Homeostasis of Electrogenic Metals (Na, K, Ca): Clinical Aspects

Valery Ivanovich Petukhov

Baltic International Academy, Latvia

*Corresponding author: Valery Ivanovich Petukhov, Baltic International Academy, LV-1003, st. Lomonosov, 4, Riga, Latvia

Summary

The article discusses the relevance of the search for necessary and sufficient criteria for sodium (Na), potassium (K) and calcium (Ca) homeostasis in the body in toto, as well as the irrelevance of using the results of hair spectrometry for an integral (in toto) assessment of the homeostasis of these metals. The author came to the conclusion (based on their own long-term studies) that the metal-ligand homeostasis (MLH) of electrogenic metals (EM) in the epidermis of healthy people (hair) is fundamentally different from a similar process in the whole body. If, in the first case, we are talking about local homeostatic control of metal traffic in the derivative of the epidermis, which, as is known, is not classified as an electrically excitable tissue, then the homeostasis of Na, K and Ca in the whole organism is determined mainly by the direct participation of these metals in maintaining water electrolyte balance and normal functioning of electrically excitable tissues (muscle and nervous).

Based on the main provisions of the self-organized criticality (SC) theory, the author put forward a hypothesis about the existence of local regulatory control of MLH at the level of the epidermis.

The term 'homeostasis' (the Greek for 'staying the same') in relation to biological systems of self-regulation means a state of unstable (dynamic) equilibrium where the main semantic component is the variability (mobility) of a process exposed to regulatory (homeostatic) factors. The main task of such factors is to keep this inconstancy to a minimum. Therefore, variability (dynamism) is the most essential feature of any homeostasis.

The reliability of self-regulation can be judged by deviations of system parameters from an average value: their minimum size indicates the maximum degree of homeostatic control. This,

idea underpins the modern method of assessing the homeostasis of electrogenic metals (EM) whose clinical use is fully justified (although the accuracy of results is far from impeccable). The essence of the method is to determine concentrations of Na^+, K^+ and Ca^{2+} in blood plasma, which for each of these ions should not go beyond a narrow range of normal values. For sodium ions, it is 135-145 mmol/l, for potassium - from 3.6 mmol/l to 6.3 mmol/l and calcium - from 2.23 mmol/l to 2.57 mmol/l.

It should be noted that making a reliable judgment on homeostasis does not necessarily require knowing the total content of metals in the body. It would suffice to have information on the dynamics of the concentration values of EMs in the extracellular fluid. This can be explained by the participation of Na^+, K^+ and Ca^{2+} ions in the work of organ systems consisting of electrically excitable tissues. Such participation implies reliable homeostatic control of the plasma level of EM during the joint work of many organs (cerebral cortex, hypothalamus, pituitary gland, adrenal cortex, kidneys, lungs, stomach, intestines), hormonal systems and working hormones: aldosterone, renin, ACTH, ADH, etc.

At the same time, it is known that in toto EM homeostasis occurs in the aquatic environment. Therefore, it is appropriate to give some details of water metabolism in the body. In humans, the volume of water ingested with food (~2.2 l) and formed as a result of metabolism (~0.3 l) totals ~2.5 l. This basically corresponds to the level of excretion of H_2O (with sweat ~0.6 l, exhaled air ~0.4 l and urine ~1.5 l). These indicators are significantly influenced by the ambient temperature, a rise in which results in an increased water loss from the body and higher water intake (due to thirst). Therefore, the indicated volume of water supplied with food (~2.2 l) should be referred to t°=18°-20°C. In total, the body of a middle-

aged individual, whose body weight is ~70 kg, contains ~42 liters of water (64% inside the cells and 36% in the extracellular space) [1].

The extracellular fluid itself (~15 l) is: a) blood plasma consisting mainly of water (~90%) with a small admixture of chemical ingredients (proteins, ions of electrogenic and other metals, Cl-, HCO3-, etc.); b) interstitium fluid (~12 l or ~17% of body weight), which is similar in chemical composition to plasma but contains less protein; c) transcellular fluid (inside the eye, in the articular and serous cavities, in the gastrointestinal tract, kidneys), which is ~ 2.5% of the total water volume or ~ 1.5% of body weight; d) water of crystallization in bone and cartilage tissues (~1.5% of the total amount of H_2O).

