FEATURES OF THE STATE OF THE NEUROENDOCRINE-IMMUNE COMPLEX AND ELECTROLYTE-NITROGENOUS EXCHANGE UNDER DIFFERENT VARIATIONS OF URIC ACID METABOLISM IN FEMALE RATS

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Abstract

Background. This article concludes the experimental part of the project "Physiological activity of uric acid". Our group previously identified 4 variants-clusters of uricemia and uricosuria both in rats and in humans. It was shown that the clusters differ from each other in the constellation of immune, autonomic and endocrine parameters as well as plasma and urine electrolytes and nitrogenous parameters, which to one degree or another correlate with uricemia and uricosuria. Because the autonomic, endocrine and immune parameters interact with each other within the neuro-endocrine-immune complex, the purpose of this study is to find out exactly which parameters of the neuro-endocrine-immune complex reflect the specificity of quantitative and qualitative clusters of uric acid metabolism. Materials and Methods. Experiment was performed on 60 healthy female Wistar rats 220-300 g. The plasma and urine levels of the uric acid, urea, creatinine, calcium, phosphates, chloride, magnesium, sodium and potassium (also in erythrocytes) as well as glucose (in plasma only) were determined. The parameters of the autonomic, endocrine and immune systems were registered. Results. 30 parameters of the neuroendocrine-immune complex and metabolism were identified, which collectively reflect the specificity of quantitative and qualitative clusters of uric acid metabolism. In addition to, by definition, uricemia and uricosuria, they represent markers of vagal tone, sympatho-vagal balance, androgenic, mineralocorticoid and glucocorticoid functions of the adrenal cortex as well as parathyroid activity; exchange of electrolytes (excretion with urine of sodium, calcium and phosphates, plasma levels of sodium, magnesium, chloride, phosphates and potassium and the latter in erythrocytes) and nitrogenous metabolites (creatininuria and plasma urea); as well as immunity parameters (the
percentage of lymphoblasts, plasma cells and macrophages in the thymocytogram, the mass of the spleen, the entropy of the splenocytogram and the percentage of macrophages and microphages in it, the percentage of Th-, B- and 0-lymphocytes in the blood immunocytogram as well as blood level of leukocytes in general). Conclusion. The quantitative and qualitative clusters of uric acid metabolism are accompanied by specific constellations of parameters of the neuro-endocrine-immune complex and the electrolytes and nitrogenous compounds.

**Keywords:** uricemia, uricosuria, neuro-endocrine-immune complex, electrolytes, nitrogenous compounds, relationships, healthy female rats.

**INTRODUCTION**

This article concludes the experimental part of the project "Physiological activity of uric acid" [26]. We previously identified 4 variants-clusters of uricemia and uricosuria both in rats [10] and in humans [21]. Using the method of discriminant analysis, it was shown that the clusters differ from each other in the constellation of immune [11-14], autonomic and endocrine [25] parameters as well as plasma and urine electrolytes and nitrogenous parameters [5], which to one degree or another correlate with uricemia and uricosuria. It is known that autonomic, endocrine and immune parameters interact with each other within the neuro-endocrine-immune complex (NEIC) [4,20,24,27,28]. The purpose of this study is to find out exactly which parameters of the NEIC reflect the specificity of quantitative and qualitative clusters of uric acid metabolism.

**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. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: mode (Mo), amplitude of the mode (AMo) and variation scope (MxDMn) as markers of the humoral channel of regulation, sympathetic and vagal tones respectively [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 as well as taking the thymus, spleen and adrenal glands.

The plasma and urine levels of the uric acid (uricase method), creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), glucose (in plasma only; glucose-oxidase method), calcium (by reaction with arsenase III), phosphates (phosphate-molybdate method), magnesium (by reaction with colgamite), chloride (mercury-rhodanidine method), sodium and potassium (both also in erythrocytes; by flaming photometry) were determined. The analyzes were carried out according to the instructions described in the manual [9].
According to the parameters of electrolyte exchange, hormonal activity was evaluated: parathyroid by coefficients (Cap/Pp)\(^0.5\) and (Cap•Pu/Pp•Cau)\(^0.25\), calcitonin by coefficients (1/Cap•Pp)\(^0.5\) and (Cau•Pu/Cap•Pp)\(^0.25\) as well as mineralocorticoid by coefficients (Nap/Kp)\(^0.5\) and (Nap•Ku/Kp•Nau)\(^0.25\), based on their classical effects and recommendations [27].

In additional, the plasma levels of the hormones of adaptation such as corticosterone, triiodothyronine and testosterone (by the ELISA) as well as daily excretion of 17-ketosteroides (by reaction with meta-dinitrobenzene) were determined.

In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope [4].

In the blood, the parameters of immunity were determined as described in the manual [23]: the percentage of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant (T-helper) and theophylline-sensitive (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline); the population of B-lymphocytes (by the test of complementary rosette formation with erythrocytes of sheep). Natural killers were identified as large granules contain lymphocytes.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytic index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [4,20,28].

The spleen and thymus were removed, weighed and made smears-imprints for counting splenocytogram and thymocytogram [3,4]. For them, as well as leukocytogram, Shannon’s entropy was calculated [24,27].

