ROLE OF NUCLEAR ANALYTICAL METHODS IN THE ENVIRONMENTAL HEALTH ELEMENTOLOGY

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Introduction

Study of changes in the content of chemical elements in an organism at all levels of its organization in response to changes of environmental conditions and detection of the role of chemical elements in the etiology and pathogenesis of different diseases is one of the subjects of new scientific discipline – Medical Elementology (from Lat. "medicina" and "elementum").[1-6] It is possible to call this part of Medical Elementology as the Environmental Health Elementology.

The science, by its definition, is a sphere of human activity whose functions include the development and theoretical ordering of objective knowledge about reality. The direct purposes of science are the description, explanation and prediction of processes and phenomena, which are the subject of its study, based on the laws discovered by the scientific process. As a rule, various scientific disciplines are characterized, first of all: 1 - by the subject of study and its corresponding name; 2 – by accepted postulates; 3 – by research methods; 4 – by methods of quality control and processing of the obtained information; 5 – by terms and definitions.

Subject of study

What is the subject of the study of Medical Elementology? Tracing basic historical stages of the development of medicine, one can detect a clear regularity - from the whole to more and more detailed, originally primary, i.e. elementary components (organism → systems, organs, tissues → cells and subcellular structures → biological molecules). The next step in the study of living organisms was investigation of the atomic level, or the level of chemical elements. From here it is possible to formulate the fundamental propositions about the subject matter of Medical Elementology.

1. Study of regularity of content and distribution of chemical elements in the human organism, its systems, organs, tissues, fluids, cells, subcellular structures and molecules under conditions of permanent contact and exchange with the environment, with regard to gender, race, nationality, physiological rhythms, profession, social status, household traditions and harmful habits of the individual;

2. Determination of the role and the degree of chemical element participation in the normal structure and function of an organism at all levels of its organization during conception, formation, maturity and ageing, in conditions of permanent contact and exchange with the environment;

3. Study of changes in the content of chemical elements in an organism at all levels of its organization in response to changes of environmental conditions, or to extreme loads or external influences, as well as in various pathological conditions;

4. Detection of the role of chemical elements in the etiology and pathogenesis of different diseases as well as the efficacy of using chemical elements for corrections of dysfunctions and treatment.

It follows from this, probably not quite full, definition of the research aims, that the basic objects of study in the new scientific discipline are **chemical elements and human organism condition** that exist in continuous and intimate contact with the environment. From here it follows that the name of this new scientific field - "Medical Elementology" is quite relevant.

Postulates of Medical Elementology

Collected knowledge allows the basic postulates of Medical and Biological Elementology to be formulated. The following six postulates are offered for discussion and acceptance.

1. Presence of chemical elements. The Biosphere, including all separated habitats, contains all the natural chemical elements have been found on the Earth (at present 90 of 109 known elements, the remaining 19 being human made). All living organisms, including humans, continuously absorb from the environment some portions and species of the medium from which they derive products necessary for their life. From this it follows that organism's tissues and fluids contain all the chemical elements available on the Earth.

2. Differential homeostasis. In all living organisms, including humans, differential homeostasis of chemical elements is carried out, i.e. at all levels of their organization (the internal environs, organs, tissues, cells, etc.) the content of chemical elements is maintained at certain levels. These levels can change with age and under the influence of various exogenous and endogenous factors, within, however, the certain ranges and limits. Differential homeostasis is a cause of irregularity in distribution and of difference in exchange velocity of chemical elements in organs, tissues, fluids and other structural formations of the organism.

3. Chemical element involvement. The processes of phylogenesis and ontogenesis began and continue under the conditions of permanent contact of living organisms with the chemical elements of their inhabitancies. Therefore none of the Earth's chemical elements can be considered as indifferent or insignificant for an organism and to be defined as an alien, or xenobiotic (from Greek: “xenos” - foreign, “biotos” - life).

4. Usefulness (essentiality, benefit) of chemical elements.
How many of the 90 naturally occurring elements are essential to life? This question has a long story – no less, at least, than from 1713 year (detection of Fe in blood) up to nowadays. After almost three centuries of increasingly refined investigation, the question still can't be answered

with certainty. A lot of the criteria for essentiality were proposed by various authors – from very hard to very formal.

