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VARIANTS OF THE STATE OF ELECTROLYTE EXCHANGE IN FEMALE RATES

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Abstract

Background. It is known that electrolyte exchange parameters are subject to the regulatory effects of a wide range of nerve, hormonal and humoral factors. The dispersion of electrolyte levels in plasma, erythrocytes and diurnal urine, first, is different, and second, variable. Therefore, there are a number of quantitative and even qualitatively different variants of the exchange of electrolytes. It is logical to assume that such diversity is conditioned by the state of the regulatory systems and/or the balance between the entering and excretion of electrolytes. We have set a goal in experiments in rats and clinical physiological observations to identify a number of variants of the state of exchange of electrolytes and their neuro-endocrine, humoral and immune support. In this article we summarize the first stage of the path to the goal. **Materials 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. The day after the completion of the drinking course in all rats the plasma and urine levels of the electrolytes were determined. **Results.** The method of cluster analysis identifies four variants of the state of electrolyte exchange. Characteristic features of the members of the major (51,7%) cluster are a moderate decrease in excretion with daily urine of sodium and chloride as well as calcium and potassium plasma levels in combination with a moderate increase in magnesium excretion. In the members of the second largest cluster (22,4%), a similar decrease in the plasma level of potassium and calcium is combined with a slight decrease in the content of potassium in erythrocytes and a decrease in its excretion with the urine. Electrolyte exchange of the third largest cluster (19,0%) is characterized by a moderate increase in the excretion of sodium and chloride and a slight increase in the excretion of potassium, magnesium, calcium and phosphate, as well as the content of potassium in erythrocytes. Finally, the minor cluster (6,9%) differs from others by drastically increasing sodium excretion, a significant increase in the excretion of chloride, calcium and phosphate, as well as the sodium content of erythrocytes in combination with a decrease in calcium (moderate), phosphate and chlorine (slight) plasma levels. **Conclusion**. Four quantitatively and qualitatively different variants of the exchange of electrolytes are revealed, which is caused, apparently, by different state of neuro-endocrine regulation.

Key words: Calcium; magnesium; phosphates; chloride; sodium; potassium; plasma; erythrocytes; urine; female rats; cluster analysis.

INTRODUCTION

It is known that electrolyte exchange parameters, on the one hand, are subject to the regulatory effects of a wide range of nerve, hormonal and humoral factors [11]. On the other hand, electrolytes have a regulatory effect on the metabolism and function of neurons, endocrinocytes, myocytes, immunocytes and other cells [9]. The dispersion of electrolyte levels in plasma, erythrocytes and diurnal urine, first, is different, and second, variable. Therefore, there are a number of quantitative and even qualitatively different variants of the exchange of electrolytes. It is logical to assume that such diversity is conditioned by the state of the regulatory systems and/or the balance between the entering and excretion of electrolytes.

One approach to studying the exchange of electrolytes is a one-time and long-term (course) introduction of them into the body by the so-called water-salt loads of animals, the natural equivalent of which is drinking balneotherapy [2,42]. As a result, changes in the parameters of exchange of not only those electrolytes that were introduced into the body with water, but also endogenous, as well as parameters of protein-nitrogen, lipid and carbohydrate metabolism [3,4,6,7,10,12,14,17-19,21-23,29,31,34,38,42,43] were revealed, which were accompanied by changes in the parameters of gastroentero-pancreatic hormonal [13,16,41], digestive [4,13,15,41], cardiovascular [4,33,35,39,40], endocrine [18,21,22,24-28,30,36,37,44], immune [7,18,22,25-28,30,31,36,37,44], autonomic and central nervous [5,7,20,24,25,27,30,31,34,36-38] systems as well as hemostasis [31].

Because in these studies, the electrolytes were out of focus, we have set a goal in experiments in rats and clinical physiological observations to identify a number of variants of the state of exchange of electrolytes and their neuro-endocrine, humoral and immune support. In this article we summarize the first stage of the path to the goal.

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 electrolytes were determined: calcium (by reaction with arsenase III), magnesium (by reaction with colgamite), phosphates (phosphate-molybdate method), chloride (mercury-rhodanidine method), sodium and potassium (both also in erythrocytes) by flamming photometry. The analyzes were carried out according to the instructions described in the manual [8]. The

analyzers “Pointe-180” ("Scientific", USA) and “Reflotron” (Boehringer Mannheim, BRD) were used with appropriate sets and a flamming spectrophotometer “СФ-47”.

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

RESULTS AND DISCUSION

Preliminary analysis revealed a wide variance of parameters. This prompted us to apply cluster analysis. Use of cluster analysis makes possible the simultaneous consideration of all the signs. Considering the totality of characteristics of persons undertaken in their relationship and conditionality of some of these (derivatives) other (main determinants) allows as to make a natural classification that reflects the nature of things, their essence. It is believed that knowledge of the essence of the object is to identify those of its quality properties that actually define the object, distinguish it from other [1,29].