The analyzers “Tecan” (Oesterreich), “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets and a flaming spectrophotometer “CΦ-47”.

Digital material statistically processed on a computer using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

As a result of the discriminant analysis [19], 30 recognition parameters were included in the model (Tables 1 and 2). In addition to, by definition, uricemia and uricosuria, they represent neuro-endocrine (6) and immune (11) systems as well as electrolytes (9) and nitrogenous metabolites (2) of blood and urine.

The identifying information contained in the 30 discriminant variables is condensed into three roots. The first root contains 56,0% of the discriminant power (r*=0,963; Wilks' \(\Lambda=0,002; \chi^2(90)=260; p<10^{-6}\)), second - 25,6% (r*=0,924; Wilks' \(\Lambda=0,028; \chi^2(58)=150; p<10^{-3}\)), third - 18,4% (r*=0,899; Wilks' \(\Lambda=0,192; \chi^2(28)=69; p<10^{-4}\)).

Calculation of the values of the discriminant roots for each animal as the sum of the products of non-standardized coefficients on the individual values of the discriminant variables together with a constant (Table 3) make it possible to visualize each rat in the information space of the roots (Fig. 1).
Table 1. Summary of the step-by-step analysis of NEIC and metabolism parameters, ranked according to the $\Lambda$ criterion

<table>
<thead>
<tr>
<th>Variable enter</th>
<th>F to enter</th>
<th>p-value</th>
<th>Lambda</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uricemia</td>
<td>45.6</td>
<td>1.0^6</td>
<td>0.290</td>
<td>45.6</td>
<td>0.000000</td>
</tr>
<tr>
<td>Parathyroid Activity</td>
<td>10.2</td>
<td>1.0^4</td>
<td>0.187</td>
<td>24.1</td>
<td>0.000000</td>
</tr>
<tr>
<td>Magnesium Plasma</td>
<td>5.61</td>
<td>0.002</td>
<td>0.142</td>
<td>18.0</td>
<td>0.000000</td>
</tr>
<tr>
<td>Macrophages Thymus</td>
<td>4.05</td>
<td>0.012</td>
<td>0.116</td>
<td>14.7</td>
<td>0.000000</td>
</tr>
<tr>
<td>Creatinine Excretion</td>
<td>4.62</td>
<td>0.006</td>
<td>0.091</td>
<td>13.2</td>
<td>0.000000</td>
</tr>
<tr>
<td>MxDMn as Vagal tone</td>
<td>3.43</td>
<td>0.024</td>
<td>0.076</td>
<td>12.0</td>
<td>0.000000</td>
</tr>
<tr>
<td>0 Lymphocytes Blood</td>
<td>3.31</td>
<td>0.027</td>
<td>0.063</td>
<td>11.1</td>
<td>0.000000</td>
</tr>
<tr>
<td>Phosphates Excretion</td>
<td>2.80</td>
<td>0.050</td>
<td>0.054</td>
<td>10.3</td>
<td>0.000000</td>
</tr>
<tr>
<td>Macrophages Spleen</td>
<td>2.50</td>
<td>0.071</td>
<td>0.047</td>
<td>9.7</td>
<td>0.000000</td>
</tr>
<tr>
<td>T helper Lymphocytes</td>
<td>2.71</td>
<td>0.056</td>
<td>0.040</td>
<td>9.2</td>
<td>0.000000</td>
</tr>
<tr>
<td>Urea Plasma</td>
<td>2.32</td>
<td>0.088</td>
<td>0.035</td>
<td>8.8</td>
<td>0.000000</td>
</tr>
<tr>
<td>Potassium Erythrocytes</td>
<td>1.86</td>
<td>0.149</td>
<td>0.031</td>
<td>8.3</td>
<td>0.000000</td>
</tr>
<tr>
<td>Phosphates Plasma</td>
<td>1.93</td>
<td>0.139</td>
<td>0.027</td>
<td>8.0</td>
<td>0.000000</td>
</tr>
<tr>
<td>Potassium Plasma</td>
<td>3.01</td>
<td>0.041</td>
<td>0.023</td>
<td>7.9</td>
<td>0.000000</td>
</tr>
<tr>
<td>B Lymphocytes Blood</td>
<td>1.80</td>
<td>0.163</td>
<td>0.020</td>
<td>7.6</td>
<td>0.000000</td>
</tr>
<tr>
<td>Entropy Splenocytogram</td>
<td>1.63</td>
<td>0.198</td>
<td>0.018</td>
<td>7.3</td>
<td>0.000000</td>
</tr>
<tr>
<td>17-Ketosteroid Excretion</td>
<td>1.80</td>
<td>0.162</td>
<td>0.016</td>
<td>7.1</td>
<td>0.000000</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>1.53</td>
<td>0.222</td>
<td>0.014</td>
<td>6.9</td>
<td>0.000000</td>
</tr>
<tr>
<td>(Ca/K)^0.5 Plasma</td>
<td>1.49</td>
<td>0.232</td>
<td>0.013</td>
<td>6.7</td>
<td>0.000000</td>
</tr>
<tr>
<td>Calcium Excretion</td>
<td>1.92</td>
<td>0.144</td>
<td>0.011</td>
<td>6.6</td>
<td>0.000000</td>
</tr>
<tr>
<td>Leukocytes Blood</td>
<td>1.41</td>
<td>0.255</td>
<td>0.010</td>
<td>6.4</td>
<td>0.000000</td>
</tr>
<tr>
<td>Plasmocytes Thymus</td>
<td>1.91</td>
<td>0.146</td>
<td>0.008</td>
<td>6.3</td>
<td>0.000000</td>
</tr>
<tr>
<td>Microphages Spleen</td>
<td>1.70</td>
<td>0.185</td>
<td>0.007</td>
<td>6.2</td>
<td>0.000000</td>
</tr>
<tr>
<td>Spleen Mass Index</td>
<td>1.43</td>
<td>0.251</td>
<td>0.006</td>
<td>6.1</td>
<td>0.000000</td>
</tr>
<tr>
<td>Mineralocorticoid Activity</td>
<td>1.75</td>
<td>0.177</td>
<td>0.006</td>
<td>6.0</td>
<td>0.000000</td>
</tr>
<tr>
<td>Sodium Excretion</td>
<td>4.10</td>
<td>0.015</td>
<td>0.004</td>
<td>6.4</td>
<td>0.000000</td>
</tr>
<tr>
<td>Lymphoblastes Thymus</td>
<td>2.04</td>
<td>0.129</td>
<td>0.003</td>
<td>6.4</td>
<td>0.000000</td>
</tr>
<tr>
<td>Uricosuria</td>
<td>1.89</td>
<td>0.153</td>
<td>0.003</td>
<td>6.4</td>
<td>0.000000</td>
</tr>
<tr>
<td>Chloride Plasma</td>
<td>1.27</td>
<td>0.305</td>
<td>0.002</td>
<td>6.3</td>
<td>0.000000</td>
</tr>
<tr>
<td>Sodium Plasma</td>
<td>1.62</td>
<td>0.208</td>
<td>0.002</td>
<td>6.3</td>
<td>0.000000</td>
</tr>
</tbody>
</table>
Table 2. Summary of the analysis of discriminant functions for NEIC and metabolism parameters, ranked by structural coefficient

