What are the Limits, If Any, of Normal Content of Electrogenic Metals (K, Na, Ca) in Epidermis?

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Abstract

The work is devoted to the problem of the norm in the quantitative measurement (atomic emission spectrometry) of electrogenic metals (K, Na, Ca) in the epidermis. The paper suggests the possibility of a physiological (bioenergetic) approach to the definition of a norm based on the theory of self-organized criticality (SC). Unfortunately, this cannot be achieved using statistical (probabilistic) estimates of the average content of electrogenic metals (EM) in the epidermal cells.

Keywords: Electrogenic metals; Normal content in epidermis; Redox status; Self-organized criticality

Introduction

Despite the obvious successes of modern bioelementology, such as the introduction of high-precision instruments for quantitative analysis of minerals in various biosubstrates and new methodological approaches, e.g., the use of hair for the spectrometry of chemical elements in epidermis, which gives elemental analysis a number of obvious advantages (noninvasiveness, storage and transportation convenience), the estimation of mineral status in a particular substrate still remains controversial and inaccurate in many respects.

This is particularly true for latent forms of mineral deficiency, e.g., latent iron deficiency (LID), which are practically impossible to diagnose by the level of serum iron (Fe). According to our data, the in formativeness of this method is 3% only, in contrast to 97%, which is the in formativeness of the serum ferritin method of LID detection [1].

Incorrect mineral status interpretations in terms of quantitative analysis of chemical elements in bio substrates can be caused by both objective and subjective reasons. The objective reasons are based on low awareness of the role of minerals in cellular homeostasis and homeostasis regulation mechanisms. The subjective reasons are related to tacit assumption of the so-called "reference norm" (see more on that in the following discussion).

It is unclear, for example, to what extent the content of electrogenic metals (EM) in a cell can change without violating the conditions of their homeostatic control. It is not very clear what provides this control at the level of the membrane and/or the cell itself. At the same time, one can easily see the dynamics of EM homeostasis using the following example with calcium (Ca).

25 mol of Ca (99% of its content in the body) is found in bone tissue, approx. 22.5 mmol in the intercellular fluid, including ~9 mmol in blood plasma. The exchange between cellular (bone) and extracellular calcium (tissue fluid, plasma) is very intensive (~500 mmol/day). Daily losses of Ca through the kidneys (~2.5 to 7.5 mmol), the intestine and skin with its appendages, along with the intake of ~ 12.5 mmol Ca per day (the necessary minimum, which increases during growth period, pregnancy, lactation), account for the fact that the extracellular pool of Ca is renewed about 33 times a day (!). It is clear that such an intensive "circuit" complicates the estimation of the true content of this metal in the body, especially when there is only one indicator, such as the level of calcium in plasma, which can grow with massive osteolysis (plasmocytoma, multiple bone metastases, etc.).

Literature Review

According to our data, the fluctuations in EM level by the results of atomic emission spectrometry of hair in 10297 practically healthy subjects (Moscow and Riga residents at the age of 2 to 85 years) were as follows (the median): potassium (K) - 0.045 µg/g to 6505.1; sodium (Na) - 0.645 µg/g to 9240 µg/g; calcium (Ca), whose content in hair differed significantly between the genders: 15.5 µg/g to 4633.7 µg/g in males, and 107.1 µg/g to 19,338.9 µg/g in females [2].

Likewise, impressive was the dispersion of individual spectrometry data values that we found in EM and other chemical elements,

whose coefficient of variation (CV) ranged from 34.1% to 226.5% (on average, 125.5±17.5%). Moreover, when we were verifying the hypothesis of the spectrometry data distribution normality, we found that the spectrometry data of 25 chemical elements including EM, had not been distributed in accordance with the normality law [3]. Later on, we did manage to show that the distribution of concentration values (at least, in EM) was of fractal nature [2].

What is the Norm?

All that raises serious questions when interpreting spectrometry data and the biggest question is: What should be considered as the norm?

The norm issue in modern bio element ology is being solved very simply: the average is taken as the average (most often, median) quantitative assessment of the content of a chemical element in a substrate of the so-called reference group consisting of practically healthy people, residents of the same region. The group size (at best) is just a few hundred, and, rarely, more than a thousand people. Such a methodical approach is widely used in clinical medicine (laboratory diagnostics), which leads to the appearance of more than strange normative indicators (such as serum ferritin level - 12 to 200 ng/ml).

