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The history of the development and use of neutron activation analysis in medical and biological research in the USSR and Russia

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Abstract

The year 2026 marks two significant anniversaries in the history of nuclear and analytical science: 100 years since the development of the tracer method, which laid the foundation for nuclear medicine, and 90 years since the discovery of neutron activation analysis (NAA), both associated with the pioneering work of György de Hevesy. To commemorate these milestones, this review examines the development and application of NAA in clinical and experimental medicine in the Soviet Union and Russia. Particular attention is given to the research centers that employed NAA, the biological materials and medical problems investigated, and the major scientific advances achieved. The review traces the development of more than 20 research nuclear reactors in the USSR and the establishment of specialized research units that applied NAA to biomedical problems. The studies are presented chronologically, covering investigations of chemical elements in biological fluids, tissues, organs, cells, and biomolecules under normal and pathological conditions, as well as applications involving stable isotope tracers, drug and contrast-agent pharmacokinetics, nanoparticles, and unique in vivo measurements. The review demonstrates that NAA provided important advantages, including multi-element analysis, high sensitivity, and the possibility of investigating trace elements in biological systems without extensive sample preparation. These capabilities contributed to advances in fundamental biomedical research and to the development of new diagnostic approaches. The historical record highlights the substantial contribution of Soviet and Russian scientists to the application of NAA in medicine and provides a basis for assessing its continuing scientific significance.

Keywords: neutron activation analysis, biomedical research, trace elements, main achievements

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1. Introduction

The year 2026 marks exactly 100 years since György de Hevesy, a professor of physical chemistry at the University of Freiburg, used radioactive phosphorus in experiments on rats.

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Using a radioactive tracer, he measured the accumulation of phosphorus in bones and teeth and determined the rate of tumor growth. This research method, known as the "labeled atom method", gave rise to a new field of science — nuclear medicine. It was for this discovery that György de Hevesy received the Nobel Prize in Chemistry in 1943. The year 2026 also marks the 90th anniversary of another discovery by this great scientist, which also made a significant contribution to the arsenal of nuclear medicine methods. In 1936, György de Hevesy, working at the Niels Bohr Institute for Theoretical Physics in Copenhagen, discovered with Hilda Levy that stable chemical elements (ChEs) become radioactive when exposed to neutrons. They concluded that the gamma radiation's energy and half-life could be used to identify the presence of a ChE in a substance, and its quantity could be determined from the radiation intensity. This method was called neutron activation analysis (NAA).

Given György de Hevesy's enormous contribution to the emergence and development of nuclear medicine methods and the scope of the ISINN-32 seminar, this review will focus on a historical overview of the use of NAA in biomedical research conducted in the USSR and the Russian Federation.

2. Sources

This review utilized PubMed, Google, and Yandex databases, as well as materials from the authors' personal archives. This review considered only the results of articles or reviews devoted to the use of NAA in biomedical research conducted in the USSR, and after the collapse of the USSR, in the Russian Federation, published between 1962 and 2026. Biomedical research was defined as studies in which NAA was used for medical purposes to determine the native ChE content in fluids, tissues, organs, and throughout the body of humans and animals, as well as in the cells, subcellular structures, and biomolecules of these organisms under normal and pathological conditions. In addition, the review included studies that used stable isotopes as tracers, followed by NAA to determine their content in fluids, tissues, organs, cells, subcellular structures, and biomolecules obtained from humans and animals. Such studies were conducted not only to investigate ChE metabolism at all levels of the body's organization, but also in pharmaceutical research, in which a stable isotope was incorporated into a contrast agent (e.g., iodine) or a therapeutic agent (e.g., cisplatin), or a stable label was specifically introduced into a pharmaceutical to simplify pharmacokinetic studies. The review also included studies on the kinetics of metal-containing nanoparticles in humans and animals, conducted using NAA. Particular attention was paid to unique in vivo NAA studies of ChE content in the whole body of humans and animals, as well as partial in vivo NAA — the determination of ChEs in tissues, organs, or body regions.

Several difficulties were encountered in searching the available bibliographic databases. For example, a PubMed search for "use of instrumental neutron activation analysis in medical research" identified only 46 articles published between 1992 and 2026. Notably, the database returned no results for the more specific query "use of instrumental neutron activation analysis in medical research in studies in which the authors were affiliated with research institutions in the USSR or Russia." In contrast, a Google search using the same general query yielded several thousand publications, many of which were unrelated to the medical applications of NAA.

To improve the relevance of the search results, the queries were progressively refined by specifying the medical field (e.g., oncology, systemic diseases, cardiovascular diseases), the tissue or biological fluid investigated (e.g., hair, blood, bone tissue), a particular chemical element, or the names of Soviet and Russian scientists known to have worked in this field. Overall, several

thousand publications were screened, and only studies conducted by scientists from the USSR and Russia were included in the final selection.

3. Results and discussion

In the USSR, NAA was applied to biomedical research at nine research centers (Table 1). However, only three — the Institute of Medical Radiology of the USSR Academy of Medical Sciences (Obninsk), the Institute of Nuclear Physics of the Uzbek SSR, and the Institute of Physics of the Georgian SSR — conducted such research on a regular basis. At the other centers, research was sporadic, with findings generally reported only in conference abstracts.

Among these three institutions, the Institute of Medical Radiology of the USSR Academy of Medical Sciences (Obninsk) was unique in being a medical institution that pursued systematic research on the medical applications of NAA for more than 60 years. This work produced substantial fundamental and applied results, including the development of new diagnostic methods. The findings were reported in more than 300 scientific publications, most of them in international journals. By comparison, researchers at the Institute of Nuclear Physics of the Uzbek SSR and the Institute of Physics of the Georgian SSR published approximately a dozen journal articles on biomedical applications of NAA, whereas the remaining centers contributed only a few journal publications.

