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RELATIONSHIPS OF UROLITHOGENICITY INDEX WITH SOME COMPONENTS OF URINE COMPOSITION IN HEALTHY OLD FEMALE RATS

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Background. Despite the long history, the results of studies of the impact of balneotherapy in the resort of Truskavets on the lithogenicity of urine are ambiguous, so the topic remains relevant. The aim of this study was to determine the relationship between the lithogenicity index of urine and a number of components of its composition. **Materials and Methods**. Experiment was performed on 60 healthy old female Wistar rats 220-300 g. Ten animals remained intact, using daily water from drinking ad libitum. Other animals for 6 days were loaded through the tube with daily and various mineral waters at a dose of 1,5 mL/100 g of body mass. The day after the completion of the drinking course collected daily urine, which determined the content of a number of components of the composition. We calculated urine lithogenicity index (Lith) by the formula: Lith = (Uric acid•Calcium/Magnesium•Creatinine)^{0,25}. **Results**. The most significant effect on the Lith is the concentration of magnesium (r=-0,730), followed by uric acid (r=0,583), calcium (r=0,352) and creatinine (r=-0,298). Medium molecular polypeptides, catalase, sodium, phosphates and urea has been identified as prolithogenic factors while tubular reabsorption of water as litholytic factor. The chemical composition of the fluids consumed by animals has little effect on the lithogenicity index of urine. **Conclusion**. Both prolithogenic and litholytic factors are present in the urine, which depend little on the chemical composition of the fluid used.
Keywords: urine lithogenicity, drinking mineral waters, Truskavets' spa, female rats.

INRODUCTION

Despite the long history, the results of studies of the impact of balneotherapy in the Truskavets' spa on the lithogenicity of urine are ambiguous [3-6,11,15-17], so the topic remains relevant. The aim of this study was to determine the relationship between the lithogenicity index of urine and a number of components of its composition.

MATERIALS AND METHODS

Experiment was performed on 60 healthy old female Wistar rats 220-300 g. Ten animals remained intact, using daily water from drinking ad libitum. Other animals for 6 days were loaded through the tube with daily and mineral waters (composition is given in Table 1) at a dose of 1,5 mL/100 g of body mass.

Table 1. Chemical composition of fresh and mineral waters (according to the Truskavetsian Hydrogeological Regime-operational station)

	Daily Water	Sofiya	Hertsa	Salt analog	Naftussya
Electrolytes, mM/L					
Na^+	0,5	156	196,7	196,7	0,6
Cl^-	3,4	142	205	205	1,0
HCO_3^-	2,9	7,5	5,6	5,6	8,2
Ca^{2+}	3,4	5,3	3,40	3,40	2,9
Mg^{2+}	0,5	4,3	3,44	3,44	2,3
K^+	0,4	0,3	0,4	0,4	0,3
SO_4^{2-}	1,2	13,1	0,1	0,1	1,0
Trace elements, mg/L					
H_2SiO_3	5	4,43	9,88	0	9,5
H_3BO_3	0,25	8,39	42,76	0	0,200
Br	8,3	6,7	21,17	0	0,034
J	0,025	1,29	6,62	0	0,004
F	0,95	0,52	0,57	0	0,160
Organic substances, mg/L					
C org	5,0	5,5	34	0	12,8
N org	0,02	0,8	0,14	0	0,33

The day after the completion of the drinking course animals were placed in individual chambers with perforated bottom for collecting daily urine. The content of the following substances in urine was determined. Electrolytes: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium; nitric metabolites: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method) and medium molecular polypeptides (by spectrophotometric method) [8]; lipid peroxidation components: diene conjugates (spectrophotometry of the heptane phase of the lipids extract [7]), malondyaldehide (in the test with thiobarbituric acid [1]) and catalase (at the rate of decomposition of hydrogen peroxide [14]), as well as amylase (Karavay's amyloclastic method with starch substrate) [8].

By the size of the diuresis and the level of creatinine in plasma and urine, glomerular filtration and tubular reabsorption were calculated.

Urine lithogenicity index (Lith) we calculated by the Tiselius' HS [18] formula modifed by Flyunt VR et al [5]: Lith = (Uric acid•Calcium/Magnesium•Creatinine)^{0,25}.

The analyzes were carried out according to the instructions. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets and a flaming spectrophotometer "СФ-47".

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

RESULTS AND DISCUSSION

For clarity, the urinary lithogenicity index was expressed as a Z-score:

Z = (Variable/Intact -1)/Cv).

Preliminary analysis revealed a wide range of lithogenicity values, and this applies even to intact animals (Fig. 1). Interestingly, among rats loaded with tap water, there were individuals with extreme levels of lithogenicity. Lithogenicity of rats of other groups also showed a pronounced variance.

This is consistent with the data of the Truskavets School of Balneology on the pronounced dispersion of the parameters of water-salt metabolism [9,10] and the ambivalence of its reactions to the use of Naftussya water [2]. It seems that the level of lithogenicity of urine is not affected by water load as such or the chemical composition of fluids. The visual impression is generally confirmed by comparing the average values (Fig. 2), with the exception of Sofiya mineral water, which significantly reduces lithogenicity.

