt will be made to supplement the immune accompaniment of each constellation of nitrogenous metabolites with parameters of the autonomic nervous and endocrine systems.

MATERIAL AND METHODS

Experiment was performed on 60 healthy female Wistar rats 220-300 g. Of these, 10 remained intact, while others received drinking water of various compositions during the week. The day after the completion of the drinking course in all rats, at first, assessed the state of autonomous regulation. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: mode (Mo), amplitude of the mode (AMo) and variational swing (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [7]. Next a sample of peripheral blood (by incision of the tip of the tail) was taken for leukocytogram analysis. Animals were then placed in individual chambers with perforated bottom for collecting daily (24h) 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: corticosterone, triiodothyronine and testosterone (by the ELISA) were determined.

Electrolytes: calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (flamming photometry) were determined in plasma and daily urine. The latter also determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene). The analyzes were carried out according to the instructions described in the manual.

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: parathyroid by coefficient $(Cap•Pu/Pp•Cau)^{0,25}$, calcitonin by coefficient $(Cau•Pu/Cap•Pp)^{0,25}$ and mineralocorticoid by coefficient $(Nap•Ku/Kp•Nau)^{0,25}$, based on their classical effects and recommendations by IL Popovych [7].

In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope [2].

In the blood, the parameters of immunity were determined as described in the manual [15]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by Jondal M et al [8], their theophylline resistant (T-helper) and theophylline sensitive (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline by Limatibul S et al [12]; the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by Bianco C [1].

Natural killers were identified as large granules contain lymphocytes. In addition, the blasttransformation reaction of T-lymphocytes by PhHA was evaluated.

About the state of the phagocytosis function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytic index, the microbial count and the killing index as well as the calculated on their basis bactericidal capacity for Staphylococcus aureus (ATCC N25423 F49) [2,3,7,10,18].

The Spleen and the Thymus weighed and made smears-imprints for counting splenocytogram and thymocytogram [2,7]. For them, as well as leukocytogram and immunocytogram, CE Shannon's entropy was calculated [17,21].

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

RESULTS AND DISCUSION

Following the algorithm discriminant analysis (method forward stepwise [9]) was conducted to identify exactly those immune and neuro-endocrine parameters, in which the nitrogenous metabolism clusters differ significantly from each other. 26 variables were selected for inclusion in the model (4 from **thymus**, 4 from **spleen**, 9 from **blood** as well as 9 neuro-endocrine) (Tables 1 and 2), while others were outside the discriminatory model (Table 3).

Table 1. Summary of Stepwise Analysis for Neuroendocrine-Immune Variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Lymphoblastes Spleen,%	6,35	0,003	0,818	6,35	0,0032
17-Ketosteroides, nM/100g•24h	5,18	0,009	0,690	5,70	0,0003
MxDMn HRV, msec	6,02	0,004	0,566	6,03	10^{-4}
Macrophages Spleen, %	4,05	0,023	0,492	5,74	10^{-5}
Reticulocytes Thymus, %	4,51	0,015	0,421	5,74	10^{-6}
Mineralocorticoid Activity	3,67	0,032	0,369	5,61	10^{-6}
Lymphocytes Thymus, %	2,58	0,086	0,335	5,31	10^{-6}
Spleen Mass Index, %	2,73	0,075	0,302	5,13	10^{-6}
Monocytes Blood, %	2,28	0,113	0,276	4,92	10^{-6}
Calcitonin Activity	1,85	0,168	0,256	4,68	10^{-6}
T helper Lymphocytes, %	1,91	0,159	0,237	4,50	10^{-6}
B Lymphocytes Blood, %	2,63	0,083	0,213	4,48	10^{-6}
Entropy Immunocytogram	1,53	0,228	0,199	4,29	10^{-6}
Amplitude Mode HRV, %	1,50	0,233	0,186	4,14	10^{-6}
T cytolytic Lymphocytes, %	1,56	0,221	0,174	4,01	10^{-6}
Fibroblastes Spleen, %	1,28	0,289	0,164	3,86	10^{-6}
Fascicular ZAC, µM	1,14	0,331	0,155	3,71	10^{-6}
Microbial Count Neutrophils, Bac/Ph	1,79	0,180	0,143	3,66	10^{-6}
Medullar ZA, µM	1,52	0,232	0,132	3,59	10^{-6}
Plasmocytes Blood, %	1,08	0,350	0,125	3,47	10^{-5}
Parathyroid Activity	1,58	0,220	0,115	3,43	10^{-5}
PMN Neutrophils Blood, %	1,05	0,361	0,109	3,32	10^{-5}
Rod shape Neutrophils Blood, %	1,65	0,207	0,100	3,30	10^{-5}
Thymus Mass Index,%	1,63	0,211	0,091	3,28	10^{-5}
Plasmocytes Thymus, %	1,24	0,302	0,084	3,22	10^{-5}
Corticosterone, nM/L	1,28	0,292	0,078	3,17	10^{-5}

