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The features of reactions to acute stress of neuro-endocrine-immune complex, metabolome, ECG and gastric mucosa in rats with various state of innate muscular endurance and resistance to hypoxia

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Annotation

Background. It is known about wide variety of individual reactions to stress explained by genetics factors. On the other hand, it is also known about aerobic fitness variability between individuals. From the above it follows the hypothesis that inter-individual differences in normal conditions determine the characteristics of the body's response to acute stress. The purpose of this study is to test the hypothesis. **Material and methods.** The experiment is at 58 rats (28 males) Wistar line. Animals were tested for resistance to hypoxic hypoxia and aerobic muscular performance by swimming test. On the basis of the received data two qualitatively equivalent groups in a ratio 10/48 were formed. After a week of recovery over the next 10 days, one animal remained intact and 5 other rats were exposed to water-immersion and restraint stress. The next day after stress, the ECG recorded and some endocrine, metabolic and immune parameters determined as well as erosive-ulcerative lesions of the gastric mucosa evaluated. **Results**. Four clusters by hypoxic and swimming tests were created retrospectively: normal resistance to hypoxia and muscular endurance (n=11); moderately reduced resistance to hypoxia and normal swimming test (n=25); drastic increased swimming test and normal hypoxic test (n=3); significantly increased resistance to hypoxia and normal swimming test (n=9). Each cluster is characterized by specific (correctness of classification 100%) post-stress changes in 6 neuro-endocrine, 12 immune, 10 metabolic and 2 ECGs parameters, as well as the index of damage to the gastric mucosa. The swimming test determines the post-stress state of the registered parameters by 63,8%, the hypoxic test - by 57,5%, and taken together - by 79,1%. **Conclusion**. The post-stress neuro-endocrine, immune and metabolic parameters as well as injuries of myocardium and gastric mucosa in rats are determined significantly by innate muscular endurance and resistance to hypoxia.

Keywords: swimming and hypoxic tests, acute stress, hormones, HRV, immunity, metabolome, ECG, gastric mucosa, rats.

INTRODUCTION

The different responses of animals and humans to an apparently equivalent stimulus are called inter-individual response variability. This phenomenon has gained more and more attention in research in recent years. Among others, this increased interest was driven by the intervention literature because the intervention-related individual differences in outcome measures have great practical relevance (e.g., in therapy, rehabilitation, health care, prevention, and sports medicine) (Kozyavkina et al, 2015; Popovych et al, 2020). Several factors constitute a potential source for inter-individual response variability. According to the literature, these factors can be categorized as follows: non-modifiable, modifiable, and other influencing factors. Non-modifiable factors comprise factors that are predetermined, such as genetics, sex, and age. There is considerable evidence highlighting the prominent influence of the *genotype* on the responsiveness of a single individual in physical performance parameters, brain structure and function etc. However, the exact influence of genetic factors on inter-individual response variability, at least for physical performance, is not yet exactly known and is currently under debate (Herold et al, 2021).

It is known about significant individual differences in stress perception, processing, and coping (Dhabhar & McEwen, 2007; Gunnar & Quevedo, 2007). Individual differences become particularly relevant while studying human subjects because stress perception, processing, and coping mechanisms can have significant effects on the kinetics and peak levels of circulating stress hormones and on the duration for which these hormone levels are elevated. Animal studies showing significant strain differences in stress reactivity and peak hormone levels (Dhabhar et al., 1993), adaptation to stress (Dhabhar et al., 1997), and in distribution and activation of adrenal steroid receptors and corticosteroid-binding globulin levels (Dhabhar et al., 1995), suggest that genetic as well as environmental factors play a role in establishing individual differences (Gomez-Serrano et al., 2001). One of the important manifestations of the body's overall resistance is its susceptibility to stress-induced damage of gastric mucosa and the myocardium. The variability of stress responses in different animal strains of the same species is well established. For example, selective breeding-based cholinergic hypersensitivity and hyposensitivity Flinders rat lines (Overstreet et Wegener, 2013); hyperanxious (HAB-M) and hypoanxious (LAB-M) mouse lines (Krömer et al, 2005); high-resistant and low-resistant to hypoxic hypoxia Wistar rats (Markova et al, 1997; Ordynskyi et al, 2017; 2019). The importance of individual vulnerability and resilience factors is increasingly acknowledged in mechanistic research and may exhibit a genetic (Savignac et al, 2011) and an epigenetic (Zannas et West, 2014) basis, and this is possibly based on “synaptic rewiring” of stress-sensitive neurons (Singh-Taylor et al, 2015). In all cases, however, it is likely that the “three-hit concept” of vulnerability and resilience persists: a genetic predisposition and early life adverse events are necessary so that a later-in-life stressor can exhibit negative health outcomes, and one or more missing may result in higher resilience (Daskalakis et al, 2013; Elsenbruch et Enck, 2017). It is known about aerobic fitness variability between individuals explained by genetics factors (Alvarez-Romero et al., 2021; Daskalakis et al., 2013; Herold et al., 2021; Overstreet & Wegener, 2013; Savignac et al., 2011; Zannas & West, 2014).

From the above it follows the hypothesis that inter-individual differences in normal conditions determine the characteristics of the body's response to acute stress. The purpose of this study is to test the hypothesis.

The close relationships between the nervous, endocrine and immune systems within the framework of the triune neuro-endocrine-immune complex are well documented (Korneva, 1993; 2020; Kozyavkina et al., 2015; Kul’chyns’kyi et al., 2017; 2017a; 2017b; Mel’nyk et al., 2019; 2022; Nance & Sanders, 2007; Pavlov et al., 2018; Popovych, 2011; Popovych et al.,

2017; 2018; 2020; Sternberg, 2006; Sydoruk et al., 2018; Thayer & Sternberg, 2010; Tracey, 2010).

Therefore, we used the parameters of neuro-endocrine-immune complex as well as metabolome and markers of gastric mucosa and myocardium injuries to quantify the body's response to stress.

MATERIAL AND METHODS

Participants. The experiment is at 58 rats Wistar line weighing 170-280 g: 28 males (Mean=216 g; SD=22 g) and 30 females (Mean=196 g; SD=19 g).

Procedure / Test protocol / Skill test trial / Measure / Instruments. At the preparatory stage, all animals were first tested for resistance to hypoxic hypoxia by the classical method of Berezovskyi (1975). To do this, each rat was placed in a pressure chamber with a transparent lid, in which the pump created a vacuum of air equivalent to a rise to a height of 12 km (20 kPa) and recorded the time of the second agonal breath or seizure. One week later, aerobic muscular performance was determined by the duration of swimming (t^0 water 26^0 C) with a load (5% of body weight) to exhaustion (falling to the bottom of the bath) (Brekhman, 1968).

After a week of recovery under light ether anesthesia for 15-20 sec recorded electrocardiogram (ECG) in standard lead II (introducing needle electrodes subcutaneously).

On the basis of the received data two qualitatively equivalent groups (equally females and males, practically identical average sizes and variances of swimming and hypoxic tests) in a ratio 10/48 were formed. Over the next 10 days, one animal remained intact and 5 other rats were exposed to water-immersion and restraint stress according to the method of Nakamura et al. (1977) in the modification of Popovych (2007), which is to reduce the duration of stay of the rat in a fixed standing position in cold water (t^0 20-21^0 C) to the level of the xiphoid process from 8 to 4 hours.

The next day after stressing, the ECG was re-recorded. Then, 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 segmental (SN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the equation derived by Popovych (2007; 2020) on the basis of the classical Shannon's (1948) equation:

$$hLCG = - (L \bullet \log_2 L + M \bullet \log_2 M + Eo \bullet \log_2 Eo + Bas \bullet \log_2 Bas + RN \bullet \log_2 RN + SN \bullet \log_2 SN)/\log_2 6.$$

The experiment was completed by decapitation of the animals in order to remove the stomach, adrenal glands, thymus, spleen, and collect the maximum possible amount of blood in which was determined some endocrine, metabolic, and immune parameters.

Among endocrine parameters determined plasma concentration of corticosterone, testosterone and triiodothyronine (by ELISA, reagents from JSC "Alkor Bio", RF) (Instructions, 2000).

On lipid metabolism judged by the level of plasma triglycerides (metaperiodate-acetylacetone colorimetric method), total cholesterol (direct method by reaction Zlatkis-Zach) and its distribution as part of α-lipoprotein (applied enzymatic method Hiller (1987) after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as well as nonα-lipoprotein (turbidometric method Burstein-Samay) as described in the handbook (Goryachkovskiy, 1998). State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract) (Gavrilov et Mishkorudnaya, 1983) and malondyaldehid (test with thiobarbituric acid) (Andreyeva et al, 1988), as well as the activity of antioxidant enzymes: catalase serum and red blood cells (by the speed of decomposition hydrogen peroxide) (Korolyuk et al, 1988) and superoxide

dismutase erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH) (Dubinina et al, 1988; Makarenko, 1988). On electrolytes metabolism judged by the level of calcium (by the reaction with arsenazo III), phosphate (phosphate molibdate method) and chloride (mercury rodanide method) in the plasma, sodium and potassium in the plasma and erythrocytes (flame photometry method) as described in the handbook (Goryachkovskiy, 1998).

Based on obtained data evaluated hormonal activities: mineralocorticoid MCA=$(Nap/Kp)^{0,5}$, parathyroid PTA=$(Cap/Pp)^{0,5}$ and calcitonin CTA=$(1/Cap•Pp)^{0,5}$, based on their classic effects and guidelines Popovych (2011; 2019).

Alanine and asparagine aminotranspherase, alkaline and acid phosphatase as well as creatine phosphokinase determined by uniform methods as described in the handbook (Goryachkovskiy, 1998).

Use analyzers "Tecan" (Oesterreich), "Pointe-180" ("Scientific", USA), "Reflotron" ("Boehringer Mannheim", BRD) and flame spectrophotometer.

The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass counted the number of ulcers and their length was measured, evaluated erosive and ulcerative damage on scale by Popovych (2007; 2011). This scale is based on the qualitative-quantitative Harrington (1965) scale.

The parameters of immunity were determined, 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 (TR) and theophylline-susceptible (TS) 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 by equation:

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

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index (percentage of cells, in which found microbes), the microbial count (number of microbes absorbed by one phagocyte) and the killing index (percentage of dead microbes) for Staphylococcus aureus (ATCC N25423 F49). According to these parameters and the content of microphages and macrophages in the blood calculated their Bactericidal Capacity (Bilas & Popovych, 2009; Bilas et al., 2020):

BCC N or M, 10^9 Bacteria/L = Leukocytes, 10^9/L•(Neutrophils or Monocytes, %)•PhI,%•MC, Bac/Phag•KI%.

The Spleen and Thymus were weighed and made smears-imprints for counting Thymocytogram and Splenocytogram (Horizontov et al., 1983; Bilas & Popovych, 2009; Bilas et al., 2020). The components of the Thymocytogram (TCG) are lymphocytes (Lc), lymphoblastes (Lb), reticulocytes (Ret), macrophages (Mac), basophiles (B), endotheliocytes (En), epitheliocytes (Ep) and Hassal's corpuscles (H). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblastes (Lb), plasma cells (Pla), reticulocytes (R), macrophages (Ma), fibroblasts (F), microphages (Mi) and eosinophils (Eo).

For them Shannon's entropy was calculated too:

$hTCG = - (Lc•\log_2 Lc+Lb•\log_2 Lb+Ret•\log_2 Ret+Mac•\log_2 Mac+B•\log_2 B+En•\log_2 En+Ep•\log_2 Ep+H•\log_2 H)/\log_2 8;$

$hSCG = - (Lc•\log_2 Lc+Lb•\log_2 Lb+Pla•\log_2 Pla+R•\log_2 R+Ma•\log_2 Ma+F•\log_2 F+Mi•\log_2 Mi+Eo•\log_2 Eo)/\log_2 8.$

Data collection and analysis / Statistical analysis. Statistical processing was performed using a softwarepackage "Microsoft Excell" and "Statistica 6.4 StatSoft Inc".

Fragments of the article were published earlier (Fil et al., 2021; Zukow et al, 2022).

RESULTS

Visualization of the sample on one plane (Fig. 1) shows significant variability of both the swimming test (range 6 ÷ 66 min, Cv = 0,672) and the hypoxic test (range 65 ÷ 317 sec, Cv = 0,515). The sex differences for the hypoxic test are completely absent (M±SD: 131±76 and 132±55 sec in males and females, respectively), while according to the swimming test males are dominated by females (M±SD: 22,6±14,1 vs 15,2±6,5 min, $p<0,05$). Obviously, this is partly due to body weight (r=0,26) and levels testosterone (r=0,31; 40,3±4,6 vs 3,53±0,53 nM/L) and corticosterone (r=-0,58; 290±114 vs 406±82 nM/L).