3.1. The beginning of NAA research

In the USSR, the first NAA research was conducted at the heavy-water research reactor (HWR), which was commissioned at the Institute of Theoretical and Experimental Physics (ITEP) in 1949. In the mid-1950s, at the urging of Prof. A. P. Aleksandrov, Academician of the USSR Academy of Sciences, the government decided to build 20 research reactors of the WWR and IRT types. By the mid-1960s, all planned reactors had been built and commissioned. Furthermore, in 1959, Europe's first fast neutron research reactor, the BR-5, was launched at the Institute of Physics and Power Engineering (IPPE) in Obninsk, and in 1960, Europe's first pulsed reactor, the IBR-1, was launched at the Joint Institute for Nuclear Research (JINR) in Dubna. The advent of research nuclear reactors gave a powerful boost to the development of NAA, as this method offered real potential for determining a large number of elements in microgram, nanogram, and even picogram quantities. However, a nuclear reactor, as a powerful neutron source, only provided activation of ChEs in relatively large quantities. Identifying ChE required traditional chemical separation methods followed by gamma radiation detection using gas-filled detectors. This method was called radiochemical NAA (RNAA). RNAA was a rather exotic method and was used primarily to determine impurities in high-purity substances used in nuclear and semiconductor technology.

In the late 1950s and early 1960s, the invention of a scintillation detector with a thallium-activated sodium iodide crystal (NaI(Tl)) made it possible to develop instrumental NAA methods. The output electrical pulse produced by a scintillation detector was directly proportional to the amount of gamma-quantum energy absorbed by the crystal during each interaction. To obtain the energy spectrum of the recorded gamma radiation, it was necessary to measure the amplitude of each pulse and construct a histogram of the amplitude distribution. The development of the electronics industry made it possible to solve this problem. As early as the 1950s, single-channel and then multi-channel pulse amplitude analyzers (PAAs) appeared. In the USSR, the AI-100 "Raduga" 100-channel pulse analyzer was developed and entered mass production in 1957. The advent of scintillation detectors and multi-channel PAAs contributed

to the rapid development of NAA instrumental methods. NAA laboratories began to emerge at many research centers with nuclear reactors.

In the late 1950s, scintillation detectors with large-volume, highly sensitive crystals became available. This allowed for the development of human radiation counters (HRCs) — special low-background chambers, usually made of steel, containing several highly sensitive scintillation detectors around the patient's bed. The advent of HRCs significantly contributed to the emergence of a new direction in NAA development in the 1960s: the in vivo determination of ChE levels in the human body, organs, and tissues.

A truly revolutionary shift in photon spectrometry, and consequently in instrumental NAA, occurred in the 1960s with the development of semiconductor detectors. The first coaxial Ge(Li) semiconductor detectors, designed to detect gamma radiation with energies above 100 keV, provided energy resolution 20–30 times better than that achieved with a conventional NaI(Tl) scintillation detector. In the 1960s, the first planar Si(Li) and Ge(Li) semiconductor detectors were developed for recording photons in the energy range from 1 to 100 keV. In the vast majority of cases, the resolution achieved with Ge(Li) and Si(Li) detectors eliminated the problem of peak overlap in the resulting energy spectrum of photons generated in the sample during interaction with neutrons.

3.2. Research centers using NAA in biomedical research

During the Soviet era, NAA was most intensively used in biomedical research at the Institute of Medical Radiology of the USSR Academy of Medical Sciences (IMR) in cooperation with the Obninsk branch of the L. Ya. Karpov Research Institute of Physics and Chemistry, as well as at the Institute of Nuclear Physics of the Uzbek SSR (Ulugbek), the Institute of Physics of the Georgian SSR (Tbilisi), the Institute of Nuclear Physics of the Kazakh SSR (Alma-Ata), the Institute of Physics of the Latvian SSR (Salaspils), the Moscow Engineering Physics Institute (Moscow), the Tomsk Polytechnic Institute, the Joint Institute for Nuclear Research (Dubna), and the Institute of Power Engineering of the Academy of Sciences of the Byelorussian SSR. After the Chernobyl disaster and, especially, after the collapse of the USSR, many research reactors were closed. In the Russian Federation, the use of NAA in biomedical research continued mainly only in the Laboratory of Neutron Physics of the Joint Institute for Nuclear Research (Table 1).

Table 1. Research centers that conducted medical and biological research using neutron activation analysis.

Scientific center Neutron source Start of work	Main collaborating centers	Main research focus	Leaders (L) and key researchers in the field of NAA
INP AS Uzbek SSR WWR-SM reactor Since 1959	TashSU TashMI RI of Regional Medicine	ChEs of hair, blood, and tissues of humans and animals in health and disease Pharmacology The accuracy of NAA	E. M. Lobanov (L) A. A. Kist (L) N. A. Kryzhenkova L. Zhuk, S. Orestova E. Flitsiyan, E. Danilova
IP AS Georg. SSR IRT-2000 reactor Since 1959	—	ChEs in tissues, organs, cells, subcellular structures, proteins, DNA and RNA	E. L. Andronikashvili (L) L. M. Mosulishvili (L) N. I. Shonia