In the strictest sense a chemical element can be classified as essential if its absence in an organism is incompatible with life. This criterion, although logically correct, is too restrictive because it is impossible to induce true “zero” concentrations in any system. So-called “zero” concentration depends on the analytical capabilities and it changes with time. It is very hard criterion and its application would hinder rather than advance element’s essentiality research. Avogadro number is $6.02 \cdot 10^{23}$ atoms in 1 mole of substance. The best detection limit of the contemporary analytical methods for chemical element contents in biological substances is on the level between $10^{-12} – 10^{-14}$. So, it means that in 1 mole of all biological substances no less than $10^{9} – 10^{11}$ atoms are out of our control. It is quite enough if remember that biological reactions may be very sensitive.

More realizable in experimental practice the criteria for essentiality include the postulate that withdrawal of an essential elements from the body must induce reproducibly the same structural abnormalities and impairment of the biological functions, regardless of species studied and that this abnormalities must be always accompanied by pertinent specific biochemical changes, reversible or preventable by supplementation. Such kind of criteria is some softer but the definition of “impairment of a biological function” is left rather vague. Also undefined is the nature of the biological function that might be impaired in a deficiency. Therefore almost any function is acceptable including behavioral functions.

Schroeder[7] has proposed definition of essentiality that is based mainly on occurrence and metabolism, but not on function. It is very formal criteria. But the absolutely formal criteria has been created by Liebscher and Smith.[8] They postulated that essential chemical elements in a population study of its contents in tissues and fluids should exhibid a “normal” distribution curve, while nonessential elements should have a “log-normal” distribution. It is interest to mark that as an example of nonessential elements they used “log-normal” distribution of As.

As usual at present researches of essentiality of chemical elements use the combinations of a few requirements. The next list of requirements for essentiality is generally accepted[9]:

- The organism can neither grow nor complete its life cycle in the absence of the element.
- The element cannot be replaced completely by any other element.
- The given element has direct influence on the organism and is involved in its metabolism.
- The element is present in tissues of different animals at comparable concentrations.
- Its withdrawal produces similar physiological or structural abnormalities regardless of species.
- Its presence reverses or prevents those abnormalities. These abnormalities are accompanied by specific biochemical changes that can be remedied or prevented when the deficiency is checked.

This list includes almost all uncertainties that were considered above.

The great previous experience shows that it is impossible to give strict definition of essentiality useful in the experimental study. The irremovable uncertainties of definition are the reason why the determination of essential or non-essential characters of some chemical elements constitutes a field of intense discussions.[10] It is clear that question will be closed with the understanding of the mechanism of chemical element action on the physiological, biochemical and chemical levels.

There is the other point of view on subject of trace element essentiality. The huge variety and specificity of biochemical reactions occurring in living organisms allow us to assume that Nature in its Majesty used all available means for their implementation, including the whole set of chemical elements. Such characteristics as "useful" or "useless" for this or that chemical element are determined only by the extent of our knowledge about the degree of its involvement in biochemical processes. We witness how new gained knowledge constantly extends the number of biologically "useful" chemical elements (Fig. 1). With such tendency in 50-70 years all 90 naturally occurring elements will be recognised as essential.