Table 2. Discriminant Function Analysis Summary for Neuroendocrine-Immune Variables ranked by Structural coefficient
Step 26, N of vars in model: 26; Grouping: 3 grps
Wilks' Lambda: 0,0782; approx. $F_{(53)}$=3,2; $p<10^{-6}$

	Clusters (n)			Parameters of Wilks' Statistics					
Variables currently in the model	**II (9)**	**III (22)**	**I (29)**	Wil-ks' Λ	Parti-al Λ	F-re-move	p-value	Tole-rancy	**Norm (10)**
MxDMn HRV as Vagal tone, msec	84 11	49 10	34 6	0,108	0,721	6,18	0,005	0,138	53 13
17-Ketosteroides, nM/100g•24h	107 17	64 9	61 5	0,139	0,565	12,3	10^{-4}	0,415	61 17
Medullar Zone Adrenals, µM	112 12	79 7	79 7	0,084	0,929	1,22	0,308	0,268	87 11
Microbial Count Neu-trophils, Bac/Phag	8,4 0,6	7,9 0,3	7,5 0,2	0,099	0,793	4,18	0,024	0,126	8,6 0,6
Entropy Immuno-cytogram	0,886 0,007	0,884 0,004	0,875 0,005	0,096	0,812	3,69	0,036	0,413	0,874 0,006
Fibroblastes Spleen, %	8,3 0,5	8,3 0,4	7,6 0,3	0,085	0,918	1,42	0,255	0,631	8,2 0,7
PMN Neutrophils Blood, %	29,2 3,1	27,6 1,5	26,8 0,9	0,100	0,785	4,39	0,021	0,271	26,0 2,2
Plasmocytes Blood, %	0,89 0,22	0,75 0,12	0,78 0,12	0,087	0,902	1,74	0,192	0,290	0,47 0,15
Corticosterone, nM/L	340 44	426 35	486 34	0,084	0,926	1,28	0,292	0,317	482 40
Monocytes Blood, %	3,67 0,70	4,32 0,59	5,45 0,38	0,092	0,851	2,80	0,076	0,154	4,80 0,95
Amplitude Mode HRV as Symp tone, %	47,3 7,3	61,8 4,8	64,5 4,2	0,087	0,896	1,86	0,172	0,128	55,8 5,5
$(Nap•Ku/Kp•Nau)^{0,25}$ as Mineralocort Activ	2,42 0,26	3,08 0,17	3,13 0,19	0,094	0,832	3,22	0,053	0,245	2,73 0,25
Thymus Mass Index,%	0,025 0,003	0,028 0,001	0,030 0,002	0,092	0,849	2,84	0,073	0,402	0,028 0,004
Rod shape Neutrophils Blood, %	3,11 0,45	3,09 0,20	3,38 0,23	0,097	0,811	3,74	0,035	0,325	3,60 0,34
Lymphoblastes Spleen,%	5,11 0,54	3,45 0,27	4,17 0,19	0,084	0,929	1,21	0,310	0,383	3,90 0,38
$(Cau•Pu/Cap•Pp)^{0,25}$ as Calcitonin Activity	1,70 0,73	1,43 0,07	1,75 0,07	0,084	0,932	1,16	0,326	0,655	1,67 0,12
Lymphocytes Thymus, %	71,2 0,8	68,6 0,5	69,1 0,5	0,089	0,883	2,12	0,136	0,400	70,3 0,8
Plasmocytes Thymus, %	1,89 0,26	1,71 0,18	2,17 0,15	0,087	0,899	1,80	0,182	0,453	1,80 0,25
$(Cap•Pu/Cau•Pp)^{0,25}$asParathyroid Activity	1,91 0,12	1,71 0,08	1,85 0,08	0,087	0,896	1,85	0,174	0,476	2,08 0,16
Fascicular Zone Adrenal Cortex, µM	400 31	387 18	425 14	0,102	0,766	4,88	0,014	0,266	402 28
B Lymphocytes Blood, %	15,9 0,6	15,4 0,6	16,3 0,7	0,098	0,799	4,02	0,028	0,326	16,0 0,9
Macrophages Spleen, %	7,89 0,51	8,95 0,37	8,03 0,34	0,122	0,644	8,85	0,001	0,110	7,90 0,50
T helper Lymphocytes, %	29,4	31,9	30,6	0,116	0,676	7,66	0,002	0,416	31,5

