# ACTIVATION BY NEUTRONS AND RELATED ANALYTICAL METHODS AS A TOOL OF MEDICAL ELEMENTOLOGY

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# Introduction

The new direction in medicine that emerged in the second half of the XX century –medical elementology – opens fundamental basis for the development and use of new methods of diagnosis and treatment of various diseases, including oncological ones, as well as for solving many other problems facing modern medicine.

At the beginning of this century, an attempt was made for the first time to characterize "Medical Elementology" as a new scientific discipline [1]. First of all, the subject of the study of "Medical Elementology" was formulated, which includes four sections.

[1] Zaichick V. Medical elementology as a new scientific discipline. J Radioanal Nucl Chem. 2006;269:303-309.

## **Subject of Medical Elementology**

• Study of patterns of content and distribution of chemical elements (ChE) in various systems of the human body, organs, tissues, fluids, cells, subcellular structures and biological molecules, under conditions of constant contact and exchange with the environment, taking into account gender, age, physiological cycles, nationality, race, profession, social status, everyday traditions, lifestyle and bad habits of the individual;

• Determination of the role and degree of participation of ChE in the construction and normal functioning of vital systems of the body at all levels of its organization during the periods of origin, formation, maturity and involution, under conditions of constant contact and exchange with the environment;

• Study of adaptive shifts in the content of ChE in the body at all levels of its organization with changing conditions in the environment, extreme loads and external influences.

• Identification of the role of ChE in the etiology and pathogenesis of various diseases, as well as the effectiveness of the use of chemical elements in corrective and therapeutic measures.

In addition, based on the accumulated facts and their systematic and practical explanations [1], it seems possible to formulate four basic postulates of "Medical Elementology", which largely determine the methodological aspects of research in this area.

• [1] Zaichick V. Medical elementology as a new scientific discipline. J Radioanal Nucl Chem. 2006;269:303-309.

#### **Postulate 1. Presence of chemical elements**

The biosphere, including all habitats, contains all natural ChE present on our planet, the Earth (there are currently 91 of them). All organisms, including humans, continuously absorb certain portions of the environment from which the products necessary to sustain life are extracted. However, organisms do not have mechanisms for the absolute selection of products necessary for life support. It follows that all ChE present in the environment and, consequently, on planet Earth are contained in human fluids, tissues, organs and the body. The number of ChE determined in biomaterials depends only on the sensitivity of the analytical technologies used. With the development of analytical methods and an increase in their sensitivity, the number of ChE available for determination increases. This conclusion was first reached by the outstanding Russian scientist and thinker Vernadsky V.I. [2,3].

[2] Vernadsky VI. Living matter. Moscow: Nauka, 1978. 358 P.
[3] Vernadsky VI. Scientific thought as a planetary phenomenon. Moscow: Nongovernmental Ecological Vernadsky V.I. Foundation, 1997. 265 P.

# Postulate 2. Homeostasis of the content of ChE in the internal environment, tissues and cells of the human body

In all organisms, including humans, differentiated homeostasis of ChE is carried out, i.e. at all levels of organization (internal environment, organs, tissues, cells, etc.) the content of ChE is maintained at certain levels. These levels can change within certain limits with age and under the influence of various exogenous and endogenous factors. Differentiated homeostasis is associated with uneven distribution and differences in the rate of exchange of ChE in organs, tissues, fluids and other structural formations of the body. The concept of the presence of homeostatic mechanisms of ChE levels in fluids, tissues and cells of the human body was first formulated at the Medical Radiological Research Center (MRRC) in the late 60s of the last century. For more than half a century, this idea has been a working hypothesis for developing new methods for assessing the state of the human body, including for diagnosing malignant neoplasms. At present, the presence of somatic and cellular homeostasis of ChE in the human body is generally recognized. Moreover, numerous studies have appeared revealing the mechanisms of intracellular metabolism of ChE and, as a result, ensuring both intracellular and tissue homeostasis of ChE.