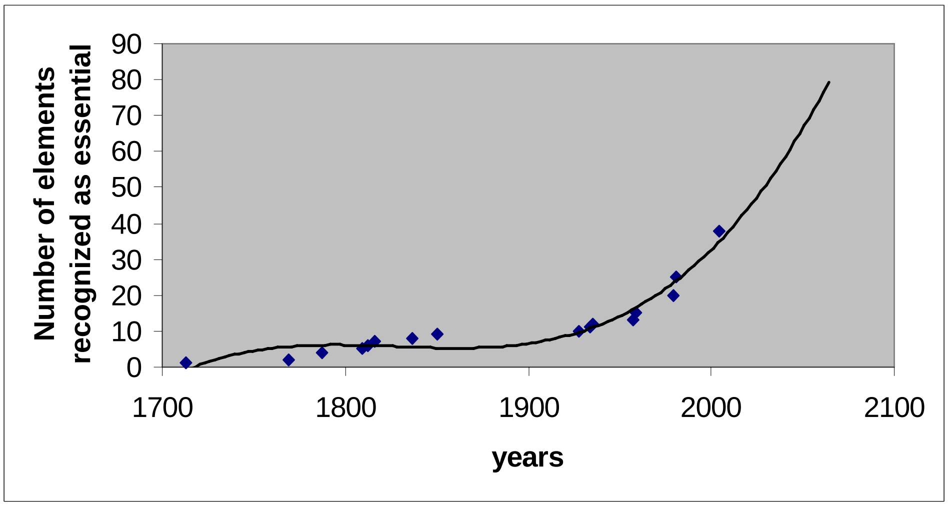

Fig. 1. Discovery of chemical elements requirements for the living organisms. The data of reviews Schwarz[11], Todhunter[12], Egger et al.[13], Mertz[14,15], Anke[16] were used.

<u>5. Harmfulness (toxicity, harm impact) of chemical elements.</u> Useful (essential) or harmful (toxic) action of a chemical element is determined only by the amount of its content in an organism. Paracelsus was right (Paracelsus, the real name is Philippus Theophrastus Bombastus von Hohenheim, 1493-1541), when he concluded, that nothing in nature is a poison - any natural substance is turned into poison only by its dose (quantity). Figs. 2a and 2b illustrate this postulate.

<u>6. Combinatorial (collective, mutual) impact of chemical elements.</u> As well as in absolute amounts, the recombination of chemical elements in their common summation has an important influence on an organism's condition. It is associated with a synergistic, antagonistic or indifferent interactions of many chemical elements. Mosaic combinations of chemical element content are formed by specific combinations of exogenous (biogeochemical peculiarities of environment, ethnic preferences in foods and so on) and endogenous (genetic heredity, acquired diseases etc.) factors.

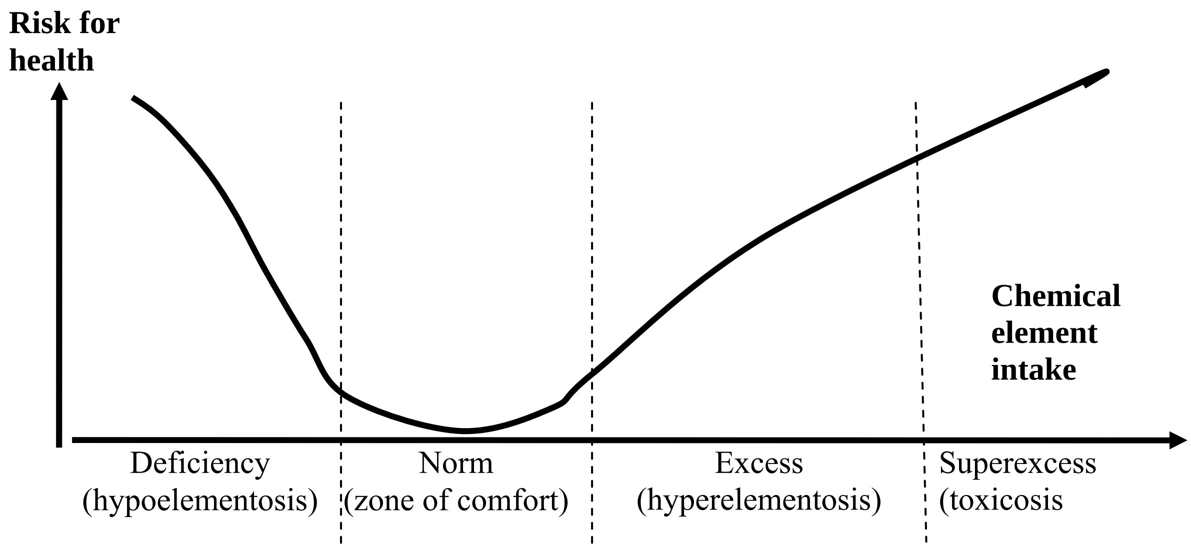

Fig. 2a. This figure illustrates the sense of this postulate. The curve of conventional risk for health versus chemical element intake has at least 4 zones: deficiency (hypoelementosis), norm (zone of comfort), excess (hyperelementosis), and superexcess (toxicosis). The minimum of the curve indicates the range where an organism enjoys its best conditions (zone of comfort).