	1,1	0,8	0,7						1,0
Reticulocytes Thymus, %	4,89 0,51	5,05 0,31	4,52 0,13	0,128	0,613	10,1	10^{-3}	0,340	4,70 0,54
Spleen Mass Index, %	0,284 0,018	0,290 0,015	0,292 0,012	0,100	0,786	4,37	0,021	0,282	0,312 0,032
T cytolytic Lymphocytes, %	16,0 0,9	16,1 0,7	15,9 0,6	0,084	0,934	1,14	0,333	0,454	16,0 0,8

Table 3. Variables of the Neuroendocrine-Immune Complex currently not in the model

	Clusters (n)			**Parameters of Wilks' Statistics**					
Variables	**II (9)**	**III (22)**	**I (29)**	Wil-ks' Λ	Parti-al Λ	F to enter	p-value	Tole-rancy	**Norm (10)**
Mode HRV as Humoral channel, ms	129 5	116 5	112 3	0,075	0,962	0,61	0,551	0,106	124 5
Testosterone, nM/L	4,20 0,61	5,02 0,39	4,56 0,42	0,076	0,976	0,38	0,684	0,585	3,93 0,34
Glomerular Zone Adrenal Cortex, µM	181 18	190 9	188 6	0,075	0,965	0,57	0,572	0,415	191 14
Reticular Zone Adrenal Cortex, µM	37,8 2,9	42,8 1,9	44,9 2,2	0,077	0,979	0,34	0,714	0,453	42,7 2,4
Adrenals Mass Index, %	0,276 0,015	0,265 0,009	0,269 0,009	0,078	0,995	0,08	0,926	0,697	0,252 0,016
Triiodothyronine, nM/L	2,07 0,14	2,21 0,10	2,34 0,06	0,078	0,994	0,09	0,917	0,111	2,14 0,18
Lymphoblastes Thymus, %	6,78 0,28	6,95 0,21	7,41 0,19	0,076	0,970	0,49	0,620	0,570	7,40 0,27
Epitheliocytes Thymus, %	7,6 0,7	10,1 0,5	9,2 0,3	0,075	0,959	0,65	0,527	0,255	8,8 0,6
Endotheliocytes Thymus,%	2,44 0,34	2,62 0,20	2,72 0,18	0,077	0,990	0,15	0,862	0,469	2,60 0,31
Macrophages Thymus, %	3,44 0,50	3,00 0,26	2,90 0,17	0,078	0,995	0,08	0,923	0,338	2,70 0,42
Lymphocytes Spleen, %	48,2 0,7	48,3 0,6	48,1 0,4	0,078	1,000	0,01	0,993	0,347	48,7 0,9
Plasmocytes Spleen, %	2,00 0,44	1,73 0,24	2,14 0,26	0,076	0,968	0,52	0,600	0,309	2,50 0,50
Leukocytes Blood, 10^9/L	10,0 0,8	11,7 1,2	12,0 0,8	0,076	0,970	0,49	0,620	0,532	12,7 1,9
Natural Killers Blood, %	14,9 0,5	15,7 0,5	16,2 0,4	0,074	0,940	0,98	0,386	0,082	15,6 0,9
0 Lymphocytes Blood, %	20,5 2,4	23,7 1,2	18,6 1,5	0,078	0,993	0,11	0,900	0,390	22,2 1,9
Microbial Count Monocytes, Bac/Phag	4,4 0,5	4,6 0,3	4,8 0,4	0,077	0,990	0,16	0,853	0,496	5,0 0,6
Bactericidal Capacity Monocytes, 10^6 B/L	51 13	74 17	103 22	0,070	0,991	0,16	0,851	0,550	122 64
Killing Index Neutrophils,%	53,0 2,8	51,1 1,4	54,5 1,1	0,078	0,997	0,04	0,961	0,438	50,7 2,0
Entropy Leukocytogram	0,583 0,017	0,594 0,013	0,557 0,012	0,076	0,934	0,38	0,564	0,602	0,596 0,018

