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Electrogenic Metals in Epidermis: Relationship with Cell Bioenergetics

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Abstract

Our previous studies have found that results of quantitative spectrometry of electrogenic metals (Ca, K, Na) in epidermal cells (hair) from healthy individuals and the liquidators of the Chernobyl accident (chronic oxidative / nitrosative stress) are conjugated. The nature of this «conjugation», like many intimate mechanisms of the metal-ligand homeostasis in the epidermis, remains unsolved. However transmembrane traffic electrogenic metals (and especially the ion Na^+) are directly related to cell bioenergetics. Therefore, intracellular bioenergetics processes in the membrane and in the mitochondrial respiratory chain, can determine homeostasis electrogenic metals. It has been shown that in the cell, which is an open dynamic system, there are signs of self-organized criticality (SC). This allows classifying electrogenic metal homeostasis among the SC-phenomena.

Keywords: Electrogenic metals; Redox status; Epidermis; Self-organized criticality; Chronic oxidative stress; Chronic nitrosative stress

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Introduction

As confirmed by numerous publications on the subject and our own observations [1,2], metal homeostasis in epidermal cells can be studied using a derivative of epidermis (hair). It is of special value for mass (screening) studies since it enables to obtain the necessary number of samples for a reliable statistical analysis.

This approach was used in the present study to confirm the close relationship between electrogenic metals (EM) and cell bioenergetics and find evidence for possible attributability of EM homeostasis to the phenomena of self-organized criticality (SC).

The above assumption is based on the following well-known facts: 1) generation and maintenance of electrochemical potential of the cell at the expense of permanent and multi-directional EM traffic across the plasma membrane; 2) participation of sodium ions (Na^+) in cellular energy exchanges as a convertible 'energy currency', complementary to adenosine triphosphate (ATP); 3) failure of the hypothesis of normal distribution of quantitative spectrometry data of metals contained in epidermis derivatives (hair) [1].

A living cell, as an open dynamic system, depends on energy from outside. However, without prior transformation of this energy into convertible forms (ATP, Na^+, H^+), easily coping with entropy, the cell would not be able to find its rational use. With this in mind, intracellular energy balance regulation (energy expenditures matching adequate energy restoration) is unquestionably relevant.

One of the possible approaches, which can help analyze successful energy regulation (in micro- as well as in macrosystems), is provided by the theory of 'self-organized criticality' ('SC') [3]. The theory may be used to explain the statistics of accidents (the Gutenberg-Richter earthquake law), numerical patterns of urban growth and urban population, word usage frequency distribution in speech and literary texts (the Zipf's law), fractal geometry, functioning of neural networks of the brain and other micro- and macrocosmic events, which are referred to as SC-phenomena.

The most visual model of SC, which has become some kind of a 'brand' of the theory, is a pile of sand formed by constant addition of minimal quantities of sand. The dynamic relationship between the grains of sand in the pile, resulting in 'avalanches' of various calibers is characterized by the degree of correlation between the size and number of avalanches within a given interval of measurements. Detection of power dependence, irrespective of the sizes of objects under study (fractality criterion), helps identify SC-phenomena in a variety of dynamic systems.

EM homeostasis in regenerating tissue cells (epidermis) has instant connection with cellular energy. Having said that, the key homeostatic mechanisms, which control the level of metals, should, most probably, be sought in the peculiarities of intracellular energy transfer since we cannot exclude the existence of common regulatory mechanisms showing signs of self-organized criticality.

Therefore, it is of interest to detect possible degree of connection between the EM content in epidermis (spectrometry) and the number of individuals belonging to a given range of quantitative estimates. It is clear that such studies should be carried out on a sufficiently extensive statistical material.

Materials and Methods

Using mathematical statistics, we have analyzed the results of atomic emission spectrometry of hair samples for Na, K, and Ca content, which were obtained at the Center for Biotic Medicine (Moscow) from 10297 healthy subjects – Moscow and Riga residents (5160 males and 5137 females) aged 2 to 85.

We considered a sample median as a parameter characterizing typical concentrations of microelements. However, the sample median is just a point estimate of the median which gives no information about the accuracy and the reliability. The sample median is determined by finite sampling of the population, and it is essentially a random variable [4]. Thus, it may strongly differ from the true median value even with the arbitrarily large *n*. Interval estimations allow us to specify the limits, within which the required parameter lies with a certain probability.