Clustering cohort of persons is realized by iterative k-means metod. In this method, the object belongs to the class Euclidean distance to which is minimal. The main principle of the structural approach to the allocation of uniform groups consists in the fact that objects of same class are close but different classes are distant. In other words, a cluster (the image) is an accumulation of points in n-dimensional geometric space in which average distance between points is less than the average distance from the data points to the rest points [1].

Typically, the number of clusters is arbitrary. We stopped at four (Table 1), because less is banal and more difficult to perceive and compare.

Table 1. Members of Clusters and Distances from Respective Cluster Center

Members of Cluster Number 1. Cluster contains 13 cases

	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
	C_2	C_5	C_12	C_18	C_19	C_20	C_21	C_23	C_26	C_40	C_49	C_54	C_55
Distance	29	22	28	29	9,6	13	29	24	27	27	17	25	14

Members of Cluster Number 2. Cluster contains 11 cases

	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
	C_1	C_4	C_9	C_16	C_29	C_30	C_34	C_36	C_37	C_46	C_51
Distance	76	12	9,2	38	39	25	29	36	14	25	25

Members of Cluster Number 3. Cluster contains 30 cases

	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
	C_39	C_41	C_42	C_43	C_44	C_45	C_47	C_48	C_50	C_52	C_53	C_56	C_57	C_58
Distance	10	16	23	11	14	18	9,9	13	22	9,7	7,4	44	19	16

Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.	Case No.
C_3	C_6	C_7	C_8	C_10	C_11	C_13	C_17	C_22	C_25	C_27	C_28	C_31	C_32	C_33	C_35
8,7	38	17	23	21	21	8,6	23	22	14	22	21	35	34	22	18

Members of Cluster Number 4. Cluster contains 4 cases

	Case No.	Case No.	Case No.	Case No.
	C_14	C_15	C_24	C_38
Distance	25,5	20	42	77

Comparison of Euclidean distances in each cluster (Table 1) and between clusters (Table 2) attests to the correctness of clustering of the cohort.

Table 2. Euclidean Distances between Clusters

Distances below diagonal. Squared distances above diagonal

	No. 1	No. 2	No. 3	No. 4
No. 1	0	2909	2038	22413
No. 2	54	0	7410	12752
No. 3	45	86	0	36594
No. 4	150	113	191	0

The maximum contribution to the distribution into clusters, judging by the criterion η^2, which reflects the proportion of intergroup variance in the total variance (Table 3), is made by sodium excretion, slightly less by chloride excretion. Phosphate and calcium excretion, as well as daily diuresis, are significantly smaller, albeit significant, in clustering. Even smaller but statistically significant contributions of sodium and potassium erythrocytes as well as excretion of the latter. Instead, magnesium excretion and the levels of all electrolytes in the plasma do not contribute to the distribution of the cohort into clusters.

Table 3. Analysis of Variance

Variables	Between SS	Within SS	η^2	R	F	signif p
Na Excretion	1599739	103631	0,939	0,969	278	10^{-6}
Cl Excretion	829567	211780	0,797	0,892	70,5	10^{-6}
P Excretion	591,45	922	0,391	0,625	11,5	10^{-5}
Ca Excretion	118,4	198,1	0,374	0,612	10,8	10^{-5}
Diurese	11,46	22,6	0,336	0,580	9,1	10^{-4}
Na Erythrocytes	362,1	959,6	0,274	0,523	6,8	**,001**
K Excretion	96971	318239	0,234	0,483	5,5	**,002**
K Erythrocytes	318,6	1936,5	0,141	0,376	3,0	**,040**
Mg Excretion	39,62	418,4	0,087	0,294	1,7	,177
K Plasma	2,84	32,7	0,080	0,283	1,6	,208
Ca Plasma	2,30	45,4	0,048	0,220	0,9	,442
P Plasma	0,59	14,3	0,040	0,199	0,7	,532
Na Plasma	36,4	1594	0,022	0,149	0,4	,745
Cl Plasma	28,3	2222	0,013	0,112	0,2	,876
Mg Plasma	0,15	15,9	0,009	0,097	0,2	,914

Note. The variance analysis parameters are calculated by the following formulas:

$\eta^2=Sb^2/(Sb^2+Sw^2)$,
$R=\eta$,
$F=[Sb^2(n-k)]/[Sw^2(k-1)]$, where
Sb^2 is the intergroup variance;
Sw^2 is intragroup variance;
n is the number of animals (58);
k is the number of groups (4).