The second phase was conducted cluster analysis of fitness variables (in **stressed** rats only). Clustering cohort of rats is realized by iterative k-means metod. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points (Aldenderfer et Blashfield, 1989).

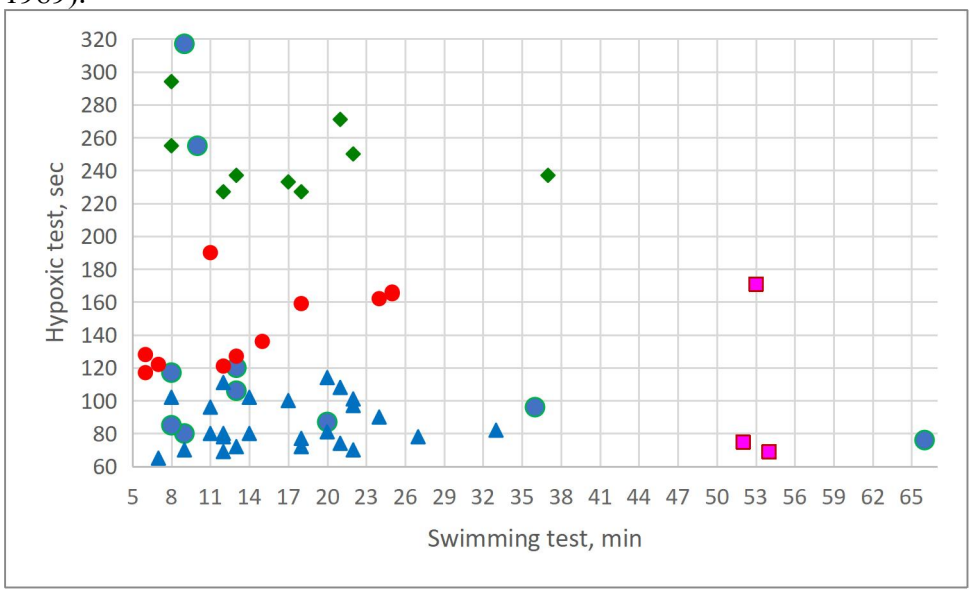


Fig. 1. Diagram of scattering of actual individual values of swimming and hypoxic tests of rats. Large circles indicate animals that have not been exposed to stress

We have identified 4 clusters (Fig. 1 and Table 1). The **first** cluster contains 9 females and 2 males, the **second** - only 3 males, the **third** - 13 males and 12 females and **fourth** cluster contains 5 males and 4 females. Note that the markers of intact rats superimposed on the plane are detected almost proportionally in each cluster. This is important given the subsequent assessment of stress-induced deviations from the norm of the registered parameters of the body.

Table 1. The average values of fitness variables intact rats and members of different clusters

Test	**Cluster (n)**	**I (11)**	**II (3)**	**III (25)**	**IV (9)**	**Intact (10)**
Hypoxic, sec	Mean	**145**	**105**	**86**	**248**	132
	SD	**24**	**57**	**15**	**22**	68
Swimming, min	Mean	**14,7**	**53,0**	**16,8**	**17,3**	18,8
	SD	**7,4**	**1,0**	**6,4**	**9,0**	12,6

For the purpose of comparative qualitative-quantitative assessment actual fitness variables (V) expressed as Z-scores (Table 2 and Fig. 2) calculated by equation:

$Z = (V/M - 1)/Cv$, where

M is Mean for the sample; Cv is Coefficient its variation.

Table 2. The average Z-scores of fitness variables intact rats and members of different clusters

Test	Cluster (n)	I H^0S^0 (11)	II H^0S^{3+} (3)	III $H^{1-}S^0$ (25)	IV $H^{2+}S^0$ (9)	Intact H^0S^0 (10)
Hypoxic, Z	Mean	+0,20	-0,39	-0,67	+1,72	+0,04
	SD	0,36	0,85	0,22	0,33	0,39
Swimming, Z	Mean	-0,32	+2,71	-0,16	-0,12	+0,03
	SD	0,58	0,08	0,51	0,71	0,46

As can be seen, the characteristics of the members of the **first** cluster are normal, both resistance to hypoxia and muscular endurance. Rats of the **second** cluster are distinguished by a drastic duration of swimming to exhaustion. The **third** cluster is characterized by moderately reduced resistance to hypoxia, and the **fourth** – its significantly increased.

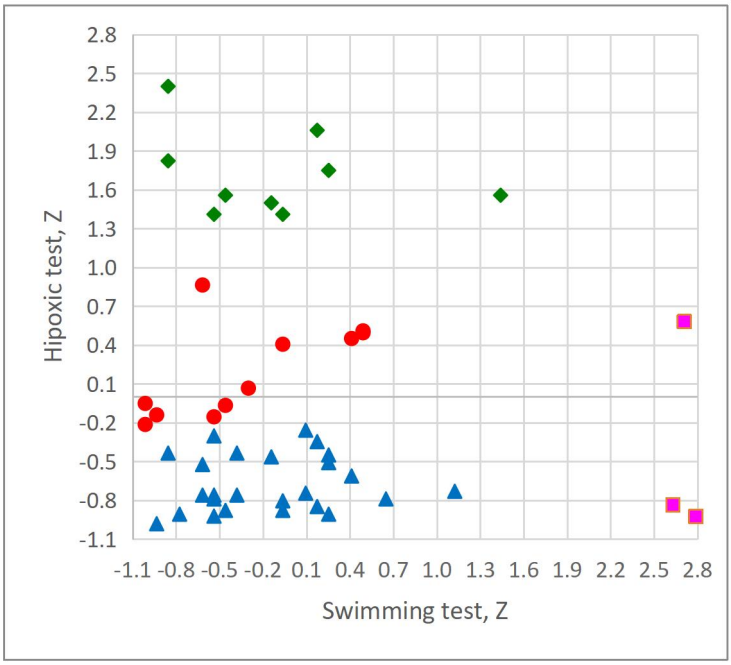


Fig. 2. Diagram of scattering of normalized individual values of swimming and hypoxic tests of rats

In order to identify exactly those post-stress parameters (variables) whose constellation is characteristic for each cluster, the available informational field was subjected to discriminant analysis by the method of forward stepwise (Klecka, 1989). To include in the model (Tables 3 and 4), the program has selected 31 variables (6 **neuro-endocrine**, 12 **immune**, 10 **metabolic**, 2 **ECGs** as well as **marker of gastric mucosa injuries.** The other registered parameters: 3 **neuro-endocrine** (Table 4), 29 **immune** (Tables 5-8), 11 **metabolic** (Table 9), 5 **ECGs** as well as 2 **markers of gastric mucosa injuries** (Table 10) were outside the discriminant model.

Table 3. Discriminant Function Analysis Summary
Step 31, N of vars in model: 31; Grouping: 4 grps; Wilks' Λ: 0,00064; appr. $F_{(93)}$=4,9; $p<10^{-6}$

	Clusters (n)				Parameters of Wilks' Statistics					
Variables currently in the model	I H^0S^0 (11)	III H^1S^0 (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)	Wilks' Λ •10^2	Partial Λ	F-remove (3,14)	p-value	Tolerancy	Norm (10)
MxDMn HRV as Vagal tone, msec	38 9	32 5	23 14	16 3	0,136	0,472	5,227	0,012	0,089	84 10
Mode HRV as Humoral Channel, msec	182 9	159 5	167 34	155 9	0,088	0,734	1,692	0,214	0,116	184 6
Corticosterone normalized by sex, Z	0,00 0,28	+0,35 0,22	-0,58 0,21	-0,40 0,31	0,110	0,587	3,277	0,053	0,127	0 0,30
Testosterone normalized by sex, Z	-0,26 0,35	-0,88 0,25	-0,63 0,42	-1,37 0,62	0,117	0,548	3,848	0,034	0,066	0 0,30
Triiodothyronine, nM/L	3,05 0,19	3,70 0,08	2,58 0,80	3,22 0,08	0,139	0,462	5,423	0,011	0,159	3,43 0,31
$(Ca/P)^{0,5}$ as Parathyroid Activity	1,81 0,08	1,62 0,07	1,35 0,06	1,50 0,08	0,119	0,540	3,98	0,030	0,032	1,53 0,07
Thymus Mass, mg	154 12	133 8	136 8	111 13	0,099	0,650	2,511	0,101	0,078	144 10
Hassal's corpuscles of Thymus, %	1,73 0,14	1,36 0,10	3,00 0,58	1,33 0,17	0,149	0,431	6,16	0,007	0,245	1,00 0,13
Fibroblastes of Thymus, %	5,36 0,43	5,68 0,31	6,00 1,15	5,67 0,62	0,109	0,588	3,263	0,053	0,184	5,33 0,65
Entropy of Thymocytogram	0,593 0,019	0,612 0,010	0,655 0,035	0,622 0,017	0,079	0,817	1,043	0,404	0,225	0,596 0,015
Lymphoblastes of Spleen, %	6,4 0,8	9,7 0,6	6,7 2,2	8,3 0,7	0,132	0,488	4,90	0,016	0,175	8,6 1,0
Macrophages of Spleen, %	1,73 0,30	2,76 0,19	2,33 0,33	3,83 0,39	0,096	0,669	2,31	0,121	0,237	2,56 0,32
Microphages of Spleen, %	10,9 1,3	12,0 0,6	11,3 1,8	12,0 0,7	0,076	0,847	0,842	0,493	0,111	12,3 0,9
Leukocytes of Blood, 10^9/L	14,93 1,42	15,60 0,97	11,70 1,18	14,57 0,98	0,078	0,828	0,966	0,436	0,075	13,81 2,09
Killing Index of Neutrophils, %	34,4 2,3	40,6 2,0	41,3 0,9	52,1 2,1	0,116	0,554	3,75	0,036	0,167	47,5 2,9
Bactericidal Capacity Neutrophils, 10^9 B/L	7,40 1,06	9,44 1,02	8,23 0,96	12,0 1,66	0,100	0,644	2,578	0,095	0,036	7,54 1,39
Phagocytic Index of Monocytes, %	6,6 0,7	6,0 0,3	5,3 0,7	4,4 0,7	0,076	0,850	0,824	0,502	0,250	5,9 0,5
Bactericidal Capacity of Monocytes, 10^6 B/L	350 121	230 31	121 33	182 75	0,115	0,559	3,685	0,038	0,067	208 37
Triglycerides, mM/L	0,99 0,03	1,08 0,02	1,17 0,07	1,07 0,01	0,092	0,696	2,04	0,154	0,186	1,07 0,02
α-LP Cholesterol, mM/L	0,69 0,05	0,81 0,03	0,84 0,11	0,69 0,03	0,127	0,505	4,58	0,020	0,146	0,84 0,05
Diene conjugates, E^{232}/mL	1,50 0,08	1,53 0,07	1,63 0,35	1,44 0,10	0,080	0,802	1,154	0,362	0,219	1,47 0,11
Malondialdehyde, μM/L	52,4 1,7	53,8 1,9	79,3 15.4	61,3 3,3	0,084	0,769	1,400	0,284	0,213	63,5 5.6
Asparagine Aminotranspherase, μKat/L	0,26 0,02	0,26 0,02	0,39 0,09	0,26 0,04	0,096	0,673	2,268	0,125	0,177	0,21 0,02
Acid Phosphatase, IU/L	30,3 1,8	40,2 2,2	36,0 5,7	33,2 9,6	0,096	0,673	2,270	0,125	0,181	31,4 1,9

Potassium Erythrocytes, mM/L	78 3	83 2	79 9	96 5	0,074	0,873	0,679	0,580	0,379	88 5
Sodium Plasma, mM/L	134,2 0,8	132,5 0,6	124,7 2,5	131,8 1,7	0,122	0,525	4,215	0,025	0,002	132,8 0,5
Chloride Plasma, mM/L	100,3 1,4	97,6 0,9	87,3 3,0	96,8 2,3	0,124	0,520	4,312	0,024	0,002	97,8 0,8
Phosphate Plasma, mM/L	1,15 0,08	1,25 0,03	1,22 0,10	1,29 0,03	0,115	0,558	3,695	0,038	0,044	1,32 0,02
q-T/R-R Ratio ECG	0,53 0,03	0,57 0,02	0,63 0,05	0,54 0,03	0,117	0,549	3,832	0,034	0,220	0,61 0,01
R wave ECG, µV	433 52	406 30	442 186	275 71	0,139	0,462	5,426	0,011	0,244	330 18
Injuries of Gastric Mucosa, points	0,41 0,07	0,29 0,05	0,20 0,15	0,11 0,05	0,135	0,477	5,117	0,013	0,087	0

Notes. In each column, the top row is the average, the bottom is the standard error. Testosterone and corticosterone levels normalized by sex (34,6±4,6 vs 3,93±0,34 nM/L and 340±45 vs 466±57 nM/L in intact male vs female respectively).