Table 1. *Continuation*

			N. E. Kuchava N. V. Bagdavadze
IP AS Lat. SSR IRT-2000 reactor Since 1961	—	ChEs of hair, blood and nails in normal and pathological conditions	L. L. Pelekis (L) N. A. Dubinskaya
IPE AS BSSR IRT-2000 reactor Since 1962	Belarusian Anti-Goiter Dispensary	Determination of uranium in the human thyroid gland	N. M. Draznin (L) A. F. Malenchenko (L) E. E. Bril
Obninsk branch of NIFHI WWR-C reactor Since 1964 14-MeV neutron generator	IMR IMBP MH USSR Priorov CITO IB MH USSR CRIDMS IRITO MEPhI, Moscow	ChEs of the thyroid gland, prostate gland, bone and other tissues, organs, and body fluids of humans and animals under normal, pathological, and extreme conditions ChEs in human food Stable tracer Kinetics of Li-6 in NCT The accuracy of NAA	L. A. Smakhtin (L) I. P. Lisovsky Yu. S. Ryabukhin (L) A. D. Melnik V. Zaichick (L) V. M. Kalashnikov A. P. Dubrovin
MEPhI, Moscow IRT-T reactor Since May 1967	IMR AUORC IB MH USSR	ChEs in human thyroid gland and bone tissue Effect of antitumor drugs on ChEs in rat tissue Exogenous cisplatin levels in rat tissue	P. L. Gruzin (L) A. M. Samonov (L) N. P. Roslyakov S. I. Sheludko
Tomsk PI IRT-T reactor Since July 1967	TSU TSMI	ChEs of hair, blood, and some organs. Hematology of the human body (after cremation)	G. G. Glukhov (L) L. P. Rikhvanov (L) N. V. Baranovskaya
INP AS Kaz. SSR WWR-K reactor Since July 1967	RIOR	ChEs in tissues and organs of rats with transplanted tumors ChEs in human hair and blood	I. V. Kazachevsky (L) V. P. Solodukhin (L) V. N. Levkovsky
FLNP of JINR IBR-30 reactor Since 1969 IBR-2 reactor Since 1984	IG RAS RIPH&HM of Sechenov MMA Pirogov NRMU DA MSU VMRRCI IM&B of TUM	Human hair and blood Pharmacokinetics of Au- and Ag-containing nanoparticles in rats ChEs in human food	M. Frontasyeva (L) S. M. Lyapunov (L) S. S. Pavlov I. Zinicovscaia (L) D. S. Grozdov A. A. Peshkova N. S. Yushin
IMR Pu–Be sources Since 1970	IMBP MH USSR Priorov CITO	In vivo NAA of the ChE contents in tissues, organs, areas and whole body of humans and animals	V. Zaichick (L) V. M. Kalashnikov A. E. Kondrashov A. P. Dubrovin

INP — Institute of Nuclear Physics, AS — Academy of Sciences, Uzbek SSR — Uzbek Soviet Socialist Republic, TashSU — Tashkent State University, TashMI — Tashkent Medical Institute, ChE — chemical elements, RI — research institute, IP — Institute of Physics, Georg. SSR — Geor-

gian SSR, Lat. SSR — Latvian SSR, IPE — Institute of Power Engineering, BSSR — Byelorussian SSR, NIFHI — Obninsk branch of the L. Ya. Karpov Research Institute of Physicochemistry and Chemistry, IMR — Institute of Medical Radiology of the USSR Academy of Medical Sciences, IMBP MH USSR — Institute of Medical and Biological Problems of the USSR Ministry of Health, Priorov CITO — N. N. Priorov Central Institute of Traumatology and Orthopedics, IB MH USSR — Institute of Biophysics of the USSR Ministry of Health, CRIDMS — Central Research Institute of Dentistry and Maxillofacial Surgery, IRITO — Irkutsk Research Institute of Traumatology and Orthopedics, NCT — neutron-capturing nuclide, MEPhI — Moscow Engineering Physics Institute, AUORC — All-Union Oncology Research Center of the USSR Academy of Medical Sciences, Tomsk PI — Tomsk Polytechnic Institute, TSU — Tomsk State University, TSMI — Tomsk State Medical Institute, Kaz. SSR — Kazakh SSR, RIOR — Research Institute of Oncology and Radiology of the Kaz. SSR, FLNP — I. M. Frank Laboratory of Neutron Physics, JINR — Joint Institute for Nuclear Research, IG RAS — Institute of Geology of the Russian Academy of Sciences, RIPH&HM — Research Institute of Public Health and Healthcare Management of I. M. Sechenov Moscow Medical Academy, Pirogov NRMU — N. I. Pirogov Russian National Research Medical University, DA MSU — Department of Anthropology of Moscow State University, VMRRCI — M. F. Vladimirsky Moscow Regional Research Clinical Institute, IM&B of TUM — Institute of Microbiology and Biotechnology of the Technical University of Moldova, Chisinau.

3.3. Main scientific forums and publications

Beginning in the 1960s, numerous meetings, symposia, and conferences were regularly held in the USSR, where, among other applications of the NAA, reports were presented on the use of this method in biomedical research:

1. All-Union Conference on the Use of Research Nuclear Reactors, chaired by Academician of the USSR Academy of Sciences, Professor A. P. Aleksandrov.
   Frequency: Every two years. A total of 15: The first conference was held in Moscow in 1960, and the last, the 15th, was held in Obninsk in 1988.
2. All-Union Conference on Activation Analysis. A total of 5: 1st in Tashkent (INP AS Uzbek SSR, Ulugbek) in 1962, 2nd in Tashkent (INP AS Uzbek SSR, Ulugbek) in 1968, 3rd in Tashkent (INP AS Uzbek SSR, Ulugbek) in 1972, 4th in Tbilisi (IP AS Georgian SSR) in 1977, 5th in Tashkent (INP AS Uzbek SSR, Ulugbek) in 1987.
3. Symposium on Methods of Determination of Microelements in Natural Objects. A total of 3: 1st in Moscow (MSU) in 1968, 2nd in Samarkand (MSU and Samarkand State University) in 1973, 3rd in Chisinau (MSU and Chisinau State University) in 1977.
4. Conference on the Use of Radionuclides and Ionizing Radiation in Scientific Research and the National Economy (Ural Polytechnic Institute, Sverdlovsk). A total of 6: 1st in 1969, 2nd in 1971, 3rd in 1973, 4th in 1975, 5th in 1979, 6th in 1983.
5. Conference on the Use of New Nuclear Physics Methods for Solving Scientific, Technical, and National Economic Problems (JINR, Dubna). A total of 4: 1st, 20–23 November 1973; 2nd, 1–4 December 1976; 3rd, 12–15 September 1978; 4th, 20–23 October 1981.
6. All-Union Conference "Nuclear-Physical Methods of Analysis in Environmental Monitoring". A total of 3: 1st, 23–26 October 1979 in Tashkent (Ulugbek); 2nd, 20–22 April 1982 in Riga; 3rd, 21–23 May 1985 in Tomsk.
7. International Workshop "Activation Analysis in Environmental Protection" (JINR, Dubna). A total of 3: 1st, 17–21 September 1990; 2nd, 5–18 September 1992; 3rd, 23–27 May 1995.