It would seem that the **selected** parameters (variables) are characteristic features of the images of the members of each of the clusters (recall that another name for the cluster analysis is the method of **image creation**). However, discriminant analysis [20], as a method of **image recognition**, gave somewhat different results (Tables 4 and 5). The forward stepwise program included only 4 variables with significant levels of η^2 in the model, while the other 4 appeared to be

out of the model (apparently as carrying excess information). Instead, plasma levels of sodium and potassium were identified as distinctive.

For the purpose of single-scale estimation of variables, they were normalized [10].

Table 4. Discriminant Function Analysis Summary

Step 6, N of vars in model: 6; Grouping: 4 grps
Wilks' Lambda: ,0214; approx. $F_{(18)}=22,3$; $p<10^{-6}$

Variables currently in the model		Clusters (n)				Parameters of Wilks' Statistics				
	Norm (10)	IV (4)	II (11)	I (13)	III (30)	Wilks' Λ	Partial Λ	F-re-move	p-level	Tole-rancy
Na Excretion µM/100g•day Cv=0,625	135 1 0	672 5,00 +6,40	276 2,05 +1,69	172 1,28 +0,45	45 0,33 -1,07	,306	,070	217,3	10^{-6}	,787
K Plasma mM/L Cv=0,167	4,23 1 0	4,12 0,97 -0,15	4,01 0,95 -0,31	3,55 0,84 -0,97	3,53 0,84 -0,99	,025	,840	3,1	,035	,714
K Excretion µM/100g•day Cv=0,650	189 1 0	161 0,85 -0,22	252 1,34 +0,52	127 0,67 -0,50	190 1,01 +0,01	,030	,710	6,7	,001	,449
K Erythrocytes mM/L Cv=0,079	87,0 1 0	85,7 0,98 -0,19	90,9 1,05 +0,58	83,7 0,96 -0,48	87,0 1,00 +0,01	,025	,856	2,8	,052	,912
Diurese mL/100g•day Cv=0,617	1,44 1 0	2,97 2,06 +1,72	1,91 1,32 +0,52	1,11 0,77 -0,37	1,63 1,13 +0,21	,028	,755	5,3	,003	,494
Na Plasma mM/L Cv=0,040	128,6 1 0	127,4 0,99 -0,24	128,0 1,00 -0,11	130,0 1,01 +0,29	129,1 1,00 +0,10	,027	,786	4,5	,008	,659

Vars currently not in model	Norm (10)	IV (4)	II (11)	I (13)	III (30)	Wilks' Λ	Partial Λ	F to enter	p-level	Tole-rancy
Cl Excretion µM/100g•day Cv=0,681	145 1 0	457 3,17 +3,18	297 2,05 +1,55	166 1,15 +0,22	65 0,45 -0,81	,020	,953	,79	,504	,291
Na Erythrocyts mM/L Cv=0,201	22,0 1 0	30,7 1,39 +1,95	23,6 1,07 +0,35	23,5 1,06 +0,32	20,9 0,95 -0,25	,021	,976	,39	,759	,740
Mg Excretion µM/100g•day Cv=0,631	3,30 1 0	2,78 0,84 -0,25	4,32 1,31 +0,49	2,90 0,88 -0,19	4,77 1,45 +0,71	,021	,970	,49	,690	,714
Ca Plasma mM/L Cv=0,305	3,35 1 0	2,18 0,65 -1,15	2,96 0,88 -0,38	2,48 0,74 -0,85	2,63 0,78 -0,71	,021	,968	,53	,661	,905
P Plasma mM/L Cv=0,585	1,04 1 0	0,74 0,71 -0,49	0,94 0,90 -0,16	0,81 0,78 -0,38	1,02 0,98 -0,03	,021	,964	,61	,615	,909
Ca Excretion µM/100g•day Cv=0,527	2,90 1 0	8,71 3,00 +3,80	3,69 1,27 +0,52	2,52 0,87 -0,25	3,60 1,24 +0,46	,020	,951	,83	,486	,586
P Excretion µM/100g•day Cv=0,671	9,38 1 0	20,3 2,17 +1,74	12,2 1,30 +0,44	6,96 0,74 -0,39	9,85 1,05 +0,08	,021	,980	,32	,810	,149
Mg Plasma mM/L Cv=0,687	0,88 1 0	0,73 0,83 -0,24	0,91 1,03 +0,05	0,90 1,03 +0,04	0,82 0,93 -0,10	,020	,946	,91	,444	,860
Cl Plasma mM/L Cv=0,064	93,8 1 0	90,9 0,97 -0,48	92,6 0,99 -0,20	93,7 1,00 -0,01	92,4 0,99 -0,22	,020	,955	,76	,522	,119