The standard parametric method for the interval estimate of the median implies the normal distribution of the population $N(0,1)$ [5]. An alternative approach is based on the method of repeated samples. The point estimate is just a single realization of the random variable. To get an idea of its distribution, one must have an ensemble of realizations. For this purpose, various artificial methods are used. In this study, we used a technique known as "bootstrapping" [6]. This method allows us to obtain the confidence intervals without any supposition about the distribution of the population.

Table 1 Age-related dynamics in K and Na levels in epidermis.

Age	Median K (mcg/g)	CI boot low	CI boot up
60-85 years	121.4	87.97	161.5
50-59 years	104.3	95.37	117.56
30-49 years	56.06	53.4	58.46
20-29 years	38.18	35.7	41.04
10-19 years	54.4	41.2	84.8
2-9 years	376.88	231.9	972.26
Age	**Median Na (mcg/g)**	**CI boot low**	**CI boot up**
60-85 years	293.9	222.9	346.7
50-59 years	228.1	211.8	241.6
30-49 years	117.71	113.4	122.6
20-29 years	82.2	78.1	86.2
10-19 years	88.9	67.1	37.6
2-9 years	324.9	233	580.7

CI – Confidence Intervals; boot – Bootstrap method

Results and Discussion

The capacity of human mitochondria, the main producers of ATP, is not the same throughout the life of an individual. Reduced ATP production in early childhood, when mitochondrial network formation is still in progress, is followed by increased energy production at puberty age achieving its maximum during the active working age (18 to 45 years). As one ages, there comes a progressive decrease in the number of mitochondria associated with the destructive action of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which ultimately leads to a decrease in the intensity of ATP synthesis [7].

Assuming that K^+ and Na^+ participate in cellular energy exchange, we may be interested not only in the possible age-related changes in the content of the said metals in epidermis, but also in comparing the metals' mitochondrial activity spectrometry data during different age periods. Therefore, using the hair spectrometry data, we found the median levels of Na and K in different age groups. The results are shown in **Table 1** and **Figures 1 and 2.**

The age-related dynamics of Na and K content in epidermis shows two periods of significant increase in the level of metals: the first – at the age of 2 to 9 years, the second - after 50 years **(Figures 1 and 2).** How can we explain such changes in the EM content?

It seems reasonable to attribute the said changes to the abovementioned insufficient ATP production in childhood and old

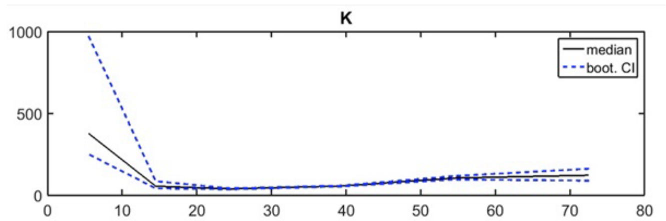

Figure 1 Age-related potassium levels in epidermal cells of healthy subjects (n=10297). The X-axis gives age (years); the Y-axis shows the K levels (μg/g). The dotted lines represent confidence intervals (the bootstrap-method).

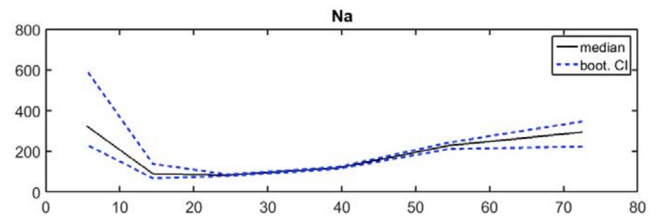

Figure 2 Age-related sodium levels in epidermal of healthy subjects (n=10297). The X-axis gives age (years), the Y-axis – the Na levels (μg/g).

Table 2 The content of metals in epidermal cells of the Chernobyl accident liquidators and healthy subjects [2].