The intracellular fluid (~27 l), containing low concentrations of Na^+, Ca^{2+}, HCO_3^-, Cl^- ions (and high concentrations of K^+, protein, organic phosphates) is directly involved in all processes of cellular metabolism, many of which are not completely investigated yet. EM, along with other electrolytes, determine the osmotic concentration (osmolality) of the aqueous medium. And the cerebral cortex and in particular, the hypothalamus (having special osmoreceptors) are very sensitive to changes in osmolality by the feedback mechanism. Therefore, EM homeostasis and water balance, which is more correctly called water-electrolyte, have common mechanisms of homeostatic regulation, including, as already mentioned, many organs and hormonal systems.

The sequence of events occurring in this case is well known: the excess of water losses over its intake leads to an increase in plasma osmolality and activity of the hypothalamic centers responsible for thirst and production of antidiuretic hormone (ADH) by the pituitary gland. This stimulates intake and ADH-dependent fluid retention (due to increased tubular reabsorption of Na^+). The involvement of EM in this process deserves a more detailed discussion.

The human body (regardless of gender) contains ~3000 mmol of sodium: 70% is the exchange pool, and 30% is in the bone tissue [1]. The extracellular fluid, as already mentioned, normally contains 135-145 mmol/l of sodium, while it is only 4-10 mmol/l in the cells. Na^+ ions are directly involved in the regulation of water and electrolyte balance, being the "main osmotic ions" of the extracellular space, where they make up 90% of all other ions. This is facilitated by the high sensitivity of the hypothalamic osmoreceptors which can be judged by a narrow range of fluctuations in the normal level of Na in plasma and by a change in the volume of extracellular fluid in response to sodium excretion within only 1% (!). It is known that 85% of sodium is excreted in the urine (with a balanced Na diet), and only 15% is excreted through the skin (sweat glands).

The content of K^+ (the main intracellular cation) in the body of an adult weighing 70 kg is ~3500 mmol. And although 90% of potassium is in free form and only 10% in bound form (erythrocytes, brain, bone tissue), the actual size of the exchange pool, due to the predominantly intracellular localization of K^+, is rather modest. In the extracellular space is only 2% of the total amount of potassium (50-60 mmol). The concentration of K^+ in the cytosol is ~110

mmol/l, in the extracellular fluid ~4 mmol/l. However, it should be borne in mind that the total volume of water in the extracellular space is almost half that in the cytosol [1].

Also significant is that even a slight disturbance of the normal fluctuations in the level of K in plasma (for example, a decrease below 3.0 mmol/l) can cause serious disturbances in cardiac activity. The polar difference in the predominant localization of the Na^+ and K^+ cations relative to the cell membrane is combined with the opposite direction of their electrochemical gradients: for Na^+, it is inside the cell, and for K^+, it is outward. The separation of cations relative to the cell membrane and the preservation of this "inequality" becomes possible due to the constant work of the ATP-dependent membrane pump - Na^+/K^+-ATPase, which pumps K^+ ions into the cell and removes Na^+ ions (in a ratio of 2:3 resp.) The resulting potential difference on opposite surfaces of the membrane (membrane potential) makes it possible to consider Na^+/K^+-ATPase as electrogenic (along with EM).

The content of calcium in the human body is noticeably higher than that of other metals (25000 mmol or 1000 g per 70 kg of body weight). 99% of Ca is in the bones of the skeleton and only 22.5 mmol is in the extracellular fluid. There is very little of this metal inside the cell - only 50 nmol/l - several orders of magnitude less than in plasma or interstitial fluid, where $[Ca^{2+}]$ ~2.5 mmol/l. The impressive "difference" of the electrochemical gradient that exists in Ca^{2+}, which is necessary for the generation of an action potential, is mainly provided by the actively working membrane Ca^{2+}-ATPase due to the energy of ATP molecules.