8. International Meeting on Monitoring Environmental Pollution by Natural and Artificial Radionuclides and Heavy Metals (JINR, Dubna). A total of 2: 1st, 2–5 November 1999; 2nd, 3–6 October 2000.
9. International Seminar on Neutron–Nucleus Interactions (FLNP JINR, Dubna). Held annually. A total of 32: from the first in 1993 to the 32nd in 2026.
10. Numerous periodic meetings of young scientists organized at the IMR AMS USSR and the INP AS Uzbek SSR, starting in 1967.
11. All-Union School on Activation Analysis and Radiochemistry, organized by INP AS Uzbek SSR and held from May 21 to 30, 1976 in the Bostanlyk region of the Uzbek SSR (Khumsan-Aktash zone of the Chatkal National Reserve, Khumsan village, "Crystal" boarding house).

In addition to the regular multidisciplinary meetings on the use of NAA in applied fields, the USSR also held forums specifically focused on the use of neutrons in medicine. For example, in 1976, the All-Union Conference "Use of Neutrons in Medicine" was held in Obninsk at the IMR. In 1978, the IMR organized the All-Union Conference "Nuclear-Physical Methods of Elemental Analysis in Biology and Medicine", which was held in Moscow. Furthermore, papers on the use of NAA in medicine were presented at the periodic "Radiation and the Organism" conferences held at the IMR. This conference was first organized in 1967 and was held every 2–3 years until 1986. Then, there was a fairly long hiatus of almost 30 years, and in 2015, it was decided to resume the tradition of holding this conference. It is now held annually at the Medical Radiological Research Center of the Ministry of Health of the Russian Federation (MRRC, Obninsk, former IMR).

Reports on the use of NAA in medicine were also presented at numerous purely medical forums: the 10th All-Union Congress of Radiologists (November 22–25, 1977, Yerevan), the 3rd All-Union Congress of Oncologists (April 9–15, 1979, Tashkent), the International Scientific Conference on Measurement Problems in Medicine and Biology (September 15–17, 1981, Suzdal), the 9th Transcaucasian Conference of Oncologists on Current Issues in Oncology (November 16–18, 1982, Yerevan), the First All-Union Conference "Diagnostics, Treatment and Organization of Oncological Care for Patients with Head and Neck Tumors" (June 22–23, 1983, Tomsk), the 11th All-Union Congress of Radiologists (October 2–4, 1984, Tallinn), the 2nd All-Union Conference on Physiology of Extreme Conditions and Individual Human Protection (December 2–3, 1986, Moscow), the 1st All-Union Conference on Pharmacokinetics (September 3–4, 1987, Kaunas), the All-Union Symposium with international participation "Human Microelementoses" (November 15–17, 1989, Moscow), and some others.

After the collapse of the USSR, work on the use of NAA in medicine within the country, in addition to the above-mentioned meetings at JINR, was also presented at the 1st and 2nd congresses of the Russian Society of Nuclear Medicine (respectively, June 9–12, 1997, Dubna and October 23–27, 2000, Obninsk), the International Scientific School on Nuclear Medicine and Radiopharmaceuticals (December 4–14, 2006, Obninsk) and, since 2015, at the annual conferences "Radiation and the Organism" held at the MRRC, as well as on the 32 editions of the International Seminar on Interaction of Neutrons with Nuclei (ISINN, Dubna).

Since 1991, it has been possible to present the achievements of Russian scientists in the development and application of NAA in biomedical research at regular international meetings, such as Nuclear Analytical Methods in the Life Sciences (NAMLS), Modern Trends in Activation Analysis (MTAA), Nuclear Analytical Chemistry (NAC), Trace Elements in Man and Animals (TEMA), Methods and Applications of Radioanalytical Chemistry (MARC), meetings of the

International Society for Trace Element Research in Humans (ISTERH), Workshop on Macro and Trace Elements (Friedrich-Schiller-Universität, Jena, Germany), Trace Elements in Human: New Perspectives (National and Kapodistrian University of Athens, Greece), and many others.

During the Soviet era, the All-Union Conference "Nuclear-Physical Methods of Elemental Analysis in Biology and Medicine", held in Moscow in 1978, brought together over 100 participants. Forums such as the All-Union Conference on Activation Analysis, held four times in Tashkent and once in Tbilisi, sometimes attracted over 300 participants, while the "NAA in Medicine and Biology" section included at least several dozen researchers. After the collapse of the USSR, research on the use of NAA in medicine and biology declined sharply, and only a few scientists continued to work in this area of science.

In addition to the proceedings of the aforementioned forums, results on the use of NAA in medicine and biology were published in domestic scientific journals, primarily "Atomic Energy", "Medical Radiology", "Journal of Analytical Chemistry", "Issues of Oncology", "Issues of Medical Chemistry", and others. After 1991, Russian researchers were given the opportunity to publish in international scientific journals: "Journal of Radioanalytical and Nuclear Chemistry. Applied Radiation and Isotopes", "The Analyst", "Biological Trace Element Research", "Journal of Trace Elements in Medicine and Biology", "Journal of Trace and Microprobe Techniques", and many others. According to information obtained from the PubMed database, the peak of publications on NAA in medicine occurred in the late 1980s, and by 2026, such publications had become rare.

