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ROLE OF THE LIPID PEROXIDATION IN IMMUNOMODULATING EFFECTS OF THE NITROGENOUS METABOLITES IN RATS

Anatoliy I. Gozhenko[1], Igor L. Kuchma[1], Sofiya V. Ruzhylo[2], Galyna Y. Kovalchuk[2], Walery Zukow[3], Igor L. Popovych[1,4]

[1]Ukrainian Scientific Research Institute for Medicine of Transport, Odesa, Ukraine prof.gozhenko@gmail.com; igorkuchma@ukr.net;
[2]Ivan Franko Pedagogical University, Drohobych, Ukraine doctor-0701@ukr.net
[3]Nicolaus Copernicus University, Torun, Poland w.zukow@wp.pl
[4]Bohomolets' OO Institute of Physiology of National Academy of Sciences, Kyïv, Ukraine i.popovych@biph.kiev.ua

Abstract

Background. We have previously shown that nitrogenous metabolites have immunomodulatory effects, but the question of mediators of the immunomodulation remains open. We hypothesized the mediating role of mediators of the autonomic nervous system and adaptation hormones as well as reactive oxygen species. Mediating role in the immunomodulation of neuroendocrine factors is analyzed in a previous article. The **aim** of this study is to analyze the relationships between the parameters of nitrogenous metabolites and lipid peroxidation as well as between latter and immune parameters subordinate modulations by nitrogenous metabolites. **Material and methods**. Experiment was performed on 60 healthy female Wistar rats. The plasma level and urinary excretion of the nitrogenous metabolites as well as parameters of lipid peroxidation (diene conjugates, malonic dyaldehid, superoxide dismutase, catalase) and neuroendocrine-immune complex were determined. **Results**. According to the canonical correlation analysis, the constellation of nitrogenous metabolites determines the state of lipid peroxidation by 38,8%. The latter, in turn, determines the constellation of immune parameters (subject to modulation by nitrogenous metabolites) by 61,4%. On the other hand, the coefficient of determination between nitrogenous metabolites and neuroendocrine parameters is 71,5%, and between the latter and immune status – 89,6%. Taken together, neuroendocrine parameters and lipid peroxidation parameters determine the pool of immune parameters subject to modulation by nitrogenous metabolites by 96,7%. It was previously shown that the coefficient of determination between nitrogenous metabolites and a number of immune parameters is 95,8%. **Conclusion**. The obtained results, taken together with the previous ones, prove that uric acid, bilirubin, urea and creatinine realize

their immunomodulatory effects both directly through receptors of immunocytes (aryl hydrocarbon, adenosine and TL) and with the participation of mediators of autonomic nervous and endocrine systems and lipid peroxidation.

Key words: uric acid, creatinine, urea, bilirubin, lipid peroxidation, ANS, hormones, immunity, relationships, rats.

INTRODUCTION

We have previously shown that nitrogenous metabolites have immunomodulatory effects, both in healthy rats [7,8,25] and in humans exposed to pathogenic influences [9,10,15,29]. The immunomodulatory effect of bilirubin is probably mediated through aryl hydrocarbon receptors, and uric acid through TL- and adenosine receptors of immune cells. The question of mediators of the immunomodulatory action of urea and creatinine remains open. Standing on the positions of the concepts of functional-metabolic continuum [6] and neuroendocrine immunomodulation [11,18-20,22,24,26-28,30-32] we hypothesized the mediating role of mediators of the autonomic nervous system and adaptive hormones. In an experiment on rats, we showed that the modulating effects of nitrogenous metabolites on neuroendocrine parameters are quite pronounced and almost identical in terms of bilirubin (R=0,603), creatinine (R=0,602), uric acid (R=0,599) and urea (R=0,586). Taken together, nitrogenous metabolites determine neuroendocrine parameters by 71,5% (R=0,845). Triiodothyronine, fascicular and medullar areas of the adrenal glands, vagal tone and calcitonin activity were the most susceptible to nitrogenous metabolites. In turn, neuroendocrine parameters determine the parameters of immunity, subject to exposure to nitrogenous metabolites, by 95,8% (R=0,979) [17]. Thus, immunomodulatory effects of nitrogenous metabolites are realized, perhaps, through the factors of the autonomic nervous and endocrine systems.