The groundlessness of this approach becomes apparent when one answers the question: What part of a reference group is composed of people having preexistent (explicit or hidden) elemental imbalances who cannot be considered as healthy? Besides, one cannot exclude that in countries where depopulation has been the "norm of life" for many years (for example, Latvia) the entire reference group can be regarded as unhealthy.

The features of metal-ligand homeostasis (MLH) in the epidermis of 947 healthy subjects and 954 liquidators of the Chernobyl accident do not speak in favor of the "reference norm" either. The fact is that the accident`s liquidators had multidirectional changes in MLH, above all, an increase in the concentration of EM and other metals, which, according to the authors, can be explained by the oxidative/nitrosative stress that exists in the accident`s liquidators [4]. It is essential that nitrosative stress, i.e., increased production of reactive nitrogen species is closely related to oxidative stress. Therefore, it seems appropriate to use the term "oxidative/nitrosative stress".

At the same time, 171 subjects (18.1%) of the control group had identical shifts in MLH (in magnitude and direction), which is indicative of the existence of similar stress (not of radiation origin) in some healthy individuals.

Discussion

How to avoid distortions in MLH evaluation when composing the reference group? Unfortunately, there is no adequate answer to this question. What is clear though it is hardly possible to solve the norm problem by means of a simple quantitative criterion. It requires different understanding of normality, which would come from the notion of the necessary and sufficient conditions for normal regulation of MLH, the essence of this regulation and

the mechanisms for its implementation at the cell level (although the need for quantitative estimates in this approach is by no means excluded).

The study of homeostasis of EM in the epidermis in 10 297 healthy persons is indicative in this respect [2]. In most of the subjects we found fractal distribution within certain ranges of concentration values in K, Na and Ca (for men and women separately). In **Figures 1-4** these values are approximated by a straight line in a double logarithmic scale, which points to the presence of a power-law relationship between the metal content in the cell and the number of individuals within a given range of quantitative estimates. This allows to regard EM homeostasis in epidermis like self-organized critical phenomenon (SC), and the segments in the graphs (**Figures 1-4**), which are approximated by a straight line, as an indication of the critical state of the system.

One must also review the origin of the segments before and after the approximated curve fragments since the detection of signs of SC-phenomenon in EM homeostasis may mean that EM level homeostatic regulation is based on the universal nature law of
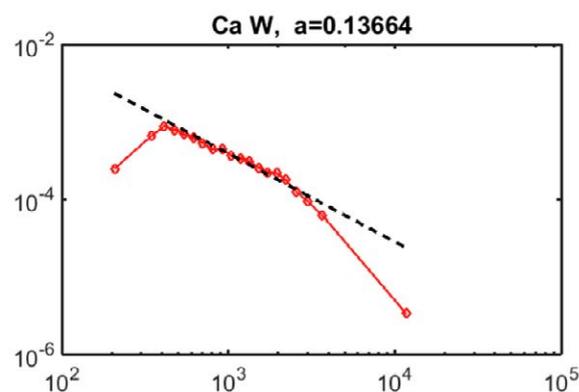

Figure 1 Estimate of the probability density function (PDF) (y-axis) of Ca levels in epidermis in μg/g (x-axis) for women (red curve) and its approximation by PDF of the Pareto distribution (black curve) [2].
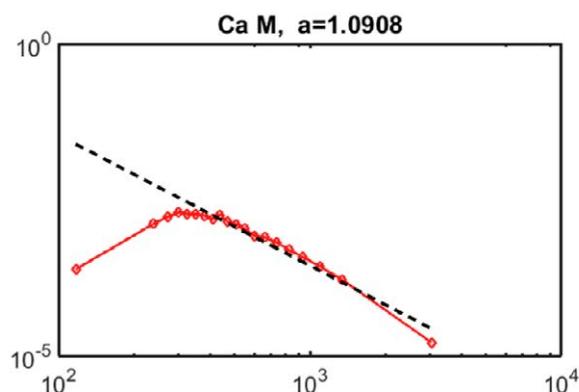