The distinctive information contained in the 26 discriminant variables is condensed into two roots. The first root contains 70% of the discriminatory potential (r*=0,891; Wilks'

Λ=0,078; $\chi^2_{(52)}$=113; $p<10^{-6}$) and second root 30% (r*=0,787; Wilks' Λ=0,381; $\chi^2_{(25)}$=43; p=0,014).

Calculating the values of the discriminant root for each animal as the sum of the product of the raw coefficients on the individual values of the discriminant variables together with a constant (Table 4) makes it possible to visualize each rat in the information space of the roots (Fig. 1).

Table 4. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Lymphoblastes Spleen,%	-0,215	-0,488	-0,179	-0,405
17-Ketosteroides, nM/100g•24h	1,120	-0,290	0,030	-0,008
MxDMn HRV, msec	1,506	-0,601	0,035	-0,014
Macrophages Spleen, %	1,831	0,967	1,046	0,552
Reticulocytes Thymus, %	1,164	-0,314	0,976	-0,264
Mineralocorticoid Activity	-0,844	-0,435	-0,912	-0,470
Lymphocytes Thymus, %	0,594	-0,140	0,240	-0,057
Spleen Mass Index, %	-0,674	-0,801	-9,994	-11,88
Monocytes Blood, %	0,302	-1,201	0,131	-0,520
Calcitonin Activity	0,112	-0,388	0,308	-1,066
T helper Lymphocytes, %	-0,280	1,075	-0,076	0,293
B Lymphocytes Blood, %	0,162	-0,981	0,051	-0,311
Entropy Immunocytogram	0,702	0,319	30,12	13,69
Amplitude Mode HRV, %	-0,229	-1,115	-0,010	-0,050
T cytolytic Lymphocytes, %	0,110	0,470	0,035	0,149
Fibroblastes Spleen, %	0,378	0,161	0,223	0,095
Fascicular ZAC, µM	0,845	-0,708	0,011	-0,009
Microbial Count Neutrophils, B/Ph	1,077	-1,081	0,806	-0,808
Medullar ZA, µM	-0,139	-0,635	-0,004	-0,019
Plasmocytes Blood, %	-0,159	-0,717	-0,262	-1,184
Parathyroid Activity	0,186	0,554	0,465	1,384
PMN Neutrophils Blood, %	0,908	-0,471	0,140	-0,073
Rod shape Neutrophils Blood, %	-0,847	0,139	-0,736	0,121
Thymus Mass Index,%	-0,671	0,170	-82,56	20,91
Plasmocytes Thymus, %	0,439	-0,336	0,542	-0,415
Corticosterone, nM/L	0,505	-0,222	0,0030	-0,0013
	Constants		-67,44	8,66
	Eigenvalues		3,87	1,63
Cumulative Proportions			0,704	1

In Table 5, interspersed with the parameters included by the program in the discriminant model, we also inserted other parameters that carry identifying information, but were outside the model, apparently due to the redundancy of this information.