Step 30, N of vars in model: 30; Grouping: 4 grps; Wilks' Λ: 0.0021; approx. F(01)=6,3; p<10^-6

<table>
<thead>
<tr>
<th>Variables currently in the model</th>
<th>Clusters of Uric acid Exchange (n)</th>
<th>Parameters of Wilks' Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-Un (15)</td>
<td></td>
</tr>
<tr>
<td>Uricemia, μM/L</td>
<td>259</td>
<td>0,007 0,290 22,0 10^-6 0,380 662</td>
</tr>
<tr>
<td>Uricosuria, μM/100g*d</td>
<td>3,31</td>
<td>0,002 0,838 1,74 0,182 0,236 5,72</td>
</tr>
<tr>
<td>MxDMn HRV, msec</td>
<td>26</td>
<td>0,003 0,753 2,96 0,050 0,219 53</td>
</tr>
<tr>
<td>0- Lymphocytes, %</td>
<td>15,5</td>
<td>0,002 0,748 3,03 0,047 0,430 22,2</td>
</tr>
<tr>
<td>K Erythrocyt, mM/L</td>
<td>85,0</td>
<td>0,002 0,826 1,89 0,155 0,439 87,0</td>
</tr>
<tr>
<td>Na Excr, μM/100 g*d</td>
<td>118</td>
<td>0,003 0,620 5,52 0,004 0,104 135</td>
</tr>
<tr>
<td>Urea Plasma, mM/L</td>
<td>9,39</td>
<td>0,003 0,690 4,04 0,017 0,255 7,42</td>
</tr>
<tr>
<td>Plasmodocytes Thym, %</td>
<td>2,40</td>
<td>0,003 0,748 3,03 0,046 0,414 1,80</td>
</tr>
<tr>
<td>Macrophages Thy, %</td>
<td>3,07</td>
<td>0,003 0,714 3,61 0,026 0,347 2,70</td>
</tr>
<tr>
<td>Macrophages Spleen, %</td>
<td>7,87</td>
<td>0,002 0,925 0,73 0,544 0,204 7,90</td>
</tr>
<tr>
<td>Phosph E, μM/100 g*d</td>
<td>9,3</td>
<td>0,003 0,720 3,49 0,029 0,061 9,4</td>
</tr>
<tr>
<td>17-KS, nM/100g24h</td>
<td>55</td>
<td>0,002 0,886 1,16 0,344 0,067 61</td>
</tr>
<tr>
<td>Creatin E, μM/100g*d</td>
<td>8,6</td>
<td>0,002 0,863 1,43 0,257 0,115 8,7</td>
</tr>
<tr>
<td>T helper Lymphoc, %</td>
<td>30,5</td>
<td>0,003 0,665 4,54 0,010 0,419 31,5</td>
</tr>
<tr>
<td>Entropy Splenocytegr</td>
<td>0,752</td>
<td>0,003 0,716 3,57 0,027 0,389 0,753</td>
</tr>
<tr>
<td>Microphag Spleen, %</td>
<td>13,8</td>
<td>0,003 0,635 5,16 0,006 0,352 13,0</td>
</tr>
<tr>
<td>B Lymphocytes, %</td>
<td>16,3</td>
<td>0,003 0,592 6,20 0,002 0,324 16,0</td>
</tr>
<tr>
<td>Mineralocort Activity</td>
<td>3,12</td>
<td>0,004 0,490 9,36 10^-4 0,081 2,73</td>
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<tr>
<td>Mg Plasma, mM/L</td>
<td>0,45</td>
<td>0,003 0,677 4,29 0,013 0,360 0,88</td>
</tr>
<tr>
<td>Phosphat Plas, mM/L</td>
<td>0,46</td>
<td>0,002 0,827 1,89 0,155 0,105 0,72</td>
</tr>
<tr>
<td>Corticosterone, nM/L</td>
<td>437</td>
<td>0,003 0,677 4,28 0,013 0,225 482</td>
</tr>
<tr>
<td>Spleen Ml, μg/g BM</td>
<td>264</td>
<td>0,003 0,793 2,35 0,095 0,403 312</td>
</tr>
<tr>
<td>Ca Excr, μM/100 g*d</td>
<td>2,89</td>
<td>0,002 0,864 1,42 0,259 0,151 2,90</td>
</tr>
<tr>
<td>K Plasma, mM/L</td>
<td>3,25</td>
<td>0,002 0,834 1,79 0,172 0,208 4,23</td>
</tr>
<tr>
<td>Na Plasma, mM/L</td>
<td>127,4</td>
<td>0,002 0,848 1,63 0,208 0,042 128,6</td>
</tr>
<tr>
<td>Cl Plasma, mM/L</td>
<td>90,7</td>
<td>0,003 0,802 2,22 0,109 0,036 94,3</td>
</tr>
<tr>
<td>Lymphoblasts Thy, %</td>
<td>7,27</td>
<td>0,002 0,861 1,45 0,251 0,443 7,40</td>
</tr>
<tr>
<td>Leukocytes Blood,μL/L</td>
<td>11,35</td>
<td>0,002 0,843 1,68 0,195 0,425 12,68</td>
</tr>
<tr>
<td>Parathyroid Activity</td>
<td>2,15</td>
<td>0,002 0,866 1,39 0,267 0,077 2,08</td>
</tr>
<tr>
<td>(Cap/Kp) as S/V bal</td>
<td>0,92</td>
<td>0,002 0,872 1,32 0,289 0,125 0,89</td>
</tr>
</tbody>
</table>
Table 3. Standardized and raw coefficients and constants for NEIC and metabolism parameters included in the discriminant model