In recent years, it has become popular (along with the described dynamic approach) to quantify EM homeostasis by the content of Na, K, and Ca in such a bio substrate as hair. Moreover, such an assessment receives the greatest confidence from researchers who for the most part are not clinicians. The non-invasive nature, the possibility of long-term storage of samples, as well as the use of modern high-tech methods of spectrometric analysis: atomic absorption spectrometry (AAS), plasma atomic emission spectrometry (ICP-AES), plasma mass spectrometry (ICP-MS), etc., provided by the approach is undeniably attractive. It is no coincidence that spectrometric analysis of hair for the assessment of the elemental status of the whole body (not only electrogenic but also other metals and non-metals) was willingly adopted by commercial medicine in many countries. In Moscow, for example, more than a dozen of medical centers emerged where the elemental status of the whole body is judged by the results of hair spectrometry.

It is, of course, legitimate to use the level of chemical elements in any particular bio-substrate to assess the elemental status of the whole organism but only on the condition that you know the answer to the key question: is it possible to extrapolate the results of the analysis to the whole organism or one should limit the assessment of bio-elemental homeostasis to the given substrate alone? There is still no definitive answer to this question in relation to the epidermis and its derivative (hair). But there are serious objections to such extrapolation that require discussion.

The number of publications on this topic that have appeared in recent years (monographs, journal articles, scientific reports) is so large that it is problematic to cite them in this review. Let us only refer to those that deserve attention [2-7]. All these works are united by the total reliance of the authors on the adequacy of their chosen criterion (the content of one or another mineral in the hair) for the integral assessment of elemental homeostasis in the whole organism. But what is the basis for such reliance if no necessary evidence is provided to justify this extrapolation? The inconsistency of such an assessment is most easily shown by the example of EM homeostasis, which is, in fact, the objective of this work, although these arguments may also be true with respect for other metals.

The fact is that EM homeostasis, closely related to the processes in the nervous (excitation-inhibition) and muscle (contraction-relaxation) tissues, as well as the regulation of water and 3 electrolyte balance, already has its own evaluation criterion. This is the concentration of metals in the extracellular fluid (plasma), which has proven to be quite appropriate for clinical use. True, if you rely only on the results of this test, you can make an incorrect conclusion about the homeostasis of EM. Therefore, additional laboratory studies should be carried out in parallel (plasma osmolality and osmolarity, the level of the main plasma anions - chlorine and bicarbonate, the volume of extracellular fluid, the level of protein, glucose, urea, etc.). Of particular value are clinical data (including case history), as well as the experience and erudition of the doctor. All this allows minimizing the number of diagnostic errors but does not reduce the significance of the analysis of the concentration of EMs in plasma as a criterion for the homeostasis of these metals in toto. To support this point, let us remind you of a very modest spread in each of the EMs of the normal values of their plasma concentration. Let, us now address the results of hair spectrometry in healthy individuals taking the scatter of normal values as a criterion for the stability or balance of EM homeostasis.

At the Centre for Biotic Medicine (Moscow), measurements of the level of sodium (Na), potassium (K) and calcium (Ca) in hair were made using inductively coupled plasma mass spectrometry (ICP-MS) on a NexION 300D spectrometer (Perkin Elmer Inc., Shelton, CT, USA) [8]. Under observation were practically healthy residents of Moscow aged 20 to 49 years (n=9991). Among them, 4999 (50.04%) are men and 4992 (49.96%) are women. It should be emphasized that all these measurements were carried out in practically healthy individuals with no pathological symptoms. At the same time, the spread of individual values of metals was impressive: for Na, from 0.645 mcg/g to 9240 mcg/g; for K, from 0.045 mcg/g to 6505.1 mcg/g; for Ca, from 15.5 mcg/g to 19338.9 mcg/g.

Here explanations are required for the spectrometric data, expressed in mcg of metal per 1 g of hair. What is behind these numbers? Taking into account the universality of cellular organization, it can be assumed that:

1.   For sodium, this is the amount of metal that is located mainly outside the epidermal cell (interstitial fluid), where $[Na^+]$ is ~10 times higher than $[Na^+]$ in the cytosol.

2.   for potassium, this is mainly an intracellular pool, because $[K^+]$ in the cell ~30 times higher than extracellular $[K^+]$;

3. $[Ca^{2+}]$ in the cell is so small (50 nmol/l) that the level of calcium in the hair, which the spectrometer shows, actually reflects the size of the extracellular pool of this metal.

