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## CLUSTER ANALYSIS OF URIC ACID EXCHANGE PARAMETERS IN FEMALE RATS

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### Abstract

**Background.** Uric acid is traditionally considered as the final product of human and primate DNA/RNA degradation, devoid of useful physiological activity. However, there is an opinion that the uric acid molecule, by analogy with methylxanthines (caffeine, theophyllin, theobromine) has physiological activity. The acquired experience allows us to offer a topic for future research: "Neurotropic and immunotropic activity of endogenous uric acid". Working hypothesis. Uric acid, interacting with A<sub>1</sub> and A<sub>2a</sub> adenosine receptors as well as phosphodiesterase and Na,K-ATPase of neurons, modulates the activity of nerve centers, which in turn modulate immunocytes. Perhaps the direct effect of uric acid on immunocytes, since the existence of theophylline-resistant and theophylline-sensitive subpopulations of T-lymphocytes has long been known. The proposed article is the first swallow of the announced project. **Material and Methods.** Experiment was performed on 58 healthy female Wistar rats 220-300 g. Among them 10 animals remained intact, using tap water from drinking ad libitum. The rats of others groups for 6 days administered through the tube various fluids at a dose of 1,5 mL/100 g of body mass. The day after the completion of the drinking course the plasma

and urine levels of the uric acid (uricase method) were determined. **Results.** We obtained a wide range of uric acid metabolism parameters, divided into four clusters, quantitatively and qualitatively different from each other. On the basis of the accepted criteria, 29,3% of animals state normouricemia in combination with normal or slightly increased excretion of uric acid. In 24,1% of rats, hypouricemia is combined with normal or slightly reduced uricosuria. In 31,0% of animals normal or slightly elevated levels of plasma uric acid are accompanied by a very marked dispersion of uricosuria. The remaining 15,5% of rats expressed hyperuricemia combined with normal or slightly increased uricosuria. In the following articles, functional relationships of uricemia and uricosuria with the parameters of autonomic regulation, immunity and metabolism will be analyzed.

**Key words: uricemia; uricosuria; female rats**

## **INTRODUCTION**

Uric acid is traditionally considered as the final product of human and primate DNA/RNA degradation, devoid of useful physiological activity. Moreover, uric acid is a recognized cause of gout and urolithiasis. Recently, hyperuricemia is considered a predictor of an increased risk of mortality from cardiovascular disease in men, but not in women. At the same time, the antioxidant properties of uric acid have been noted.

However, Efroimson VP [2] considered hyperuricemia to be one of the factors of increased mental activity (even genius), based on the abnormally high incidence of gout and urolithiasis among prominent individuals. As another proof, the author cites the similarity of the molecule of uric acid (2,6,8-trioxipurine) to the molecules of methylxanthines: caffeine (2,6-dioxi-1,3,7-trimethylpurine) and theophylline (2,6-dioxi-1, 3-dimethylpurine).

This article prompted Ivassivka SV, Popovych IL, Aksentyuchuk BI and Flyunt IS to conduct a large-scale study of the physiological activity of endogenous uric acid, the results of which are summarized in the monograph [4].

Observing 66 women and 298 men treated at the Truskavets' spa (Ukraine), authors found a wide range of physiological activity of endogenous uric acid. In particular, uricemia correlates with the parameters of protein-nitrogen metabolism (levels in the blood of albumins and alpha<sub>1</sub>-, alpha<sub>2</sub>-, beta- and gamma-fractions of globulins, as well as urea), exchange of electrolytes (plasma and erythrocyte levels of sodium and potassium, calcium, magnesium, chloride and phosphate plasma, activity of Na,K-ATPase, Ca-ATPase and Mg-ATPase of erythrocyte shadows), exchange of lipides and lipid peroxidation, as well as glucose tolerance

test. On the same contingent of patients, authors found connections between the level of uricemia and the parameters of immunity (blood level of CD4<sup>+</sup>, CD8<sup>+</sup>, CD19<sup>+</sup> lymphocytes, serum level of Igg M, G and A as well as CIC, phagocytosis of neutrophils and monocytes). The third battery of tests was the parameters of hemostasis. It is important that not only the magnitude of correlation coefficients, but even their sign, is conditioned by the phase of chronic calculous pyelonephritis and/or the type of general adaptation reaction. Changes in the level of uricemia under the influence of balneotherapy are accompanied by changes in the listed parameters of metabolism, immunity and hemostasis. On a different contingent of patients, authors found the connections between balneotherapy-induced changes in the level of uricemia, on the one hand, and the parameters of heart rate variability and hemodynamics, on the other hand.