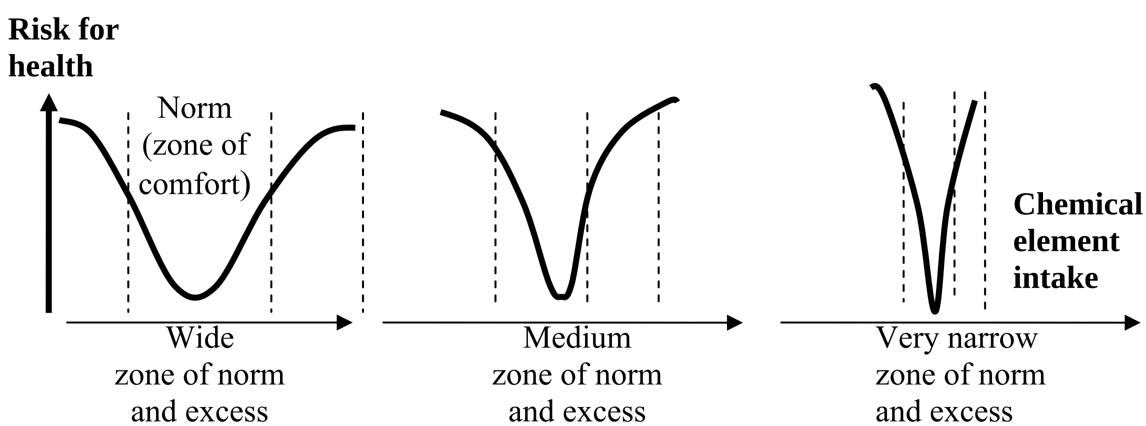

Fig. 2b. The minimum of the curve of conventional risk for health indicates the range where an organism enjoys its best conditions (zone of comfort). The wider this range the less likely it will be disturbed by an external influence. It is clear from this why there is an historical definition of some chemical elements as toxic. These elements are characterized by very narrow zones of “norm” and “excess” and, therefore it is easy to pass from the zone of comfort to the zone of toxicity.

Research methods

The basic information unit in Medical and Biological Elementology is the quantitatively expressed content of a chemical element in a studied biological object. The presence of chemical elements is inherent for all objects, alive or lifeless, and hence it is not specific for living organisms only. Only their quantity, ratios and nature of changes in levels of content during their development and existence can be specific. However only in the last decades of the 20^{th} century there appeared really quantitative and metrologically sound (quality assured) methods for analysis of chemical elements in biological objects which allowed to start studying the content of a large number of chemical elements in the human organism, practically at all levels of its organization. Among these methods, it is necessary first to mention spectral methods such as atomic absorption spectrophotometry (AAS), inductively coupled plasma atomic emission spectrometry (ICPES) and inductively coupled plasma mass spectrometry (ICPMS), and nuclear-physical methods - neutron activation analysis (NAA), X-ray fluorescent analysis (XRF), proton induced X-ray emission (PIXE), proton induced gamma emission (PIGE), synchrotron radiation induced X-ray fluorescent (SRXRF). Their combination in an instrumental variant (without preliminary concentration or separation) allows the determination of up to 60 or more chemical elements that present in biological samples.

Application of nuclear-physical methods not only expands the number of chemical elements accessible to estimation, but also opens additional unique opportunities to study biological objects. These methods do not demand destruction and utilization of a sample. They also avoid the possibility of additional introduction of chemical elements into the sample or losses of chemical elements during the sample preparation. These features make nuclear-physical methods among the most reliable ones and give them the referent status. Besides that, as the sample remains intact, nuclear-physical methods can be successfully combined with other analytical methods. Besides estimation of total quantities of chemical elements, such nuclear-physical methods as electron probe X-ray fluorescent microanalysis (EPXRMA), proton induced X-ray emission (PIXE) and ion probe microscopy based on secondary ion mass spectrometry (SIMS) also provide the capability to investigate chemical elements' distribution by their morphological structures. The absolute prerogative of nuclear-physical methods is the possibility of measuring the content of some chemical elements in the human organism *in vivo*. Current developments in neutron activation and X-ray fluorescent analysis already allow the content of elements such as H, C, N, O, Na, P, Cl, K, Ca, Zn, Sr, Cd, I, Pb to be determined *in vivo*, either in the whole body, or in certain organs and tissues. There are theoretical and technological preconditions for significant expansion of *in vivo* methods in the near future.