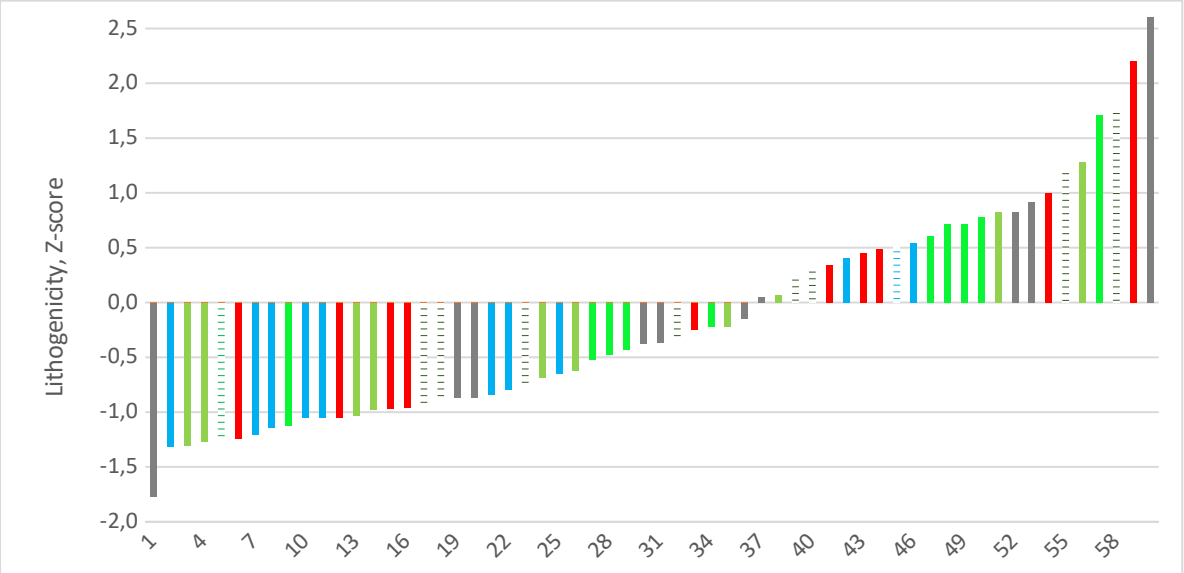


Fig. 1. Profile of urine lithogenicity index. Intact rats - columns with patterned filling, loaded with tap water - gray columns, Naftussya water - red, Sofiya water - blue, native Hertsa water - light green, its salt analog - green columns

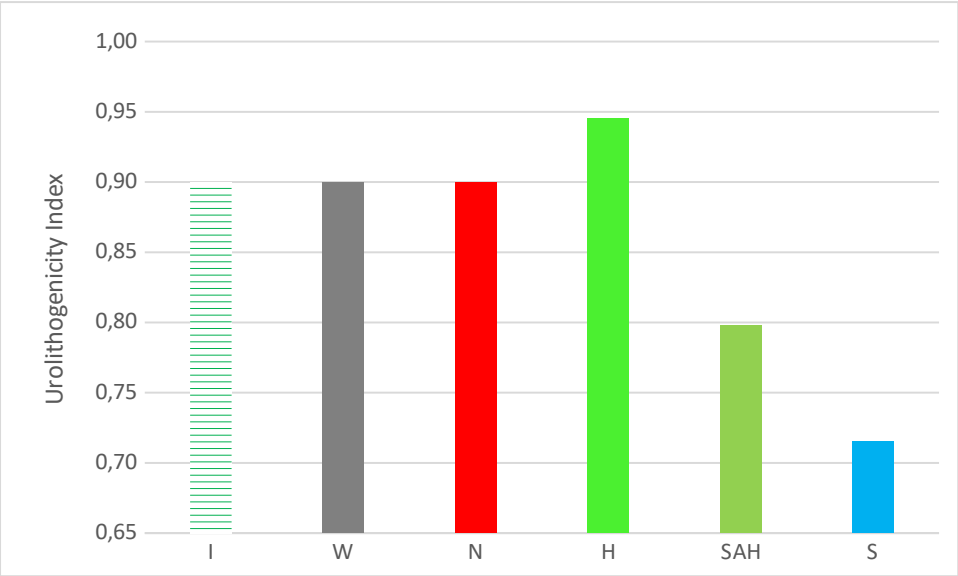


Fig. 2. Average values (Mean±SE) of urine lithogenicity index. Intact rats - column with patterned filling, loaded with tap water - gray, Naftussya water - red, native Hertsa water - light green, its salt analog - green, Sofiya water – blue columns

The difference in lithogenicity in rats loaded with native Hertza mineral water and its artificial salt analogue is noteworthy. Screening of correlations between lithogenicity and components of chemical composition of fluids consumed by animals revealed marginal significance (for a sample with n=60 critical level |r|=0,255) downregulating effects of calcium (r=-0,25) and sulfate (r=-0,25), as well as, to some extent, organic nitrogen (r=-0,21). Instead, the effects of organic carbon (r=0,21) and metasilicic acid (r=0.20) are upregulating. This at least partially clarifies the nature of the litholytic action of Sophia water and the prolithogenic action of Hertza native water.

At the next stage, the screening of correlations between the lithogenicity index of urine and its components - on the one hand, and other recorded parameters of urine and urination - on the other hand. Only the coefficients worth noting are included in the matrix (Table 2).