## Postulate 3. Involvement, usefulness and harmfulness of ChE in the human body

Since the processes of phylogenesis and ontogenesis occur in constant contact of organisms with all ChE of the environment, none of the natural ChE can be considered as indifferent to the organism and, moreover, be designated as alien or xenobiotic. The gigantic diversity and specificity of biochemical reactions occurring in organisms allows us to assume that Her Majesty Nature used all available resources, including the entire set of ChE, for their implementation. Such characteristics as "usefulness" or "uselessness" for a particular ChE are determined only by the current level of information. Our knowledge in this area is still very limited, and we are witnessing how, as new data are acquired, the range of biologically "useful" ChE is constantly expanding. The harmful (toxic) effect of ChE is determined only by the level of its content in the body. At the end of the 60s of the last century, the MRRC proposed to consider that the presence of all ChE in certain quantities and ratios in the body's fluids, tissues and cells is, at the very least, not indifferent to its existence. From this followed the need for simultaneous determination of all ChE available for analysis in biomaterials.

### Postulate 4. Combinatoric (combinative) influence of ChE on the human body

In addition to the absolute quantities of chemical elements in the body, their recombinations in the totality are of great importance for life. This is due to the synergism and antagonism of the relationships of many ChE. By now, the mechanisms of synergism and antagonism of several trace elements have been identified, for example, Cu and Zn, Cu and Fe, Se and I, Cr and Cu, Cr and Fe, Zn and Cd, and some others. However, it is obvious that the range of interelement interactions is much wider, more diverse and more complex.

The definition of the subject of the study and the formulated postulates allow us to assess the scale of the goals and objectives of medical elementology. It is also obvious that the goals and objectives of medical elementology are inextricably linked with many sections of medicine, including medical radiology, oncology, medical ecology, pharmacology, medical biochemistry, physiology, dietetics, toxicology, occupational hygiene, etc.

# Nuclear and related analytical methods of ChE determination in medical and biomaterials

# **1.** Nuclear physics methods (NPM) that do not require destruction and preliminary preparation of the medical or biosample:

- INAA instrumental neutron-activation analysis
- GAA gamma-ray activation analysis
- EDXRF energy dispersive X-ray fluorescence analysis
- SIXRF synchrotron induced X-ray fluorescence analysis
- TXRF total reflection X-ray fluorescence analysis (for liquid samples)

The indestructibility of the studied samples and the multi-element nature of these methods determine their accuracy and productivity. Nondestructive analysis allows for a consistent combination of some NPMs, which expands the number of elements to be determined and allows for the control of the accuracy of the data obtained by comparing the results for the same elements obtained by different methods. Moreover, the preservation of samples after elemental analysis allows for combining NAMs with morphological and biochemical studies of the same samples, which opens up new possibilities in medical elementology.

#### 2. NPM that require dissolution of the bio-sample:

ICP-AES - inductively coupled plasma atomic emission spectrometry ICP-MS - inductively coupled plasma mass spectrometry

The combination of non-destructive and destructive NPMs significantly expands the list of elements available for determination in medical and biological samples.. For example, the combination of EDXRF+INAA+ICP-AES+ICP-MS methods allows the determination of up to 70 ChE in the same bio-sample.

Such methods as INAA, EDXRF, TXRF, ICP-AES and ICP-MS have high sensitivity. This allows them to be used as a basis for developing micromethods in which a sample weighing several milligrams is sufficient to determine the content of ChE. Samples with such a mass in modern medicine can be obtained from many organs and tissues using puncture biopsies.

#### 3. *In vivo* NPM

The absolute prerogative of NPM is the ability todetermine ChE in tissues and organs in vivo, i.e. without extracting a sample. *In vivo* methods are of exceptional interest for the development of non-invasive diagnostic methods in clinical medicine.

# Examples of the use of NPM in medicine 1. Clinical medicine

# **Oncology:**

1. Differential diagnostics of benign and malignant neoplasms by the level of ChE content in the material of percutaneous puncture biopsy. In these methods, a significant difference in the ChE content in benign and malignant lesions plays the role of a cancer biomarker.

2. Screening for early detection of malignant neoplasms using ChE levels in biofluid samples. In these methods, a significant deviation of the content of CE in the studied sample of biofluid from the level characteristic of a healthy person acts as a biomarker for cancer.