Metals	Healthy subjects (n = 947) μg/g	Liquidators of the Chernobyl accident (n = 954) μg/g	Healthy subjects (*n*=947). μg/g Group I	Group II
			$r_{K\text{-}Zn}$ = - 0.01 (n = 253)	$r_{K\text{-}Zn}$ = - 0.43 (n = 171)
K	277.4<**317.7**<361.1	365.8<**394.8**<422.4*	82.6<**127**<181.9	715.3<**894**<1094.8*
Na	427.9<**480.9**<542.9	757.5<**822.3**<892.4*	159.7<**197.8**<245.4	1020.6<**1233.9**<1506.5*
Ca (w)	1348.1<**1439.6**<1528.4	832.0<**927.4**<1032.8*	1494.6<**1627.8**<1770.8	788.8<**946.8**<1101.1*
Ca (m)	529.1<**620.1**<763.1	560.0<**577.6**<601.5	755.0<**934.7**<1152.5	621.4<**856.9**<1111.6
V (w)	0.058<**0.06**<0.07	0.10<**0.12**<0.13*	0.05<**0.06**<0.07	0.08<**0.09**<0.11*
V (m)	0.08<**0.09**<0.1	0.11<**0.116**<0.12*	0.05<**0.06**<0.07	0.09<**0.11**<0.13*
Zn	181.5<**185.2**<189.3	162.5<**165.8**<169*	196.0<**204.3**<213.5	143.2<**150.7**<158.3*
Cu	19.06<**20.7**<22.3	10.6<**10.99**<11.4*	18.6<**21**<23.5	14.3<**16**<18.2*
Cd	0.04<**0.05**<0.06	0.2<**0.25**<0.3*	0.027<**0.03**<0.04	0.05<**0.06**<0.08*
Fe	19.3<**21.07**<23.1	22.4<**23.7**<25.07**	15.0<**17.8**<21.5	20.0<**22.5**<24.9**
Al	8.1<**8.8**<9.5	19.3<**20.1**<20.9*	4.3<**5.2**<6.2	10.8<**12.3**<14.2*
Cr	0.48<**0.51**<0.54	0.85<**0.9**<0.92*	0.35<**0.39**<0.42	0.62<**0.72**<0.83*
Pb	1.04<**1.1**<1.27	1.5<**1.8**<2.2*	0.68<**0.95**<1.3	1.1<**1.4**<1.6**
Li	0.03<**0.04**<0.05	0.05<**0.06**<0.062*	0.02<**0.023**<0.03	0.046<**0.05**<0.06*

Mean values are shown in bold. Plain script shows confidence interval (bootstrap-method); Statistical significance of differences is marked with asterisks: *p < 0.001; **p < 0.05.

age. Sodium potential, as a convertible energy 'currency', seems to be able of compensating the lack of ATP in the aforesaid age periods. Such increased production of ROS/RNS in elderly and senile persons should lead to more intense activity of membrane pumps (primarily, Na^+/K^+-ATPases) due to oxidative modification and/or S-nitrosylation of these proteins. In this case ROS/RNS play an important role of signaling molecules that help maintain the required level of power cell and (consequently) an increase in life expectancy of an individual.

Evidently, a crucial role in extending the life of an individual cell and the whole body is played rather by the energy conservation potential on the cellular or whole-body level than by the presence or absence of prooxidant shift in the redox status. And if this goal is served by the sodium potential, which is capable of intertransformation other 'energy currencies' (ATP and H^+) and whose realization involves ROS/RNS and Na^+/K^+-ATPase activity, then it is reasonable to expect a prooxidant shift and a sodium level increase in aging body cells.

This fact confirms the known ROS/RNS ability of accumulating in old age and challenges the universality of the free-radical aging theory, where free radicals are viewed as the main factors of aging [8].

It is not very clear what stimulates Na^+/K^+-ATPase activity in children, although there are reports of prooxidant shift in the redox status of children aged 7-11 years, which are explained (by the authors of the reports) by deficit of natural antioxidants and adaptive disorders [9].

The connection between EM homeostasis and bioenergetics justifies the participation of self-organized criticality in the control of EM intracellular content. It was, therefore, of interest to identify the nature of the relationship between the EM level (spectrometry data) and the number of individuals in the specified range of measured EM values. Idenification of power dependence between these parameters (the SC criterion) would enable to classify EM homeostasis as a SC-phenomenon.

According to our previous studies, the calcium content in female hair was significantly higher than in male hair [1]. Therefore, statistical data processing spectrometry for Ca are held separately for men and women. The study results are represented in **Figures 3-6.**

Figures 3-6, that are carried out in a double logarithmic scale, present a model of power-series distribution density (the number of columns = 20):

$y(x) = \frac{ab^a}{x^{a+1}}$, where $x \geq b$

The linear function of $\ln y = A \ln x + B$, where $A = -a - 1$; $B = \ln a + a \ln b$, is fit by the least square method of the last 9 columns. The straight line approximates the data visually on a logarithmic scale very well. On a linear scale, however, this is not the optimal solution in terms of least squares.