Table 4. Neuro-endocrine variables currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	**I** H^0S^0 **(11)**	**III** H^1S^0 **(25)**	**II** H^0S^{3+} **(3)**	**IV** $H^{2+}S^0$ **(9)**	Wilks' Λ •10^2	Partial Λ	F to enter	p-value	Tolerancy	Norm (10)
AMo HRV as Sympathetic tone, %	55 7	67 4	54 15	73 6	0,053	0,822	0,935	0,452	0,187	46 5
$(Ca•P)^{-0,5}$ as Calcitonin Activity	0,50 0,02	0,51 0,02	0,61 0,03	0,53 0,03	0,056	0,870	0,649	0,597	0,010	0,51 0,03
$(Nap/Kp)^{0.5}$ as Mineralocorticoid Activity	6,30 0,20	6,01 0,14	5,11 0,06	6,03 0,34	0,053	0,822	0,935	0,452	0,187	5,75 0,17
Adrenals Mass, mg	65 4	58 3	50 2	62 3	0,063	0,977	0,101	0,958	0,324	55 5

Table 5. Variables of the Thymus currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	**I** H^0S^0 **(11)**	**III** H^1S^0 **(25)**	**II** H^0S^{3+} **(3)**	**IV** $H^{2+}S^0$ **(9)**	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy	Norm (10)
Reticulocytes of Thymus, %	3,41 0,40	3,87 0,29	5,82 1,33	5,54 0,33	0,061	0,951	0,224	0,878	0,095	4,16 0,74
Macrophages of Thymus, %	5,91 0,44	6,88 0,40	6,33 0,33	7,00 0,37	0,057	0,890	0,538	0,664	0,127	5,39 0,50
Lymphocytes of Thymus, %	66,6 1,5	65,0 0,8	62,0 2,6	61,2 1,4	0,063	0,977	0,101	0,958	0,324	65,8 1,3
Lymphoblastes of Thymus, %	6,0 0,5	6,6 0,3	7,0 1,5	7,3 0,5	0,063	0,977	0,101	0,958	0,324	7,5 1,0
Epitheliocytes of Thymus, %	8,4 0,9	7,8 0,5	7,5 1,6	7,6 0,9	0,057	0,890	0,538	0,664	0,127	8,0 0,8
Basophiles of Thymus, %	2,64 0,45	2,80 0,31	2,33 0,88	4,33 0,54	0,053	0,822	0,935	0,452	0,187	2,78 0,39

Table 6. Variables of the Spleen currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	I H^0S^0 (11)	III $H^{1-}S^0$ (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy	Norm (10)
Spleen Mass, mg	668 41	663 29	807 80	753 35	0,057	0,890	0,538	0,664	0,127	773 58
Plasmocytes of Spleen, %	3,00 0,54	1,80 0,22	3,00 0,58	1,75 0,17	0,063	0,977	0,101	0,958	0,324	1,67 0,22
Lymphocytes of Spleen, %	70,6 2,3	66,7 1,1	67,3 3,5	66,3 1,1	0,063	0,977	0,101	0,958	0,324	68,4 1,6
Reticulocytes of Spleen, %	3,36 0,34	3,08 0,17	4,00 1,15	2,33 0,17	0,053	0,822	0,935	0,452	0,187	2,67 0,22
Rod-shaped Neutrophils of Spleen, %	1,91 0,34	2,04 0,16	1,00 0,00	1,67 0,17	0,053	0,822	0,935	0,452	0,187	1,78 0,26
Eosinophiles of Spleen, %	2,18 0,42	1,92 0,26	4,33 0,88	3,00 0,60	0,061	0,951	0,224	0,878	0,095	2,00 0,39
Entropy of Splenocytogram	0,517 0,028	0,559 0,012	0,567 0,035	0,576 0,012	0,057	0,890	0,538	0,664	0,127	0,533 0,019

Table 7. Variables of the Leukocytogram and Phagocytosis currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	I H^0S^0 (11)	III $H^{1-}S^0$ (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy	Norm (10)
Eosinophils of Blood, %	3,61 0,64	3,67 0,50	3,33 0,33	3,22 0,43	0,061	0,951	0,224	0,878	0,095	4,90 0,72
Rod-shaped Neutrophils, %	2,59 0,28	2,60 0,42	2,67 0,33	2,89 0,39	0,057	0,890	0,538	0,664	0,127	2,20 0,25
Polymorphonuclearry Neutrophils, %	40,2 2,7	40,3 1,1	37,0 2,6	38,9 2,0	0,063	0,977	0,101	0,958	0,324	34,7 1,1
Pan Lymphocytes of Blood, %	47,6 2,6	47,8 0,9	51,3 2,2	49,9 1,9	0,063	0,977	0,101	0,958	0,324	51,8 1,5
Monocytes of Blood, %	5,95 0,54	5,12 0,32	5,67 1,20	5,00 0,58	0,053	0,822	0,935	0,452	0,187	6,20 0,73
Entropy of Leukocytogram	0,669 0,013	0,663 0,008	0,669 0,014	0,660 0,012	0,053	0,822	0,935	0,452	0,187	0,682 0,003
Phagocytic Index of Neutrophils, %	57,3 2,4	56,4 1,7	60,3 4,3	56,3 3,0	0,053	0,822	0,935	0,452	0,187	55,2 1,8
Microbial Count of Neutrophils, Bac/Ph	5,8 0,2	6,1 0,2	7,2 0,2	6,5 0,3	0,063	0,978	0,097	0,960	0,044	5,5 0,3
Microbial Count of Monocytes, B/Ph	4,8 0,8	4,6 0,4	3,7 0,9	4,7 0,8	0,063	0,977	0,101	0,958	0,324	4,45 0,2

Table 8. Variables of the Immunocytogram currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	I H^0S^0 (11)	III $H^{1-}S^0$ (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy	Norm (10)
Theophylline-resistant T-Lymphoc, %	30,5 0,9	31,6 0,5	31,0 0,6	31,3 1,0	0,061	0,951	0,224	0,878	0,095	29,7 0,3
Theophylline-susceptible T-Lymph, %	13,6 0,9	13,0 0,6	13,0 0,6	13,4 1,2	0,057	0,890	0,538	0,664	0,127	15,3 1,1
B-Lymphocytes of Blood, %	12,3 0,9	12,6 0,4	12,3 0,3	12,4 0,9	0,053	0,822	0,935	0,452	0,187	13,4 0,8
Plasmocytes of Blood, %	0,06 0,06	1,12 0,33	0,00 0,00	0,19 0,19	0,057	0,890	0,538	0,664	0,127	0,40 0,26
NK-Lymphocytes of Blood, %	6,3 0,5	6,3 0,3	6,7 0,1	6,6 0,5	0,053	0,822	0,935	0,452	0,187	5,3 0,3
0-Lymphocytes of Blood, %	36,9 1,0	37,3 1,9	35,4 1,0	36,0 1,8	0,024	0,991	0,06	0,979	0,417	35,9 1,6
Entropy of Immunocytogram	0,792 0,010	0,815 0,008	0,799 0,004	0,800 0,010	0,063	0,977	0,101	0,958	0,324	0,807 0,009

Table 9. Metabolic variables currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	I H^0S^0 (11)	III $H^{1-}S^0$ (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy	**Norm (10)**
Alanine Aminotransferase, µKat/L	0,59 0,04	0,62 0,04	1,00 0,26	0,68 0,13	0,063	0,977	0,101	0,958	0,324	0,53 0,05
Alkaline Phosphatase, IU/L	331 29	426 50	514 35	465 36	0,057	0,890	0,538	0,664	0,127	418 51
Creatine Phosphokinase, IU/L	1,83 0,19	1,83 0,02	1,73 0,08	1,91 0,06	0,055	0,853	0,745	0,544	0,262	1,68 0,10
Superoxide Dismutase Erythrocytes, un/mL	59,5 4,2	63,1 2,8	45,3 1,3	59,2 5,5	0,053	0,822	0,935	0,452	0,187	61,8 5,4
Katalase Serum, µM/L•h	146 10	132 9	119 5	160 26	0,055	0,853	0,745	0,544	0,262	143 12
Katalase Erythrocytes, µM/L•h	245 19	220 13	227 6	286 33	0,061	0,942	0,264	0,850	0,428	227 17
Nonα-LP Cholesterol, mM/L	0,92 0,08	0,79 0,06	0,81 0,10	0,79 0,10	0,063	0,976	0,106	0,955	0,546	1,04 0,07
Calcium Plasma, mM/L	3,68 0,18	3,32 0,16	2,20 0,00	2,98 0,34	0,063	0,972	0,123	0,945	0,011	3,18 0,27
Potassium Plasma, mM/L	3,48 0,22	3,79 0,15	4,78 0,04	3,82 0,32	0,061	0,951	0,224	0,878	0,095	4,10 0,20
Sodium Erythrocytes, mM/L	24,0 3,1	26,1 2,1	27,4 1,7	24,0 4,4	0,057	0,890	0,538	0,664	0,127	27,4 3,0

Table 10. Variables of gastric mucosa injuries and EEG currently not in the model

	Clusters (n)				Parameters of Wilks' Statistics					
Variables	I H^0S^0 (11)	III $H^{1-}S^0$ (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy	**Norm (10)**
Gastric Ulcers Length, mm	5,4 1,3	2,7 0,6	1,7 1,7	0,6 0,3	0,063	0,979	0,094	0,962	0,050	0
Gastric Ulcers Amount	2,6 0,7	1,4 0,3	1,0 1,0	0,6 0,3	0,061	0,951	0,224	0,878	0,095	0
T wave ECG, μV	82 19	101 9	88 35	105 12	0,057	0,890	0,538	0,664	0,127	131 3
S-T joint ECG, μV	29 12	44 7	39 39	47 13	0,053	0,822	0,935	0,452	0,187	54 5
S wave ECG, μV	137 19	153 22	65 30	136 27	0,057	0,890	0,538	0,664	0,127	136 16
qRS interval ECG, msec	30,5 0,5	29,7 0,7	29,7 1,4	29,9 1,2	0,055	0,853	0,745	0,544	0,262	29,5 0,1
P-q interval ECG, msec	52,1 0,5	47,7 1,4	49,3 5,8	51,0 3,7	0,057	0,892	0,527	0,671	0,099	55,6 0,8

Table 11. Summary of Stepwise Analysis for Variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Hassal's corpuscles of Thymus, %	9,98	10^{-4}	0,595	9,98	10^{-4}
Killing Index of Neutrophils, %	7,61	10^{-3}	0,389	8,65	10^{-6}
Triglycerides, mM/L	5,24	0,004	0,283	7,74	10^{-6}
Macrophages of Spleen, %	3,94	0,015	0,220	7,01	10^{-6}
Lymphoblastes of Spleen, %	3,75	0,018	0,171	6,61	10^{-6}
α-LP Cholesterol, mM/L	3,44	0,026	0,135	6,32	10^{-6}
$(Ca/P)^{0,5}$ as Parathyroid Activity	4,02	0,014	0,103	6,31	10^{-6}
Potassium Erythrocytes, mM/L	2,96	0,045	0,083	6,11	10^{-6}
Triiodothyronine, nM/L	2,59	0,068	0,068	5,91	10^{-6}
Bactericidal Capacity of Neutrophils, 10^9 B/L	3,18	0,036	0,054	5,90	10^{-6}
Chloride Plasma, mM/L	2,67	0,063	0,043	5,81	10^{-6}
Corticosterone normalized by sex, Z	2,63	0,066	0,035	5,76	10^{-6}
Microphages of Spleen, %	3,85	0,018	0,026	5,98	10^{-6}
Phagocytic Index of Monocytes, %	2,78	0,058	0,020	6,01	10^{-6}
Sodium Plasma, mM/L	2,53	0,076	0,016	6,01	10^{-6}
R wave ECG, μV	2,85	0,055	0,012	6,10	10^{-6}
MxDMn HRV as Vagal tone, msec	1,67	0,196	0,011	5,95	10^{-6}
Fibroblastes of Thymus, %	1,68	0,194	0,009	5,83	10^{-6}
Phosphate Plasma, mM/L	1,47	0,246	0,008	5,68	10^{-6}
Injuries of Gastric Mucosa, points	1,78	0,178	0,006	5,62	10^{-6}
q-T/R-R Ratio ECG	1,54	0,230	0,005	5,52	10^{-6}
Thymus Mass, mg	1,97	0,146	0,004	5,54	10^{-6}
Testosterone normalized by sex, Z	1,83	0,171	0,003	5,53	10^{-6}
Diene conjugates, E^{232}/mL	1,90	0,160	0,003	5,56	10^{-6}
Bactericidal Capacity of Monocytes, 10^6 B/L	1,35	0,287	0,002	5,46	10^{-6}
Leukocytes of Blood, 10^9/L	1,16	0,351	0,002	5,32	10^{-6}
Asparagine Aminotranspherase, μKat/L	1,17	0,347	0,002	5,20	10^{-6}
Acid Phosphatase, IU/L	1,78	0,188	0,001	5,25	10^{-6}
Malondialdehyde, μM/L	1,15	0,360	0,001	5,13	10^{-6}
Mode HRV as Humoral Channel, msec	1,17	0,356	0,001	5,03	10^{-6}
Entropy of Thymocytogram	1,04	0,404	0,001	4,90	10^{-6}