The obtained results allowed authors to assume that the uric acid molecule, by analogy with methylxanthines (caffeine, theophyllin, theobromine) has physiological activity, which is realized by blocking both A<sub>1</sub> and A<sub>2a</sub> adenosine receptors, as well as modulating phosphodiesterase, Na,K-ATPase activity and Na/Ca exchanger.

The cited study is not without its limitations. First, the authors recorded only the level of uric acid in the plasma in the morning, whereas its excretion with daily urine is more informative. Second, the immunotropic activity of uric acid is estimated only by the parameters of the blood immunogram, so the question of its effect on the parameters of the thymocytogram and splenocytogram remains open. Third, the neurotropic activity of uric acid was evaluated only against the autonomic nervous system, ignoring the CNS.

Previously, we found the relationship between the heart rate variability parameters, on the one hand, and the EEG parameters on the other hand [9, 10]. In line with the concept of the immunological homunculus Tracey KJ [13], we showed that different immunity parameters correlate with EEG activity in individual loci. The connections between the parameters of EEG and HRV, on the one hand, and the parameters of cellular and humoral immunity and phagocytosis, on the other hand, as well as the changes in these two parameter sets caused by balneotherapy, have been revealed [5-8, 11, 12].

The acquired experience allows us to offer a topic for future research: “Neurotropic and immunotropic activity of endogenous uric acid”. Working hypothesis. Uric acid, interacting with A<sub>1</sub> and A<sub>2a</sub> adenosine receptors as well as phosphodiesterase and Na,K-ATPase of neurons, modulates the activity of nerve centers, which in turn modulate immunocytes. Perhaps the direct effect of uric acid on immunocytes, since the existence of

theophylline-resistant and theophylline-sensitive subpopulations of T-lymphocytes has long been known.

The object of the study will be healthy rats as well as patients with chronic pyelonephritis and cholecystitis in the phase of remission. Subject of research: plasma and daily urine uric acid, EEG ("NeuroCom Standard"), HRV ("Cardiolab+VSR"), immunity (blood level of CD4<sup>+</sup>, CD8<sup>+</sup>, CD56<sup>+</sup>, CD22<sup>+</sup> lymphocytes, serum level of Igg M, G and A as well as CIC, activity, intensity and completeness of phagocytosis by neutrophils *E. coli* and *Staph. aureus*).

It will build bridges between biochemistry, neurophysiology and immunology. Evidence will be obtained about the neurotropic and immunotropic activity of endogenous uric acid, as well as factors conditioning the severity and directivity of this activity (sex, age, phase of the chronic disease, constellation of EEG, HRV and Immunity, and so on). Ultimately, the uric acid molecule can stand in a row with NO, CO, H<sub>2</sub>S molecules as a signal molecule.

The proposed article is the first swallow of the announced project.

## **MATERIAL AND METHODS**

Experiment was performed on 58 healthy female Wistar rats 220-300 g. Among them 10 animals remained intact, using tap water from drinking ad libitum. The rats of others groups for 6 days administered through the tube various fluids at a dose of 1,5 mL/100 g of body mass.

The day after the completion of the drinking course animals were 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 and urine levels of the uric acid (uricase method) were determined. The analyzes were carried out according to the instructions described in the manual [3]. The analyzers "Pointe-180" ("Scientific", USA) were used with appropriate sets.

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

## **RESULTS AND DISCUSSION**

Preliminary analysis revealed a wide variance of uric acid exchange parameters. As we can see, the correlation between the two parameters is statistically significant, but only

mediocre (Fig. 1). This indicates the need to record both parameters of uric acid to adequately assess its state of metabolism.