How can one explain such "tolerance" (insensitivity) of epidermal cells to pronounced (several orders of magnitude!) quantitative shifts of Na, K, and Ca, which predominantly occur (with the exception of potassium) in the interstitial space of the epidermis? Recall that much more modest fluctuations in the plasma level of the same metals are fraught with serious (fatal) "failures" in the operation of the most important functional systems. While answering this question, one cannot ignore the fact that epidermal cells, unlike neurons and myocytes, do not have electrical excitability. And the physiological processes in the nervous and muscular tissues with the participation of EM are far from equivalent to those in the epidermis, which, apparently, lacks special "alarm receptors" (similar to the hypothalamic osmoreceptors), which are highly sensitive to disturbances in EM homeostasis. It cannot be ruled out that throughout the entire period of evolution, tissues that do not have electrical excitability simply did not need this kind of signal structures. The final conclusion from these considerations can be as follows: the observed changes in the EM content in the hair, which are not accompanied by any pathological symptoms and do not affect the health of the individual, cannot be used as a criterion for assessing EM homeostasis at the level of the whole organism and should be considered as probable signs of the homeostasis at the local level.

It is appropriate to ask the question: Is there any homeostatic regulation of EM traffic in epidermal cells? And if so, what "regulators" are involved in it? In the course of our recent studies in search of answers to these questions, signs of EM homeostasis at the level of the epidermis belonging to the phenomena of self-organized criticality (SC) were found. We tend to consider this fact as evidence of the possible existence of homeostatic control in relation to EM. Let's talk about each of these features in detail.

Power-law Relationship between the Content of EMs in the Epidermis (hair) and the Number of Individuals

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) [9]. 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

calibres, 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.

It should be noted that there are much more signs of similarity between the "pile of sand" model and metal homeostasis in the epidermis than it seems at first glance. In both cases, we are talking about the events of the so-called "phase transition" in open dynamic systems on the border of Chaos and Order, when anti-entropy energy-consuming systems (in a heap of sand - this is the weight of grains of sand and the force of their cohesion - "static friction", and in a cell - ATP -dependent membrane pumps working against the electrochemical gradient), which try to maintain - in one case (heap of sand) its structural order, and in the other - (epidermis) metal-ligand homeostasis.

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 Centre for Biotic Medicine (Moscow) from 10297 healthy subjects – Moscow and Riga residents (5160 males and 5137 females) aged 2 to 85. According to our previous studies, the calcium content in female hair was significantly higher than in male hair [10]. Therefore, statistical data processing spectrometry for Ca are kept separately for men and women.

The straight line approximates the data visually on a logarithmic scale very well [11]. All the curves had pieces of different 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, 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) [12-14].

The presence of a power-law relationship between the metal content and the number of individuals makes it possible to explain the predominance of individuals with low and medium levels of metal (according to hair spectrometry data) by the features of the power-law distribution itself, which may not depend on the supply of this metal to the whole organism. Figuratively speaking, the power law in the sand-pile model works regardless of the size of the sand grains (or the size of the pile). Therefore, it is natural to expect the results of hair spectrometry to be independent of the total content of metals in the body. It is essential that the power-law distribution of metals at the level of individuals (being fractal, independent of the scale of the system) will also take place at the cellular level. This greatly simplifies the task of studying metal-ligand homeostasis (MLH) in a particular tissue.

The idea of the prevalence of critical processes in living systems and/or synchronization (as a special case of criticality) helps to take a fresh look at many physiological processes. For example, on peristalsis: rhythmic, sequential contractions of the muscles of hollow organs (including the heart and blood vessels), the immanent mechanism of which remains unsolved.

The phenomenon of synchronization (criticality) can also be detected at the molecular level between water dipoles, which, at a close distance (~1 angstrom), due to weak electromagnetic interaction, are able to combine into synchronized oscillatory systems [15]. This leads to the formation of spherical structures ~0.1 μm in size, the so-called coherence domains (CDs). At room temperature, CDs occupy ~40% of the total volume, at 100°C the number of CDs tends to zero, and when cooled to a temperature close to the water crystallization point (0°C), the total volume of CDs becomes predominant. In contrast to incoherent water, CDs have not only powerful resonant energy (the amplitude of coherent oscillations in the CD center reaches 109 V/cm), but also a sharply reduced viscosity. A significant increase in the rate of diffusion of protein molecules (including enzymes) in CD makes it possible to explain the "lightning-fast" nature of enzymatic reactions in a living organism.