The first PhD thesis on the use of NAA in the study of biomedical samples was defended in 1964 by Alexander Alekseevich Kist [1]. Based on the results of the development and use of NAA in research directly related to medicine, Liguri Mikhailovich Mosulishvili defended his doctoral dissertation (DSc) in 1985 [2], and Vladimir Efimovich Zaichick [3] defended his doctoral dissertation in 2011. The number of PhD dissertations on this topic is measured in dozens.

3.4. Main advantages of NAA

The use of NAA in biomedical research offers a number of advantages over other analytical methods:

1. The ability to determine the ChE content in a sample without any preliminary processing (the analytical technology is fully instrumental);
2. Preservation of the analyzed sample (non-destructive analytical technology);
3. Relatively high sensitivity for determining many ChEs in biomedical samples;
4. The ability to simultaneously determine many ChEs (the analytical technology is multi-element);
5. The speed of NAA when using gamma spectrometry of ultra- and short-lived radionuclides formed in the sample under neutron exposure (the method is rapid);
6. The ability to simultaneously irradiate the sample and reference standard with neutrons, which guarantees complete identity of the irradiation conditions (neutron flux, neutron energy spectrum, irradiation duration);
7. The possibility of non-invasive in vivo determination of the content of certain ChEs in organs, tissues, areas and throughout the body of humans and animals.

These advantages of NAA are important in biomedical research for many reasons. The fully instrumental nature of the analytical technology ensures high reliability of the obtained information, which is particularly valuable when used for diagnostic purposes. This reliability is achieved by eliminating any preliminary sample processing, which is a potential source of error, with instrumental NAA. The integrity of the analyzed sample allows NAA to be combined with morphological examination of the same sample, or to continue determining the ChE content in the same sample using other analytical methods. Some ChEs that play an important role in the body, such as halogens (Cl, Br, and I), are readily determined in biomedical samples using NAA, while their determination by other methods presents certain difficulties. The ability to simultaneously determine many ChEs is of particular interest, since life support requires not only certain ChE levels but also the quantitative ratios of these ChEs. The multielement nature of NAA ensures precise control of these ratios. The rapidity of the method is important in cases where, for example, a tissue sample is obtained during surgery and the further course of the operation depends on the analysis result of this sample. As noted above, the accuracy of the result is particularly valuable in biomedical research. In other analytical methods, standards and international certified reference materials (ICRMs) used to control the quality of the obtained results are measured separately from the sample. Some instability in the measurement conditions of the sample, standards, and ICRMs can introduce uncertainty into the obtained result. In NAA, when the sample, standards, and ICRMs are irradiated simultaneously, identical conditions for determining ChEs are ensured during the neutron activation stage. Of particular note is the unique ability of NAA to determine the content of certain ChEs in organs, tissues, areas, and whole body of humans and animals in vivo. This unique capability enables non-invasive studies of ChE metabolism in the human body and opens up prospects for the development of new diagnostic methods in which ChE levels are used as a marker.

3.5. Main areas of NAA use in biomedical research

Considering the number of published scientific papers conducted using NAA, the main areas of research can be categorized as follows:

1. Determination of ChE content in light (hair, nails, urine, mixed saliva) or relatively easily accessible samples (whole blood, serum, and blood plasma) collected from healthy humans and those with various pathologies. This study pursued several goals:
   a) to determine the "elemental status of the body" and identify the presence of ChE deficiency or excess;
   b) to elucidate the role of ChEs in the etiology and pathogenesis of the disease under study;
   c) to identify the presence of ChE markers, the levels of which can be used for diagnostic purposes.

   Such studies were conducted at many nuclear centers in collaboration with medical institutes. As an example, NAA studies of hair [4, 5], blood [6], nails [7], urine [8], and mixed saliva [9, 10] can be used.
2. Determination of ChE content in tissue and fluid samples (lymph, cerebrospinal fluid, prostate juice, gastric juice, etc.) under normal and various pathological conditions. In the case of oncological diseases, ChE content is also determined in the lesion tissue, and in the presence of a neoplasm, in the tumor itself and adjacent visually healthy tissue. The goals pursued in this case are identical to those outlined in point 1.

Systematic studies of tissues and relatively hard-to-reach fluids of the human body were conducted only at the IMR AMS USSR. At the INP AS Uzbek SSR, the IP AS Georgian SSR, and the Moscow Engineering Physics Institute (Moscow), such studies were sporadic. The following publications illustrate the work conducted in this area at the IMR: teeth and bone tissue [11–28], prostate gland [29–53], thyroid gland [39–53], mammary gland [54–56], and lymph [57].