Notes. For each indicator, the first line reflects the actual values, the second - their proportions relative to the average of intact animals (V/N), the third - the normalized values Z=(V/N-1)/Cv, where Cv is the coefficient of variation in intact animals

Table 5. Summary of Stepwise Analysis

Variables	F to enter	p-level	Λ	F-value	p-level
Na Excretion	278	10^{-6}	,061	278	10^{-6}
K Excretion	7,1	,0004	,043	67	10^{-6}
Diurese	5,7	,002	,033	43	10^{-6}
K Erythrocytes	2,4	,080	,029	32	10^{-6}
Na Plasma	2,0	,122	,025	26	10^{-6}
K Plasma	3,1	,035	,021	22	10^{-6}

The distinctive information contained in the 6 discriminant variables is condensed into three roots. The first root contains 95,5% of the discriminatory potential (r*=0,977; Wilks' Λ=0,021; $x^2_{(18)}$=200; p<10^{-6}), second root only 3,0% (r*=0,626; Wilks' Λ=0,462; $x^2_{(10)}$=40; p<10^{-4}), and the third root is even smaller - 1,5% (r*=0,491; Wilks' Λ=0,759; $x^2_{(4)}$=14; p=0,006), however significant.

The sum of the products of the raw coefficients (Table 6) for the values of the discriminant variables (Table 5) together with the constant (Table 6) gives the values of the discriminant roots for each animal and makes it possible to visualize its condition in the roots information field (Figs. 1 and 2).

Table 6. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Na Excretion, µM/100g•day	-1,110	,026	,182	-,0253	,0006	,0042
K Plasma, mM/L	-,402	-,419	-,034	-,5175	-,5389	-,0436
K Excretion, µM/100g•day	-,357	-,947	,845	-,0047	-,0123	,0110
K Erythrocytes, mM/L	-,033	-,589	-,298	-,0055	-,0984	-,0497
Diurese, mL/100g•day	-,023	,281	-1,389	-,0359	,4346	-2,148
Na Plasma, mM/L	,491	,493	,037	,0903	,0907	,0068
		Constants		-4,286	,3055	4,458
		Eigenvalues		20,59	,644	,317
	Cumulative Properties			,955	,985	1,000

In the Table 7 gives the complete structural coefficients, ie correlation coefficients between the discriminant root and the variables. The structural coefficient indicates how closely related variables and discriminant functions are, that is, what proportion of root information is embedded in that variable. The centroid of the clusters of all three roots and the mean Z-values of the variables are also given.

The extreme left position along the axis of the first root of the members of the fourth cluster reflects their drastically increased excretion of sodium in combination

with a completely normal level of plasma potassium. Instead, the members of the **third** cluster are localized in the extreme right axis of the root, characterized by a qualitatively opposite state - hyponatriuria in combination with a moderate decrease in the level of potassium. The intermediate positions of the members of the other two clusters reflect the intermediate values of these variables, which are negatively related to the first root.

Table 7. Correlations Variables-Canonical Roots, Centroides of Roots and Means of changes in Variables

	Root 1	Root 2	Root 3	IV	II	I	III
Root 1(95,5%)				**-13,3**	**-3,5**	**+0,2**	**+3,0**
Na Excretion, Z	**-,864**	,350	,101	**+6,40**	**+1,69**	**+0,45**	**-1,07**
K Plasma, Z	**-,058**	-,169	,029	**-0,15**	**-0,31**	**-0,97**	**-0,99**
Root 2(3,0%)				+0,90	**-1,32**	**+0,97**	-0,06
K Excretion, Z	-,012	**-,677**	-,142	-0,22	**+0,52**	**-0,50**	+0,01
K Erythrocytes, Z	-,013	**-,498**	-,068	-0,19	**+0,58**	**-0,48**	+0,01
Root 3(1,5%)				**-0,93**	+0,46	+0,74	-0,37
Diurese, Z	-,116	-,252	**-,776**	**+1,72**	+0,52	-0,37	+0,21
Na Plasma, Z	,023	,115	**,108**	**-0,24**	-0,11	+0,29	+0,10

Along the axis of the second root, opposite positions are occupied by members of the **second** (lower) and **first** (upper) clusters, reflecting their maximum/minimum levels for sampling of kaliuria and kalihistia.