The possibility of the existence of not only purely aqueous, but also mixed CDs was shown, in the formation of which, along with water, metal ions, proteins, amino acids, peptides, and other biomolecules, whose spectrum (about 12 eV) coincides with the spectrum of H2O, equally participate. At the same time, it is not ruled out that it is in mixed CDs that the most important synthetic reactions occur that require energy-consuming, for example, the formation of tertiary and quaternary protein structures [16].

Linear Relationship between [Na] and [K] in hair

We have already reported on a reliable relationship (Pearson) between [Na] and [K] in hair in the earliest works on the problem of MLH [10,17]. However, the coefficient r K-Na, which was 0.6-0.7 in different samples, looked too "modest" for synchronous (critical) operation of membrane pumps (as a whole). Therefore, it was interesting to find out what leads (directly or indirectly) to a possible "lowering" of the r K-Na level. One of the parameters of sodium and potassium homeostasis in the human body is the ratio of [Na+] and [K+] concentrations in the cell and in the extracellular environment in terms of the entire body, which is normally ~1.3 [1]. The ratio of the average values of [Na] and [K] in the hair of 947 healthy individuals, according to our data, was almost identical - 1.5 [11].

Under observation were practically healthy residents of Moscow aged 20 to 49 years (n=9991). Among them, 4999 (50.04%) men and 4992 (49.96%) women. Measurements of sodium (Na) and potassium (K) content in hair were carried out in the laboratory of the Centre for Biotic Medicine (Moscow) using inductively coupled plasma mass spectrometry (ICP-MS) on a NexION 300D spectrometer (Perkin Elmer Inc., Shelton, CT, USA). A correlation analysis (Pearson) of the obtained data was carried out with the determination of the correlation coefficient r K-Na (pair correlations between the concentration values of [K] and [Na] in the substrate).

The normal distribution hypothesis was tested using the Jarque-Bera test [18] and the Kolmogorov-Smirnov test [19]. As a result of this verification, it was possible with a high probability to refute the hypothesis of the normal distribution of chemical elements. Therefore, an alternative approach (bootstrap method) was used, which does not require a normal distribution of the a priori ensemble [20]. The distribution of individuals depending on the value of the [Na]/[K]-ratio was as follows: [Na]/[K] <1 was found in 1834 people. (18.4%) - group I; [Na]/[K] from 1 to 5 in 6884 people. - (68.9%) - II group; [Na]/[K] from 5 to 10 in 893 people. (8.9%) - group III; [Na]/[K] >10 in 380 people (3.8%) - IV group. The correlation coefficient r (Pearson) between [Na] and [K] in each of these groups was found, which was: r K-Na = 0.86 (I group); r K-Na = 0.87 (group II); r K-Na =0.98 (III group); r K-Na=0.9 (group IV). The same indicator in the general group (n=9991) in total: r K-Na = 0.61[8].

It was necessary to find out which of the presented fragments of the general sample to a greater extent (than the others) contributes to the "underestimation" of r in the general population. Group I with [Na]/[K]-coefficient <1 caused the greatest "suspicions" in this regard, which fundamentally distinguished it from all the others. Therefore, we decided to find out how the presence of group I in the total sample and in combination with other groups affects the value of r.

It was found that the addition of group I (with Na/K <1) to each of the other three (individually and in various combinations) led to a noticeable (almost twofold) decrease in the value of r K-Na. It is quite likely that it is the presence of individuals with Na/K <1 (I group) in the total sample that explains the "underestimated" (only 0.61) value of r K-Na for the general population. A high level of r K-Na (~ 0.9) is, in our opinion, a sign of the synchronous (critical) mode of operation of Na+/K+-ATPase. At the same time, the high values of this indicator in group I (r K-Na = 0.86; p <0.05) with the "inverted" Na/K-coefficient (<1), which indicated the synchronous operation of Na+/K+- pumps in these individuals turned out to be unexpected and required an answer to the question: why, despite the pronounced tightness of the K-Na bond (0.86), the presence of group I ([Na]/[K]<1) leads to a noticeable decrease in r K- Na in the total sample? One of the possible explanations for this fact may be as follows.