Table 5. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables	Correlations Variables-Roots		I (29)	III (22)	II (9)
Root 1 (70%)	**Root 1**	**Root 2**	**-1,55**	**+0,39**	**+4,05**
MxDMn HRV as Vagal tone, msec	**0,209**	-0,021	**-0,46**	**-0,09**	**+0,77**
17-Ketosteroides urine, nM/100g•24h	**0,205**	-0,137	**0,00**	**+0,04**	**+0,84**
Medullar Zone Adrenal, µM	**0,152**	-0,122	**-0,21**	**-0,22**	**+0,69**
Mode HRV as Humoral channel, msec	currently not in model		**-0,79**	**-0,54**	**+0,34**
Microbial Count Neutrophils, Bac/Phag	**0,119**	0,001	**-0,55**	**-0,39**	**-0,08**
Entropy Immunocytogram	**0,094**	0,071	**+0,06**	**+0,54**	**+0,70**
Fibroblastes Spleen, %	**0,085**	0,102	**+0,93**	**+1,01**	**+1,02**
Polymorphonucleary Neutrophils Blood, %	**0,066**	-0,001	**+0,12**	**+0,24**	**+0,47**
Plasmocytes Blood, %	**0,028**	-0,044	**+0,66**	**+0,61**	**+0,90**
Macrophages Thymus, %	currently not in model		**+0,15**	**+0,22**	**+0,56**
Corticosterone, nM/L	**-0,154**	-0,022	**+0,03**	**-0,45**	**-1,12**
Amplitude Mode HRV as Sympathetic tone, %	**-0,132**	0,056	**+0,50**	**+0,35**	**-0,49**
$(Nap•Ku/Kp•Nau)^{0,25}$ as Mineralocorticoid Activity	**-0,130**	0,080	**+0,52**	**+0,45**	**-0,40**
Reticular Zone Adrenal Cortex, µM	currently not in model		**+0,29**	**+0,02**	**-0,65**
Triiodothyronine, nM/L	currently not in model		**+0,35**	**+0,12**	**-0,13**
Monocytes Blood, %	**-0,146**	-0,085	**+0,22**	**-0,16**	**-0,38**
Thymus Mass Index,%	**-0,104**	0,005	**+0,13**	**0,00**	**-0,28**
Lymphoblastes Thymus, %	currently not in model		**+0,02**	**-0,53**	**-0,74**
Leukocytes Blood, 10^9/L	currently not in model		**-0,12**	**-0,16**	**-0,45**
Endotheliocytes Thymus,%	currently not in model		**+0,13**	**+0,02**	**-0,16**
Microbial Count Monocytes, Bac/Phag	currently not in model		**-0,10**	**-0,22**	**-0,29**
Natural Killers Blood, %	currently not in model		**+0,21**	**+0,03**	**-0,27**
Bactericidal Capacity Monocytes, 10^6 Bac/L	currently not in model		**-0,11**	**-0,28**	**-0,42**
Root 2 (30%)	**Root 1**	**Root 2**	-0,80	**+1,61**	-1,36
$(Cau•Pu/Cap•Pp)^{0,25}$ as Calcitonin Activity	-0,056	**-0,321**	+0,22	**-0,62**	+0,10
$(Cap•Pu/Cau•Pp)^{0,25}$ as Parathyroid Activity	0,012	**-0,155**	-0,47	**-0,75**	-0,34
Fascicular Zone Adrenal Cortex, µM	-0,070	**-0,140**	+0,26	**-0,16**	-0,02
Lymphoblastes Spleen,%	0,106	**-0,332**	+0,23	**-0,37**	+1,01
Lymphocytes Thymus, %	0,136	**-0,186**	-0,51	**-0,71**	+0,39
Plasmocytes Thymus, %	-0,079	**-0,165**	+0,47	**-0,11**	+0,11
B Lymphocytes, %	-0,036	**-0,100**	+0,12	**-0,22**	-0,04
Rod shape Neutrophils Blood, %	-0,048	**-0,065**	-0,21	**-0,47**	-0,45
Plasmocytes Spleen, %	currently not in model		-0,23	**-0,49**	-0,32
Killing Index Neutrophils,%	currently not in model		+0,60	**+0,07**	+0,36
Testosterone, nM/L	currently not in model		+0,59	**+1,02**	+0,25
Macrophages Spleen, %	0,007	**0,212**	-0,08	**+0,66**	-0,01
T helper Lymphocytes, %	-0,038	**0,175**	-0,30	**+0,12**	-0,67
Reticulocytes Thymus, %	0,068	**0,125**	-0,11	**+0,20**	+0,11
Spleen Mass Index, %	-0,014	**0,057**	-0,20	**-0,13**	-0,28
T cytolytic Lymphocytes, %	0,010	**0,022**	-0,06	**+0,04**	0,00
Epitheliocytes Thymus, %	currently not in model		+0,20	**+0,68**	-0,63
0 Lymphocytes Blood, %	currently not in model		-0,58	**+0,25**	-0,27
Entropy Leukocytogram	currently not in model		-0,66	**-0,02**	-0,21
Lymphocytes Spleen,%	currently not in model		-0,22	**-0,14**	-0,18