3. A study of the effect of radiation and other extreme effects (hypokinesia, heat, cold, etc.) on the content of ChEs in tissue and fluid samples of humans and animals. Such studies using NAA were conducted only at the IMR AMS USSR [58–61].
4. A study of the pharmacokinetics of antitumor drugs based on platinum [62] and metal-containing nanoparticles [63–70].
5. A study of the content of ChEs in cells, subcellular structures, proteins, amino acids, DNA, and RNA in humans and animals with malignant neoplasms. The use of NAA for these purposes was mainly made by the IP AS Georgian SSR, headed by E. L. Andronikashvili [71–76]. The results obtained are most fully reflected in the doctoral dissertation of L. M. Mosulishvili [2].
6. Study of ChE levels in tissues and body fluids in malignant tumors and the transformation of these levels during chemotherapy (animal studies) [77–79].
7. Use of a combination of a stable isotope tracer and NAA for diagnostic purposes. Such studies were conducted only at the IMR AMS USSR [8, 80].
8. Determination of ChE intake by the human body with food. Studies of ChE content in food using NAA were conducted at the IMR AMS USSR [81, 82] and the FLNP JINR [83].
9. The effect of food additives on the ChE content in tissues and body fluids and the role of food additives in the etiology of oncological and other diseases. Similar studies were conducted only at the IMR AMS USSR together with the IB MH USSR [84, 85].
10. Study of the accuracy of ChE content determination in biomedical samples. The accuracy of the NAA for the determination of ChE content in biomedical samples was addressed to a greater or lesser extent in all the centers listed in Table 1. It was clear that the greatest contribution to the uncertainty of the results obtained was made by procedures associated with sample collection, processing, and storage. Systematic studies in this regard, covering the entire technological chain of sample collection and preparation for NAA, were conducted only at the IMR AMS USSR [86–92]. Research on accuracy issues directly related to the NAA technology is illustrated by work on the standardization and automation of the method [86, 92–94].
11. Development of micromethods for the NAA of biomedical objects in order to ensure the possibility of studying vital biopsy material. Such methods were developed at the INP AS Uzbek SSR [95], the IP AS Georgian SSR [96], and the IMR AMS USSR [97, 98].
12. Development of in vivo methods for the NAA of ChE content in tissues, organs, areas, and whole body of humans and animals. Research in this area was conducted only at the IMR AMS USSR [12, 98–105].

3.6. Key achievements of NAA use in biomedical research

The development and use of NAA in biomedical research has not only significantly expanded our knowledge of the role of ChEs in life support, but it has also demonstrated for the first

time that information on ChE levels in human tissues and body fluids can be used for diagnostic purposes. More specifically, the achievements of NAA use in biomedical research can be summarized as follows.

3.6.1. Expanding the spectrum of analyzable chemical elements

The development and use of NAA in biomedical research has significantly expanded the spectrum of ChEs available for quantitative determination. The advent of this analytical tool has also led to an increase in the number of ChEs whose necessity for the normal functioning of the human body has been identified. If at the beginning of the 1960s only fifteen ChEs (H, C, N, O, Ca, P, S, Na, Cl, K, Fe, I, Zn, Cu, Co) were considered "vital", then by the beginning of the 21st century the number of such elements had exceeded thirty [106, 107]. This increase confirmed the postulate that all ChEs of natural origin are involved to one degree or another in life processes and that with the advent of new, more sensitive and precise analytical methods the number of ChEs considered vital will increase [106, 107]. In addition, if at the beginning of the 1960s elements such as As and Se were among the five most "toxic" ChEs, then by the beginning of the 21st century they had migrated to the group of "essential" ChEs. This radical transformation confirmed the postulate that only the level of ChE content in the body makes it useful or harmful [106]. It also followed that the division of ChEs into "essential" and "toxic" is archaic and incorrect.

3.6.2. Improving the accuracy of chemical element determination

Non-destructive NAA allowed us to identify a number of sources of error associated with sample collection and preparation for analysis. The preservation of the sample during NAA allowed us to analyze the same sample before and after any exposure, as well as under different storage conditions and for different storage periods. It was shown that sources of error can include the use of stainless steel instrumentation, the effect of temperature during drying or ashing of samples, the use of chemical reagents during their dissolution, and the material of the container in which the sample is stored [86–92].

Significant improvement in the accuracy of ChE determination results in biological samples was achieved by combining non-destructive NAA with other analytical methods [108–116]. Determining the same ChE by different methods allowed us to verify the correctness of the results obtained in such studies.

The International Atomic Energy Agency (IAEA), which promoted the development and use of NAA in solving various applied problems, was the first to note the wide range of results for determining the ChE content in the same biological samples analyzed in different laboratories (Table 2). Following IAEA recommendations, 10 subsamples were prepared from the submitted sample in each laboratory involved in the study and the mass fractions of all ChEs available for analysis were measured. The laboratory sent the results obtained for the 10 subsamples to the IAEA, which statistically processed the results, calculating the arithmetic mean, median, geometric mean, and other parameters of the ChE mass fractions for each respondent separately. The results of the statistical processing of the data received from each analytical center for all ChEs studied separately were presented in IAEA publications [117–120]. From these publications, for each ChE, we took the arithmetic mean values of mass fractions (M_i), obtained separately for all respondents involved in the study, and ranked the selected M_i for the same ChE in ascending order. From the resulting series of mean values of the mass fraction of a specific ChE, calculated based on data from different laboratories, we selected the minimum ($M_{\min}$) and maximum ($M_{\max}$) values. The $M_{\max}/M_{\min}$ ratio characterized the spread of data on

the mass fraction of a specific ChE, obtained by comparing the results of analysis of the same sample in different laboratories. The results of analyses of the M_{max}/M_{min} ratio for the studied ChEs in animal muscle, human hair, animal bone, and animal blood samples are presented in Table 2. From the results in Table 2 it follows that, for example, the discrepancy in the results of determining the mass fraction of Cl, K and Na in different laboratories $M_{0\,max}/M_{min}$ is in the range from 1.4 to 2.0, and when determining Ba, Br, Ca, Ce, Cs, Cu, Fe, Rb and S, the differences can reach a mathematical order.

Table 2. Results of analyses of mean mass fractions ratio (M_{max}/M_{min}) obtained from of the same biological samples, prepared by the IAEA as prospective standard reference material and conducted in different laboratories worldwide.