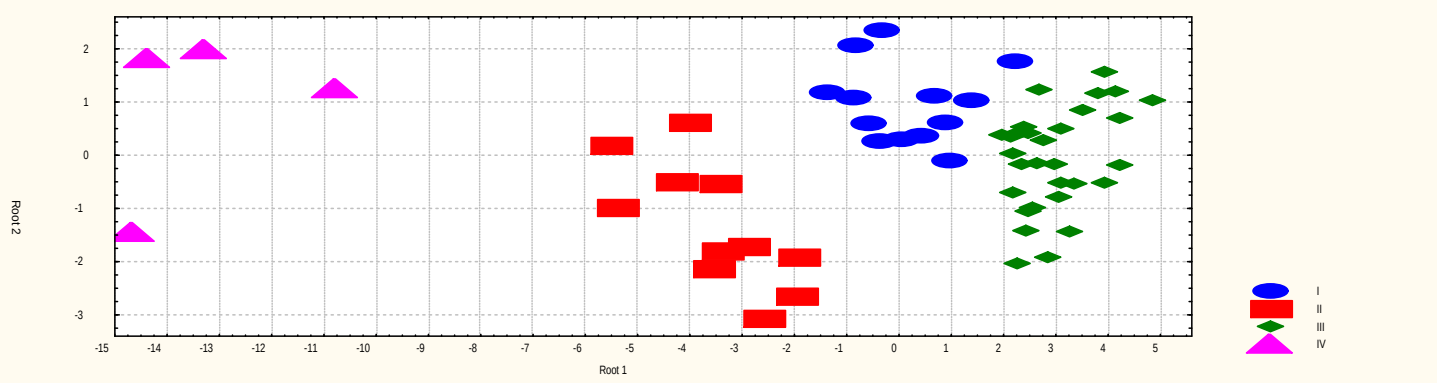

Fig. 1. Individual values of the first and second roots, in which condensed information about the parameters of the exchange of electrolytes in rats of different clusters

The members of the **fourth** cluster are delimited (but not clearly) with other clusters along the axis of the third root, occupying the lower position (Fig. 2). This reflects their maximum level of diuresis in combination with the minimum sodium content in erythrocytes.

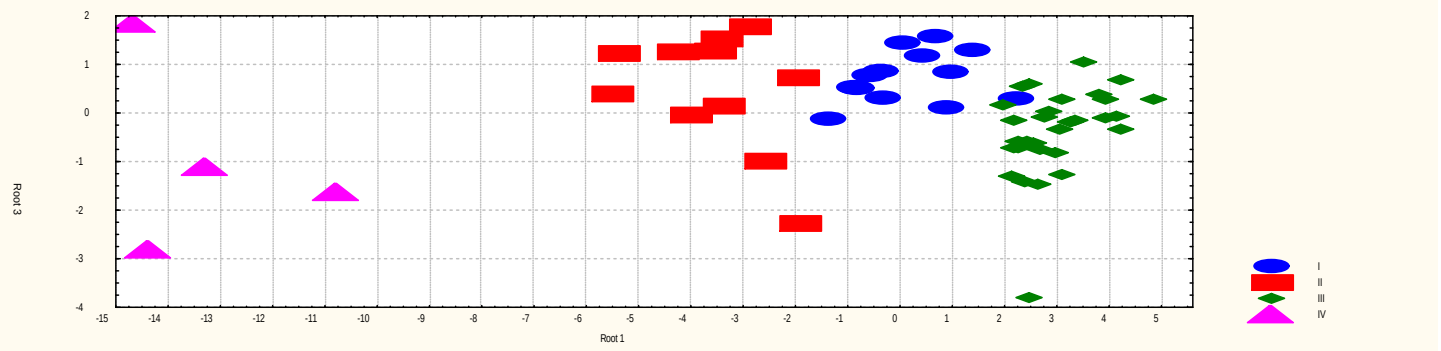

Fig. 2. Individual values of the first and third roots, in which condensed information about the parameters of the exchange of electrolytes in rats of different clusters

Cluster delineation is more clearly illustrated by visualizing the localization of their centroids (Figs. 3-5).

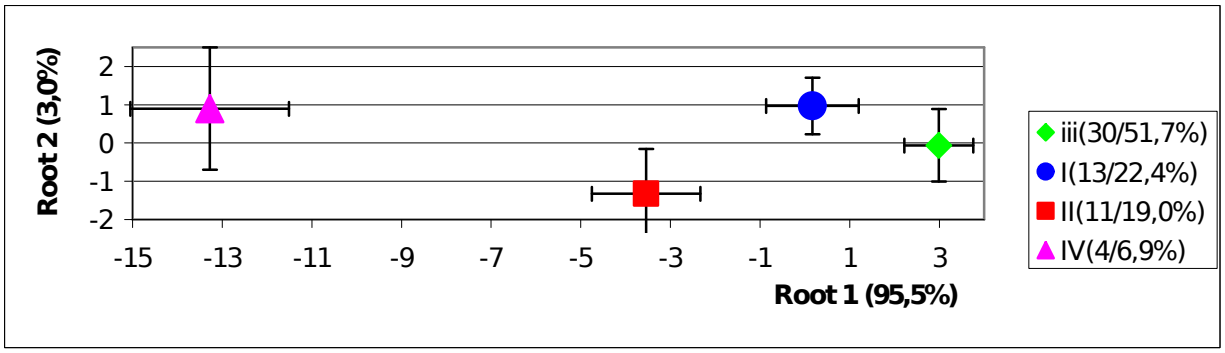

Fig. 3. Means of the first and second roots, in which condensed information about the parameters of the exchange of electrolytes in rats of different clusters