3. Study of the role of ChE in the etiology of malignant neoplasms with the aim of developing preventive measures and new treatment methods

# 1. Differential diagnostics

Differential diagnostics of osteomyelitis and osteogenic sarcoma, as well as benign and malignant bone tumors based on the content of calcium, phosphorus, sodium and chlorine in the lesion

 The levels of concentration of Ca, P, Cl, Na and their ratios in biopsy material from a bone lesion, quickly determined using NAA with short-lived radionuclides, are informative biomarkers for the differential diagnosis of osteomyelitis and bone tumors, as well as various benign and malignant tumors of bone tissue.



# Differential diagnostics of prostate cancer by zinc levels in puncture biopsy material taken from the lesion

1. Normal tissue, 2. Benign hyperplasia, 3. Cancer



Differential diagnostics of thyroid cancer using iodine levels in puncture biopsy material taken from the nodule 1. Normal tissue, 2. thyroid benign nodules , 3. thyroid malignant nodules



A method for diagnosing latent thyroid cancer by determining the iodine content in puncture biopsy material from affected regional lymph nodes in the head and neck area

Metastases of latent thyroid cancer accumulate iodine. This accumulation is not as pronounced as in normal thyroid tissue but is significantly higher than the levels characteristic of other tissues and organs (except for the salivary glands and mammary gland). It has been shown that if the mass fraction of iodine in the tissue of the affected lymph node exceeds the threshold of 3.6  $\mu$ g/g of fresh tissue, then this is a metastasis of latent thyroid cancer.



# 2. Screening for early detection of malignant neoplasms

Zaichick V, Zaichick S. Population-based screening for prostate cancer by measuring zinc levels in prostatic fluid. International Journal of Medicine Sciences 2020;2(1):17-24

1. Norm, 2. Chronic prostatitis, 3. Benign hyperplasia, 4. Cancer



# **3. Study of the role of ChE in the cancer etiology**

For the first time, it was shown that an increase in zinc concentration in prostate tissue is a pathological process leading to benign hyperplasia and cancer of the gland.

Zaichick V., Zaichick S. Wynchank S. Intracellular zinc excess as one of the main factors in the etiology of prostate cancer. Journal of Analytical Oncology, 2016, 5(3), 124-131.

For the first time using NPM, the content of about 70 ChE in the tissues of the thyroid, prostate and mammary glands was studied in the norm, with benign lesions and malignant neoplasms, and a multiple excess of concentrations of many ChE in cancerous tissues was revealed.

Zaichick V, Wynchank S. Reference man for radiological protection: 71 chemical elements' content of the prostate gland (normal and cancerous). Radiat Environ Biophys 2021; 60: 165–178.

Zaichick V. Distinguish Thyroid Malignant from Benign Alterations using Trace Element Contents in Nodular Tissue determined by Neutron Activation and Inductively Coupled Plasma Mass Spectrometry. Journal of Clinical and Diagnostic Pathology 2022;1(4):18-33.

# Systemic and autoimmune diseases Diagnostic methods Osteoporosis

 A method and devices for in vivo NAA have been developed that allow determining the level of calcium content in the hand, foot and two sections of the spine - thoracic and lumbar. Under gravity, these areas of the skeleton are loaded differently and therefore the rate of demineralization of bone tissue in them may be different.



#### Diagnostic methods Periodontosis

Periodontosis is an autoimmune disease. For its successful treatment, it is necessary to detect the disease at an early stage. For early diagnosis of this disease, a method has been developed based on determining the concentration of Na, Mg, Cl, K and Ca in mixed unstimulated saliva. With the development of periodontosis, the content of these electrolytes in saliva increases significantly. Using modern devices for TXRF, it is possible to determine the concentrations of all these electrolytes in a drop of saliva within 1 minute.