As you can see, nine rats of the second cluster, which are characterized by significantly increased levels of urea and creatinine excretion, as well as plasma uric acid, are located in the extreme right zone of the axis of the first root. This reflects their above-average or normal

but maximum sampling levels of four neuro-endocrine and six immune parameters, correlated with root **directly**, combined with five neuro-endocrine and eight immune parameters that were reduced or minimal for sampling, connected to the root **inversely**.

At the opposite pole of the axis are the rats of the first cluster, which are characterized by moderately elevated plasma urea and upper borderline creatinineemia in combination with lower borderline uricemia. This state of nitrogen metabolism is accompanied by opposite (**reduced/minimum** or **increased/maximum**, respectively) levels of the same parameters of the neuro-endocrine-immune complex.

The intermediate position on the axis of the first root is occupied by the members of the third cluster, but its delimitation with the first cluster is not quite clear (there are interpenetrations). However, additional delimitation of these clusters occurs along the axis of the second root. The top position of the third cluster reflects below-average or minimum for sampling levels of three endocrine and seven immune parameters that correlate with the root **inversely**, combined with elevated testosterone levels and normal but maximum sampling levels of nine immune parameters that represent the root **direct**.

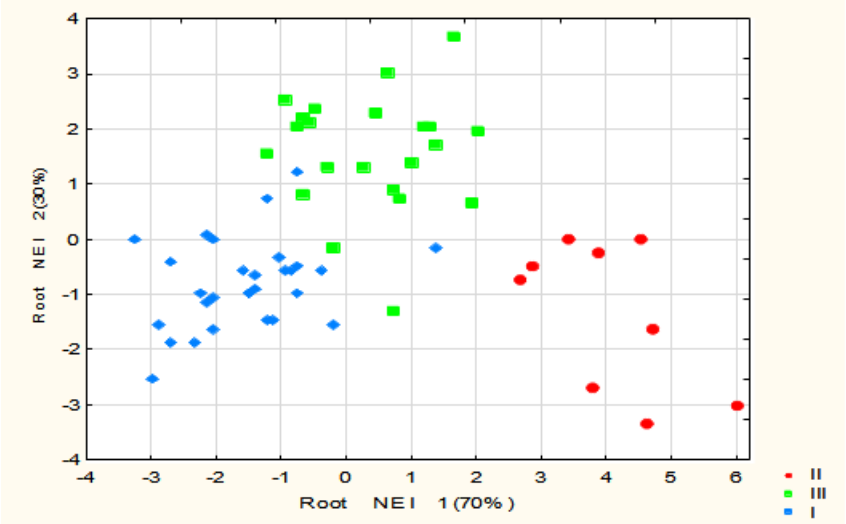

Fig. 1. Scatterplot of rats of differ clusters in space of Roots containing the information about discriminant neuro-endocrine-immune parameters

In general, in the information space of the two discriminant roots, all clusters are clearly separated from each other, that is, they differ from each other in terms at least of 26 parameters of neuro-endocrine-smmune complex. This distinction is documented by the calculation of the Mahalanobis distances between clusters (Table 6).