In a parallel study in this project, we showed that in patients with postradiation encephalopathia constellation of nitrogenous metabolites (primarily plasma urea and creatinine) determines the state of lipids peroxidation by 44,0% (R=0,663). The inclusion in factor structure of canonical correlation between the HRV markers of ANS and parameters of Immunity the parameters of lipids peroxidation increases the degree of determination from 89,6% (R=947) to 94,6% (R=0,973) [14,16]. Therefore, lipids peroxidation plays a role in the mechanism of the immunomodulatory effect of nitrogenous metabolites.

The **aim** of this study is to analyze the relationships between the parameters of nitrogenous metabolites and lipids peroxidation as well as between latter and immune parameters subordinate modulations by nitrogenous metabolites.

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 assessed the state of autonomous regulation by the parameters of the HRV [2]. Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible. The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine and testosterone (by the ELISA [12]); as well as parameters of lipids peroxidation: diene

conjugates (spectrophotometry of the heptane phase of the lipids extract [4]) and malonic dyaldehid (in the test with thiobarbituric acid [1]), antioxidant enzymes: superoxide dismutase erythrocytes (according to the degree of inhibition of reduction of nitroblue tetrazolium in the presence of N-methylphenazonium metasulphate and NADH [3,20]) and catalase plasma (at the rate of decomposition of hydrogen peroxide [13]). Electrolytes: calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), sodium and potassium (flamming photometry) were determined in plasma and daily urine. The analyzes were carried out according to the instructions described in the manual [5].

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 [11,26].

In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope [11,26].

Methods for determining nitrogenous metabolites and immune parameters are given in previous article [25].

Digital material is statistically processed on a computer using the software package "Statistica 20".

RESULTS AND DISCUSION

In the first stage, the screening of the links between nitrogenous metabolites, on the one hand, and the parameters of lipid peroxidation, on the other hand, was performed (Table 1).

Table 1. Correlation matrix for nitrogenous metabolites and Lipid Peroxidation parameters

Variable	Cr Ex	Cr P	Urea Ex	UA Ex	Bilir	Urea P	UA P
SOD	-0,21	0,35	-0,23	-0,03	-0,03	0,17	-0,23
Katalase	0,16	0,32	0,43	0,26	0,42	0,25	-0,04
MDA	-0,14	0,14	-0,07	0,23	0,10	-0,03	0,28
DC	-0,08	-0,11	0,11	0,10	-0,08	-0,24	0,41

Based on the obtained matrix, regression models were further created by stepwise exclusion to reach the maximum level of Adjusted R^2.

It was found that the activity of plasma Catalase is determined by nitrogenous metabolites by 29,9% (Table 2), and erythrocyte Superoxide dismutase by only 18,3% (Table 3).

Table 2. Regression Summary for Katalase Plasma
$R=0,589$; $R^2=0,347$; Adjusted $R^2=0,299$; $F_{(4,6)}=7,3$; $p<10^{-4}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	0,060	0,017	3,51	0,001
Urea Excretion, µM/24h•100 g	**0,43**	0,360	0,115	0,00010	0,00003	3,13	0,003
Bilirubinemia, µM/L	**0,42**	0,280	0,117	0,0061	0,0026	2,39	0,020
Creatininemia, mM/L	**0,32**	0,391	0,201	0,547	0,281	1,94	0,057
Urea Plasma, mM/L	**0,25**	-0,206	0,202	-0,0032	0,0031	-1,02	0,313