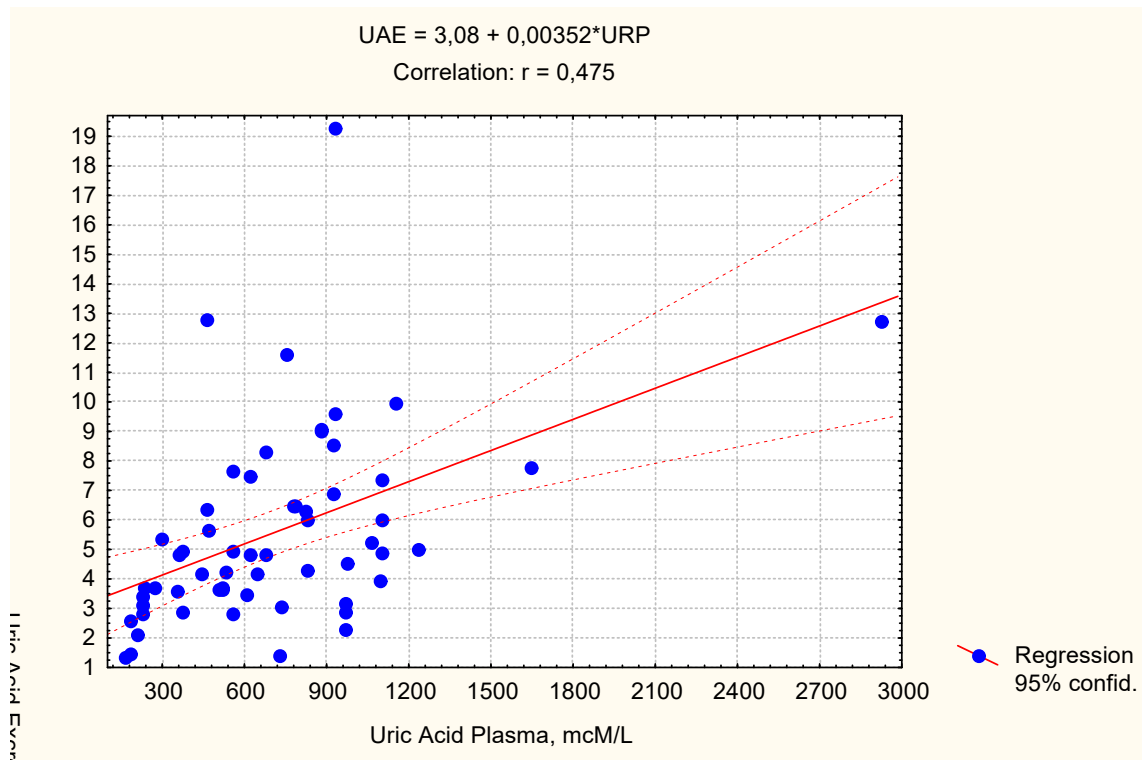


Fig. 1. Scatterplot of correlation between Uric Acid Plasma (X-line) and its Excretion with daily Urine (Y-line)

To create homogeneous groups, we applied cluster analysis. Clustering cohort of rats is realized by iterative k-means method. 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 [1].

Typically, the number of clusters is arbitrary. We stopped at four, because less is banal and more difficult to perceive and compare.

Clusters are clearly distinguished by the totality of uricemia and uricosuria (Table 1).

Table 1 - Euclidean Distances between Clusters Distances below diagonal. Squared distances above diagonal

Clusters	No. 1	No. 2	No. 3	No. 4
Pn+U-+ (18)	0	50854	48988	182339
P++Un+ (9)	226	0	199666	425778
PnUn+ (17)	221	447	0	42304
P-Un- (14)	427	653	206	0

Uricemia was a major parameter in clustering (Table 2).

Table 2 - Analysis of Variance

Parameters of Uric Acid Exchange	Between SS	Within SS	$\eta^2$	R	F	signif. p
Uric Acid Plasma	1037653 1	555208	0,949	0,974	248	$10^{-6}$
Uric Acid Excretion	153	447,7	0,255	0,505	4,51	0,003

In order to qualitatively evaluate the status of uric acid metabolism in different clusters, we calculated the mean values of uricemia and uricosuria as well as their variability in intact rats. For uricemia, they are  $662 \mu\text{M/L}$  ( $Cv=0,516$ ), for uricosuria  $5,72 \mu\text{M}/100\text{g Body Mass}\cdot 24\text{h}$  ( $Cv=0,939$ ). Taking the narrowed norm range  $-0,5\sigma \div 0,5\sigma$ , we will obtain for uricemia a range of  $490 \div 830 \mu\text{M/L}$ , for uricosuria  $3,0 \div 7,5 \mu\text{M}/100\text{g BM}\cdot 24\text{h}$  (Table 3 and Fig. 2).

Table 3 - Cluster Means

Parameters of Uric Acid Exchange	Clusters of Uric Acid Exchange (n)				
	Norm (10)	P-Un- (14)	PnUn+ (17)	Pn+U-+ (18)	P++Un+ (9)
Uric Acid Plasma, $\mu\text{M/L}$	662	263	554	867	1379
Uric Acid Excretion, $\mu\text{M}/100\text{g BM}\cdot 24\text{h}$	5,72	3,29	5,46	6,73	7,00