As a hypothesis, we propose the following interpretation of the obtained data. All the curves **(Figures 3-6)** have pieces of different

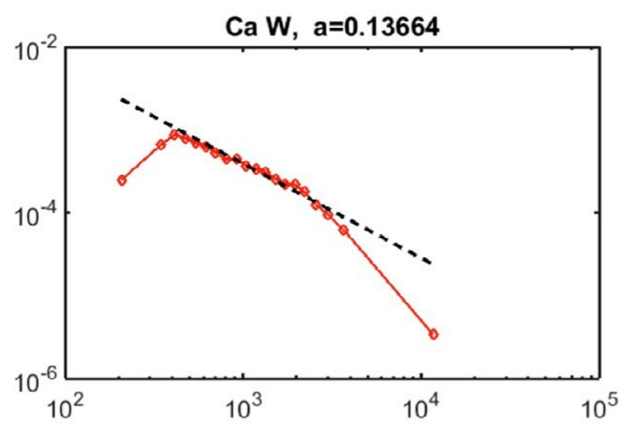

Figure 3 Estimate of the probability density function (PDF) (Y-axis) of Ca levels in epidermis in μg/g (X-axis) for women (red curve) and its approximation by PDF of the Pareto distribution (black curve).

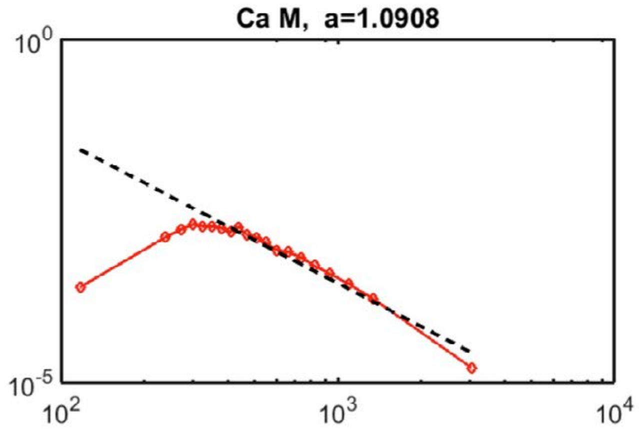

Figure 4 Estimate of PDF (Y-axis) of Ca levels in epidermis in µg/g (X-axis) for men (red curve) and its approximation by PDF of the Pareto distribution (black curve).

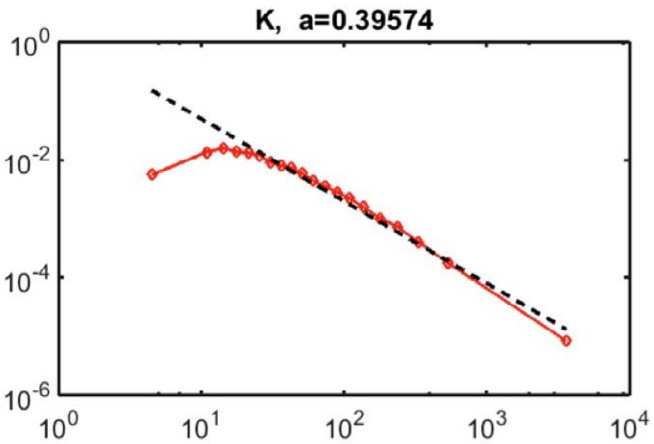

Figure 5 Estimate of PDF (Y-axis) of Potassium levels in epidermis in µg/g (X-axis) (red curve) and its approximation by PDF of the Pareto distribution (black curve).