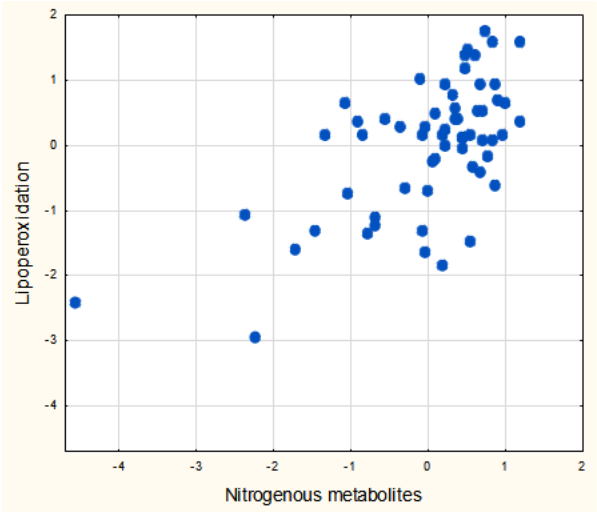
Table 3. Regression Summary for SOD Erythrocytes
R=0,488; R^2=0,239; Adjusted R^2=0,183; $F_{(4,6)}$=4,3; p=0,004

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	55,2	4,70	11,8	10^{-6}
Creatininemia, mM/L	**0,35**	0,641	0,216	192,5	64,9	2,96	0,004
Urea Plasma, mM/L	**0,17**	-0,375	0,221	-1,232	0,727	-1,69	0,096
Urea Excretion, µM/24h•100 g	**-0,23**	-0,199	0,122	-0,011	0,007	-1,63	0,109
Uricemia, µM/L	**-0,23**	-0,142	0,126	-0,003	0,003	-1,13	0,262

Lipid peroxidation products are even less bound to nitrogenous metabolites. In general, nitrogenous metabolites determine the constellation of lipoperoxidation parameters by 38,8% (Table 4 and Fig. 1).

Table 4. Factor load on canonical roots of nitrogenous metabolites (left set) and Lipid Peroxidation parameters (right set)

Left set	**Root 1**
Urea Excretion, µM/24h•100 g	**-0,942**
Bilirubinemia, µM/L	**-0,432**
Uricemia, µM/L	**-0,248**
Creatininemia, mM/L	**0,132**
Right set	**Root 1**
Katalase, nM/h•mL	**-0,659**
Superoxide Dismutase, un/mL	**0,520**
Diene conjugates, E^{232}/mL	**0,070**
Malonic Dyaldehid, nM/mL	**0,065**



R=0,623; R^2=0,388; $\chi^2_{(20)}$=65; p=10^{-6}; Λ Prime=0,301
Fig. 1. Scatterplot of canonical correlation between the nitrogenous metabolites (X-line) and lipid peroxidation parameters (Y-line) in female rats

In the future, a matrix for Lipid Peroxidation and Immunity is created (Table 5).

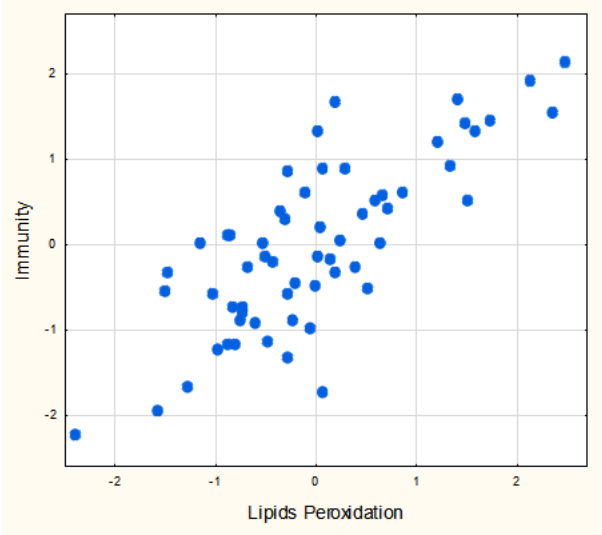
Table 5. Correlation matrix for Lipid Peroxidation and Immunity parameters (only subordinate modulations by nitrogenous metabolites are included)