It can be assumed that a decrease in the content of Na with a predominance of K in the biosubstrate (hair) is caused (at least in some individuals with the lowest numbers [Na] and the highest - [K]) by significant shifts in the distribution of these metals inside and outside the cell. It cannot be ruled out that such a disproportion can polarity change the direction of the electrochemical gradient of Na+ and K+ ions or, in other words, cause a reversal of the pumping function of Na+/K+ATPase ("pumping out" of K+ ions from the cell and "pumping in" of Na+ ions). At the same time, the known proportion of ion exchange (2:3) for this pump may be maintained. Note that such a reversion (due to changes in the potential and content of sodium and calcium in the cell) occurs in the work of the sodium–calcium exchanger (NCX) [21].

Conjugated (cluster) Nature of Shifts in the MLH of the Epidermis against the background of Oxidative/Nitrosative Stress

It is known that the regulation of transmembrane transport of EMs (in particular, Ca2+ in cardiomyocytes) involves reactive oxygen species (ROS) and reactive nitrogen species (RNS). Nitric oxide (NO) and superoxide anion radical (O2.-) constantly formed in the cell act as oxidizing agents (redox modifiers). An increase in the production of NO and O2.- (oxidative / nitrosative stress) can, apparently, lead to even greater activation of P-type ATPases (due to the involvement of a larger number of protein molecules in the process of transmembrane transfer and/or the participation of more aggressive RNS (ONOO-, NO2.) as redox modifiers. Therefore, under conditions of oxidative/nitrosative stress, we have the right to expect quantitative shifts in the MLH of not only electrogenic (K, Na, Ca), but also other metals (Cd, Zn, Pb, Cu, Co, Ag), whose traffic through the outer membrane is carried out by the P1B-type pump from the ATPase superfamily (P-type) [13].

The reality of such events is indicated by hair spectrometry data obtained by us from the liquidators of the accident at the Chernobyl nuclear power plant [10]. The choice of the latter was not accidental. The fact is that it was in them that one could expect increased activity of ROS and RNS. Studies of the redox status confirmed the presence of a prooxidant shift in them, which could not be completely eliminated even after a long-term intake of antioxidants [22]. Using the method of quantitative EPR spectroscopy using in vitro diethyldithiocarbamate (DETC) as a "trap" for nitroxyl radicals, we were able to measure the NO radical activity in a bio-substrate such as hair obtained from liquidators of the Chernobyl accident (n=45) and healthy individuals (n=40). It turned out that the level of the NO signal on the EPR spectrogram in the liquidators of the accident (n=45) significantly (p<0.05) exceeded the normal values (25.9±1.8 vs 20.7±2.5) [23].

Along with this, the mineral composition of the hair was analyzed by inductively coupled argon plasma atomic emission spectrometry (ICP-AES). In the Centre for Biotic Medicine (Moscow) in 947 healthy individuals (238 men and 709 women aged 2 to 86 years), as well as in 954 liquidators of the Chernobyl accident - residents of Moscow (213 women and 741 men aged 37 up to 82 years) [10].

When statistically processing the spectrometry data, we did not use standard statistical methods (t-test), because as a result of testing the hypothesis that the sample belongs to the normal distribution for each of the chemical elements, it was possible to reject the hypothesis of normal distribution with a high probability. Therefore, we used alternative approaches (bootstrap methods) not requiring normal distribution of the a priori ensemble [20]. When identifying possible gender differences in the mineral composition of hair, it was found that the content of calcium (Ca) and vanadium (V) depended on gender.

The results of EM spectrometry in the hair of healthy individuals and liquidators of the accident turned out to be the following

(bold font – mean M value in mcg/g, normal – confidence interval boundaries – bootstrap method):

1. in healthy people – K=277.4<317.7<361.1; Na=427.9<480.9<542.9; Ca (without gender) =

1176.8<1249<1318.9;

2. in liquidators of the accident – K=365.8<394.8<422.4; Na=757.5<822.3<892.4;

Ca=623.4<654.8<685.2.