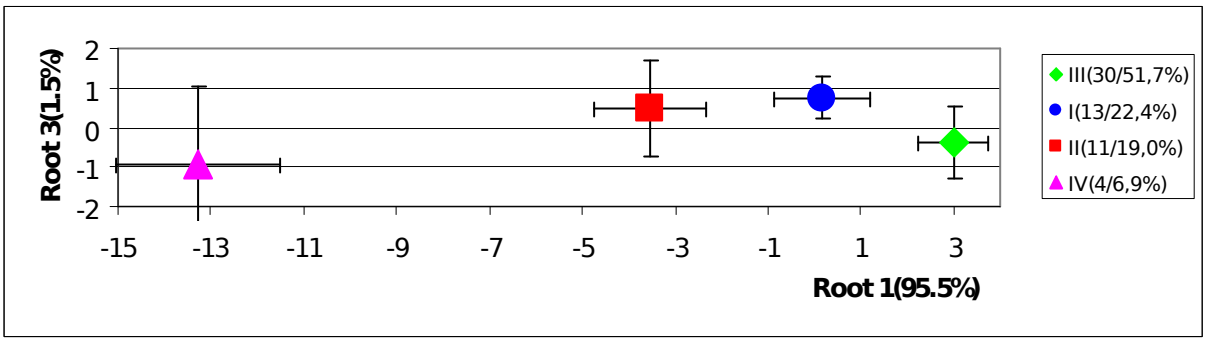

Fig. 4. Means of the first and third roots, in which condensed information about the parameters of the exchange of electrolytes in rats of different clusters

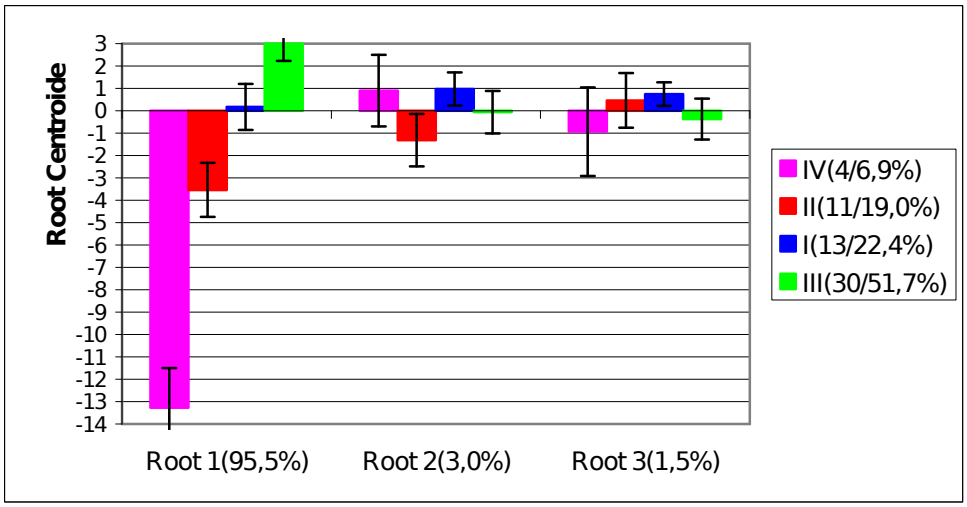

Fig. 5. Means of the discriminant roots, in which condensed information about the parameters of the exchange of electrolytes in rats of different clusters

The visual impression that the members of all four clusters are clearly distinguished in the information space of the three canonical roots is documented by the computation of Mahalanobis distances between the centroids of the clusters (Table 8).

Table 8. Squared Mahalanobis Distances, F-values and p-levels

Clusters	I	II	III	IV
I	0,0	20	11	197
II	17 $<10^{-6}$	0,0	48	109
III	14 $<10^{-6}$	54 $<10^{-6}$	0,0	286
IV	72 $<10^{-6}$	38 $<10^{-6}$	117 $<10^{-6}$	0,0

The same discriminant variables can be used to identify whether a rat is a member of a particular cluster. This purpose of discriminant analysis is realized by means of classification (discriminant) functions (Table. 9).

These functions are special linear combinations that maximize group differences and minimize intra-group variance. The coefficients of the classification functions are not standardized and therefore not interpreted. The object belongs to the group with the maximum value of the function, calculated by summing the product of the variables by the coefficients of the classification functions plus a constant.