Root Variable	Correlations			
	SOD	Katal	MDA	DC
MCN	0,029	0,253	0,334	0,184
PhIM	-0,205	-0,117	-0,025	-0,053
PhIN	-0,046	0,190	0,091	0,149
Spleen MI	-0,023	0,006	-0,050	-0,057
LbS	-0,027	0,373	0,112	0,216
MacPhaS	-0,041	-0,231	0,018	-0,156
MicPhaS	0,142	-0,222	-0,255	-0,105
EosS	-0,101	-0,150	0,022	-0,039
FibrS	-0,158	-0,110	-0,026	0,023
LCT	0,039	0,233	0,076	0,147
LbT	0,158	0,291	0,133	-0,066
RetT	-0,048	-0,138	-0,102	-0,009
EndT	-0,250	0,029	0,061	0,077
MacPhT	-0,209	0,208	-0,094	-0,104
HassT	-0,051	0,074	0,067	-0,028
StubN B	0,053	0,111	-0,023	0,001
Eos B	0,053	0,088	-0,236	-0,182
Mon B	0,101	-0,314	-0,475	-0,299
Leukoc B	0,211	-0,125	0,045	0,055
Th B	0,295	0,215	0,012	-0,050
NK B	0,036	-0,326	-0,381	-0,227
H LCG	-0,041	-0,320	-0,112	0,093
H SCG	-0,052	0,218	-0,043	-0,086

Canonical analysis shows that the constellation of lipid peroxidation parameters determines the constellation of immunity parameters subject to modulation by nitrogenous metabolites by 61,4% (Table 6 and Fig. 2).

Table 6. Factor load on canonical roots of Lipid peroxidation (left set) and Immunity (right set)

Left set	Root 1
Katalase, nM/h•mL	**0,668**
Malonic Dyaldehid, nM/mL	**0,643**
Diene conjugates, E^{232}/mL	**0,401**
Superoxide Dismutase, un/mL	**-0,478**
Right set	**Root 1**
Monocytes Blood, %	**-0,626**
Natural Killers Blood, %	**-0,525**
Microbial Count Neutrophils	**-0,437**
Entropy Leukocytogram	**-0,284**
Leukocytes Blood, 10^9/L	**-0,215**
Macrophages Spleen, %	**-0,141**
Eosinophiles Blood, %	**-0,127**
Th Lymphocytes Blood, %	-0,041
Eosinophiles Spleen, %	-0,029
Lymphoblastes Spleen, %	**0,384**
Macrophages Thymus, %	**0,245**
Endotheliocytes Thymus, %	**0,242**
Phagocytic Index Neutrophils, %	**0,241**
Reticulocytes Thymus, %	**0,229**
Lymphocytes Thymus, %	**0,204**
Lymphoblastes Thymus, %	**0,192**
Entropy Splenocytogram	**0,172**
Hassal's corpuscles Thymus, %	**0,138**
Stub Neutrophils Blood, %	0,076
Spleen Mass Index, g/100g	0,063
Phagocytic Index Monocytes, %	0,039
Fibroblastes Spleen, %	0,013



R=0,783; R^2=0,614; $\chi^2_{(76)}$=110; p=0,006; Λ Prime=0,096

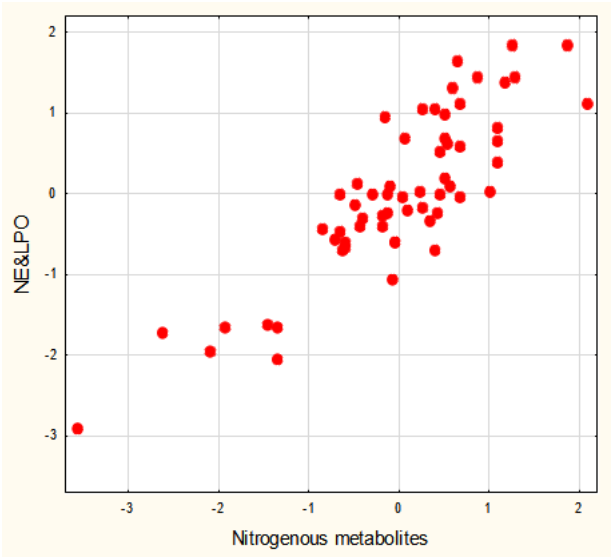
Fig. 2. Scatterplot of canonical correlation between the Lipids Peroxidation parameters (X-line) and Immunity parameters (Y-line) in female rats

As a result of the analysis of the canonical correlation between nitrogenous metabolites and all presumed mediators of their immunomodulatory effects, katalase, malonic dyaldehid and diene conjugates appeared in the constellation of parameters of the first neuroendocrine

root, subordinate to upregulation by bilirubin, uric acid, urea urine and creatinine plasma while downregulation by creatinine urine (Table 7 and Fig. 3).