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>Standardized</th>
<th>Raw</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Root 1</td>
<td>Root 2</td>
<td>Root 3</td>
</tr>
<tr>
<td>Uricemia</td>
<td>1.303</td>
<td>-0.429</td>
<td>-0.408</td>
</tr>
<tr>
<td>Parathyroid Activity</td>
<td>-0.690</td>
<td>-0.101</td>
<td>1.262</td>
</tr>
<tr>
<td>Magnesium Plasma</td>
<td>0.080</td>
<td>0.415</td>
<td>0.959</td>
</tr>
<tr>
<td>Macrophages Thymus</td>
<td>-0.716</td>
<td>0.271</td>
<td>-0.597</td>
</tr>
<tr>
<td>MxDMn as Vagal tone</td>
<td>0.796</td>
<td>-0.778</td>
<td>0.171</td>
</tr>
<tr>
<td>0 Lymphocytes Blood</td>
<td>0.648</td>
<td>0.434</td>
<td>0.208</td>
</tr>
<tr>
<td>Phosphates Excretion</td>
<td>0.335</td>
<td>2.273</td>
<td>-0.243</td>
</tr>
<tr>
<td>Macrophages Spleen</td>
<td>-0.212</td>
<td>0.456</td>
<td>-0.429</td>
</tr>
<tr>
<td>T helper Lymphocytes</td>
<td>-0.505</td>
<td>0.755</td>
<td>0.310</td>
</tr>
<tr>
<td>Urea Plasma</td>
<td>-0.785</td>
<td>0.622</td>
<td>0.626</td>
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<tr>
<td>Potassium Erythrocytes</td>
<td>-0.053</td>
<td>0.363</td>
<td>-0.589</td>
</tr>
<tr>
<td>Phosphates Plasma</td>
<td>-0.141</td>
<td>0.084</td>
<td>1.422</td>
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<tr>
<td>Potassium Plasma</td>
<td>0.537</td>
<td>0.057</td>
<td>0.811</td>
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<tr>
<td>D Lymphocytes Blood</td>
<td>0.703</td>
<td>-0.819</td>
<td>0.533</td>
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<td>Entropy Splenocytogram</td>
<td>-0.212</td>
<td>0.495</td>
<td>-0.770</td>
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<tr>
<td>17-Ketosteroids Excretion</td>
<td>0.215</td>
<td>-1.362</td>
<td>-0.327</td>
</tr>
<tr>
<td>Creatinine Excretion</td>
<td>0.452</td>
<td>0.415</td>
<td>1.026</td>
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<tr>
<td>Corticosterone</td>
<td>0.723</td>
<td>-0.968</td>
<td>0.432</td>
</tr>
<tr>
<td>(Ca/K)½ Plasma</td>
<td>0.111</td>
<td>-0.371</td>
<td>-1.052</td>
</tr>
<tr>
<td>Calcium Excretion</td>
<td>-0.280</td>
<td>-0.526</td>
<td>0.855</td>
</tr>
<tr>
<td>Leukocytes Blood</td>
<td>0.512</td>
<td>0.316</td>
<td>0.229</td>
</tr>
<tr>
<td>Lymphocytes Thymus</td>
<td>-0.017</td>
<td>-0.834</td>
<td>0.138</td>
</tr>
<tr>
<td>Microphages Spleen</td>
<td>0.499</td>
<td>-0.880</td>
<td>0.420</td>
</tr>
<tr>
<td>Spleen Mass Index</td>
<td>0.459</td>
<td>-0.551</td>
<td>0.272</td>
</tr>
<tr>
<td>Mineralocorticoid Activity</td>
<td>1.473</td>
<td>-2.235</td>
<td>-0.045</td>
</tr>
<tr>
<td>Sodium Excretion</td>
<td>1.047</td>
<td>-1.758</td>
<td>-0.069</td>
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<tr>
<td>Lymphoblasts Thymus</td>
<td>-0.309</td>
<td>0.442</td>
<td>0.267</td>
</tr>
<tr>
<td>Uricosuria</td>
<td>-0.697</td>
<td>0.436</td>
<td>-0.301</td>
</tr>
<tr>
<td>Chloride Plasma</td>
<td>0.696</td>
<td>-0.993</td>
<td>-2.268</td>
</tr>
<tr>
<td>Sodium Plasma</td>
<td>-0.450</td>
<td>0.560</td>
<td>1.991</td>
</tr>
<tr>
<td><strong>Constants</strong></td>
<td><strong>-3.586</strong></td>
<td><strong>-13.865</strong></td>
<td><strong>-2.813</strong></td>
</tr>
<tr>
<td><strong>Eigenvalues</strong></td>
<td><strong>12.73</strong></td>
<td><strong>5.824</strong></td>
<td><strong>4.194</strong></td>
</tr>
<tr>
<td><strong>Cumulative Proportion</strong></td>
<td><strong>0.560</strong></td>
<td><strong>0.816</strong></td>
<td><strong>1.000</strong></td>
</tr>
</tbody>
</table>