# 2. Environmental and occupational medicine

Over the last 100-150 years, there have been significant changes in the extraction and use of ChE in industry and chemistry. In addition to the general pollution of the biosphere, this has led to the largely uncontrolled use of ChE in agriculture, the food industry, medicine, pharmaceuticals, cosmetology, and even in military affairs. As a result, the evolutionarily established levels of ChE intake into the human body with inhaled air, drinking water, and food have been disrupted. In cases where homeostasis mechanisms cannot cope with the perverted flows of ChE into the body, pathological processes occur, leading to various diseases. The current situation has stimulated the search for methods to control the saturation of the body with ChE, as well as studies of the role of ChE in the etiology of various diseases.

As a rule, readily available samples such as hair, nails, urine and blood are used for these purposes.

#### Study of the content of ChE in hair

By now, several thousand articles have been published on the topic of studying the content of hair ChE in order to establish the level of saturation of the body with ChE (elemental status of the body) or the association between the elemental composition of hair and a particular disease. However, our studies have shown that the elemental composition of hair does not reflect the true content of ChE in the body, since it depends largely on external influences.

1. Zaichick S., Zaichick V. Commercial hair analysis: science or quackery? Trace Elements and Electrolites 2010; 27(3):150-180.

2. Zaichick S., Zaichick V. The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans. Biol Trace Elem Res 2010; 134(1): 41-54.

3. Zaichick S., Zaichick V. Is scalp hair valid indicator for the assessment of chemical element contents in human body? In: Neutron Spectroscopy, Nuclear Structure, Related Topics. Joint Institute for Nuclear Research, Dubna, Moscow Region, Russia, 2010, pp.125-132.

Zaichick S., Zaichick V. The scalp hair as a monitor for trace elements in biomonitoring of atmospheric pollution. Int. J. Environment and Health, 2011; 5(1/2):106-124.

#### Study of the content of ChE in blood

Although taking a blood sample to determine the content of ChE requires venipuncture, such studies are also numerous, since blood is a routine object in medical research.

An example of such a study is the association we found between some blood ChE and the risk of low body weight in Moscow women. The study showed that the risk of developing low body weight in women is independently and reliably associated with the nature and degree of anthropogenic chemical exposure. Antimony has the greatest information content, an increase in the concentration of which, even in the subtoxic range, leads to an increase in the risk by 1.56 times. With an increase in the concentration of lead and cobalt in the blood of women, a tendency towards an increase in the risk of developing low body weight is also noted.

Ilychenko IN, Bylova NA, Frontasyeva MV, Lyapunov SM, Okina OL, Gorbunov AV, Pavlov SS, Kulikova O, Arutyunov GP. The blood concentrations of toxic, potentially toxic, and essential elements in Moscow women and the risk of low body weight (a pilot study). Profilac Medicine 2010; 13(1):7-12.

### Study of the content of ChE in organs and tissues

It should be noted, however, that the content of many ChE in the blood is subject to significant fluctuations and strongly depends on the food consumed the day before the study. For this reason, there are still no reference values for the content of ChE for the blood, with the exception of the main electrolytes.

The only way to accurately determine the level of saturation of the body, organs and tissues with ChE is to determine their content directly in the organs and tissues, and above all in the target tissues and organs that accumulate the main fraction of the ChE contained in the human body. Such target organs and tissues are primarily bone and muscle tissue, liver, lungs, kidneys (a lot of ChE), as well as the kidneys (Cd) and the thyroid gland (I). The influence of the environment on the elemental status of the human body is most clearly traced in the study of agerelated changes in the elemental composition of target organs and tissues. We have conducted a number of similar studies, determining age-related changes in the content of CE in bone tissue, prostate, mammary and thyroid glands.

# The most significant results of the study of ChEs contents in organs and tissues

1. Age-related accumulation of rare earth elements in bone tissue has been discovered for the first time.

Zaichick S., Zaichick V., Karandashev V., Nosenko S. Accumulation of rare earth elements in human bone within the lifespan. Metallomics 2011; 3: 186–194.

2. Age-related accumulation of uranium in the prostate gland has been detected for the first time.

Zaichick V., Zaichick S. Global contamination from uranium: insights into problem based on the uranium content in the human prostate gland. J Environ Health Sci 2015; 1(4): 1-5. 3. Age-related accumulation of Br, Ca, Co, Mg, Rb, and Zn was detected for the first time in the thyroid gland of women. It was suggested that inadequate levels of these elements play a role in the etiology of subclinical hypothyroidism in women.