Next, the 31-dimensional space of **discriminant variables** transforms into 3-dimensional space of a **canonical discriminant functions** (canonical roots), which are 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 (clusters) and a discriminant function. It is for Root 1 0,970 (Wilks' Λ=0,00064; $\chi^2_{(93)}$=217; $p<10^{-6}$), for Root 2 0,961 (Wilks' Λ=0,01084; $\chi^2_{(60)}$=133; $p<10^{-6}$), for Root 3 0,927 (Wilks' Λ=0,14022; $\chi^2_{(29)}$=58; p=0,0011). The first root contains 46,7% of discriminative opportunities, the second is 35,2% and the third 18,1%.

Table 12 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.

Table 12. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized			Raw		
Variables	**Root 1**	**Root 2**	**Root 3**	**Root 1**	**Root 2**	**Root 3**
Hassal's corpuscles of Thymus, %	-0,225	-1,535	-0,339	-0,442	-3,019	-0,666
Killing Index of Neutrophils, %	1,676	0,097	-0,155	0,194	0,011	-0,018
Triglycerides, mM/L	1,202	0,127	-0,553	16,39	1,727	-7,538
Macrophages of Spleen, %	0,900	0,816	0,159	0,930	0,844	0,164
Lymphoblastes of Spleen, %	1,301	1,184	-0,225	0,491	0,447	-0,085
α-LP Cholesterol, mM/L	-1,693	0,572	-0,670	-12,56	4,244	-4,964
$(Ca/P)^{0,5}$ as Parathyroid Activity	-2,701	0,932	2,760	-10,45	3,604	10,67
Potassium Erythrocytes, mM/L	0,379	0,377	-0,282	0,035	0,035	-0,026
Triiodothyronine, nM/L	-0,865	-1,602	-0,603	-1,130	-2,090	-0,790
Bactericidal Capacity of Neutrop, 10^9 B/L	-3,040	-0,293	1,098	-0,652	-0,063	0,236
Chloride Plasma, mM/L	-15,96	-2,559	2,714	-3,102	-0,497	0,527
Corticosterone normalized by sex, Z	-0,573	1,578	-0,861	-0,586	1,612	-0,879
Microphages of Spleen, %	-0,295	-1,095	0,465	-0,094	-0,347	0,148
Phagocytic Index of Monocytes, %	0,035	0,292	-0,777	0,019	0,160	-0,425
Sodium Plasma, mM/L	15,90	2,915	-2,852	4,456	0,817	-0,799
R wave ECG, μV	-0,980	-0,758	-0,946	-0,0055	-0,0042	-0,0053
MxDMn HRV as Vagal tone, msec	-2,306	-0,064	-1,029	-0,091	-0,002	-0,040
Fibroblastes of Thymus, %	0,822	-0,930	-0,964	0,536	-0,607	-0,629
Phosphate Plasma, mM/L	-3,184	0,553	-0,481	-18,85	3,271	-2,849
Injuries of Gastric Mucosa, points	-2,240	-0,199	-1,218	-9,710	-0,862	-5,279
q-T/R-R Ratio ECG	-1,128	-0,367	-0,919	-11,48	-3,734	-9,354
Thymus Mass, mg	-0,475	-0,269	-2,214	-0,012	-0,007	-0,057
Testosterone normalized by sex, Z	-0,438	0,074	2,790	-0,371	0,063	2,358
Diene conjugates, E^{232}/mL	-0,169	0,563	-0,826	-0,484	1,613	-2,366
Bactericidal Capacity of Monoc., 10^6 B/L	0,863	0,835	2,464	3,549	3,433	10,14
Leukocytes of Blood, 10^9/L	0,293	-0,583	-1,490	0,066	-0,132	-0,336
Asparagine Aminotranspherase, μKat/L	-0,411	-0,626	-1,242	-4,450	-6,773	-13,45
Acid Phosphatase, IU/L	-0,711	0,946	0,764	-0,071	0,095	0,077
Malondialdehyde, μM/L	0,945	-0,476	0,206	0,092	-0,047	0,020
Mode HRV as Humoral Channel, msec	1,293	0,439	0,800	0,043	0,015	0,027
Entropy of Thymocytogram	0,550	0,628	0,438	10,46	11,95	8,324
			Constants	-262,8	-66,98	78,22
			Eigenvalues	15,86	11,93	6,132
			Cumulative Proportion	0,467	0,819	1

The third discriminant parameter is the **full structural coefficients** (Table 13), 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 portion of information about the discriminant function (root) contained in this variable.

Then variables obtained after acute stress (SV) expressed as Z-scores calculated by formula:

Z = (SV/NV – 1)/Cv, where

NV is Norm (obtained from intact rats) Variable, Cv is Coefficient its variation in intact rats.

This approach allows us to compare the variables expressed in different units (μKat, %, nM/L, msec etc) in one scale.

Table 13 shows, in addition to those included in the model, extramodel variables, which still carry differentiating information.

Table 13. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables	Correlations Variables-Canonical Roots			I H^0S^0 (11)	III H^1S^0 (25)	II H^0S^{3+} (3)	IV $H^{2+}S^0$ (9)
Root 1 (46,7%)	**Root 1**	**Root 2**	**Root 3**	**-4,7**	-0,7	+2,6	**+6,8**
Injuries of Gastric Mucosa	**-0,113**	-0,002	0,011	**+0,46**	+0,33	+0,23	**+0,12**
Gastric Ulcers Length				**+1,57**	+0,80	+0,48	**+0,16**
Gastric Ulcers Number				**+1,40**	+0,75	+0,55	**+0,31**
R wave ECG	**-0,073**	-0,017	-0,058	**+1,08**	+0,80	+1,18	**-0,57**
T wave ECG				**-1,87**	-1,11	-1,64	**-0,97**
S-T joint ECG				**-0,98**	-0,36	-0,58	**-0,24**
Killing Index of Neutrophils	**0,171**	0,028	0,032	**-1,40**	-0,74	-0,66	**+0,50**
Macrophages of Spleen	**0,136**	0,081	-0,028	**-0,82**	+0,20	-0,22	**+1,26**
Microphages of Spleen	**0,021**	0,030	-0,025	**-0,50**	-0,13	-0,35	**-0,12**
Entropy of Thymocytogram	**0,094**	-0,030	-0,030	**-0,06**	+0,32	+1,19	**+1,35**
Macrophages of Thymus				**+0,33**	+0,95	+0,60	**+1,02**
Bactericidal Capacity of Neutrophils	**0,078**	0,037	0,015	**-0,03**	+0,43	+0,16	**+1,01**
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	**-0,108**	0,022	0,102	**+1,29**	+0,41	-0,85	-0,16
Calcium Plasma				**+0,57**	+0,16	-1,13	-0,23
Thymus Mass	**-0,082**	-0,029	-0,002	**+0,23**	-0,37	-0,25	**-1,08**
Phagocytic Index of Monocytes	**-0,108**	0,005	-0,015	**+0,45**	+0,09	-0,30	**-0,84**
Bactericidal Capacity of Monocytes	**-0,059**	-0,000	0,063	**+1,20**	+0,18	-0,74	-0,22
Phosphate Plasma	**0,064**	0,042	-0,035	**-2,56**	-1,02	-1,47	**-0,46**
Potassium Erythrocytes	**0,098**	0,036	0,047	**-0,57**	-0,32	-0,52	**+0,46**
MxDMn HRV as Vagal tone	**-0,078**	0,009	-0,004	**-0,66**	-0,75	-0,88	**-0,97**
Testosterone normalized by sex	**-0,048**	-0,039	0,032	**-0,26**	-0,88	-0,63	**-1,37**
1/Mo HRV as Circulating Catecholamines	**0,066**	0,057	-0,055	**+0,04**	+0,63	+0,43	**+0,74**
AMo HRV as Sympathetic tone				**+0,28**	+0,61	+0,25	**+0,84**
Root 2 (35,2%)	**Root 1**	**Root 2**	**Root 3**	-2,6	**+2,4**	**-10,1**	-0,2
Corticosterone normalized by sex	-0,036	**0,076**	-0,030	0,00	**+0,35**	**-0,58**	-0,40
Triiodothyronine	-0,000	**0,042**	-0,080	-0,38	**+0,27**	**-0,86**	-0,21

Lymphoblastes of Spleen	0,040	**0,139**	-0,111	-0,69	**+0,35**	**-0,57**	-0,07
Leukocytes of Blood	-0,019	**0,060**	0,018	+0,17	**+0,27**	**-0,32**	+0,11
Plasmocytes of Blood				-0,41	**+0,87**	**-0,47**	-0,24
Superoxide Dismutase Erythrocytes				-0,13	**+0,08**	**-0,97**	-0,16
Acid Phosphatase	0,004	**0,078**	-0,132	-0,19	**+1,48**	**+0,78**	+0,31
Theophylline-resistant T-Lymphocytes				+0,80	**+1,14**	**+0,78**	+0,98
Hassal's corpuscles of Thymus	-0,009	**-0,225**	-0,111	+1,67	**+0,83**	**+4,60**	+0,77
Malondialdehyde	0,104	**-0,139**	-0,103	-0,63	**-0,55**	**+0,89**	-0,12
Root 3 (18,1%)	**Root 1**	**Root 2**	**Root 3**	+2,6	-1,4	**-5,4**	+2,5
$(Cap•Pp)^{-0,5}$ as Calcitonin Activity				-0,08	+0,01	**+0,95**	+0,22
Potassium Plasma				-0,96	-0,48	**+1,07**	-0,43
Triglycerides	0,089	-0,008	**-0,215**	-1,53	+0,18	**+1,83**	-0,05
Alkaline Phosphatase				-0,54	+0,05	**+0,60**	+0,29
Reticulocytes of Thymus				-0,32	-0,12	**+0,71**	+0,59
Asparagine Aminotranspherase	0,021	-0,083	**-0,083**	+0,65	+0,65	**+2,59**	+0,73
Alanine Aminotranspherase				+0,39	+0,57	**+3,10**	+1,01
Diene conjugates	-0,011	-0,010	**-0,050**	+0,07	+0,17	**+0,47**	-0,09
Fibroblastes of Thymus	0,016	-0,001	**-0,036**	+0,02	+0,17	**+0,33**	+0,16
NK-Lymphocytes of Blood				+0,89	+0,92	**+1,29**	+1,17
Microbial Count of Neutrophils				+0,25	+0,58	**+1,55**	+0,93
Phagocytic Index of Neutrophils				+0,36	+0,22	**+0,90**	+0,20
α-LP Cholesterol	-0,012	0,034	**-0,183**	-0,98	-0,21	**+0,03**	-1,03
Sodium Erythrocytes				-0,36	-0,24	**+0,00**	-0,35
q-T/R-R Ratio ECG	0,019	-0,016	**-0,103**	-1,17	-0,63	**+0,26**	-0,91
Adrenals Mass				+0,69	+0,19	**-0,37**	+0,51
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity				+1,03	+0,48	**-1,20**	+0,53
Sodium Plasma	-0,079	0,100	**0,166**	+0,07	-0,01	**-0,39**	-0,05
Chloride Plasma	-0,075	0,087	**0,164**	+0,16	-0,02	**-0,69**	-0,07
Katalase Serum				+0,07	-0,28	**-0,62**	+0,45

In order to facilitate perception, individual variables are grouped into patterns. The first pattern combines variables that reflect stress-induced erosive-ulcerative damage to the gastric mucosa and dystrophic-necrotic damage to the myocardium (the negative sign for T wave and S-T joint is reversed for adequate damage assessment). As we can see (Fig. 3, Table 13), the maximum damage occurs in rats of the H^0S^0 cluster, while the minimum damage occurs in the rats of the $H^{2+}S^0$ cluster, and the $H^{1-}S^0$ and H^0S^{3+} clusters are located between them (though not on the same line). The next 4 patterns are almost mirror images of the damage pattern. One of them demonstrates that the most severe damage to cells of the mucosa and myocardium is accompanied by a maximum decrease in the content of potassium in erythrocytes (as a marker of general hypokaliumhistia) and phosphate in plasma, while in rats with minimal stress damage these parameters do not differ from those in intact animals. Such metabolic effects of acute stress are accompanied by congruent changes in the content of macro- and microphages in the spleen and the killing activity of blood neutrophils/microphages (Fig. 3, Table 13).