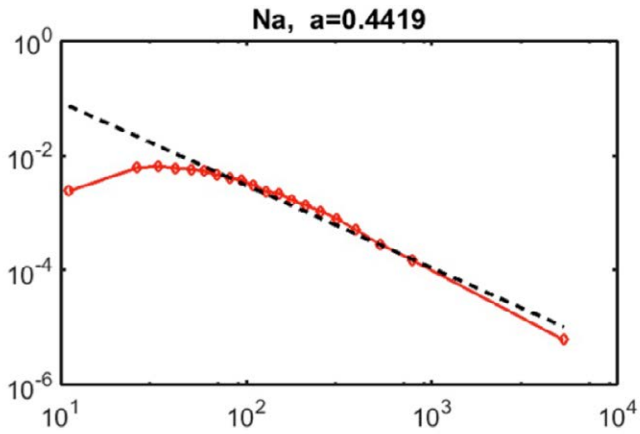

Figure 6 Estimate of PDF (Y-axis) of Sodium levels in epidermis in µg/g (X-axis) (red curve) and its approximation by PDF of the Pareto distribution (black curve).

sizes, almost coinciding with the straight line, which, according to SC theory, is indicative of the power nature of relationship between the studied parameters (the EM level and the number of subjects) and/or of the critical state (branching parameter σ =1) in the activity of ATPases. In other words, within the numerical values of EM, which are approximated by a straight line in the double logarithmic scale **(Figures 3-6),** there is synchronization (as a subcase of critical state) not only of Na^+/K^+-ATPases, but, apparently, other membrane pumps of the ATPase superfamily (P-type), which are in charge of the traffic of Ca, Zn and other metals [10-12]. This assumption may be justidied by the below-listed facts.

Our previous correlation analysis of K and Na levels in epidermis (by atomic emission spectrometry) showed a clear linear relationship (Pearson) between these parameters (***r*** K-Na=0.6-0.8). The studies were conducted in healthy subjects (n=947) and liquidators of the Chernobyl accident (n=954) with signs of oxidative/nitrosative stress [13, 14]. The presence of reliable K-Na correlation is indicative of synchronous operation of membrane Na^+/K^+-ATPases, which operate with much greater intensity under the conditions of oxidative/nitrosative stress. The aforesaid assumption is further confirmed by our finding of a significant increase in the Na and K levels in epidermis of the Chernobyl liquidators versus the control group. As an indirect evidence of intensified Na^+/K^+-ATPase activity under oxidative/nitrosative stress, one can mention the simultaneous and significant reduction of Ca in the epidermis of the Chernobyl victims, which is most probably related to the activaion of Na^+/Ca^{2+}-exchanger (NCX), antiporter for Ca^{2+} [1].

The fact is that Na^+/K^+-ATPase along with Ca^{2+}-ATPase of plasma membrane, which removes Ca^{2+} from cells, and the family of Ca^{2+}-ATPase endo- and sarcoplasmic reticulums (SERCA), which pump Ca^{2+} into intracellular structures, belongs to the so-called primary systems of active transport ions. The secondary active transport of ions is carried out through the energy of movement in the direction of Na^+ electrochemical gradient and depends on the efficient operation of Na^+/K^+-pump, which ensures the existence of this gradient. An example of secondary transport is the Na^+/Ca^{2+}-exchanger, which removes one calcium ion (Ca^{2+}) due to the input of three sodium ions (Na^+) into the cell. Therefore, the NCX activation, which leads to Ca decrease in the cell, points to intensified Na^+/K^+-ATPases activity.

To verify the fact that the synchronization of the membrane pump work is inherent in all the superfamily of ATPases (P-type) in the Chernobyl accident liquidators and healthy subjects, the linear correlation ratio by the ***r*** coefficient (Pearson) was determined between the concentration values of potassium (K) and zinc (Zn). As a result, another criterion of distinction was found: in the Chernobyl accident liquidator's $r_{K\text{-}Zn}$ (-0.42; $p < 0.05$) appeared to be by 50% greater than in healthy subjects ($r_{K\text{-}Zn}$= -0.28; $p < 0.05$).

It was found that the absolute majority of the accident liquidators (88%) showed a negative and significant K-Zn correlation (in 205 persons $r_{K\text{-}Zn}$= -0.62; $p < 0.05$; in 634 persons $r_{K\text{-}Zn}$= -0.41; $p < 0.05$). In 12% of the Chernobyl accident liquidators (115 persons), it was not detected (***r*** = -0.03).

The K-Zn correlation was absent (***r*** = -0.01) in 253 healthy subjects (26.7%), feebly marked ($r_{K\text{-}Zn}$= -0.22; $p<0.05$) in 523 persons (55.2%) and obviously marked ($r_{K\text{-}Zn}$= -0.43; $p < 0.05$) in 171 persons (18.1%) only.