Table 6. Squared Mahalanobis Distances between clusters (above the diagonal), F-values (df=26,3) and p-levels (under the diagonal)

Clusters	II	III	I
II	0	**22**	**32**
III	**3,1** 0,001	0	**9,6**
I	**4,7** 10^{-4}	**2,6** 0,006	0

The use of classification functions (Table 7) makes it possible to retrospectively identify clusters II without error and the other two with two errors (Table 8), ie the overall recognition accuracy is 93,3%.

Table 7. Coefficients and Constants for Classification Functions

Clusters	II	III	I
Variables	p=,150	p=,367	p=,483
Lymphoblastes Spleen,%	-10,30	-10,85	-9,526
17-Ketosteroides urine, nM/100g•24h	1,910	1,778	1,739
MxDMn HRV as Vagal tone, msec	2,153	1,982	1,948
Macrophages Spleen, %	83,98	81,80	78,44
Reticulocytes Thymus, %	86,06	81,70	80,45
(Nap•Ku/Kp•Nau)^{0,25} as Mineralocorticoid Activity	-62,94	-61,01	-58,11
Lymphocytes Thymus, %	33,88	32,83	32,50
Spleen Mass Index, %	-670,2	-669,0	-620,9
Monocytes Blood, %	27,12	25,09	26,10
(Cau•Pu/Cap•Pp)^{0,25} as Calcitonin Activity	29,86	25,56	27,54
T helper Lymphocytes, %	-8,100	-6,946	-7,507
B Lymphocytes Blood, %	15,22	14,11	14,76
Entropy Immunocytogram	4960	4891	4799
Amplitude Mode HRV as Sympathetic tone, %	-0,846	-0,957	-0,817
T cytolytic Lymphocytes, %	0,643	0,959	0,532
Fibroblastes Spleen, %	15,79	15,26	14,60
Fascicular Zone Adrenal Cortex, µM	0,996	0,931	0,932
Microbial Count Neutrophils, Bac/Ph	82,89	77,53	77,92
Medullar Zone Adrenals, µM	-0,519	-0,561	-0,506
Plasmocytes Blood, %	-62,88	-65,45	-62,08
(Cap•Pu/Cau•Pp)^{0,25} as Parathyroid Activity	28,74	31,16	26,91
PMN Neutrophils Blood, %	13,31	12,58	12,49
Rod shape Neutrophils Blood, %	-70,67	-67,61	-66,48
Thymus Mass Index,%	-7349	-6984	-6875
Plasmocytes Thymus, %	67,88	64,66	64,61
Corticosterone, nM/L	0,363	0,348	0,346
Constants	-4709	-4428	-4318

Table 8. Classification Matrix for clusters
Rows: Observed classifications; Columns: Predicted classifications

Clusters	Percent Correct	II p=,150	III p=,367	I p=,483
II	100	**9**	0	0
III	90,9	0	**20**	**2**
I	93,1	0	**2**	**27**
Total	93,3	9	22	29

Thus, the variety of states of exchange of nitrogenous metabolites is accompanied by specific constellations of 26 parameters of neuro-endocrine-immune complex. This situation does not seem to be accidental, as it is known that immunocytes express Ah-, TL- and Adenosine receptors, the agonists of which, among others, may be bilirubin and uric acid

[4,13,14,16,20]. We did not find information on the presence of receptors on immunocytes for other nitrogenous metabolites - creatinine and urea - on PubMed and PMC resources. Based on the presence of significant correlations between plasma and urine urea and creatinine levels, on the one hand, and immune parameters, on the other, we hypothesized that such connections are mediated by hormones and neurotransmitters of the autonomic nervous system.

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 carrying out of experiments was approved by the Ethics Committee of the Ukrainian Scientic Research Institute of Medicine for Transport. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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