Table 9. Coefficients and Constants for Classification Functions

Clusters	I	II	III	IV
Variables	p=,224	p=,190	p=,517	p=,069
Na Excretion, µM/100g•day	-,118	-,027	-,195	,216
K Plasma, mM/L	-8,256	-5,091	-9,112	-1,188
K Excretion, µM/100g•day	-,124	-,082	-,137	-,079
K Erythrocytes, mM/L	2,064	2,323	2,205	2,227
Diurese, mL/100g•day	13,02	12,76	14,86	17,07
Na Plasma, mM/L	5,169	4,625	5,323	3,937
Constants	-398,6	-391,2	-418,9	-437,8

Table 10 demonstrates the virtually complete accuracy of retrospective recognition.

Table 10. Classification Matrix

Rows: Observed classifications; Columns: Predicted classifications

	Clusters	I	II	III	IV
Clusters	Correct, %	p=,224	p=,190	p=,517	p=,069
I	92,3	12	0	1	0
II	100	0	11	0	0
III	100	0	0	30	0
IV	100	0	0	0	4
Total	98,3	12	11	31	4

Using the axis of the discriminant roots as abscissa and deferring the normalized values of all the registered parameters of the exchange of electrolytes along the axis of ordinates, we created a number of patterns (Fig. 6-10).

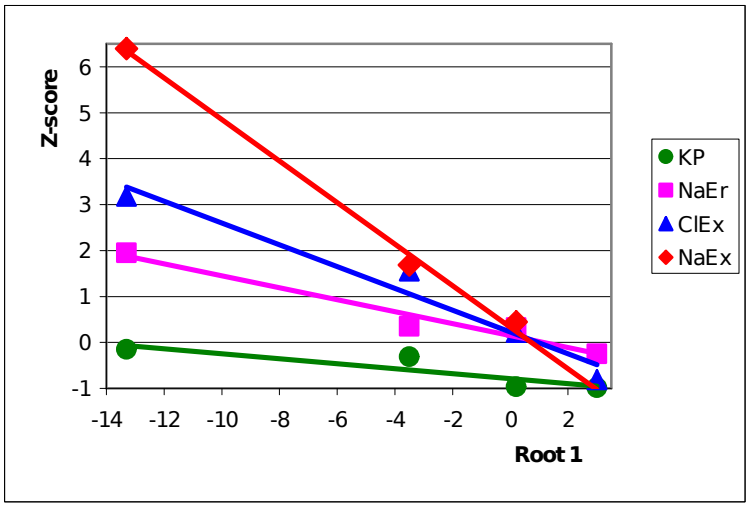

Fig. 6. Pattern of parameters of exchange of electrolytes inversely associated with the first root in rats of different clusters

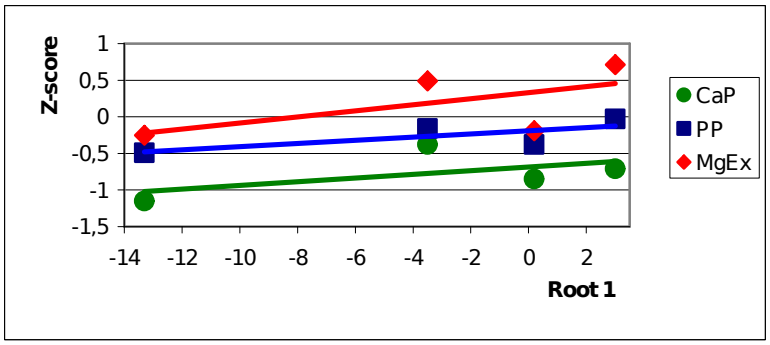

Fig. 7. Pattern of parameters of exchange of electrolytes positively associated with the first root in rats of different clusters

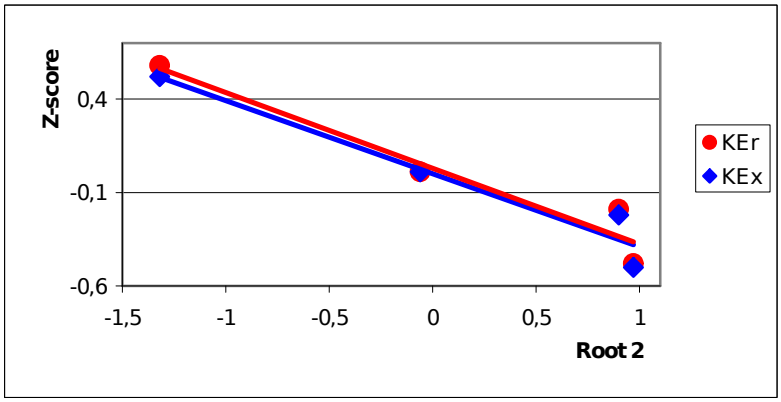

Fig. 8. Pattern of parameters of exchange of electrolytes inversely associated with the second root in rats of different clusters