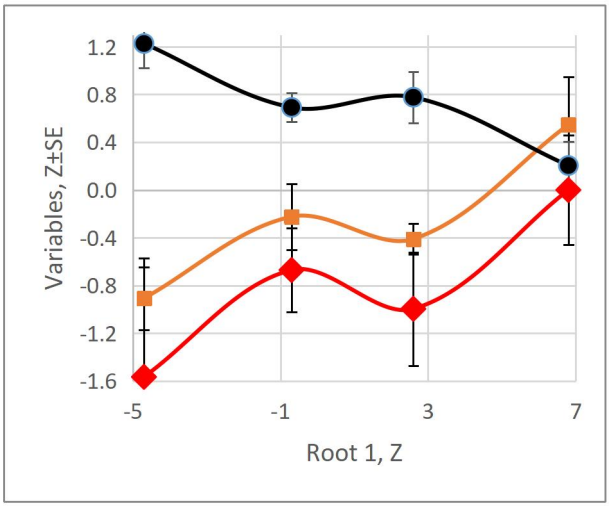


Fig. 3. The first series of patterns of parameters related to the first root.
Cluster localization order: H^0S^0, $H^{1-}S^0$, H^0S^{3+}, $H^{2+}S^0$. Damage to the gastric mucosa and myocardium (circles); the content of macro- and microphages in the spleen and the killing activity of blood neutrophils (diamonds); the content of phosphate in plasma and potassium in erythrocytes (squares)

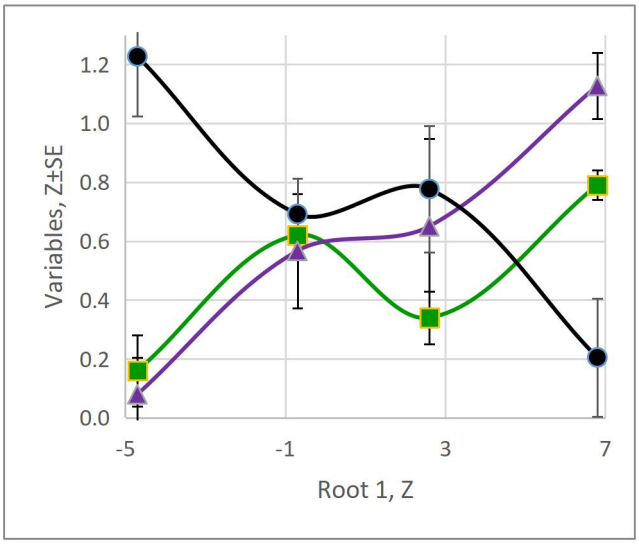


Fig. 4. The second series of patterns of parameters related to the first root.
Damage to the gastric mucosa and myocardium (circles); entropy of the thymocytogram and its content of macrophages aa well as bactericidal capacity of blood neutrophils (triangles); levels of sympathetic tone and circulating catecholamines (squares)

However, with maximum stress damage and inhibition of the killing activity of blood neutrophils, their bactericidal capacity remains normal due to an increase in the intensity of phagocytosis and the total content of neutrophils in the blood. On the other hand, these same parameters are due to the post-stress increase of bactericidal capacity of neutrophils in rats of other clusters above the normal level.

The following pattern shows that maximal stressor damage is accompanied by completely normal levels of sympathetic tone (marker – AMo HRV) and circulating catecholamines (marker – 1/Mode HRV), while with minimal damage sympatho-adrenomedullary activity is maximal (Fig. 4, Table 13).

The next two patterns, unlike the previous ones, reflect post-stressor changes that are unidirectional with the severity of stomach and heart damage (Fig. 5, Table 13). The first pattern illustrates that the most severe damage is accompanied by a minimal for the sample decrease in vagal tone in the absence of changes in the testosterone level. Less pronounced damage in rats of the next two clusters is accompanied by a significant decrease in the levels

of both neuro-endocrine factors, which reach a minimum in rats with minimal damage. The next pattern is not as congruent as the previous one. In particular, the most severe damage is accompanied by an increase in both parathyroid activity (assessed by an increase in the plasma level of calcium with a simultaneous decrease in the level of phosphate) and phagocytic activity of blood monocytes in the absence of a decrease in thymus mass. Lighter damage in $H^{1}S^{0}$ cluster rats is accompanied by normal levels of parathyroid and macrophage activity, while similar damage in $H^{0}S^{3+}$ cluster rats is accompanied by maximally reduced levels of these parameters, which, in turn, remain at the same low level in $H^{2+}S^{0}$ cluster rats with minimal post-stressor damage.

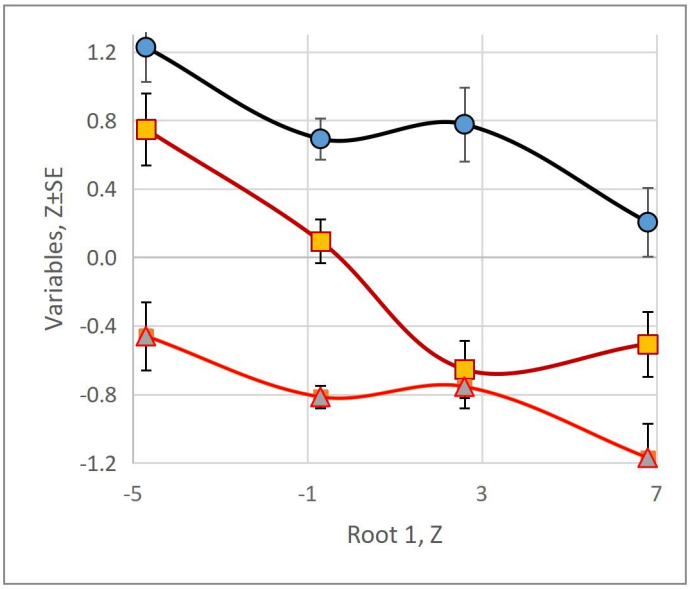


Fig. 5. The third series of patterns of parameter associated with the first root.
Damage to the gastric mucosa and myocardium (circles); parathyroid activity, thymus mass and bactericidal capacity of blood monocytes (squares); vagal tone and testosterone levels (triangles)

The variables associated with the second root are grouped into three patterns. The first pattern reflects the maximum post-stressor decrease in plasma levels of corticosterone and triiodothyronine in rats of the $H^{0}S^{3+}$ cluster, while in rats of the $H^{1}S^{0}$ cluster the levels of these hormones exceed those of intact animals. The second pattern reflects a similar trend of levels of superoxide dismutase in erythrocytes, leukocytes and plasma cells in the blood, as well as lymphoblastes in the spleen. Intermediate positions are occupied by rats of the other two clusters with quasi-normal levels of the listed parameters (Fig. 6, Table 13).

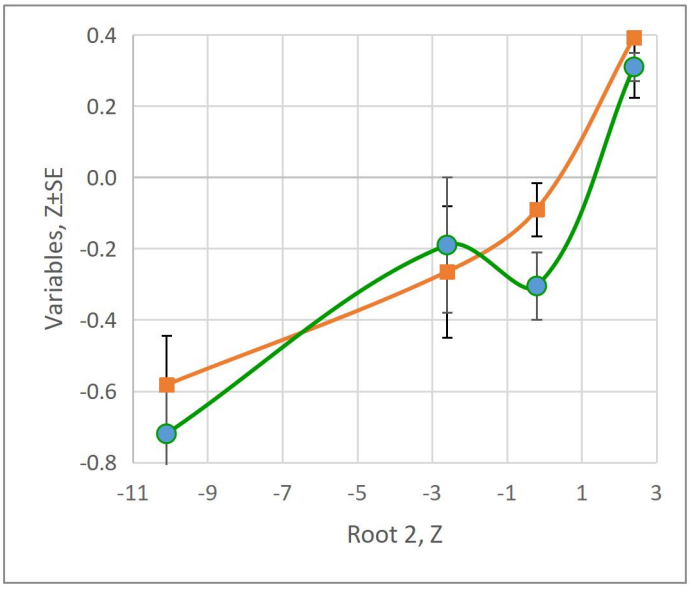


Fig. 6. Pattern of parameters directly related to the second root.
Cluster localization order: H^0S^{3+}, H^0S^0, $H^{2+}S^0$, $H^{1-}S^0$. Levels of corticosterone and triiodothyronine in the plasma (circles); SOD in erythrocytes, leukocytes and plasma cells in the blood, lymphoblastes in the spleen (squares)

The other two variables show the opposite trend (Fig. 7, Table 13).

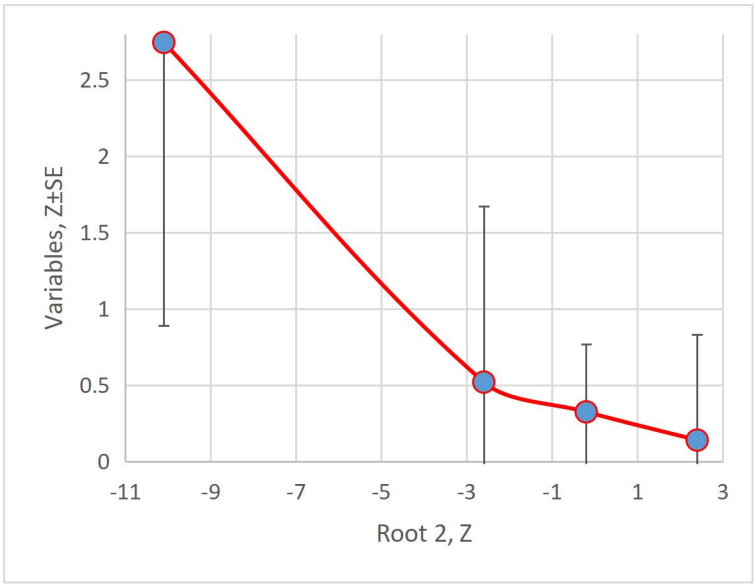


Fig. 7. Pattern of parameters inversely related to the second root.
Levels of malondialdehide in the plasma and Hassal's corpuscles in the tymus

Another four patterns are formed from 8 variables associated with the third discriminant root, as well as 12 variables not included in the discriminant model, but with similar trends (Figs. 8 and 9, Table 13).

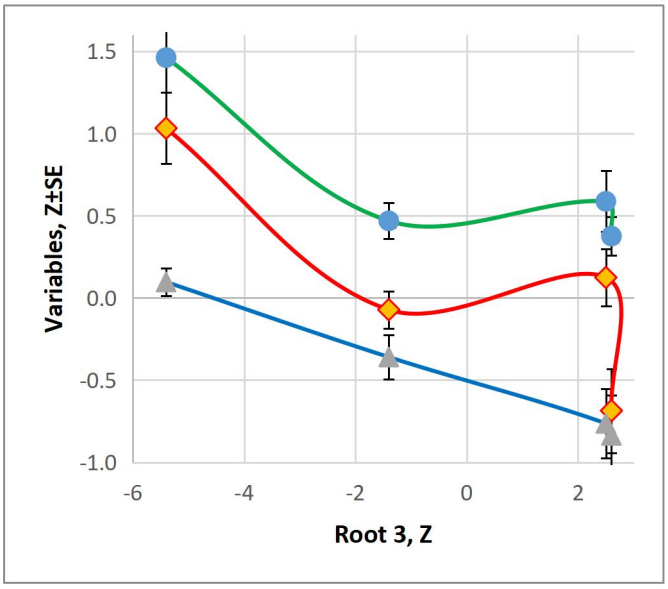


Fig. 8. Pattern of parameters inversely related to the third root
Cluster localization order: H^0S^{3+}, $H^{1-}S^0$, $H^{2+}S^0$, H^0S^0.