Table 7. Factor load on first canonical roots of nitrogenous metabolites (left set) and neuroendocrine and lipid peroxidation parameters (right set)

Left set	**Root 1**
Bilirubinemia, µM/L	**-0,486**
Uricosuria, µM/24h•100 g	**-0,458**
Urea Excretion, µM/24h•100 g	**-0,422**
Uricemia, µM/L	**-0,181**
Creatininemia, mM/L	**-0,159**
Creatinineuria, µM/24h•100 g	**0,309**
Right set	**Root 1**
Triiodothyronine, nM/L	**0,747**
Fascicular ZAC, µM	**0,674**
Mineralocorticoid Activity	**0,414**
Testosterone, nM/L	**0,273**
Reticular ZAC, µM	**0,263**
Glomerular ZAC, µM	**0,170**
Medullar ZA, µM	**-0,657**
Katalase, nM/h•mL	**-0,494**
Calcitonin Activity	**-0,451**
MxDMn HRV, msec	**-0,408**
Malonic Dyaldehid, nM/mL	**-0,316**
Diene conjugates, E^{232}/mL	**-0,276**
Adrenals Mass, mg/100 g	**-0,118**



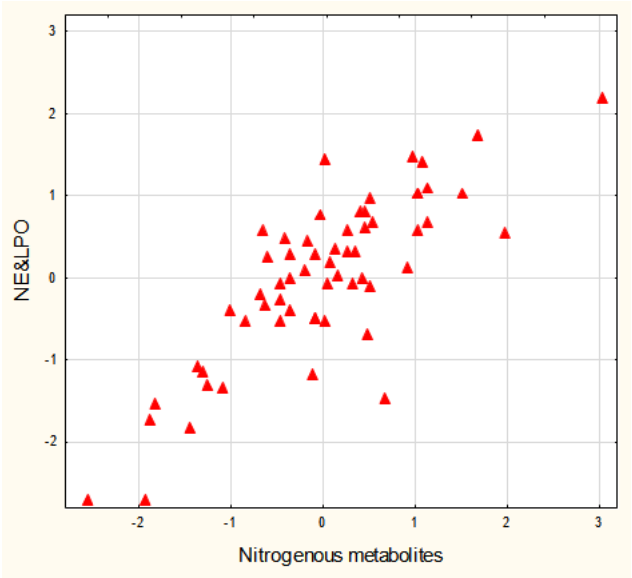
R=0,863; R^2=0,745; $\chi^2_{(112)}$=225; p<10^{-6}; Λ Prime=0,008

Fig. 3. Scatterplot of canonical correlation between the nitrogenous metabolites (X-line) and neuroendocrine and lipid peroxidation parameters (Y-line) in female rats. First pair of Roots

Instead, superoxide dismutase entered the factor structure of only the second neuroendocrine root, surrounded by parameters subject to downregulation by uric acid urine and urea plasma while upregulation by creatinine and bilirubin plasma (Table 8 and Fig. 4).

Table 8. Factor load on second canonical roots of nitrogenous metabolites (left set) and neuroendocrine and lipid peroxidation parameters (right set)

Left set	**Root 2**
Uricosuria, µM/24h•100 g	**-0,408**
Urea Plasma, mM/L	**-0,133**
Creatininemia, mM/L	**0,234**
Bilirubinemia, µM/L	**0,220**
Right set	**Root 2**
Parathyroid Activity	**-0,497**
Mineralocorticoid Activity	**-0,482**
Reticular ZAC, µM	**-0,179**
Medullar ZA, µM	**0,294**
Diene conjugates, E^{232}/mL	**0,228**
Fascicular ZAC, µM	**0,208**
Corticosterone, nM/L	**0,198**
Triiodothyronine, nM/L	**0,190**
Katalase, nM/h•mL	**0,170**
Adrenals Mass, mg/100 g	**0,157**
Superoxide Dismutase, un/mL	**0,138**
Malonic Dyaldehid, µM/L	**0,129**
MxDMn HRV, msec	**0,120**
Glomerular ZAC, µM	**0,101**