Table 4 demonstrates that the characteristic features of the S-Un cluster are a combination of moderate hypouricemia and lower border line uricosuria with reduced vagal tone and the level of 0-lymphocytes in the blood, lower border line potassium level and a normal, but minimal for the sample, level of natriuria, on the other hand, a moderately increased percentage of plasma cells in the thymus and a maximum for a sample elevated level of urea in the plasma. Such a constellation of parameters is visualized by the localization of cluster members in the extreme left zone of the first root axis (Fig. 1).

At the opposite pole of the axis are members of the S+Un+ cluster, which are characterized by a combination of pronounced hyperuricemia and normal, but maximal for the sample, uricosuria with moderately increased levels of vagal tone and natriuria and normal, but maximal for the sample, levels of 0-lymphocytes in the blood and potassium in erythrocytes, instead, a moderately reduced level of urea in the plasma and a normal, but minimal for sampling content of plasma cells in the thymus.

Members of the other two clusters occupy an intermediate position along the axis of the first root and are mixed. Their separation occurs along the axis of the second root. The top position is occupied by the rats of the Sn+U+ cluster, in which the upper limit level of uricemia is combined with the maximum for the sample upper limit levels of macrophages in
the thymus and spleen as well as urinary excretion of phosphates, creatinine and 17-ketosteroids while normal levels of T-helpers and splenocytogram entropy. Instead, they have a reduced level of macrophages in the spleen, and the level of B-lymphocytes in the blood and mineralocorticoid activity are normal, but minimal for the sample, while in the members of the  **SnUn+**  cluster, the lower borderline level of uricemia is accompanied to a greater or lesser extent by lower/higher levels of the listed parameters.

Additional delimitation of these clusters occurs along the axis of the third root (Fig. 2). The top position is occupied by rats of the  **SnUn+**  cluster, which reflects their upper limit plasma levels of magnesium, phosphates, corticosterone and calcium excretion as well as normal, but maximum for the sample, levels of chloride in the plasma, leukocytes in the blood, lymphoblastes in the thymus, and mass index of the spleen in combination with the maximally reduced electrolyte markers of parathyroid activity and sympatho-vagal balance, while the rats placed below the  **Sn+U+-**  cluster are characterized to a greater or lesser extent by lower/higher levels of the listed parameters.