Figure 2 Estimate of PDF (y-axis) of Ca levels in epidermis in μg/g (x-axis) for men (red curve) and its approximation by PDF of the Pareto distribution (black curve) [2].
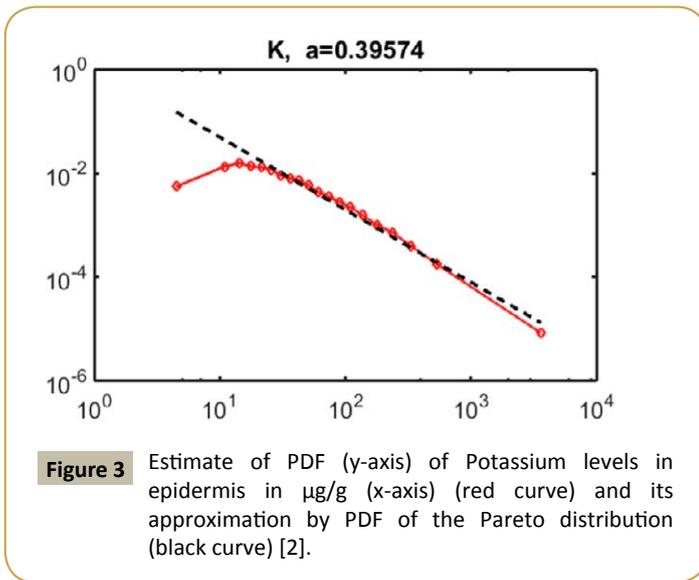
Figure 3 Estimate of PDF (y-axis) of Potassium levels in epidermis in µg/g (x-axis) (red curve) and its approximation by PDF of the Pareto distribution (black curve) [2].
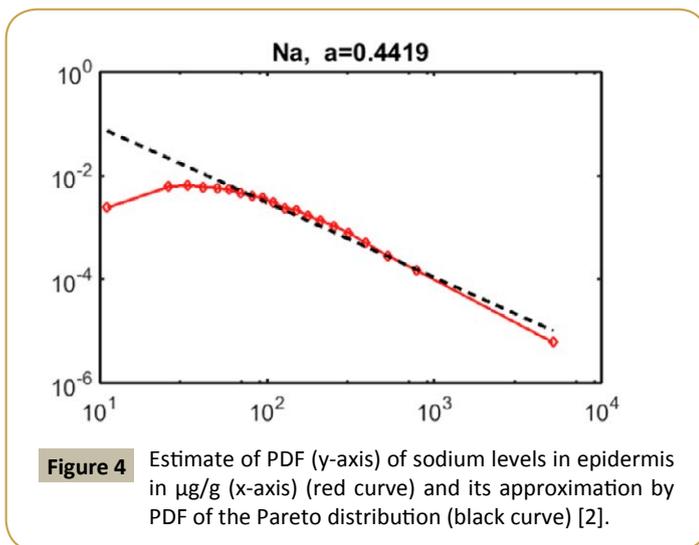

Figure 4 Estimate of PDF (y-axis) of sodium levels in epidermis in µg/g (x-axis) (red curve) and its approximation by PDF of the Pareto distribution (black curve) [2].

self-organized criticality enabling the cell (as an open dynamical system) to resist entropy. In this respect, a living cell does not differ much from a pile of sand, which is being constantly replenished with sand in minimum quantities (a kind of 'brand' of the SC-theory) [5]. The very possibility of regulating homeostasis should, apparently, be associated with the critical state of a system. Let us explain this in detail.

In accordance with the theory of SC, a dynamic system in the process of self-organization can have the following states: subcritical, critical and supercritical. The main distinguishing feature and the basic condition of staying in each of the states is the value of the so-called branching parameter σ. This indicator, which reflects the process of propagation of the exciting pulse (energy) among the functionaries of the dynamic system, is numerically equal to the average number of the nearest "neighbors" that could receive this impulse. The critical state corresponds to $\sigma = 1$; subcritical – $\sigma < 1$, and supercritical – $\sigma > 1$.

As shown in the graph, the deviations from the straight line, which for Na and K are located in the beginning of the abscissa

axis **(Figures 3 and 4),** and for Ca **(Figures 1 and 2)** in the end (since with increasing of NO production in the cell, i.e., nitrosative stress [4], the level of Ca in the cell decreases) correspond to the *subcritical* state ($\sigma < 1$). The transition to the supercritical state of the system ($\sigma > 1$) can only be guessed by the slight deviation from the straight line in the end of the abscissa axis on the K and Na graphs and clearly seen in Ca at the beginning of the abscissa axis. The critical state ($\sigma = 1$), as already mentioned, corresponds to the part of the graph in **Figures 1-4,** which is clearly approximated by a straight line and represents a certain series of numerical values of EM, which, in our opinion, deserve to be regarded as normative.