R=0,813; R^2=0,661; $\chi^2_{(90)}$=161; p<10^{-5}; Λ Prime=0,033

Fig. 4. Scatterplot of canonical correlation between the nitrogenous metabolites (X-line) and neuroendocrine and lipid peroxidation parameters (Y-line) in female rats. Second pair of Roots

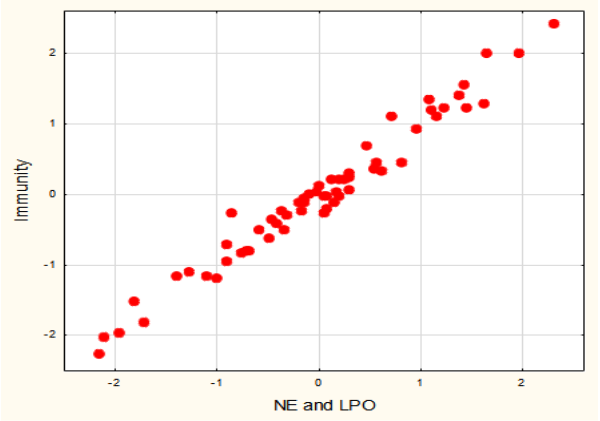
At the final stage, the analysis of the canonical correlation between all the presumed mediators of the immunomodulatory action of nitrogenous metabolites, on the one hand, and the constellation of immune parameters subject to the influence of the latter, on the other hand.

Two neuroendocrine-immune pairs of canonical roots are formed. The first pair of roots reflects the immunomodulatory effect, primarily of triiodothyronine and glucocorticoids, to a lesser extent - mineralocorticoids, androgens and parathyroid hormone, as well as, conversely,

catecholamines, vagus, calcitonin, katalase, malonic dyaldehid and diene conjugates (Table 9). The degree of determination of immunity is 96,7% (Fig. 5). Therefore, lipid peroxidation parameters increase neuroendocrine determination of immunity by only 0,9% [17].

Table 9. Factor load on first canonical roots of Neuroendocrine and Lipid peroxidation parameters (left set) and Immune parameters (right set)

Left set	**Root 1**
Triiodothyronine, nM/L	**0,953**
Fascicular ZAC, µM	**0,609**
Mineralocorticoid Activity	**0,339**
Reticular ZAC, µM	**0,310**
Parathyroid Activity	**0,216**
Testosterone, nM/L	**0,156**
Medullar Zone Adrenals, µM	**-0,427**
Katalase, nM/h•mL	**-0,404**
MxDMn HRV, msec	**-0,384**
Calcitonin Activity	**-0,344**
Malonic dyaldehid, nM/mL	**-0,313**
Diene conjugates, E^{232}/mL	**-0,233**
Right set	**Root 1**
Natural Killers Blood, %	**0,911**
Monocytes Blood, %	**0,893**
Macrophages Spleen, %	**0,272**
Phagocytic Index Monocytes, %	**0,253**
Reticulocytes Thymus, %	**0,229**
Hassal's corpuscles Thymus, %	**0,138**
Eosinophiles Spleen, %	**0,128**
Fibroblastes Spleen, %	**0,127**
Stub Neutrophils Blood, %	**0,076**
Spleen Mass Index, g/100g	**0,063**
Microbial Count Neutrophils	**-0,884**
Phagocytic Index Neutrophils, %	**-0,639**
Lymphoblastes Spleen, %	**-0,415**
Lymphocytes Thymus, %	**-0,286**
Lymphoblastes Thymus, %	**-0,247**
Th Lymphocytes Blood, %	**-0,187**
Entropy Splenocytogram	**-0,181**
Macrophages Thymus, %	**-0,112**
Eosinophiles Blood, %	**-0.071**