### Table 4. Structural coefficients of NEIC and metabolism parameters, their average Z-values and centroids of discriminant roots for clusters

<table>
<thead>
<tr>
<th></th>
<th>Correlations</th>
<th>Variables-Roots</th>
<th>S-Un</th>
<th>Sn+U-</th>
<th>SnUn+</th>
<th>S+Un+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Root 1 (56.0%)</strong></td>
<td>Root 1</td>
<td>Root 2</td>
<td>Root 3</td>
<td>-4.30</td>
<td>-0.43</td>
<td>+0.63</td>
</tr>
<tr>
<td>Uricemia</td>
<td>.393</td>
<td>.191</td>
<td>-.250</td>
<td>-1.18</td>
<td>+0.59</td>
<td>-0.32</td>
</tr>
<tr>
<td>Uricosuria</td>
<td>.106</td>
<td>.126</td>
<td>-.010</td>
<td>-0.45</td>
<td>+0.17</td>
<td>-0.05</td>
</tr>
<tr>
<td>Mx/DMn as Vagal tone</td>
<td>.168</td>
<td>-.057</td>
<td>-.055</td>
<td>-0.63</td>
<td>-0.34</td>
<td>-0.18</td>
</tr>
<tr>
<td>0 Lymphocytes Blood</td>
<td>.097</td>
<td>.122</td>
<td>.044</td>
<td>-1.08</td>
<td>-0.10</td>
<td>-0.07</td>
</tr>
<tr>
<td>Potassium Erythrocytes</td>
<td>.062</td>
<td>.024</td>
<td>-.040</td>
<td>-0.29</td>
<td>+0.04</td>
<td>-0.12</td>
</tr>
<tr>
<td>Sodium Excretion</td>
<td>.040</td>
<td>.041</td>
<td>-.023</td>
<td>-0.19</td>
<td>+0.59</td>
<td>+0.17</td>
</tr>
<tr>
<td>Urea Plasma</td>
<td>-.125</td>
<td>.039</td>
<td>.116</td>
<td>+1.15</td>
<td>+0.78</td>
<td>+1.09</td>
</tr>
<tr>
<td>Plasmocytes Thymus</td>
<td>-.081</td>
<td>-.067</td>
<td>-.027</td>
<td>+0.76</td>
<td>+0.04</td>
<td>+0.10</td>
</tr>
<tr>
<td><strong>Root 2 (25.6%)</strong></td>
<td>Root 1</td>
<td>Root 2</td>
<td>Root 3</td>
<td>-2.40</td>
<td>+3.25</td>
<td>-0.46</td>
</tr>
<tr>
<td>Macrophages Thymus</td>
<td>-.086</td>
<td>.137</td>
<td>-.003</td>
<td>+0.27</td>
<td>+0.64</td>
<td>+0.16</td>
</tr>
<tr>
<td>Macrophages Spleen</td>
<td>-.004</td>
<td>.129</td>
<td>-.012</td>
<td>-0.02</td>
<td>+0.76</td>
<td>+0.17</td>
</tr>
<tr>
<td>Phosphates Excretion</td>
<td>-.009</td>
<td>.120</td>
<td>.006</td>
<td>-0.02</td>
<td>+0.52</td>
<td>+0.16</td>
</tr>
<tr>
<td>17-Ketosteroids Excretion</td>
<td>.013</td>
<td>.114</td>
<td>.014</td>
<td>-0.11</td>
<td>+0.40</td>
<td>+0.14</td>
</tr>
<tr>
<td>Creatinine Excretion</td>
<td>.065</td>
<td>.044</td>
<td>.076</td>
<td>-0.03</td>
<td>+0.90</td>
<td>+0.88</td>
</tr>
<tr>
<td>T helper Lymphocytes</td>
<td>-.052</td>
<td>.101</td>
<td>.062</td>
<td>-0.31</td>
<td>+0.14</td>
<td>-0.07</td>
</tr>
<tr>
<td>Entropy Splenocytogram</td>
<td>-.051</td>
<td>.100</td>
<td>-.002</td>
<td>-0.03</td>
<td>+0.25</td>
<td>-0.08</td>
</tr>
<tr>
<td>Microphages Spleen</td>
<td>.010</td>
<td>.188</td>
<td>-.010</td>
<td>+0.56</td>
<td>-0.82</td>
<td>+0.08</td>
</tr>
<tr>
<td>B Lymphocytes Blood</td>
<td>.007</td>
<td>-.075</td>
<td>.032</td>
<td>+0.09</td>
<td>-0.32</td>
<td>+0.12</td>
</tr>
<tr>
<td>Mineralocorticoid Activity</td>
<td>-.005</td>
<td>-.053</td>
<td>.022</td>
<td>+0.50</td>
<td>+0.12</td>
<td>+0.46</td>
</tr>
<tr>
<td><strong>Root 3 (18.4%)</strong></td>
<td>Root 1</td>
<td>Root 2</td>
<td>Root 3</td>
<td>-1.23</td>
<td>-0.89</td>
<td>+3.10</td>
</tr>
<tr>
<td>Magnesium Plasma</td>
<td>.098</td>
<td>.050</td>
<td>.300</td>
<td>-0.72</td>
<td>-0.17</td>
<td>+0.64</td>
</tr>
<tr>
<td>Phosphates Plasma</td>
<td>.086</td>
<td>.196</td>
<td>.203</td>
<td>-0.57</td>
<td>+0.85</td>
<td>+1.02</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>-.051</td>
<td>.008</td>
<td>.187</td>
<td>-0.36</td>
<td>-0.47</td>
<td>+0.40</td>
</tr>
<tr>
<td>Spleen Mass Index</td>
<td>.062</td>
<td>.012</td>
<td>.126</td>
<td>-0.48</td>
<td>-0.25</td>
<td>+0.09</td>
</tr>
<tr>
<td>Calcium Excretion</td>
<td>-.008</td>
<td>.097</td>
<td>.103</td>
<td>-0.01</td>
<td>+0.83</td>
<td>+0.90</td>
</tr>
<tr>
<td>Potassium Plasma</td>
<td>.048</td>
<td>.076</td>
<td>.099</td>
<td>-1.39</td>
<td>-0.69</td>
<td>-0.51</td>
</tr>
<tr>
<td>Sodium Plasma</td>
<td>.039</td>
<td>.010</td>
<td>.096</td>
<td>-0.23</td>
<td>+0.02</td>
<td>+0.43</td>
</tr>
<tr>
<td>Chloride Plasma</td>
<td>.049</td>
<td>-.004</td>
<td>.095</td>
<td>-0.50</td>
<td>-0.33</td>
<td>+0.07</td>
</tr>
<tr>
<td>Lymphoblastes Thymus</td>
<td>-.048</td>
<td>-.010</td>
<td>.094</td>
<td>-0.16</td>
<td>-0.41</td>
<td>+0.01</td>
</tr>
<tr>
<td>Leukocytes Blood</td>
<td>.006</td>
<td>-.004</td>
<td>.026</td>
<td>-0.22</td>
<td>-0.21</td>
<td>-0.12</td>
</tr>
<tr>
<td>Parathyroid Activity</td>
<td>-.068</td>
<td>-.166</td>
<td>-.260</td>
<td>+0.13</td>
<td>-0.75</td>
<td>-1.08</td>
</tr>
<tr>
<td>(Ca/K)ex Plasma</td>
<td>-.012</td>
<td>-.068</td>
<td>-.144</td>
<td>+0.17</td>
<td>-0.27</td>
<td>-0.58</td>
</tr>
</tbody>
</table>
The apparent clarity of the demarcation of the four clusters in the information space of the three canonical discriminant roots is documented by the calculation of the Mahalanobis distances between the clusters (Table 5).