It was found that the limits of normative values (median) of K level in epidermis (with some approximation) were as follows: 50 to 1000 µg/g **(Figure 3);** Na: 80 to 1000 µg/g **(Figure 4);** Ca_{male}: 400 to 2000 µg/ g **(Figure 2);** Ca_{female}: 400 to 3000 µg/g **(Figure 1)** [1].

The presented numerical values of the EM level in epidermis correspond to the critical state, which, in all likelihood, should be attributed to the cell's ability of controlling (regulating) EM homeostasis. This ability can serve as a kind of criterion for normal functioning of the cell. Below we will explain this in more detail.

To generate the electrical potential (EP) and maintain the intracellular ion concentration at a constant level, the cell emits active transport mechanisms to counteract the electrochemical gradient (primary and secondary active transport), which allows the rest potential to remain unchanged.

The primary transport uses the ATP hydrolysis energy, for example, Na^+/K^+-ATPase, which, due to the cleavage energy of one ATP molecule, transfers three Na^+ ions outward and two K^+ ions into the cell, thereby changing the total transmembrane charge per unit with each such transfer. Primary active ion transport systems include Ca^{2+}-ATPases of the plasma membrane, which release Ca^{2+} from the cell, and the family of Ca^{2+}-ATPases of endo- and sarcoplasmic reticulum (SERCA), which inject Ca^{2+} into intracellular structures.

The secondary active transport of ions is due to the energy of movement of Na^+ in the direction of its electrochemical gradient and depends on the effective operation of the Na^+/K^+ pump, which ensures the existence of this gradient. An example of a secondary transport Na^+/Ca^{2+} exchanger, which outputs a calcium ion (Ca^{2+}) due to the entry in the cell of three sodium ions (Na^+). Na^+/K^+-pump, Ca^{2+}-ATPase and H^+/K^+-ATPase are the ATPase superfamily of the P-type.

It can be assumed that the normal homeostasis of EM in epidermal cells provides for a critical (or close to it) mode of operation of membrane ATPases that participate in the traffic of these metals and represent an oscillatory system of protein molecules with a weak interaction between them. At the same time (as in any system of oscillators), when a certain density of activated molecules is reached, spontaneous synchronization of their oscillations (the critical state of the system) can occur. This assumption may also be true for other metals in which the ATPase superfamily (P-type) takes part [6].

Thus, the criticality of the operation of membrane pumps for EM should be understood as the synchronous nature of their functioning, since synchronization is a specific instance of the critical state. At the same time, if the Na+/K+-ATPases of the outer membrane of the epidermocytes work synchronously (in the critical mode), then the K+ and Na+ levels in the cell should show a correlation relationship. It must be remembered that each Na+/K+-pump transfers three Na+ ions outward and two K+ ions inside the cell.

It is significant that this very statement, which is often found in modern literature, presents an a priori acknowledgment of the existing proportionality of the intracellular content of Na+ and K+ ions. Meanwhile, such proportionality can only occur when the membrane Na+/K+-pump operation is synchronous.

The existence of direct correlation between [Na] and [K] could be clarified by measuring the content of these metals directly in the epidermal cells if it were not for the methodological difficulties related to such an approach. However, if EM homeostasis is indeed a SC- phenomenon, then the [Na] - [K] correlation (as well as the relationship between the number of cells and the content of EMs in them) should have fractality (independence from the scale of the system) and be detected not only at the cell level but also at the body level. In other words, in most study subjects (in a given range of numerical EM values) one can detect not only the power relationship between the results of spectrometry and the number of individuals **(Figures 3 and 4),** but also a direct correlation between the level of Na and K in epidermis by correlation analysis (Pearson) at the level of individuals.

The linear positive Na-K bond (r_{Na-K}=0.6-0.8; $p<0.05$) was detected by us both in healthy individuals (n=947) and in the liquidators of the Chernobyl accident (n=954), in which the signs of oxidative/nitrosative stress have been found [4].