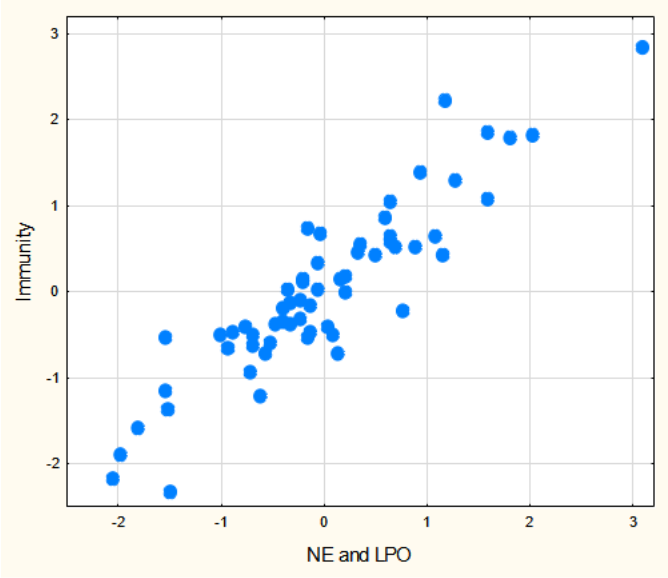
R=0,983; R^2=0,967; $\chi^2_{(345)}$=514; p<10^{-6}; Λ Prime<10^{-5}

Fig. 5. Scatterplot of canonical correlation between the Neuroendocrine and Lipid peroxidation parameters (X-line) and immune parameters (Y-line) in female rats. First pair of Roots

The second neuroendocrine root is poorly structured and reflects the modulating effect of vagus, parathyrin, calcitonin and corticosterone as well as diene conjugates and katalase, whith represent the root inversely, and steroid hormones as well as superoxide dismutase, which represent the root directly, on another constellation of immune parameters. The degree of determination of immunity is 83,4% (Table 10 and Fig. 6).

Table 10. Factor load on second canonical roots of Neuroendocrine and Lipid peroxidation parameters (left set) and Immune parameters (right set)

Left set	**Root 2**
MxDMn HRV, msec	**-0,490**
Parathyroid Activity	**-0,353**
Diene conjugates, E^{232}/mL	**-0,195**
Katalase, nM/h•mL	**-0,152**
Calcitonin Activity	**-0,144**
Corticosterone, nM/L	**-0,101**
Superoxide dismutase, un/mL	**0,354**
Glomerular ZAC, µM	**0,295**
Testosterone, nM/L	**0,260**
Mineralocorticoid Activity	**0,160**
Fascicular ZAC, µM	**0,144**
Right set	**Root 2**
Macrophages Spleen, %	**0,598**
Leukocytes Blood, 10^9/L	**0,358**
Th Lymphocytes Blood, %	**0,191**
Eosinophiles Spleen, %	**0,145**
Eosinophiles Blood, %	**0,056**
Microphages Spleen, %	**-0,251**
Endotheliocytes Thymus, %	**-0,241**
Phagocytic Index Monocytes, %	**-0,208**
Entropy Leukocytogram	**-0,202**
Fibroblastes Spleen, %	**-0,132**
Macrophages Thymus, %	**-0,104**



R=0,913; R^2=0,834; $\chi^2_{(308)}$=380; p=0,003; Λ Prime<10^{-4}

Fig. 6. Scatterplot of canonical correlation between the Neuroendocrine and Lipid peroxidation parameters (X-line) and immune parameters (Y-line) in female rats. Second pair of Roots

Therefore, lipid peroxidation parameters increase neuroendocrine determination of another pool immunity parameter by 10,5% [17].

It seems that nitrogenous metabolites modulate the activity of the autonomic nervous system, the adrenal, thyroid and parathyroid glands as well as lipid peroxidation, mediators which, in turn, have an immunomodulatory effect.

The obtained results, taken together with the previous ones, prove that uric acid, bilirubin, urea and creatinine realize their immunomodulatory effects both directly through receptors of immunocytes (aryl hydrocarbon, adenosine and TL) and with the participation of mediators of autonomic nervous and endocrine systems and lipid peroxidation [33,34].

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the Ukrainian Scientific Research Institute for Medicine of 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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