Any additional methods of analysis should necessarily be combined with the basic methods for research carried out in the field of Medical and Biological Elementology. As additional methods it is possible to list/include: all existing inorganic, biological and bioinorganic chemistry methods of separation and identification of various biosubstrates and macromolecules; all epidemiological, physiological and clinical methods of estimation of health state both for a population as a whole and also at the level of an individual organism, its organs, systems, tissues, cells and subcellular structures; all fundamental experimental and ecological methods used for investigation of biota - animals (mostly mammalians), plants, micro-organisms, bacteria etc.

The quality control methods and processing of the acquired information

The definition of a science underlines the necessity of obtaining an objective knowledge. The criterion of knowledge objectivity is fine reproducibility of research results. In analytical chemistry a special system of measures of analytical quality control was developed to guarantee required reproducibility of results. This complex provides regular intra-laboratory monitoring of quality and inter-laboratory or external control, as well as the obligatory usage of national or international standard or certified reference materials (SRM or CRM). Unfortunately, the necessity of carrying out these actions in the field of Medical and Biological Elementology is not always carried out in practice.[3,5]

This conclusion is well-illustrated by the following historical example. In the late 1970s/early1980s the IAEA, concerned by poor reproducibility of chemical element analysis in biological samples, decided to develop a number of actions for assessing measurement quality including the development of standard reference materials (SRM) from natural biological substances. For this purpose large amount of certain biological materials (for example, human hair, animal blood and tissues, etc.) were dried, homogenized, packaged, and sent to key analytical laboratories of the world for the quantitative analysis of chemical element content. Analyses of the same element from the same biological sample, received from various laboratories differed by many mathematical orders.[17-19] Such divergence of results testified to the extremely pitiable condition of analytical practice and stimulated many countries to establish national and international centres especially aimed at developing a wide nomenclature of standard reference samples, including biological ones; and to providing different analytical laboratories with these reference materials. Therefore, to guarantee reproducibility of results obtained by different laboratories and to ensure valid comparison of data, it is necessary for the investigational practice of Medical and Biological Elementology for laboratories to make obligatory monitoring of the quality of analytical results.

Another difficulty in obtaining objective knowledge in medical elementology concerns the dependence of chemical element content of biological objects on many exogenous (environment) and endogenous (health condition, age, gender, physiological rhythms, nationality, individual habits etc.) factors. A review of the literature on the chemical element content of the hair and blood of healthy humans clearly demonstrates how strong an influence these factors can have.[3,5]

A failure to account for the main exogenous and endogenous factors, together with low analytical quality, generates data with a high dispersion of values that hampers objective estimation and practical application of measured information. In order always to allow for the influence of the main factors and, as far as possible, to account for the role of numerous secondary influences, it will be necessary to adopt the following measures:

i) - to regulate methodological approaches to planning full-scale and experimental research, depending on their scales, aims and tasks,

ii) - to unify methods of obtaining, storaging and preparating samples of each kind of biological object, having rejected the old technological methods, inevitably leading to distortion of results in estimating content of chemical elements.

Definitions and terms

Many terms, historically existing in various scientific disciplines and used in medical elementology, have no exact definitions or are generally wrong or unhelpful:

1) Examples of terms not having exact definitions **- macro-, micro- and ultramicroelements, trace elements, traces, biological elements, biotics, atomovites, heavy metals, metals of life, essential elements, toxic elements.**

2) Examples of wrong terms **- biogenic elements, mineral elements, xenobiotic elements, and essential heavy metals.**

3) Examples of wrongly used terms, i.e. the terms having strict definitions, but occupied in other scientific disciplines **- minerals, bioindicators, biomonitoring**.

Therefore it is necessary to conduct a revision of the terms in use and to remove all ambiguous and incorrect definitions.[1,3,5] It is also necessary to give precise formulations to all basic concepts.

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