Table 2. Matrix of correlations between urine components

Variable	Correlations N=60				
	Lith	CrU	MgU	CaU	UaU
Lithogen	1,00	-0,30	-0,73	0,35	0,58
NaU	0,20	-0,19	-0,17	0,06	0,06
ClU	0,02	0,00	-0,12	-0,22	0,06
Diuresis	0,04	-0,25	-0,22	-0,07	-0,26
CrU	-0,30	1,00	-0,01	-0,16	0,08
GF	-0,17	0,18	-0,24	-0,33	-0,16
CReabs	-0,26	0,64	-0,15	-0,43	-0,02
UreaU	0,17	-0,05	-0,31	0,23	-0,22
KU	-0,15	0,28	0,03	-0,45	0,20
MgU	-0,73	-0,01	1,00	0,04	-0,30
CaU	0,35	-0,16	0,04	1,00	0,00
PU	0,19	-0,43	0,06	0,32	-0,01
UaU	0,58	0,08	-0,30	0,00	1,00
Amyl U	-0,04	0,39	0,07	0,00	0,21
MMMU	0,36	-0,44	-0,16	0,24	0,05
KatalU	0,34	-0,24	-0,22	0,39	0,07
MDAU	-0,17	0,36	0,05	-0,20	0,07
DCU	-0,08	0,11	-0,07	-0,19	-0,12

The most significant effect on the lithogenicity index of urine is the concentration of magnesium in it (Fig. 3), followed by uric acid (Fig. 4), calcium (Fig. 5) and creatinine (Fig. 6).

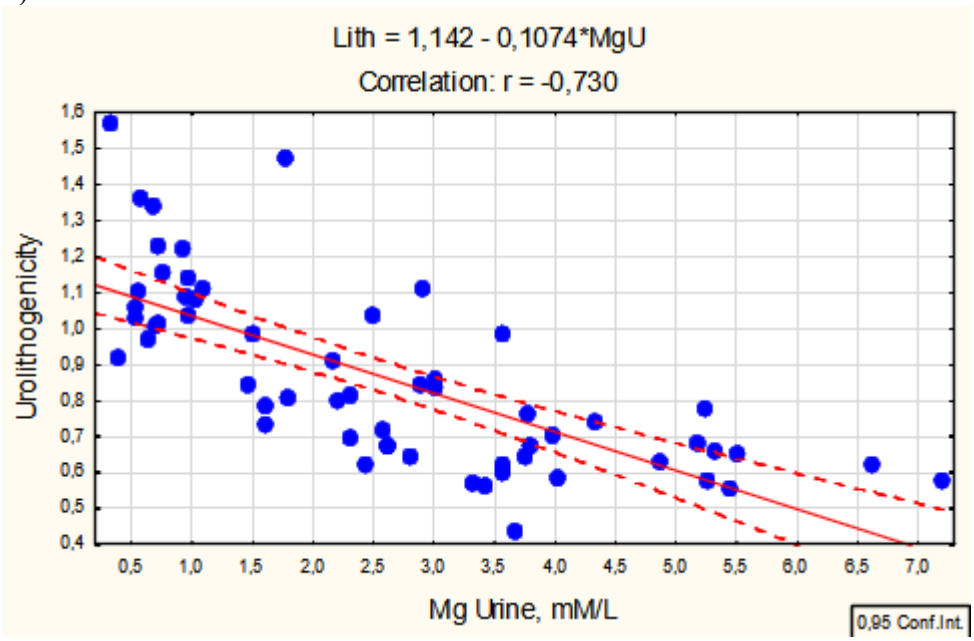


Fig. 3. Scatterplot of correlation between the magnesium urine (X-line) and lithogenicity index (Y-line)

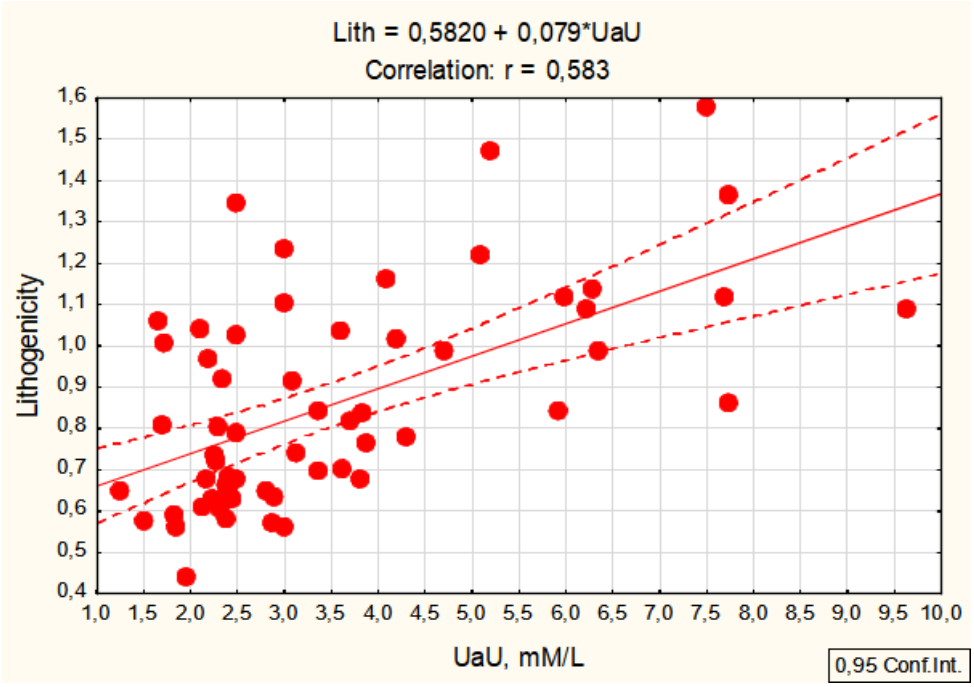


Fig. 4. Scatterplot of correlation between the uric acid urine (X-line) and lithogenicity index (Y-line)