The concentration values of EM in the epidermis of the liquidators of the accident significantly (p<0.05) differed from the norm. In addition, equally significant differences with the norm were found in them with respect to such metals as Al, Cd, Cr, Cu, Fe, Li, Pb, V, and Zn [10]. The identified shifts in the MLH of the liquidators of the accident can be symbolically written: K($\uparrow$), Na($\uparrow$), Ca($\downarrow$), Al($\uparrow$), Cd($\uparrow$), Cr($\uparrow$), Cu($\downarrow$),Fe($\uparrow$) , Li($\uparrow$), Pb($\uparrow$), V($\uparrow$), Zn ($\downarrow$), where the arrow indicates the direction of the shift relative to the norm.

Since these changes in MLH (and not only in EMs) do not look random and are apparently caused by the increased production of ROS and RNS in "Chernobyl" victims, it was interesting to find out if these shifts are associated? In other words, how unique is the K-Na connection? Are there similar correlations for other metals?

To test this hypothesis, we found the so-called synchronization index (SI), which is numerically equal to the area on the graph under the curve of the coefficients r (Pearson) in paired correlations ("each with each") of the concentration values of metals: Al, Cd, Cr, Cu, Fe, Li, Pb, V, and Zn [24]. The required area was measured using the trapezoidal method.

The SI value in the liquidators of the Chernobyl accident was almost three times higher than in healthy individuals (6.13 versus 2.17 resp.), which indicates the conjugate (cluster) nature of quantitative changes in the MLH of the epidermis against the background of oxidative/nitrosative stress. This conclusion was confirmed in subsequent observations [25].

Homeostasis of Metals in the Epidermis as an SC Phenomenon

To explain the data obtained, it is advisable to use the main provisions of the theory of self-organized criticality, according to which membrane pumps - ATPases (P-type), which carry out metal traffic in the epidermis, being oscillators, are capable of self-organization - transition to a critical (synchronous) mode of operation. The role of activators of these pumps can be played by ROS and RNS in particular NO or, more precisely, its derivatives: nitrosonium (NO+) and nitroxyl (NO-) ions. The main source of these ions, capable of S-nitrosylating thiol groups of proteins and facilitating their interaction with other bioactive molecules, is a three-component chemical system constantly functioning in the autocatalysis mode (according to the Belousov-Zhabotinsky reaction type), consisting of NO, nonheme Fe2+ and low molecular weight thiols [26].

In this system, the purpose of which is to prolong the activity of nitroxide, there is a constant mutual transformation of dinitrosyl iron complexes (DNIC) formed in it with thiol-containing ligands ({(RS-)2 Fe2+(NO+)2}+) and S-nitrosothiols (RS-NO), which, with a sufficient supply of the necessary components (NO, thiols, Fe2+), makes it a permanent generator of periodic NO+ oscillations, which, due to the activating effect of NO+ on membrane ATPases, can lead to conjugate shifts in the MLH of the epidermis. The activation of Na+, K+-ATPase itself occurs during the modification of the SH-groups of cysteine in the protein molecule of this enzyme with the participation of ROS (oxidation) and/or RNS (nitrosylation). As a result, not only the number of actively working transporters (ATPases) increases, but also the size of the pools of the transported Na+ and K+ ions themselves.

An increase in the production of ROS and RNS in the epidermal derivative should inevitably lead to an increase in the density (number) of activated ATPases (P-type) on the cell membrane, thereby contributing to the self-starting of the synchronization process. It cannot be ruled out that for the normal functioning of ATP-dependent membrane pumps in the epidermis, it is the synchronization mode (as a special case of a critical state) that is the main regulatory (controlling) mechanism of MLH.

In conclusion, we would like to emphasize that the very fact of the power-law (fractal) distribution of hair spectrometry data (as evidence of criticality) not only allows us to attribute MLH in the epidermis to SC phenomena that are widespread in living systems, but, in our opinion, can also warn clinicians from unjustified extrapolations when assessing MLH in toto.

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