Range of M_{max}/M_{min} ratios	Animal muscle H-4 [117]	Human hair HH-1 [118]	Animal bone H-5 [119]	Animal blood A-13 [120]
$1.4 - <2.0$	Cl, K, Na	Ag, F, Mg, Sr,	—	Br, Cl, S
$2.0 - <10$	Ba, Br, Ca, Ce, Cs, Cu, Fe, Rb, S	Ca, I, La, Mn, Na, Rb, S, Zn	Ba, Ca, F, Mo	Au, Ba, Ni, Rb, Sr
$10 - <10^2$	As, Cr, Hg, I, La, Mg, Mn, Mo, Ni, P, Pb, Sc, Se	Al, Au, Ba, Br, Co, K, Ni, P, Sb, Se, Ti, V	K, Pb, Rb, S, Si, Sr	Cr, Cu, Fe, K, Mg, Mo, Na, P, Pb, Se, V, Zn
$10^2 - <10^3$	Al, Cd, Co, Sb	As, Cd, Cl, Cu, Fe, Mo	Al, Cd, Cl, Mg, Na, Sb, Zn	Ca, Cd, Co
$10^3 - <10^4$	Au	Cr, Hg	As, Br, Fe	As, Sb
$10^4 - <10^5$	—	Pb	Cr, Mn, Ni, P, Se	Hg, Mn
$10^5 - <10^6$	—	—	Co, Cu, Hg	—
$>10^6$	—	—	V	—

IAEA — the International Atomic Energy Agency.

The data presented in Table 2 demonstrated the enormous variation in results obtained when analyzing the same samples in different laboratories. This variation was due solely to the imperfections of the analytical technologies used in these laboratories. The analytical errors identified by the IAEA largely explained the variability in the published results for determining the ChE content in internal tissues and body fluids. An example of such a comparison is presented in Table 3. In this table, the uncertainty of the ChE determination result in bone tissue associated with analytical errors is compared with the results of a literature review on the ChE content in human bone tissue. The analytical errors were estimated based on the results of a study conducted by the IAEA [119], and the literature review data illustrating the variability in published data on the ChE content in human bone tissue were taken from a previously published paper [121].

A comparison of the data presented in Table 3 (columns 2 and 3) demonstrates the significant contribution of analytical errors to the variability of results for many ChEs content in human bone tissue.

Table 3. Comparison of the variations in the M_{max}/M_{min} ratios of the elemental mean mass fractions in bone arising from analytical uncertainty alone, as well as from analytical and elemental variability combined.

Range of M_{max}/M_{min} ratios	Reference material H-5	Various human bones
	Analytical uncertainty alone [119]	Analytical and elemental variability combined [121]
$1.0 - <1.5$	—	—
$1.5 - <2.0$	—	H
$2.0 - <10$	Ba, Ca, F, Mo	Dy, Hf, Lu, N, O, Ta, Tl, Ra
$10 - <10^2$	K, Pb, Rb, S, Si, Sr	Be, C, Cl, Er, Eu, Ga, Ho, I, Mg, Na, Nd, Po, Pr, S, Sm, Sn, Tb, Tm, Y, Yb
$10^2 - <10^3$	Al, Cd, Cl, Mg, Na, Sb, Zn	B, Ba, Cr, Cs, F, Hg, K, La, Ni, Sb, Th, Zn
$10^3 - <10^4$	As, Br, Fe	As, Ca, Co, Fe, Li, P, Pb, Rb, Se, Si, Ti, V, Zr
$10^4 - <10^5$	Cr, Mn, Ni, P, Se	Ag, Al, Br, Cd, Cu, Mn, Mo, Sr, W
$10^5 - <10^6$	Co, Cu, Hg	Au, Sc
$>10^6$	V	U

The studies conducted by the IAEA and the results obtained stimulated the creation of international certified reference materials (CRMs), which significantly increased the reliability and accuracy of ChE determination in biological samples. The IAEA, with the participation of many laboratories, conducted a special study to find the main sources of errors and came to the conclusion that they are the sampling and sample preparation [122]. Synthetic multi-element standards also played a significant role in enhancing the reliability of NAA results for biomaterials; these standards were developed and produced in small batches at the Institute of Physics of the Georgian SSR and came into widespread use across research centers in the USSR [86, 93].

3.6.3. Confirmation of the postulate of elemental tissue homeostasis

The reliability of results obtained using NAA in combination with other modern analytical methods confirmed the postulate of elemental tissue and cellular homeostasis [106]. For example, a review of literature data on ChE content in human bone tissue revealed exceptionally wide variability in published results (Table 3, column 3), which was largely due to analytical errors (Table 3, column 2). Indeed, the results of a study of ChEs in human rib bone tissue conducted in Russia and China using NAA in combination with other modern analytical methods (EDXRF, ICP-AES, and ICP-MS), as well as with the accuracy of the obtained results verified using CRMs, showed good agreement (Table 4). The good agreement between ChE levels in the ribs of Russian and Chinese subjects confirmed the presence of homeostasis of ChE content in bone tissue, while the significant discrepancies identified for some trace elements could be attributed to the geochemical characteristics of the territories of Russia and China and the differences in the diets of the inhabitants of these countries.

Table 4. Comparison of the variations in the elemental mass fractions in rib bone of Russians and Chinese, presented by Zaichick [121] and Zhu et al. [123], respectively.

Range of means ratio	Results presented by Zaichick (2013) and Zhu et al. (2010)
1.0 – <1.1	Ca, Cr, Cu, Fe, Hg, Mg, Na, P, Th, Y
1.1 – <1.2	Zn
1.2 – <1.3	Dy, K, Pb, Se
1.3 – <1.5	Cl, Ga, Yb, Pt, Sc
1.5 – <2.0	Al, Ba, Ce, Cs, Er, Mo, Nd, Pr, Sb, Sm, Ti, U, V
2.0 – <10	Ag, Au, B, Be, Br, Cd, Eu, Gd, Ho, La, Lu, Mn, Ni, Rb, S, Sn, Sr, Tb, Tl, Tm, Zr
10 – $<10^2$	As, Bi, Co

Evidence of the presence of elemental homeostasis in bone and other tissues and organs of the human body stimulated research on the intracellular metabolism of ChEs and led to the discovery of a number of intracellular ChE transporters.