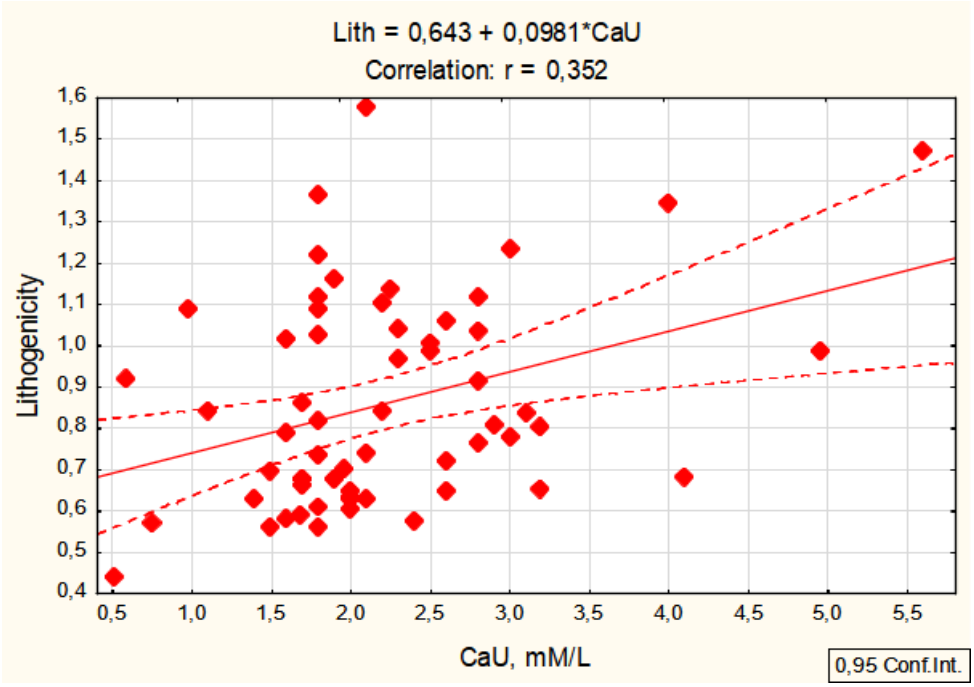


Fig. 5. Scatterplot of correlation between the calcium urine (X-line) and lithogenicity index (Y-line)

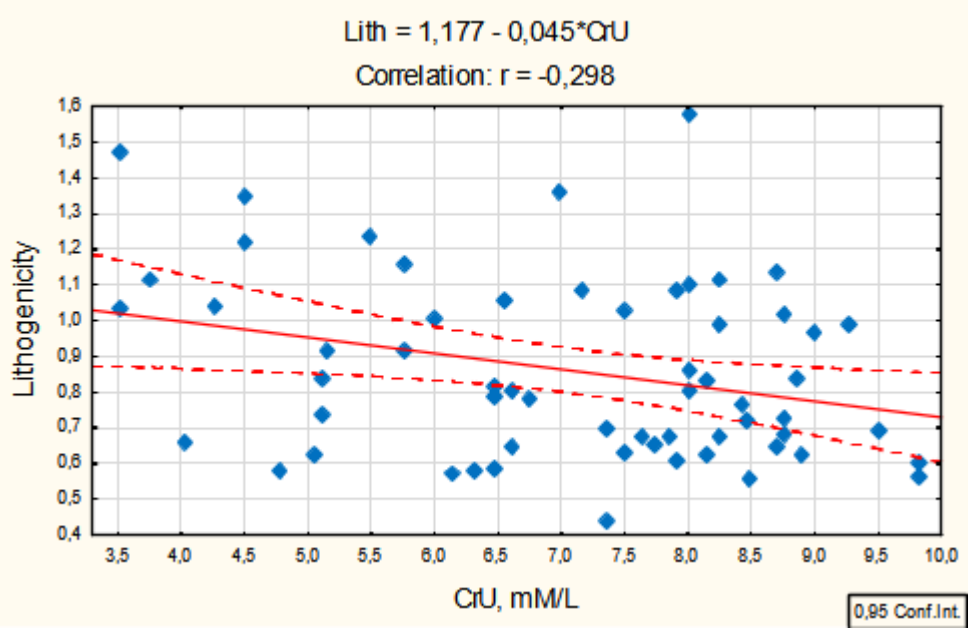


Fig. 6. Scatterplot of correlation between the creatinine urine (X-line) and lithogenicity index (Y-line)

Fig. 7 shows the relationship of the lithogenicity index with its major components, and in Fig. 8 - with all four, built on the parameters of the regression model (Table 3).