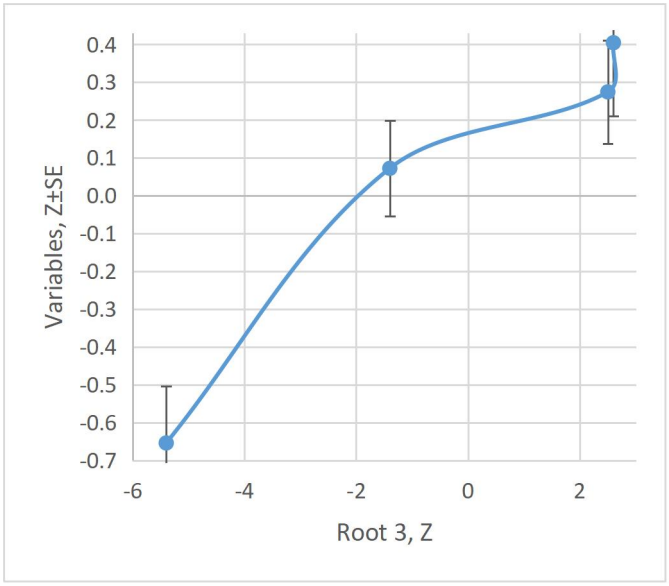


Fig. 9. Pattern of parameters directly related to the third root.
The mass of adrenal glands and their mineralocorticoid activity, levels of Na and Cl in plasma, catalase activity

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.

The localization of the members of the **first** cluster in the extreme left zone of the axis of the first root (Fig. 9) reflects their maximum sample levels of parameters that are inversely related to the root, and minimum sample levels of parameters that are directly related to the root. At the opposite pole of the first root axis, there are rats of the **fourth** cluster with the minimum/maximum levels of these parameters, respectively. The intermediate positions of the animals of the other two clusters reflect, as a rule, the intermediate levels of the same parameters. As you can see, the demarcation of all clusters is quite clear (only 4 rats out of 48 intersect).

Additional demarcation of the **second** and **third** clusters occurs along the axis of the second root.

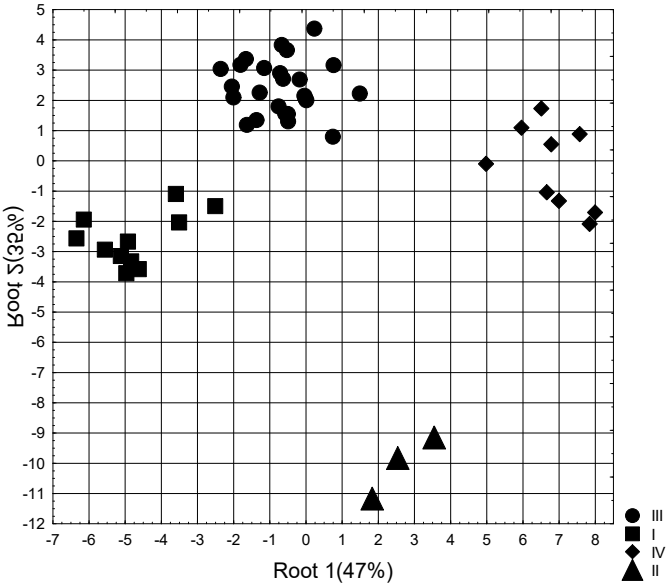


Fig. 9. Diagram of scattering of individual values of first and second Roots of rats of different clusters

The number of mixings of members of the **third** and **fourth** clusters along the axis of the second root decreases to two along the axis of the third root (Fig. 10).

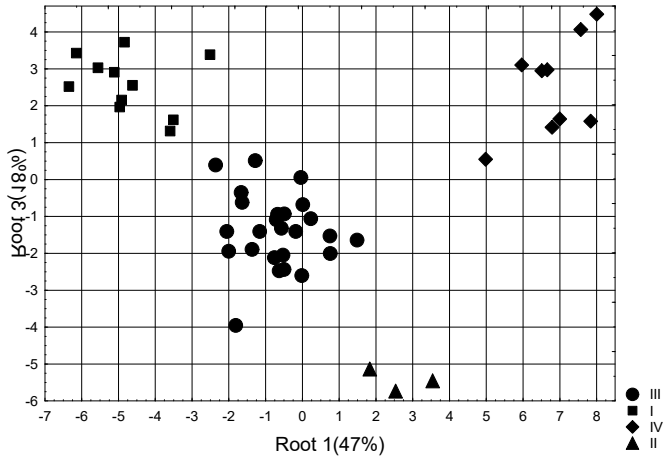


Fig. 10. Diagram of scattering of individual values of first and third Roots of rats of different clusters

The same discriminant parameters can be used to identify (classify) the belonging of one or another animal to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 15). 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.

Table 15. Coefficients and Constants for Classification Functions

Clusters	III $H^{1+}S^{0}$	I $H^{0}S^{0}$	IV $H^{2+}S^{0}$	II $H^{0}S^{3+}$
Variables	p=0,521	p=0,229	p=0,187	p=0,063
Hassal's corpuscles of Thymus, %	-199,8	-185,5	-197,7	-160,9
Killing Index of Neutrophils, %	31,65	30,73	33,00	32,22
Triglycerides, mM/L	9812	9706	9900	9875
Macrophages of Spleen, %	233,8	226,5	239,2	225,8
Lymphoblastes of Spleen, %	217,8	213,2	219,9	214,2
α-LP Cholesterol, mM/L	-3658	-3649	-3783	-3733
$(Ca/P)^{0,5}$ as Parathyroid Activity	-3944	-3877	-3990	-4067
Potassium Erythrocytes, mM/L	5,797	5,371	5,866	5,582
Triiodothyronine, nM/L	-577,7	-565,8	-583,7	-552,2
Bactericidal Capacity of Neutrophils, 10^9 B/L	-153,0	-149,1	-156,8	-155,4
Chloride Plasma, mM/L	-1794	-1777	-1814	-1800
Corticosterone normalized by sex, Z	251,2	242,0	239,1	232,7
Microphages of Spleen, %	-93,06	-90,35	-92,26	-89,64
Phagocytic Index of Monocytes, %	49,69	47,11	47,74	49,48
Sodium Plasma, mM/L	2628	2602	2656	2635
R wave ECG, μV	-1,906	-1,883	-1,956	-1,849
MxDMn HRV as Vagal tone, msec	-34,55	-34,33	-35,38	-34,65
Fibroblastes of Thymus, %	280,0	278,3	283,1	291,9
Phosphate Plasma, mM/L	-5402	-5354	-5563	-5494
Injuries of Gastric Mucosa, points	-2341	-2319	-2433	-2341
q-T/R-R Ratio ECG	-4507	-4479	-4619	-4460
Thymus Mass, mg	3,472	3,328	3,175	3,748
Testosterone normalized by sex, Z	-226,0	-215,4	-219,7	-237,5
Diene conjugates, E^{232}/mL	-363,3	-378,9	-380,4	-375,4
Bactericidal Capacity of Monocytes, 10^6 B/L	1185	1194	1242	1113
Leukocytes of Blood, 10^9/L	-37,64	-38,60	-38,12	-34,42
Asparagine Aminotranspherase, μKat/L	-2115	-2116	-2183	-1990
Acid Phosphatase, IU/L	-40,54	-40,42	-41,02	-42,27
Malondialdehyde, μM/L	41,91	41,85	42,81	42,72
Mode HRV as Humoral Channel, msec	22,05	21,91	22,43	21,90
Entropy of Thymocytogram	3366	3297	3445	3218
Constants	-84377	-82677	-85886	-84799

The accuracy of classification (retrospective recognition) is **100%**.

The apparent clear demarcation of clusters is documented by calculating Mahalanobis distances (Table 15).

Table 15. Squared Mahalanobis Distances between clusters (above the diagonal), F-values (df=31,1) and p-levels (under the diagonal)

Clusters	III	I	IV	II
III $H^{1+}S^{0}$	0	57,6	78,7	183
I $H^{0}S^{0}$	4,5 0,0022	0	139	175
IV $H^{2+}S^{0}$	5,3 0,0009	7,1 0,0002	0	178
II $H^{0}S^{3+}$	5,0 0,0012	4,2 0,0031	4,1 0,0036	0

At the final stage of the analysis, the role of muscular endurance and resistance to hypoxia in determining the post-stress state of immunity was clarified. For this, a correlation matrix was first created (Tables 16-18), and then regression models were built by stepwise elimination until the maximum Adjusted R^2 levels were reached.

Table 16. Matrix correlations between Swimming&Hypoxic testes and post-stress parameters currently not in the factor structure of canonical Roots

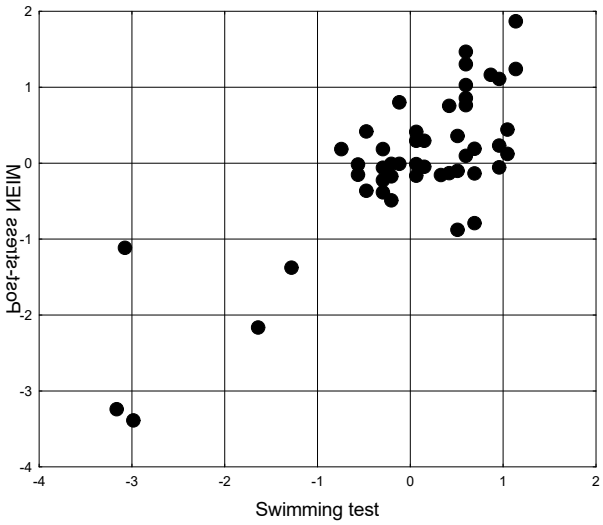
Variables	Swimming	Hypoxic
MxDMn HRV as Vagal tone	-0,05	-0,28
Testosterone actual	**0,29**	-0,09
Adrenals Mass	-0,28	0,14
Hassal's corpuscles of Thymus	**0,42**	-0,09
Rod-shaped Neutrophils of Spleen	**-0,37**	-0,15
Eosinophils of Spleen	**0,33**	0,16
Microbial Count of Neutrophils	**0,39**	0,12
Sodium Plasma	**-0,38**	-0,00
Chloride Plasma	**-0,37**	0,01
Malondialdehyde	**0,49**	0,13
Katalase Erythrocytes	-0,01	**0,30**

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 48 observations critical value of correlation coefficient module at $p<0,05$ ($t>2,01$) is 0,29, at $p<0,02$ ($t>2,40$) is 0,34, at $p<0,01$ ($t>2,68$) is 0,37.

Interestingly, variables with insignificant correlation coefficients entered the regression model of the swimming test, while 8 others were left out of the model despite a significant correlation with the test. In total, the swimming test determines the post-stress state of the registered parameters of the Immunity by 63,8% (Table 17 and Fig. 11).

Table 17. Regression Summary for Swimming test
$R=0,799$; $R^2=0,638$; Adjusted $R^2=0,541$; $F_{(10,4)}=6,5$; $p<10^{-5}$

N=48		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(37)}$	p-level
Variables	r		Intercpt	-12,2	20,6	-0,59	0,556
Alanine Aminotranspherase	**0,41**	0,161	0,139	7,06	6,13	1,15	0,257
Asparagine Aminotranspherase	**0,38**	0,236	0,148	28,03	17,55	1,60	0,119
Triglycerides	**0,39**	0,266	0,116	35,60	15,48	2,30	0,027
q-T/R-R Ratio ECG	0,25	0,138	0,107	15,83	12,29	1,29	0,206
Reticulocytes of Thymus	0,24	-0,142	0,120	-1,03	0,88	-1,18	0,247
Reticulocytes of Spleen	0,23	0,345	0,109	3,91	1,24	3,15	0,003
Corticosterone actual	**-0,43**	-0,319	0,108	-0,04	0,01	-2,96	0,005
S wave ECG	-0,27	-0,190	0,119	-0,02	0,01	-1,60	0,118
Phagocytic Index of Monocytes	-0,27	-0,237	0,108	-1,39	0,63	-2,20	0,034
Triiodothyronine	-0,26	-0,181	0,118	-3,33	2,17	-1,53	0,134



R=0,798; R^2=0,638; $\chi^2_{(10)}$=42; p<10^{-5}; Λ Prime=0,362

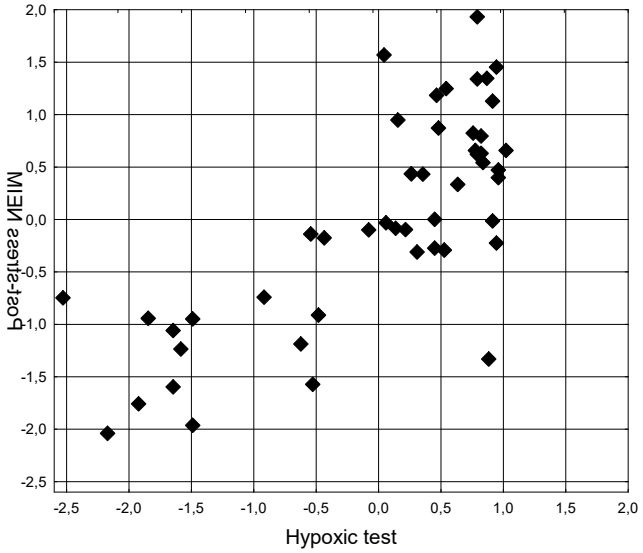
Fig. 11. Scatterplot of canonical correlation between Swimming test (X-line) and Post-Stress parameters (Y-line) in rats

The conditioning effect of innate resistance to hypoxia refers to the post-stress state of other parameters and, in general, is significantly inferior to such innate muscular endurance, accounting for only 57,5% (Table 18 and Fig. 12).