The concentration values of Zn and electrogenic metals (K, Na, Ca) in the accident liquidators' epidermis had significant ($p < 0.05$) distinctions compared to the control group, and in the Chernobyl accident liquidators they were as follows: Zn=162.5<**165.8**<169; K=365.8<**394.8**<422.4; Na=757.5<**822.3**<892.4; $Ca_{(fem.)}$=832.0<**927.4**<1032.8. In healthy subjects: Zn=181.5<**185.2**<189.3; K=277.4<**317.7**<361.1; Na=427.9<**480.9**<542.9; $Ca_{(fem.)}$=1348.1<**1439.6**<1528.4 (the boldface characters are the medium value (M) in mcg/g; the normal type – the confidence limits – the bootstrap-method).

The analysis of electrogenic metals and zinc homeostasis in epidermal cells of healthy subjects at the extreme values of $r_{K\text{-}Zn}$ (-0.01 and -0.43) has shown that shifts in metal homeostasis of healthy persons with $r_{K\text{-}Zn}$= -0.43 (*vs.* $r_{K\text{-}Zn}$= -0.01) are, by their directionality number of cases and by their magnitude, similar to those of the total group of the Chernobyl accident liquidators (*n*=954), when compared with the total group of controls (*n*=947) [2]. One can draw a conclusion, (which seems obvious although requiring verification) about the presence of oxidative/nitrosative stress of non-radiation nature in healthy subjects with $r_{K\text{-}Zn}$= -0.43 **(Table 2).**

Similar changes in concentration values of electrogenic metals, which are dependent on the value of $|r|_{K\text{-}Zn}$, were also found in the Chernobyl accident liquidators [2].

If the negative correlation ratio K-Zn in the setting of oxidative/nitrosative stress really reflects the synchronous operation of the superfamily of membrane ATPases (which is indirectly suggested by the spectrometry results), the revealed 'sensitivity' of electrogenic metals to increased production of ROS/RNS, which, alongside with the growth of $|r|_{K\text{-}Zn}$, is accompanied by notable shifts in intracellular level of Ca^{2+}, K^{+}, Na^{+}, must inevitably lead to the conclusion that a normal (without fail) operation of membrane pumps for metals must take place in the synchronous mode, that is, in the mode of self-organized criticality.

As already mentioned above, synchronization (*critical state* in terms of SC) takes the form of a straight line in **Figures 3-6,** which means that whithin the given interval of numerical EM values, limited by the straight line, there is a power dependence between the EM level and the number of test subjects. The existence of such relation is, most probably, responsible for the contingency (synchronism) of the membrane ATPase activity.

The deviations from the straight line in the Graph, which are located in the beginning of the X-axis for Na and K, and in the end portion for Ca (i.e. an increase in $|r|_{K\text{-}Zn}$ leads to reduction of Ca level in a cell), correspond to a subcritical state (σ < 1) according to the SC theory.

One may only guess about the system transition into supercritical state (σ > 1) judging by the slight deviation from the straight line in the end portion of the X-axis in the Graphs for K and Na and the marked one in the beginning of the X-axis for Ca. It must be noted that being in a sub- or supercritical state for a system means desynchronization of its operation.

The intervals of numerical EM values (spectrometry data of 947 healthy subjects **(Table 2)**), which correspond to asynchronous (sub- and supercritical state with $\mathbf{r}_{K\text{-}Zn}$ = - 0.01) and synchronous operation of membrane pumps (critical state with $\mathbf{r}_{K\text{-}Zn}$ = -0.43), are similar to the data obtained from a tenfold sampling (n=10297) **(Figures 3-6).**

The aforesaid opens new opportunities of diagnosing critical states (synchronous operation of ATPases) by the metal content in epidermis (spectrometry). Besides, it may by worthwhile to use (both clinically and experimentally) the analysis of metal-ligand homeostasis, which would take account of synchronization/desynchronization processes in membrane pumps operation.

Conclusion

The ROS/RNS-assisted activation and normal functioning of membrane ATPases suggests synchronous (critical) nature of transmembrane traffic of metals. The synchronous operation of membrane pumps is confirmed by the negative K-Zn relation (Pearson) and signs of '*self-organized criticality'* (SC) according to the data of mathematical analysis. The obtained results point to the probable attributability of electrogenic metals homeostasis in epidermis to SC-phenomena.

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