A reliable Na-K correlation, indicating synchronous operation of membrane Na+/K+-ATPase, was independent of the sample size, but was closely related to the current mode of membrane Na+/K+-ATPase functioning (critical, sub- and supercritical) or, in other words, from the synchronous (critical status) and asynchronous (sub- and supercritical state) operation of these pumps. This is well illustrated by the values of r_{Na-K} in the general group and at the concentration values of K and Na, which correspond to the sub- and supercritical state **(Table 1).**

As shown in **Table 1** above, the correlation coefficient r_{K-Na} (Pearson) in the subcritical and supercritical states is reduced

Table 1 The correlation coefficient r K-Na (Pearson): the link between the potassium (K) and sodium (Na) levels in epidermis.

Study groups	r K-Na
The general group (n=10,012)	0.61 (p <0.05)
Subcritical state	
Na < 80 µg/g	0.1
K < 10 µg/g (n=424)	
Supercritical state	
Na> 1500 µg g	0.17
K_{median}=915.8 µg/g (n=260)	

sharply in comparison with the general group, which, in our opinion, reflects the asynchronous operation of membrane Na+/K+-ATPase inherent in these states.

The possible mechanism of synchronization (the critical state) and desynchronization (the sub- and supercritical state) of the operation of membrane pumps in the transmembrane transport of EM in epidermal cells requires discussion.

An important place in this process belongs to redox processes involving active forms of oxygen (ROS) and nitrogen (NOS), since the state of the thiol groups of cysteine in the protein molecule of membrane pumps, that are easily subjected to redox modification (oxidation with formation of disulfide bonds, S-nitrosation), determines the conduction channel ATPase in the cell. The role of oxidizers (redox modifiers) is played by nitric oxide (NO), nitrosonium (NO^+), nitroxyl (NO^-), superoxide anion radical (O_2^{-}), peroxynitrite ($NOOO^-$), etc., which are constantly produced in the cell. Interestingly, NO, NO^+, NO^- (HNO) are generated in the cytosol by an auto oscillatory system containing NO, free thiols and non-heme iron, in the oscillator mode (the Belousov-Zhabotinsky reaction) [7].

The production of ROS/NOS, in which the density of activated ATPase on the cell membrane is sufficient to trigger the synchronization process, will cause the critical state of the system. If the density of the activated membrane ATPases is insufficient, such a start-up can be difficult or impossible (the subcritical state). Hyperproduction of ROS/RNS is able to cause the supercritical state of the system. It should be emphasized that "regulation" (if this term in this case is legitimate) of transmembrane traffic EM provides exactly the critical ($\sigma =1$), or close to it, mode of operation of membrane ATPases, at which the exciting (information) pulse is capable of instantaneous (without distortion) spread for relatively long distances, not only within the cell, but probably (according to cell-cell contacts) in the whole cell population. Such a possibility is unavailable for the asynchronous (sub- and supercritical) modes of operation. Therefore, the numerical values of EM that correspond to the critical (synchronous) mode of operation of ATPases should be recognized as normative.

It was interesting to compare the normative indicators of EM level in epidermis as obtained by different methodological approaches: 1) by the so-called alternative method of interval estimation of the average for non-normal distribution of the *a* priori ensemble (bootstrap method) [8]; and 2) by taking into account the possible belonging of EM homeostasis to SC phenomena.

The comparative analysis results are given in **Table 2.**

As follows from **Table 2,** the limits of the norm for EM on the basis of the theory of SC are much higher than those obtained using the interval estimation of the mean (bootstrap method).

Conclusion

From the above analysis, it is clear that the applicability and accuracy of each of the discussed approaches can only be verified in clinical context. But even now we can mention one of the

Table 2 Normative indicators of electrogenic metal levels in epidermis as obtained by various methodological approaches.

Electrogenic metals	Interval estimation of the average (bootstrap method) µg/g	Self-organized criticality µg/g
K	277.4 < **317.7** < 361.1	50 − 1000
Na	427.9 < **480.9** < 542.9	80 − 1000
Ca$_{male}$	529.1 < **620.1** < 763.1	400 − 2000
Ca$_{female}$	1348.1 < **1439.6** < 1528.4	400 − 3000
Note: The average value (M) is in bold type, the borders of the confidence intervals (bootstrap method) are in normal font		

advantages of the SC-evaluation, namely, the possibility of its physiological (bioenergetic) justification, which, unfortunately, cannot be achieved using statistical (probabilistic) estimates of an average EM content in epidermal cells.

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