Zaichick V. Zaichick S. Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine 2018; 6(1): 47-61.

4. Age-related accumulation of arsenic was detected in the mammary glands of women for the first time.

Zaichick V, Dogadkin D, Tyurin D, et al. Association between trace element contents in normal human breast and age investigated using inductively coupled plasma mass spectrometry. World Journal of Advanced Research and Reviews 2024;21(03):158–170.

# Method for *in vivo* determination of Ca, Zn, Pb, and Sr in anterior teeth

The greatest interest in environmental and occupational medicine is in vivo determination the content of ChE in organs and tissues. One of such example is the method and device for determining the content of Ca, Zn, Pb, and Sr in anterior teeth. The skeleton and teeth are the main pool of Ca, Pb, and Sr, and a significant fraction of Zn. The ratio of Ca and Sr in teeth plays an important role in the etiology of caries. The content of Zn in teeth show the adequacy of this element's intake, and the level of Pb can be used to assess the degree of contamination of the body with this element.



# **3. Experimental medicine**

In the past two decades, extensive research has been conducted on the use of inorganic metal nanoparticles (NPs) mainly made of gold, silver, iron, zinc, titanium, thallium, platinum, cerium, and copper for the prevention and treatment of aging and related diseases including neurodegenerative diseases, cancer, cardiovascular diseases, and diabetes. However, before using nanoparticles for preventive and therapeutic purposes, it is necessary to conduct a thorough study of the pharmacokinetics of nanoparticles taking into account the type of metal, as well as their shape and mass. Metals such as gold, silver, and zinc are quite well determined even in low concentrations by NPM, and therefore these methods are widely used in such studies.

An example of such research is a study conducted in the Neutron Physics Laboratory of the Joint Institute for Nuclear Research with gold nanoparticles (AuNPs).

#### **Experiment design**



To study uptake of AuNPs (purchased purchased from the M9 Company (Tolyatti, Russia)) both for mothers and the offspring experimental females were drinking the AuNPs solution with concentration of 25  $\mu$ g/ml since one week before pregnancy and to the end of lactation (one month after birth).

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#### 4. Space medicine

Two main problems in space medicine are related to skeletal demineralization and redistribution of body water spaces in zero gravity conditions. To develop methods for preventing these changes and their consequences, both groundbased experimental models and studies conducted on board a spacecraft are used.

To assess changes occurring in the skeleton in ground-based experiments, we used the *in vivo* calcium NAA complex, developed for the diagnosis of osteoporosis. To assess changes occurring with the volume of extracellular fluid in the body of astronauts, we developed a method using a stable bromine label. Saliva and urine samples are collected during spaceflight and analyzed upon return using EDXRF or TXRF.



#### Military and disaster medicine

Our studies revealed high sensitivity of zinc content in prostate juice to external gamma irradiation. This allowed us to develop a new method of biological dosimetry.

In the first days after irradiation, a sample of prostate juice is obtained using transrectal massage and the zinc content is determined using EDXRF or TXRF. The dose of external gamma irradiation is determined by the deviation from the normal level.

The method is suitable for emergency situations at nuclear facilities, as well as for examining a military contingent subjected to a nuclear attack.



# Conclusion

The development of civilization is inexorably connected with the everincreasing anthropogenic pollution of the biosphere, including ChE in quantities and ratios unusual for the biosphere. This pollution causes a great danger to the health of the planet's population. For example, despite the great successes of modern medicine, the incidence and mortality from oncological diseases is steadily increasing. It is well known that only about 10% of oncological diseases are caused by hereditary reasons. The remaining 90% are associated with external factors, including pollution of the environment with ChE. Extrapolation of the situation allows us to conclude that in the near future humanity will have to monitor the content of ChE in the air, drinking water, and all food products. Moreover, for the effective treatment of many diseases, it will be necessary to study the content of ChE in the organs, tissues and fluids of the human body. All this will require a huge resource of analytical methods and NPM, especially *in situ* and *in vivo*, will certainly find a worthy place in it.

# Thank you very much, indeed, for your attention !



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