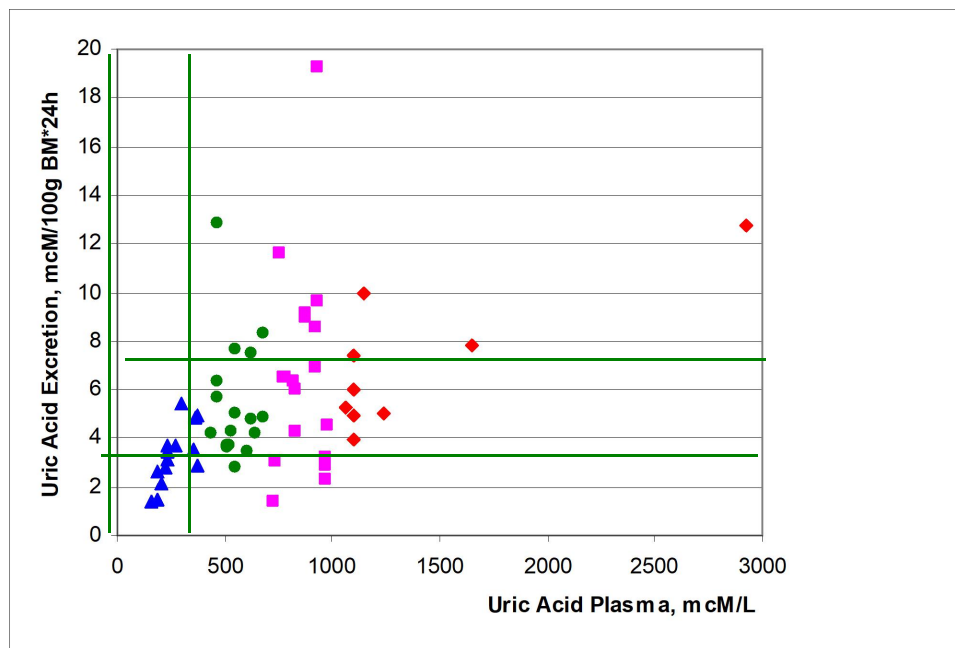


Fig. 2. Clusters of female rats with different uric acid metabolism.

Vertical lines indicate the limits of uricemia, horizontal lines limit the range of uricosuria ( $-0,5\sigma \div 0,5\sigma$ )

On the basis of the accepted criteria, 29,3% of animals state normouricemia in combination with normal or slightly increased excretion of uric acid (**PnUn+** cluster). In 24,1% of rats, hypouricemia is combined with normal or slightly reduced uricosuria (**P-Un-** cluster). In 31,0% of animals normal or slightly elevated levels of plasma uric acid are accompanied by a very marked dispersion of uricosuria (**Pn+Un-** cluster). The remaining 15,5% of rats expressed hyperuricemia combined with normal or slightly increased uricosuria (**P++Un+** cluster).

Because the variability of uricosuria is almost twice that of uricemia, we consider the Z-values of these parameters, calculated by the formula  $Z=(V/N-1)/Cv$ , to be more informative in terms of physiology (Fig. 3).

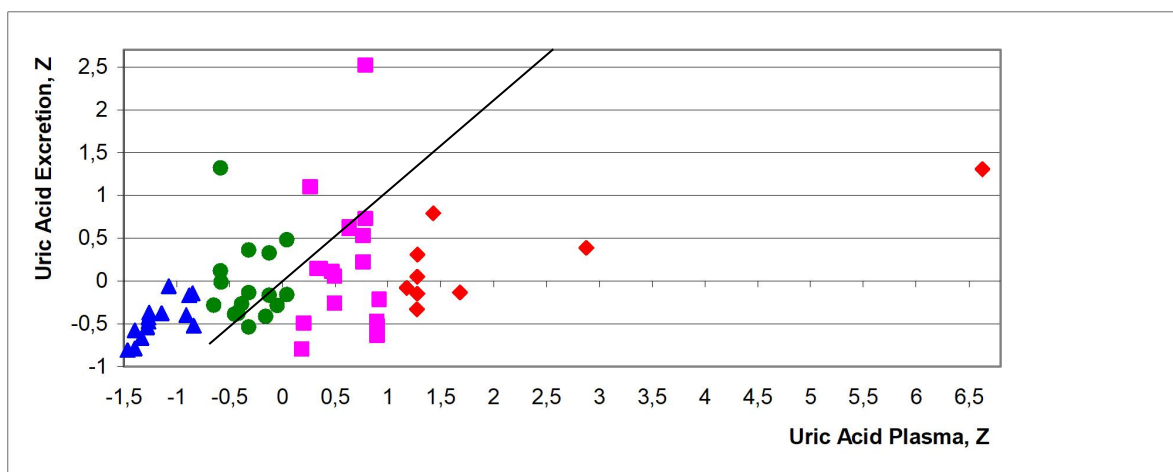


Fig. 3. Normalized levels (Z-scores) of uricemia (X-line) and uricosuria (Y-line) in female rats of different clusters

Therefore, we obtained a wide range of uric acid metabolism parameters in rats, divided into four clusters, quantitatively and qualitatively different from each other. In the following articles, functional relationships of uricemia and uricosuria with the parameters of autonomic regulation, immunity and metabolism will be analyzed.

### CONFORMITY TO ETHICAL STANDARDS

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

The carrying out of experiments was approved by the Ethics Committee of the Horbachevskyyi Ternopil' National Medical University. 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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