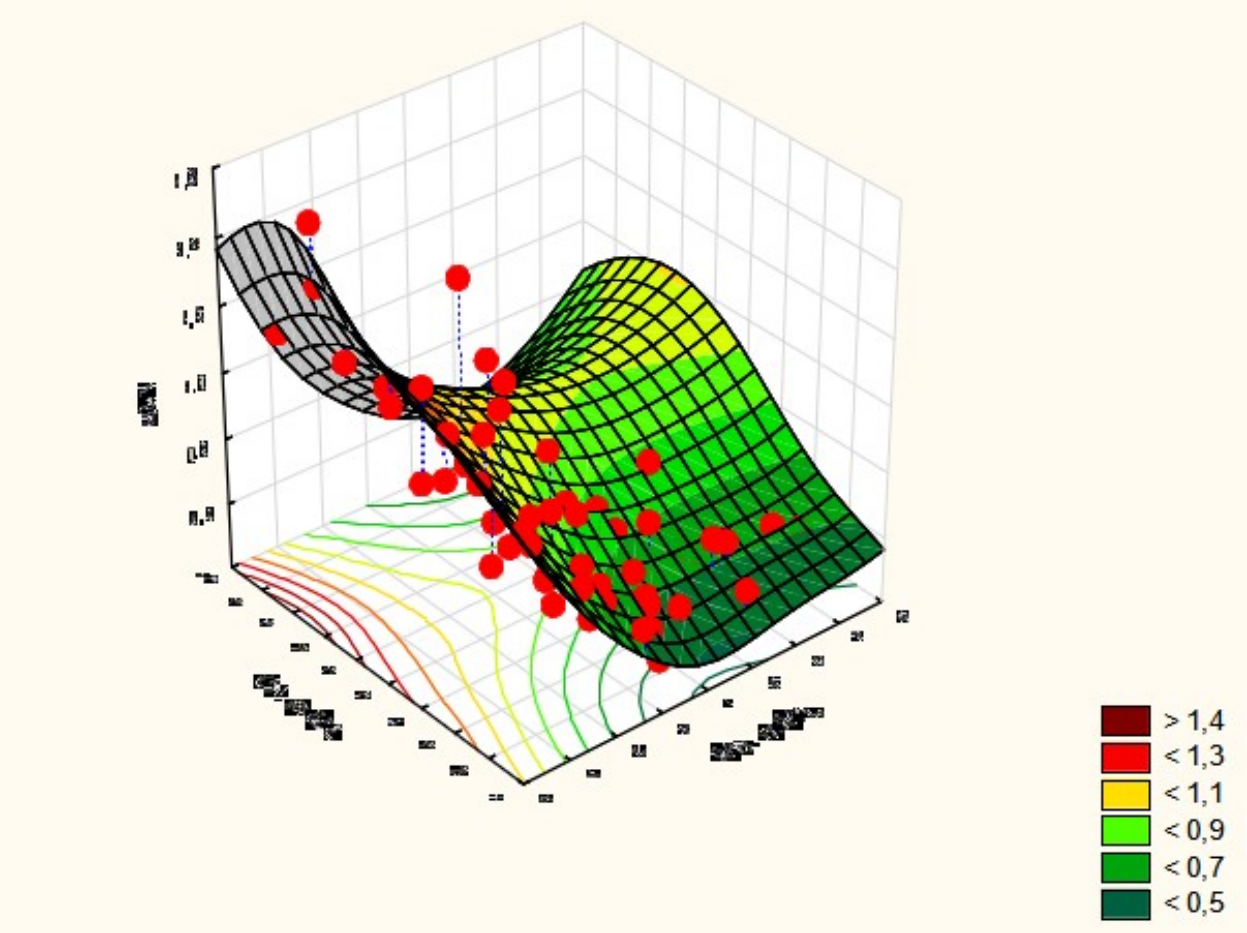
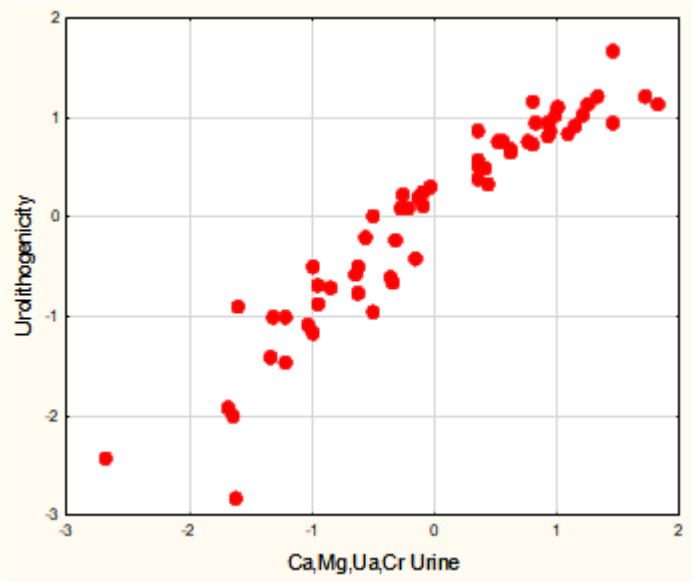


Fig. 7. Scatterplot of correlations between the urine magnesium (X-line), uric acid (Y-line) (Y-line) and lithogenicity index (Z-line)

Table 3. Regression Summary for Urolithogenicity index
R=0,947; R^2=0,897; Adjusted R^2=0,890; $F_{(4,6)}$=120; $p<10^{-4}$

		Beta	St. Err. of Beta	B	SE of B	$t_{(55)}$	p-level
Variables	r		Intercpt	1,002	0,0647	15,5	10^{-6}
Magnesium, mM/L	**-0,730**	-0,622	0,045	-0,0914	0,0067	-13,7	10^{-6}
Creatinine, mM/L	**-0,298**	-0,285	0,044	-0,0428	0,0066	-6,49	10^{-6}
Uric acid, mM/L	**0,583**	0,415	0,045	0,0560	0,0061	9,13	10^{-6}
Calcium, mM/L	**0,352**	0,332	0,044	0,0923	0,0122	7,57	10^{-6}



R=0,947; R^2=0,897; $\chi^2_{(4)}$=128; $p<10^{-6}$; Λ Prime=0,103
Fig. 8. Scatterplot of canonical correlation between urine lithogenic and litholytic substances (X-line) and urolithogenicity index (Y-line)