Table 5. Squares of Mahalanobis distances between clusters (above the diagonal) and F-criteria (df=30,3) and p-levels (below the diagonal)

<table>
<thead>
<tr>
<th>Clusters</th>
<th>S-Un-</th>
<th>S+Un+</th>
<th>SnUn+</th>
<th>Sn+U-</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Un-</td>
<td>0</td>
<td>126</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>S+Un+</td>
<td>11,4</td>
<td>0</td>
<td>67</td>
<td>82</td>
</tr>
<tr>
<td>SnUn+</td>
<td>6,0</td>
<td>6,3</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Sn+U-</td>
<td>6,3</td>
<td>8,0</td>
<td>4,4</td>
<td>0</td>
</tr>
</tbody>
</table>

The use of classification functions (Table 6) enables error-free retrospective identification of all members of the four clusters (Table 7).
Table 6. Coefficients and constants of classification functions for neuroendocrine-immune and metabolic support of uric acid metabolism clusters

<table>
<thead>
<tr>
<th>Variable</th>
<th>S-Un- p=,250</th>
<th>S+Un+ p=,150</th>
<th>SnUn+ p=,283</th>
<th>Sn+U+ p=,317</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uricemia</td>
<td>0,051</td>
<td>0,114</td>
<td>0,074</td>
<td>0,082</td>
</tr>
<tr>
<td>Parathyroid Activity</td>
<td>-88,49</td>
<td>-114,23</td>
<td>-82,93</td>
<td>-96,85</td>
</tr>
<tr>
<td>Magnesium Plasma</td>
<td>80,91</td>
<td>81,80</td>
<td>93,16</td>
<td>87,73</td>
</tr>
<tr>
<td>Macrophages Thymus</td>
<td>22,98</td>
<td>15,89</td>
<td>17,68</td>
<td>21,61</td>
</tr>
<tr>
<td>Creatinine Excretion</td>
<td>-16,21</td>
<td>-15,24</td>
<td>-14,60</td>
<td>-15,25</td>
</tr>
<tr>
<td>MxDMn as Vagal tone</td>
<td>-0,229</td>
<td>-0,015</td>
<td>-0,150</td>
<td>-0,261</td>
</tr>
<tr>
<td>0 Lymphocytes Blood</td>
<td>1,55</td>
<td>2,61</td>
<td>2,27</td>
<td>2,28</td>
</tr>
<tr>
<td>Phosphates Excretion</td>
<td>12,19</td>
<td>13,12</td>
<td>13,15</td>
<td>14,88</td>
</tr>
<tr>
<td>Macrophages Spleen</td>
<td>-9,45</td>
<td>-10,52</td>
<td>-10,60</td>
<td>-8,53</td>
</tr>
<tr>
<td>T helper Lymphocytes</td>
<td>3,39</td>
<td>1,85</td>
<td>3,48</td>
<td>4,06</td>
</tr>
<tr>
<td>Urea Plasma</td>
<td>-5,03</td>
<td>-8,21</td>
<td>-5,01</td>
<td>-4,78</td>
</tr>
<tr>
<td>Potassium Erythrocytes</td>
<td>7,07</td>
<td>7,06</td>
<td>6,73</td>
<td>7,33</td>
</tr>
<tr>
<td>Phosphates Plasma</td>
<td>-90,20</td>
<td>-96,01</td>
<td>-77,31</td>
<td>-89,24</td>
</tr>
<tr>
<td>Potassium Plasma</td>
<td>62,32</td>
<td>69,37</td>
<td>70,43</td>
<td>65,78</td>
</tr>
<tr>
<td>B Lymphocytes Blood</td>
<td>-2,62</td>
<td>-0,36</td>
<td>-1,30</td>
<td>-3,17</td>
</tr>
<tr>
<td>Entropy Splenocytogram</td>
<td>2911</td>
<td>2837</td>
<td>2755</td>
<td>2989</td>
</tr>
<tr>
<td>17-Ketosteroids Excretion</td>
<td>0,209</td>
<td>0,261</td>
<td>0,134</td>
<td>0,034</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>-0,0231</td>
<td>0,0214</td>
<td>-0,0017</td>
<td>-0,0383</td>
</tr>
<tr>
<td>(Ca/K)^0,5 Plasma</td>
<td>329,6</td>
<td>340,8</td>
<td>300,6</td>
<td>317,1</td>
</tr>
<tr>
<td>Calcium Excretion</td>
<td>2,45</td>
<td>0,74</td>