Table 18. Regression Summary for Hypoxic test

R=0,759; R^2=0,575; Adjusted R^2=0,513; $F_{(6,4)}$=9,3; p<10^{-5}

N=48		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(41)}$	p-level
Variables	r		Intercpt	7,5	68,8	0,11	0,914
α-LP Cholesterol	**-0,37**	-0,333	0,109	-150,7	49,5	-3,05	0,004
Plasmocytes of Blood	**-0,31**	0,418	0,104	2,67	0,67	4,01	10^{-3}
Acid Phosphatase	-0,21	-0,447	0,111	-22,28	5,54	-4,02	10^{-3}
Killing Index of Neutrophils	**0,34**	-0,171	0,110	-1,06	0,69	-1,55	0,129
P-q interval ECG	0,22	0,260	0,105	2,22	0,89	2,48	0,017
AMo as Sympathetic tone	0,22	0,366	0,114	1,10	0,34	3,21	0,003



R=0,759; R^2=0,575; $\chi^2_{(6)}$=37; p<10^{-5}; Λ Prime=0,425

Fig. 12. Scatterplot of canonical correlation between Hypoxic test (X-line) and Post-Stress parameters (Y-line) in rats

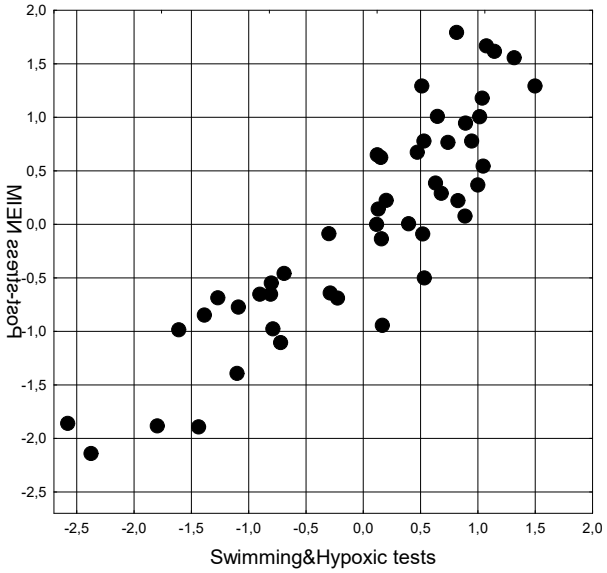
At the final stage, following the accepted algorithm, the canonical correlation between the two innate parameters of cardiorespiratory fitness (CRF), on the one hand, and the post-stressor parameters - on the other hand, is analyzed. As a result of canonical analysis, two pairs of canonical roots were formed.

The canonical root of the CRF of the first pair receives factor loadings with the same sign. The factor structure of the resulting root contains parameters that are subject to downregulation by **both CRF components**, by **swimming test only**, by **hypoxic test only**, upregulation by **both CRF components**, by **swimming test only**, by **hypoxic test only**.

Taken together, both innate factors of cardiorespiratory fitness determine the acute stress-induced changes in neuro-endocrine-immune complex and metabolome of rats by 79,1% (Table 19 and Fig. 13).

Table 19. Factor load on first pair of canonical roots

Left set	**Root 1**
Swimming test	**-0,649**
Hypoxic test	**-0,728**
Right set	**Root 1**
Phagocytic Index of Monocytes	**0,438**
Plasmocytes of Blood	**0,449**
Triiodothyronine	**0,364**
Corticosterone actual	**0,393**
S wave ECG	**0,284**
α-LP Cholesterol	**0,287**
Reticulocytes of Thymus	**-0,331**
Alanine Aminotranspherase	**-0,333**
Asparagine Aminotranspherase	**-0,245**
Triglycerides	**-0,225**
Killing Index of Neutrophils	**-0,347**
AMo as Sympathetic tone	**-0,145**



R=0,890; R^2=0,791; $\chi^2_{(32)}$=94; p<10^{-6}; Λ Prime=0,082

Fig. 13. Scatterplot of canonical correlation between Swimming&Hypoxic tests (X-line) and Post-Stress parameters (Y-line) in rats. First pair of Roots

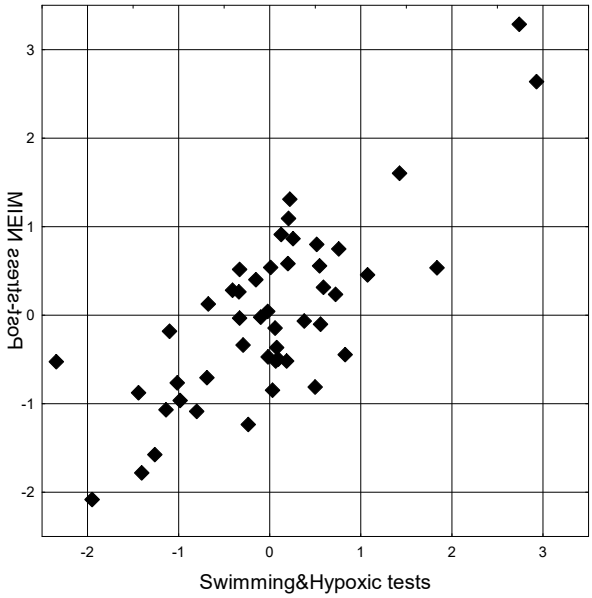
While the canonical root of the CRF of the second pair receives factor loadings with opposites signs. The factor structure of the resulting root contains parameters that upregulated

by **swimming test only** and **hypoxic test only**, in return downregulated by **swimming test only** and **hypoxic test only**.

Such a constellation of post-stress parameters is determined by innate factors of cardiorespiratory fitness by 60,8% (Table 19 and Fig. 13).

Table 20. Factor load on second pair of canonical roots

Left set	**Root 2**
Swimming test	0,761
Hypoxic test	-0,685
Right set	**Root 2**
Triglycerides	**0,438**
Asparagine Aminotranspherase	**0,409**
Alanine Aminotranspherase	**0,361**
Reticulocytes of Spleen	**0,390**
q-T/R-R Ratio ECG	**0,365**
P-q interval ECG	**-0,286**
AMo as Sympathetic tone	**-0,227**
Killing Index of Neutrophils	**-0,222**
Corticosterone actual	**-0,337**
S wave ECG	**-0,215**
α-LP Cholesterol	**0,347**
Acid Phosphatase	**0,284**



R=0,780; R^2=0,608; $\chi^2_{(15)}$=35; p=0,002; Λ Prime=0,392

Fig. 14. Scatterplot of canonical correlation between Swimming&Hypoxic tests (X-line) and Post-stress parameters (Y-line) in rats. Second pair of Roots

DISCUSSION

We found inter-individual variability, on the one hand, between innate two parameters of cardiorespiratory fitness (CRF), and responses of neuro-endocrine, immune, metabolic and ECGs parameters as well as markers of gastric mucosa damage - on the other hand. In addition, we have shown that both aerobic muscular endurance (to a greater extent) and resistance to hypoxia (to a lesser extent) determine not only the severity but also the direction of stress-induced reactions of the autonomic nervous and endocrine systems, which in turn

cause immunomodulation as well as damage to the myocardium and gastric mucosa, the severity of which differs significantly in rats of different clusters.

Our data, in principle, are consistent with existing provision that cardiorespiratory fitness (CRF), an objective and more reproducible measure, reflects the functional consequences of physical activity habits of the individual, and therefore may provide a better exposure with which to evaluate associations with relevant health outcomes.

Thus, low or unhealthy CRF is a strong, independent predictor of cardiovascular disease and all-cause mortality in adults. In youth, CRF is a predictor of a number of health indicators including cardiometabolic health and premature cardiovascular disease. Studies have investigated the relationship between CRF and various non-modifiable and modifiable factors including genetics, age, sex, race/ethnicity, physical activity and dietary patterns, obesity, sedentary time, built environment, and socioeconomics (Ross et al, 2016; Raghuveer G et al, 2020). Cheng et al (2000) have shown that active men had a significantly reduced risk for duodenal ulcers (relative hazard for the active group 0,38 vs 0,54 for the moderately active group). No association was found between physical activity and gastric ulcers for men or for either type of ulcer for women. Authors concluded that physical activity may provide a non-pharmacologic method of reducing the incidence of duodenal ulcers among men. Peel et al (2009) have found that a low level of fitness is an independent predictor of digestive cancer mortality and morbidity.

It is especially interesting to compare our data with the data of Lu et al (2019) who studied gastroprotective effects of the adaptogen Kangfuxin (KFX) against water-immersion and restraint stress (WIRS)-induced gastric ulcer in rats. They showed that pre-treatment with KFX could effectively reduce the area of gastric ulcers and improve the pathological changes of ulcerated tissue. Moreover, KFX increased the prostaglandin E2 (52%) and cyclooxygenase-1 (30%) levels, and improved malondialdehyde (54%), superoxide dismutase (58%), catalase (39%), and nitric oxide (11%) and TNF-α (9%), IL-6 (11%), MMP-9 (54%) and MMP-2 (53%) of ulcer tissue. Furthermore, pre-treatment with KFX dramatically increased IGF-1, PTEN, and Akt protein expression. Thus, results suggest that KFX has protective effects on WIRS-induced gastric ulcer via inflammatory reactions, oxidative stress inhibition, and pro-survival action.

In this regard, it is interesting to give the latest ideas about the mechanisms of WIRS-induced damage to the gastric mucosa (review: Zhao D. Q. et al, 2020). Some studies have found that RWIS leads to the elevation of blood corticosterone and adrenocorticotropic hormone levels in rats. This seems to indicate that the activity of the hypothalamic-pituitary-adrenal (HPA) axis is enhanced during RWIS. However, severing the subphrenic vagus nerves or consuming atropine can significantly alleviate and even cure RWIS-induced gastric mucosa lesion (GML), but removing the pituitary glands and adrenal glands or administering adrenergic α-receptor blocker has little impact on RWIS-induced GML, gastric hyperkinesia and RWIS-induced gastric acid secretion. This suggests that the HPA axis does not play a major role in RWIS-induced GML [on the contrary, Filaretova et al (1998; 2008) consider corticosterone a gastroprotective factor] and the peripheral nervous mechanism of RWIS-induced GML is mainly through the enhanced parasympathetic activity. Therefore, the nervous mechanism of RWIS-induced gastrointestinal dysfunction in rats is mainly the "enhanced activity of parasympathetic nervous system", rather than the traditional ideas of the "enhanced activity of sympathetic-adrenal medulla system" and "HPA axis". The dorsal vagal complex (DVC) and vagal efferent play an outstanding role in the regulation of gastric mucosal resistance to injury. However, the role of the vagal nerve is likely to be dual, as it can mediate both mucosal damaging and protective effects. Biochemical and pharmacological studies have demonstrated that the mechanisms of vagal-mediated gastroprotective effects

may be due to the activation of vagal cholinergic pathways, secretion of gastric prostaglandins and production of NO.

Gastrointestinal excitatory motor neurons release excitatory transmitters, such as ACh and SP, thus promoting gastrointestinal smooth muscle contraction and glandular secretion. On the contrary, inhibitory motor neurons release inhibitory transmitters, such as NO and VIP, thus suppressing gastrointestinal smooth muscle contraction and glandular secretion. All these gastrointestinal excitatory and inhibitory motor neurons can interact with each other under a complex and delicate balance. If this balance is broken, gastrointestinal dysfunction may be induced.