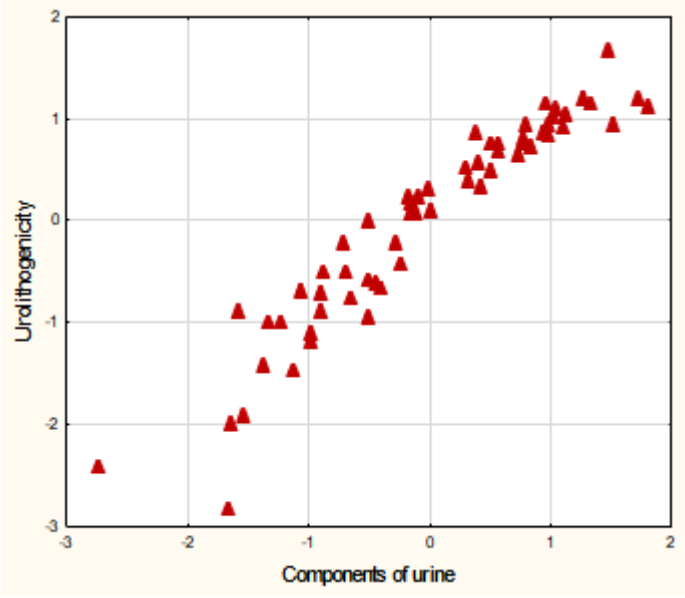
Next, the canonical correlation between the lithogenicity index of urine and all its registered parameters is analyzed. In the factor structure of urinary metabolic root, the program included, by definition, components of the lithogenicity index, medium molecular polypeptides, catalase, sodium, phosphates and urea as prolithogenic factors and tubular reabsorption of water as litholytic factor (Table 4).

Table 4. Factor structure of canonical roots of urine components

Variable	Root 1
Na U	-0,215
CReab	0,278
Urea U	-0,178
P U	-0,200
MMM U	-0,376
Katal U	-0,356
Cr U	0,314
Ca U	-0,371
Ua U	-0,613
Mg U	0,769

Interestingly, the additional inclusion of metabolic parameters in the factor structure of the urinary root had almost no effect on the value of the canonical correlation coefficient (Fig. 9).

We interpret this as evidence of redundancy/duplication of information contained in these parameters.



R=0,950; R^2=0,902; $\chi^2_{(10)}$=123; $p<10^{-6}$; Λ Prime=0,098
Fig. 9. Scatterplot of canonical correlation between components of urine (X-line) and its lithogenicity (Y-line)

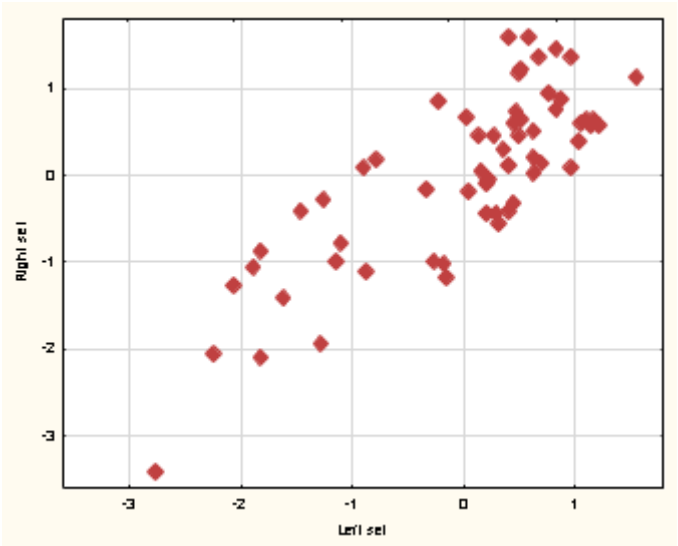
This statement is supported by the close links between the two sets of urinary metabolites (Table 5 and Fig. 10).

Table 5. Results of the canonical correlation between two sets of urine components

Variable	Factor Structure, left set Root 1
NaU	-0,207
CReab	0,909
Urea U	-0,094
P U	-0,623
MMM U	-0,559
Katal U	-0,425
MDA U	0,457

Variable	Factor Structure, right set Root 1
CrU	0,895
MgU	-0,117
CaU	-0,563
UaU	-0,006

Root Removed	Correlations, left set with right set			
	CrU	MgU	CaU	UaU
NaU	-0,187	-0,166	0,059	0,064
CReab	0,642	-0,146	-0,426	-0,016
Urea U	-0,047	-0,308	0,228	-0,219
P U	-0,435	0,060	0,321	-0,014
MMM U	-0,438	-0,162	0,240	0,049
Katal U	-0,236	-0,219	0,393	0,070
MDA U	0,357	0,051	-0,200	0,067



R=0,809; R^2=0,655; $\chi^2_{(28)}$=85; p<10^{-6}; Λ Prime=0,202

Fig. 10. Scatterplot of canonical correlation between two sets of urine components

Another approach to achieve this goal is discriminant analysis (Tables 6-8).

Table 6. Discriminant Function Analysis Summary
Step 8, N of Variables currently in the model: 8; Grouping: 3 groups
Wilks' Lambda: 0,1819; approx. $F_{(16,1)}$=8,40; $p<10^{-6}$

Variables currently in the model	Groups (n)				Parameters of Wilks' Statistics				
	Intact rats (10)	Lith - (13)	Lith ± (40)	Lith + (7)	Wilks' Λ	Partial Λ	F-remove (2,50)	p-level	Tolerancy
Magnesium Urine, mM/L	2,56 1 0	4,38 1,71 +1,02	2,38 0,93 -0,10	0,82 0,32 -0,98	0,384	0,473	27,8	10^{-6}	0,623
Phosphates Urine, mM/L	6,39 1 0	5,55 0,87 -1,07	6,34 0,99 -0,06	6,69 1,05 +0,38	0,221	0,822	5,42	0,007	0,693
Katalase Activity Urine, µM/h•L	123 1 0	129 1,05 +0,24	134 1,09 +0,42	204 1,66 +2,96	0,210	0,866	3,88	0,027	0,800
Amylase Activity Urine, g/h•L	202 1 0	187 0,92 -0,28	216 1,07 +0,25	181 0,89 -0,40	0,208	0,875	3,58	0,035	0,703
Calcium Urine, mM/L	2,10 1 0	1,66 0,79 -1,17	2,27 1,08 +0,47	2,89 1,38 +2,10	0,228	0,799	6,27	0,004	0,574
Uric Acid Urine, mM/L	3,68 1 0	2,29 0,62 -0,75	3,67 1,00 0,00	5,02 1,36 +0,73	0,205	0,887	3,19	0,049	0,869
Creatinine Urine, mM/L	6,41 1 0	7,44 1,16 +0,56	7,24 1,13 +0,45	5,54 0,86 -0,47	0,205	0,889	3,13	0,052	0,655
Potassium Urine, mM/L	131 1 0	122 0,94 -0,21	125 0,96 -0,14	104 0,80 -0,68	0,190	0,958	1,10	0,340	0,671