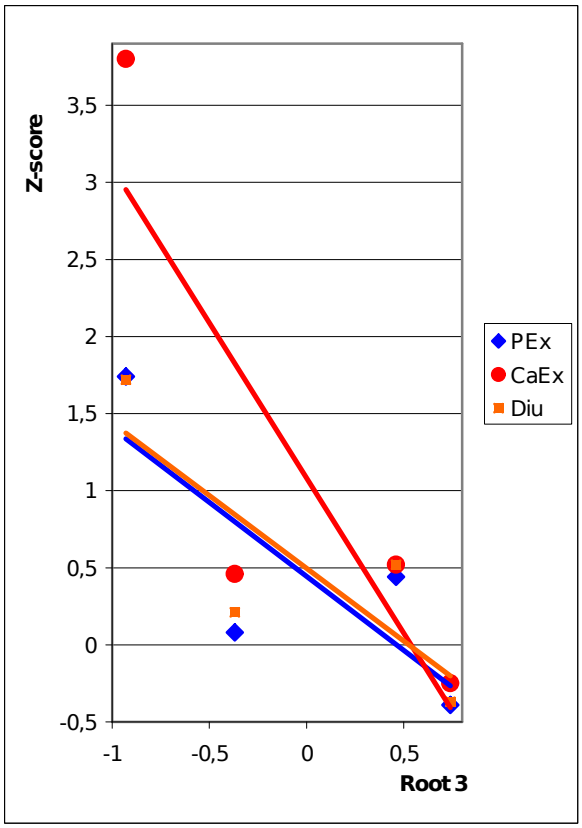

Fig. 9. Pattern of parameters of exchange of electrolytes inversely associated with the third root in rats of different clusters

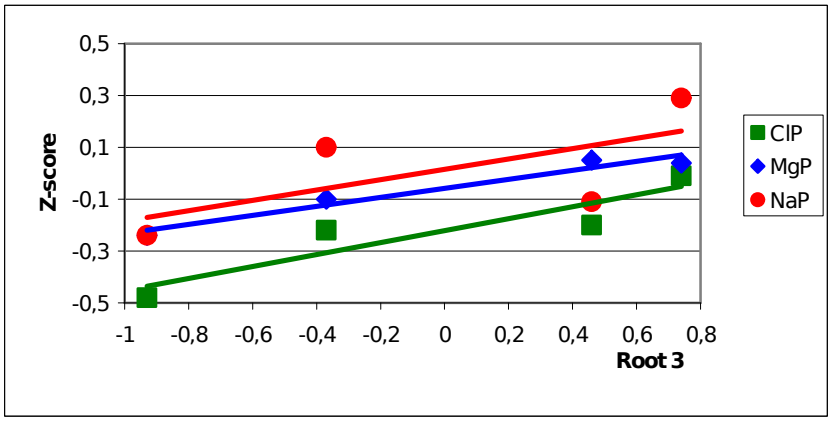

Fig. 10. Pattern of parameters of exchange of electrolytes positively associated with the third root in rats of different clusters

CONCLUSION

Thus, healthy female rats exhibit significant features of water-salt metabolism. The major changes in water-salt metabolism occur on the part of excretion. The cellular sector in ionic composition is more stable than the excretion rates of

electrolytes. The method of cluster analysis identifies four variants of the state of electrolyte exchange. Characteristic features of the members of the major (51,7%) cluster are a moderate decrease in excretion with daily urine of sodium and chloride as well as calcium and potassium plasma levels in combination with a moderate increase in magnesium excretion. In the members of the second largest cluster (22,4%), a similar decrease in the plasma level of potassium and calcium is combined with a slight decrease in the content of potassium in erythrocytes and a decrease in its excretion with the urine. Electrolyte exchange of the third largest cluster (19,0%) is characterized by a moderate increase in the excretion of sodium and chloride and a slight increase in the excretion of potassium, magnesium, calcium and phosphate, as well as the content of potassium in erythrocytes. Finally, the minor cluster (6,9%) differs from others by drastically increasing sodium excretion, a significant increase in the excretion of chloride, calcium and phosphate, as well as the sodium content of erythrocytes in combination with a decrease in calcium (moderate), phosphate and chlorine (slight) plasma levels. Four quantitatively and qualitatively different variants of the exchange of electrolytes are revealed, which is caused, apparently, by different state of neuro-endocrine regulation. This aspect will be the subject of the next article.

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 Horbachevskyi Ternopil' State 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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