Previous studies have demonstrated that NO can inhibit gastric acid secretion and neutrophil adhesion, improve gastric mucosal blood circulation and eliminate oxygen free radicals, thereby protecting the gastric mucosa from injury. It was reported that the expression level of iNOS increased significantly in the gastric mucosa of RWIS rats, while that of eNOS reduced significantly, indicating that the changes in iNOS and eNOS activities in the gastric mucosa are closely related to the incidence of GML. In stress-induced GML, NOS inhibitor can decrease the production of NO, thus exacerbating acute GML and inhibiting the healing process of chronic gastric ulcers, while NO precursor can obviously prevent the injury. Thus NO is involved in RWIS, and can promote the GML healing process.

The mechanisms of NO in protecting gastric mucosa are as follows. NO can reduce vascular permeability, inhibit platelet adhesion and aggregation in gastric mucosal vascular endothelium, and prevent thrombosis. Under physiological conditions, gastric mucosal vascular endothelium synthesizes NO, which in turn regulates vascular smooth muscle tension and maintains GMBF. In acute GML, NO increases GMBF by dilating the mucosal blood vessels, thus promoting gastric mucosal repair. In addition, the secretion of gastric acid can also be inhibited by NO as well as endogenous NO can inhibit the stimulation of histamine through parietal cells, thus reducing gastric acid secretion and protecting gastric mucosa. Gastric mucous cells promote NO synthesis by expressing high-level NOS, and enhance the mucous barrier through the NO effects of promoting mucin synthesis and secretion. RWIS-induced GMLs can weaken the synthesis and secretion of gastric mucus by reducing nNOS activity, while the NO donor can increase nNOS activity and mucus secretion.

Gozhenko et al (2000) believe that among a number of factors involved in the pathogenesis of acute gastric injury, the main ones are the activation by glucocorticoids of gluconeogenesis in the cells of the gastric mucosa, accompanied by the breakdown of proteins and increased release of ammonia, which activates acid secretion together with the vagus, also disturbance of microcirculation in the stomach wall due to vasoconstriction, upregulated by the catecholamines while downregulated by the vagus, the mediator of which is NO. The validity of the participation of these mechanisms is confirmed by the authors' data on the increase in NO synthesis in the stomach wall (in microvessels and secretory epithelium) in patients with acute ulcers.

Returning to our results, we note that minimal RWIS-induced injuries to both gastric mucosa and myocardium were found in rats with maximum resistance to hypoxia ($H^{2+}S^{0}$), which is to be expected, whereas the most severe damage occurred unexpectedly in animals with a completely normal state of cardiorespiratory fitness ($H^{0}S^{0}$). Even more unexpected was the higher than in the previous cluster stress resistance of rats with minimal resistance to hypoxia at normal aerobic performance ($H^{1-}S^{0}$). And the combination of drastic “Ethiopian-Kenyan” duration of swimming to exhaustion with normal resistance to hypoxia ($H^{0}S^{3+}$) did not guarantee the “champion” stress resistance (only “silver” for the stomach and “bronze” for the myocardium).

However, this state of affairs is not accidental, because it is accompanied by specific post-stress changes in neuroendocrine and metabolic parameters. In particular, minimal RWIS-

induced injuries are accompanied by the lowest levels of plasma testosterone (but not corticosterone) and vagal tone in combination with the highest sympathetic tone and circulating catecholamines, while in the case of the most severe injuries, deviations of these tread parameters from the levels of intact animals are minimal or absent. On the other hand, the most severe injuries are accompanied by maximally increased mineralocorticoid and parathyroid activities, while with minimal damage their changes are insignificant.

In rats of the H^0S^{3+} cluster, the protective factors are a decrease in corticosterone levels as well as mineralocorticoid and parathyroid activities in combination with increase in calcitonin activity.

Our data are consistent with the provision that norepinephrine and dopamine are important endogenous inhibitory neurotransmitters that protect the integrity of gastric mucosa during stress. Zhao et al (2020) found that catecholaminergic neurons in the nucleus of the medullary visceral center participate in the regulation of RWIS-induced GML, whereas catecholaminergic neurons in the nucleus of the anterior hypothalamus are rarely or not involved. Therefore, the neurons responsible for RWIS are not located in the anterior hypothalamus, but instead the neuronal activity in the nucleus may be regulated by medullary catecholaminergic neurons.

In a study close to ours, Ordynskyi et al (2017; 2019) simulated **chronic** stress (4 times by an hour-long immobilization of rats the back down with an interval each 24 hours). In all groups of stressed animals, macroscopic damage to the gastric mucosa was noted, but the most vulnerable were low-resistance to hypoxic hypoxia (LRH) females. It was found that in control LRH male and female rats, compared with highly resistant to hypoxic hypoxia (HRH), is dominated by sympathetic tone. Under stress in males, the level of circulating catecholamines decreases, but sympathetic tone remains higher in LRH, and parasympathetic - in HRH. In LRH females under stress, an increase in circulating catecholamines and a decrease in vagus tone. In our study, post-stress levels of both circulating catecholamines and sympathetic tone were higher in HRH (IV cluster) than in LRH (III cluster) as well as vagal tone was lower.

Stress is known to suppress immune function and increase susceptibility to infections and cancer. Paradoxically, stress is also known to exacerbate asthma, and allergic, autoimmune and inflammatory diseases, although such diseases should be ameliorated by immunosuppression. Moreover, the short-term fight-or-flight stress response is one of nature's fundamental defense mechanisms that enables the cardiovascular and musculoskeletal systems to promote survival, and it is unlikely that this response would suppress immune function at a time when it is most required for survival (e.g. in response to wounding and infection by a predator or aggressor). These observations suggest that stress may suppress immune function under some conditions while enhancing it under others. Dhabhar (2009; 2018) propose that it is important to study and, if possible, to clinically harness the immunoenhancing effects of the acute stress response, that evolution has finely sculpted as a survival mechanism, just as authors study its maladaptive ramifications (chronic stress) that evolution has yet to resolve. In view of the ubiquitous nature of stress and its significant effects on immunoprotection as well as immunopathology, it is important to further elucidate the mechanisms mediating stress-immune interactions and to meaningfully translate findings from bench to bedside.

In this study, as part of the discussion about the role of ***causes and conditions*** in pathogenesis/sanogenesis (Gozhenko, 2010), we showed that acute stress causes both adverse and favorable effects on the parameters of immunity, which are conditioned by innate two parameters of cardiorespiratory fitness. However, their conditioning effect is ambiguous.

In particular, significantly **increased** resistance to hypoxia in rats of the fourth cluster conditions a significant stress-induced increase in the content of neutrophils both in the

leukocytogram (+1,23 Z) and in the blood (the content of leukocytes does not change) in combination with increased intensity (+0,93 Z) and improvement of completeness (+0,50 Z) of the phagocytic function of neutrophils, which ultimately gives an increase in their bactericidal ability at least against gram-positive bacteria (+1,01 Z). At the same time, the bactericidal ability of blood monocytes shows a tendency even to decrease (-0,22 Z) due to the predominance of a decrease in the activity of phagocytosis (-0,80 Z) over an increase in its intensity (+0,28 Z). However, it should be borne in mind that the lion's share of monocytes/macrophages is localized in tissues, in particular in the spleen, the mass of which does not change, but there is an increase in the content of macrophages in the splenocytogram (+1,26 Z). Therefore, in rats of this phenotype (at least 1/5 of our sample), increased resistance to hypoxia with normal muscular endurance ($H^{2+}S^{0}$) is associated with their ability to respond to acute stress by increasing the bactericidal capacity of blood neutrophils and tissue macrophages as well as blood NK lymphocytes (+1,17 Z). At the same time, in such animals, acute stress reduces the mass of the thymus (-1,08 Z), but the level of macrophages in the thymocytogram increases significantly (+1,02 Z), as well as basophils (+1,27 Z), Hassal's corpuscles (+0,77 Z) and reticulocytes (+0,59 Z), which apparently reflects the activation of immunogenesis. Additional evidence is an increase in the content of T-helper lymphocytes in the blood (+0,98 Z).

Instead, moderately **reduced** resistance to hypoxia with normal muscular endurance in rats of the third cluster (half of the sample) conditions a stress-induced decrease in the killing index of neutrophils (-0,74 Z), which, despite an increase in their content in the blood and an increase in the intensity of phagocytosis, leads to only a moderate increase their bactericidal capacity (+0,43 Z). The bactericidal capacity of blood monocytes, as in the previous cluster, does not change (+0,18 Z). Instead, the mass of the spleen (-0,60 Z) and the absolute (but not relative) content of macrophages decrease moderately. On the other hand, this phenotype prevents a post-stress reduction in the mass of the thymus, while the content of macrophages and Hassal's corpuscles, but not reticulocytes and basophils, increases in the thymocytogram, as in the previous cluster. This is accompanied by the maximum for the sample increase in the blood content of T-helpers (+1,14 Z) as well as moderately increase in NK lymphocytes (+0,92 Z).

Drastic (+2,71 Z) duration of swimming to exhaustion is associated with an even more drastic (+4,60 Z) post-stress increase in the thymocytogram the level of Hassal's corpuscles, as well as, to a lesser extent, reticulocytes (+0,71 Z) and macrophages (+0,60 Z) in the absence of a significant change in thymus mass as well as **minimum** for the sample increase in the blood content of T-helpers (+0,78 Z) while maximally increase in NK content. The mass of the spleen also does not change significantly, but in the splenocytogram the content of plasma cells increases (+1,89 Z), instead, the content of lymphoblasts decreases (-0,57 Z) and there is a tendency to decrease in the content of microphages (-0,35 Z) and macrophages (-0,22 Z). The described post-stressor changes in the cytoarchitecture of the thymus and spleen are accompanied by a decrease in the intensity (-1,03 Z) and activity (-0,30 Z) of the phagocytic function and bactericidal capacity (-0,74 Z) of blood monocytes. At the same time, the bactericidal ability of blood neutrophils does not change, because the increase in the content of neutrophils in it and the intensity of phagocytosis (+1,55 Z) compensates for the deterioration of its completion (-0,66 Z). Regarding the validity of the conclusions, one should bear in mind the small number of members of this cluster (and on the other hand, there are also very few endurance champions).

Finally, in rats of the first cluster with normal, both resistance to hypoxia and muscular endurance, in response to acute stress, the bactericidal ability of blood neutrophils does not change, because the increase in the content of neutrophils in it is leveled by the weakening of the killing function (-1,40 Z). Instead, the level of natural killers (+0,89 Z) and bactericidal ability of blood monocytes/macrophages increases (+1,20 Z) due to an increase in both activity (+0,45 Z) and intensity (+0,48 Z) of phagocytosis without increasing their total content. At the same time, the content of macrophages in the splenocytogram decreases (-0,82 Z), as well as microphages (-0,50 Z), and taking into account the decrease in the mass of the spleen (-0,57 Z), the total number of both types of phagocytes in it decreases even more. The content of lymphoblasts in the spleen also decreases, instead, the content of plasma cells increases. At the same time, the mass of the thymus does not change, while the content of Hassal's corpuscles in the thymocytogram increases, but other form elements do not change significantly.

So, the features of the emergency response of the immune system to **acute** stress are determined by the features of the innate state of muscular endurance and resistance to hypoxia. A similar polyvariant was previously discovered by us regarding the immune response to **chronic** stress in rats (Polovynko et al., 2016; 2016a; 2016b; Zajats et al., 2017; 2017a; Popovych et al., 2020) and humans (Lukyanchenko et al., 2019).

Regarding ***entropy***, which is a special subject of research in our laboratory (Flyunt et al., 2008; Kostyuk et al., 2007; Gozhenko et al., 2021; Popovych et al., 2020), it is interesting to note the significant post-stress increase in entropy of the thymocytogram in rats of the second and fourth clusters, that is, with extreme levels of muscular endurance or resistance to hypoxia.

Given the well-documented neuro-endocrine-immune interrelationships (Gozhenko et al., 2021; Khaitov, 2005; Korneva, 2020; Mel'nyk et al., 2019; 2021), the features of the immune response to acute stress revealed in this study are undoubtedly related to the features of autonomic and endocrine reactions.

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 carry out of experiments was approved by the Ethics Committee of the Ukrainian Scientific Research Institute of Medicine of Transport (protocol No35; 05.10.2022). 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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