Note. In each column, the first line is the average value, the second is the fraction of the norm, and the third is the Z-score.

Table 7. Discriminant Function Analysis Summary. Variables currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Intact rats (10)	Lith- (13)	Lith± (40)	Lith+ (7)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Sodium Urine, mM/L	105 1 0	62 0,59 -0,65	108 1,02 +0,04	125 1,19 +0,30	0,178	0,981	0,48	0,624	0,938
Chloride Urine, mM/L	115 1 0	88 0,77 -0,33	118 1,03 +0,04	100 0,87 -0,19	0,179	0,982	0,45	0,643	0,798
Diuresis, mL/24h•100 g	1,44 1 0	1,87 1,30 +0,48	1,49 1,04 +0,06	2,32 1,61 +0,99	0,177	0,974	0,66	0,523	0,573
Glomerular Filtration, µL/min•100 g	86,0 1 0	143 1,66 +1,85	117 1,37 +1,02	90,7 1,05 +0,15	0,177	0,975	0,62	0,541	0,663
Canalicular Reabsorbtion, %	98,69 1 0	98,92 1,002 +0,28	98,83 1,001 +0,17	98,02 0,993 -0,83	0,178	0,976	0,59	0,558	0,455
Urea Urine, mM/L	105 1 0	100 0,95 -0,13	124 1,18 +0,47	142 1,35 +0,93	0,181	0,996	0,09	0,913	0,657
Middle Mass Molecules Urine, units	182 1 0	149 0,82 -0,62	163 0,89 -0,37	209 1,15 +0,53	0,179	0,985	0,38	0,685	0,832
Malondialdehyde Urine, µM/L	92 1 0	94 1,02 +0,04	93 1,01 +0,03	67 0,73 -0,58	0,179	0,981	0,46	0,632	0,747
Diene conjugates Urine, E^{232}/mL	1,86 1 0	1,87 1,01 +0,03	1,78 0,96 -0,12	1,58 0,85 -0,42	0,178	0,977	0,57	0,567	0,928

Table 8. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Magnesium Urine, mM/L	16,9	10^{-5}	0,628	16,9	10^{-5}
Phosphate Urine, mM/L	11,6	10^{-4}	0,444	14,0	10^{-6}
Katalase Activity Urine, µM/h•L	7,79	0,001	0,346	12,8	10^{-6}
Amylase Activity Urine, g/h•L	6,14	0,004	0,282	11,9	10^{-6}
Calcium Urine, mM/L	4,49	0,016	0,241	11,0	10^{-6}
Uric Acid Urine, mM/L	3,76	0,030	0,211	10,2	10^{-6}
Creatinine Urine, mM/L	2,80	0,070	0,190	9,43	10^{-6}
Potassium Urine, mM/L	1,10	0,340	0,182	8,40	10^{-6}

The dividing information contained in 8 variables is condensed in 2 canonical discriminant roots (Table 9). The major root contains 87% of discriminative opportunities (r*=0,860; Wilks' Λ=0,1819; $\chi^2_{(16)}$=91; p<10^{-6}) and the minor root 13% (r*=0,547; Wilks' Λ=0,7008; $\chi^2_{(7)}$=19; p=0,008).

Table 9 shows standardized (normalized) and non-standardized (raw) coefficients for discriminant variables. 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 (Fig. 11).

Table 9. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Magnesium Urine, mM/L	1,056	-0,259	0,760	-0,186
Phosphate Urine, mM/L	-0,508	0,469	-0,583	0,538
Katalase Activity Urine, µM/h•L	-0,155	-0,708	-0,004	-0,019
Amylase Activity Urine, g/h•L	-0,398	0,451	-0,011	0,013
Calcium Urine, mM/L	-0,675	0,197	-0,792	0,231
Uric Acid Urine, mM/L	-0,386	-0,259	-0,222	-0,149
Creatinine Urine, mM/L	0,431	0,329	0,268	0,204
Potassium Urine, mM/L	-0,274	0,157	-0,0062	0,0035
	Constants		5,950	-4,643
	Eigenvalues		2,852	0,427
Cumulative Proportions			0,870	1

In the Table 10 together with discriminant variables are also variables that carry identifying/ separating information, but were outside the model due to its duplication/redundancy. For ease of comparison, the values of the variables are transformed into Z-scores.