<td>3,01</td>
<td>0,82</td>
</tr>
<tr>
<td>Leukocytes Blood</td>
<td>1,19</td>
<td>2,41</td>
<td>2,07</td>
<td>2,01</td>
</tr>
<tr>
<td>Plasmocytes Thymus</td>
<td>-6,59</td>
<td>-7,38</td>
<td>-7,98</td>
<td>-12,50</td>
</tr>
<tr>
<td>Microphages Spleen</td>
<td>5,91</td>
<td>8,49</td>
<td>7,26</td>
<td>4,39</td>
</tr>
<tr>
<td>Spleen Mass Index</td>
<td>0,05</td>
<td>0,12</td>
<td>0,09</td>
<td>0,31</td>
</tr>
<tr>
<td>Mineralocorticoid Activity</td>
<td>45,31</td>
<td>61,53</td>
<td>48,14</td>
<td>38,07</td>
</tr>
<tr>
<td>Sodium Excretion</td>
<td>0,076</td>
<td>0,140</td>
<td>0,085</td>
<td>0,042</td>
</tr>
<tr>
<td>Lymphoblastes Thymus</td>
<td>5,25</td>
<td>1,73</td>
<td>5,75</td>
<td>6,67</td>
</tr>
<tr>
<td>Uricosuria</td>
<td>-6,30</td>
<td>-8,80</td>
<td>-7,61</td>
<td>-6,41</td>
</tr>
<tr>
<td>Chloride Plasma</td>
<td>-30,20</td>
<td>-28,78</td>
<td>-31,51</td>
<td>-30,78</td>
</tr>
<tr>
<td>Sodium Plasma</td>
<td>40,36</td>
<td>39,21</td>
<td>41,74</td>
<td>40,75</td>
</tr>
<tr>
<td>Constant</td>
<td>-2895</td>
<td>-2954</td>
<td>-2944</td>
<td>-2981</td>
</tr>
</tbody>
</table>

Table 7. Classification matrix for uric acid metabolism clusters
Rows: observed classifications; columns: predicted classifications

<table>
<thead>
<tr>
<th>Clusters</th>
<th>Percent Correct</th>
<th>S+Un+ p=,150</th>
<th>Sn-Un+ p=,317</th>
<th>SnUn+ p=,283</th>
<th>S-Un- p=,250</th>
</tr>
</thead>
<tbody>
<tr>
<td>S+Un+</td>
<td>100</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sn+U+</td>
<td>100</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SnUn+</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>S-Un-</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>9</td>
<td>19</td>
<td>17</td>
<td>15</td>
</tr>
</tbody>
</table>
CONCLUSION

So, the state of uric acid metabolism is closely related to the state of the neuro-endocrine-immune complex. This confirms both old [6,17,30] and modern [8,21] hypotheses about the physiological activity of uric acid.

According to the concept of our laboratory, this is due to the similarity molecule of the uric acid (2,6,8-trioxipurine) with the molecules of theophylline (2,6-dioxo-1,3-dimethylpyridine or 1,3-dimethylxanthine), caffeine (2,6-dioxo-1,3,7-trimethylpyridine or 1,3,7-trimethylxanthine) and other 2,6-dimethylxanthines, which, in turn, are structural homologues of adenosine [(2R,3R,4R,5R)-2-(6-aminopurine-3H)-5-(hydroximethyl) oxolan-3,4-diol].

It is known that the effects of adenosine are realized through its receptors (A1, A2A, A2B, A3), which express virtually all populations of immunocytes such as T, NK, B lymphocytes, macrophages, neutrophils, dendritic and endothelial cells [1,15,16,32] as well as neurons of central and autonomous neural systems [7,8,18,21,22,29].

A detailed discussion will be conducted after the publication of the results of a similar study in humans.

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 SR Institute of 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).

REFERENCES