Table 10. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots		Lith + (7)	Lith ± (40)	Lith - (13)
Root 1 (87%)	**R1**	**R2**	**-2,81**	-0,41	**+2,78**
Calcium Urine	**-0,247**	-0,071	**+2,10**	+0,47	**-1,17**
Uric Acid Urine	**-0,271**	-0,070	**+0,73**	0,00	**-0,75**
Phosphates Urine	**-0,250**	0,112	**+0,38**	-0,06	**-1,07**
Katalase Activity Urine	**-0,260**	-0,709	**+2,96**	+0,42	**+0,24**
Glomerular Filtration			**+1,85**	+1,02	**+0,15**
Canalicular Reabsorbtion			**+0,28**	+0,17	**-0,83**
Malondialdehyde Urine			**+0,04**	+0,03	**-0,58**
Diene conjugates Urine			**+0,03**	-0,12	**-0,42**
Magnesium Urine	**0,456**	0,019	**-0,98**	-0,10	**+1,02**
Creatinine Urine	**0,155**	0,380	**-0,47**	+0,45	**+0,56**
Sodium Urine			**-0,65**	+0,04	**+0,30**
Urea Urine			**-0,13**	+0,47	**+0,93**
Middle Mass Molecules			**-0,62**	-0,37	**+0,53**
Root 2 (13%)	**R1**	**R2**	**-1,37**	**+0,42**	-0,56
Amylase Activity Urine	-0,063	**0,643**	-0,40	**+0,25**	-0,28
Potassium Urine	0,043	**0,201**	-0,68	**-0,14**	-0,21
Chloride Urine			-0,33	**+0,04**	-0,19
Diuresis			+0,48	**+0,06**	+0,99

The localization of the cluster of rats with high lithogenicity index in the extreme left zone of the major root axis (Fig. 11) reflects their maximally elevated levels of the calcium and uric acid, on the one hand, and maximally reduced levels of the magnesium and creatinine, by definition, as well as phosphates and catalase as prolithogenic factors and sodium, urea and middle mass molecules as litholytic factors. At the opposite pole of the axis are rats with reduced lithogenicity, and the intermediate position, of course, is a normal cluster, ie with lithogenicity indices in the range: $-\sigma \div +\sigma$. It should be noted that our data contradict the

position that hypercalciuria and hyperphosphaturia associated with the production of reactive oxygen species. However, this occurs in the presence of urolithiasis [12].

Additional differentiation of rats occurs along the axis of the minor root, demonstrating that both reduced and increased urinary lithogenicity is accompanied by lower than normal amylase activity and potassium and chloride concentrations in combination with higher daily diuresis.

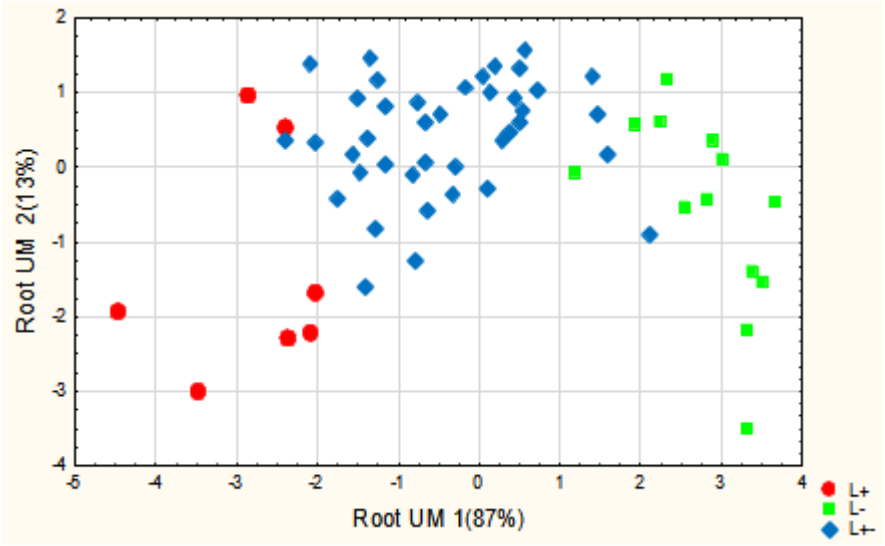


Fig. 11. Individual values of the first and second roots of the urine metabolic parameters in rats with different levels of lithogenicity

Despite separate interpenetrations, the three lithogenicity clusters differ significantly from each other, as documented by the calculation of Mahalanobis distances (Table 11).

Table 11. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=8,50) and p-levels (under diagonal)

Groups	Lith + (7)	Lith - (13)	Lith ± (40)
Lith +	0,0	31,9	9,0
Lith -	15,9 10^{-6}	0,0	11,1
Lith ±	5,9 10^{-4}	12,0 10^{-6}	0,0

The accuracy of the retrospective classification using the coefficients and constants of Table 12 is 93,3% (Table 13).

Table 12. Coefficients and Constants for Classification Functions

Variables currently in the model	Lith + (p=0,117)	Lith - (p=0,217)	Lith ± (p=0,667)
Magnesium Urine, mM/L	-4,371	-0,273	-2,881
Phosphate Urine, mM/L	13,50	10,68	13,07
Katalase Activity Urine, µM/h•L	0,144	0,104	0,099
Amylase Activity Urine, g/h•L	0,192	0,138	0,187
Calcium Urine, mM/L	5,091	0,854	3,606
Uric Acid Urine, mM/L	1,854	0,489	1,053
Creatinine Urine, mM/L	2,119	3,783	3,129
Potassium Urine, mM/L	0,084	0,052	0,075
Constants	-99,75	-68,78	-87,33

Table 13. Classification Matrix
Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	Lith + (7)	Lith - (13)	Lith ± (40)
		p=,117	p=,217	p=,667
Lith + (7)	71,4	**5**	0	2
Lith - (13)	92,3	0	**12**	1
Lith ± (40)	97,5	0	1	**39**
Total	93,3	5	13	42

CONCLUSION

Both prolithogenic and litholytic factors are present in the urine, which depend little on the chemical composition of the fluid used.

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 Horbachevskyi 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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