3.6.4. NAA of chemical element content in intravital biopsy material

The high sensitivity of NAA allowed for the first time in the world to develop micromethods for determining ChE content in intravital biopsy material, including puncture biopsies [95–98]. This enabled the use of new diagnostic methods in clinical practice, in which ChE levels act as tumor markers.

3.6.5. Chemical element levels as tumor markers

For the first time in the world, NAA micromethods were used to demonstrate that ChE levels in puncture biopsy material of the lesion can be used as tumor markers [27, 28, 38, 45, 50, 52, 53, 124–126]. This confirmed the postulate that pathological conditions can be caused by a disruption of elemental homeostasis or, conversely, that a disruption of such homeostasis can be a consequence of pathological processes [106].

3.6.6. NAA in the study of interstitial localization of chemical elements

NAA does not require destruction of the test sample. This advantage allowed, for the first time in the world, to demonstrate that instrumental NAA in combination with morphological examination made it possible to determine ChE content and morphometric parameters in the same tissue sample. Comparison of data on ChE content in a tissue sample with its morphometric parameters made it possible to determine the interstitial localization of ChE [127–130].

3.6.7. NAA of chemical element content in proteins, DNA, and RNA

Using highly sensitive NAA, ChE content in proteins, DNA, and RNA was determined. It was shown that their structure, in addition to the bulk ChEs of proteins and nucleic acids (H, C, N, O, P and S), also includes many microelements [71–76, 131]. The findings obtained at the Institute of Physics of the Georgian SSR provided the basis for the hypothesis that coordination compounds of metals with structural components of nucleic acids may play a role in malignant growth.

3.6.8. Noninvasive in vivo NAA of the chemical element content in bone tissue

NAA enabled the world's first development and clinical implementation of diagnostic methods based on the noninvasive in vivo determination of certain ChE levels in bone tissue [98, 99].

Noninvasive in vivo NAA of Ca content in the hand, foot, and spine was also used in space medicine research, where ground-based experiments on volunteers were used to test methods for preventing calcium metabolism disorders in zero-gravity conditions [100–105].

3.6.9. NAA and the elemental status of the body

Several nuclear centers, particularly the INP AS Uzbek SSR, have devoted considerable attention to the NAA of ChE content in human hair and blood. Studies of the elemental composition of other tissues were exceedingly rare. For example, researchers at the Institute of Nuclear Physics of the Academy of Sciences of the Uzbek SSR used NAA to determine the concentrations of ChEs in ocular tissues [132]. The preferential use of hair and blood as study materials was primarily attributable to the accessibility of these biosamples. NAA-based determination of ChE concentrations in hair and blood pursued two main objectives. The first is to assess the so-called "elemental status of the body", or, in other words, to determine whether the element being studied is deficient or abundant in the body based on ChE levels in hair. The second goal is to elucidate the role of ChEs in the etiology and pathogenesis of human diseases [133, 134].

By definition, the elemental status of the body is an indicator that evaluates the quantity of various ChEs in the body, i.e., their state of deficiency, excess, or imbalance. With the help of NAA, it was proven that the levels of ChEs in hair and blood do not reflect the "elemental status of the body", since the content of ChEs in hair strongly depends not only on many internal factors (diet, hair color, gender and age of the individual), but also on external influences — the level of pollution of the environment and hygiene habits (frequency of hair washing, type of shampoo, hair coloring, use of hairspray, etc.), and the level of ChEs in the blood — on the food eaten the day before [106, 135–138].

Table 5. Comparison of variation ranges (max/min values) of chemical elements content in hair of healthy humans for the variation arising from analytical uncertainty and individual variability.

Range of max/min ratio of mean values	Level of variation arising from analytical uncertainty by the example of "Human Hair" IAEA reference material [118]	Level of variation arising from both analytical uncertainty and individual variability of chemical elements content in hair of healthy humans, according to the literature [121]
$1.4 - < 2.0$	Ag, F, Mg, Sr	—
$2.0 - < 10$	Ca, I, La, Mn, Na, Rb, S, Zn	—
$10 - < 10^2$	Al, Au, Ba, Br, Co, K, Ni, P, Sb, Se, Ti, V	Be, Li, P, Rb, S
$10^2 - < 10^3$	As, Cd, Cl, Cu, Fe, Mo	B, Ba, Cu, Fe, Mg, Mo, Tl
$10^3 - < 10^4$	Cr, Hg	Ag, Al, Br, Ca, Cd, Co, Cr, Hg, I, K, Mn, Sb, Si, V, Zn
$10^4 - < 10^5$	Pb	As, Na, Ni, Pb, Se, Sn, Sr, U

AEA — the International Atomic Energy Agency.

For the above reasons, it followed that correcting a "deficiency" or "excess" of ChEs in the body based on hair chemical analysis lacked scientific basis and could cause harm (Table 5). For this reason, the diagnosis of microelementoses based on hair ChE levels was banned in some developed countries [106, 135–138]. It has been shown that assessment of the "elemental status of the body" requires determining the concentrations of chemical elements in the major body pools (skeleton, muscle, and adipose tissue) or in specific target organs, such as the thyroid gland (I), liver (Cu), and kidneys (Cd). [106, 135–138].

Conclusions

The development and application of NAA in biomedical research have substantially advanced our understanding of the role of ChEs in vital physiological processes and have expanded the potential clinical applications of information on their concentrations in tissues and body fluids. Although powerful alternative techniques, such as ICP-AES and ICP-MS, are now widely available for the determination of chemical elements in biological samples, NAA retains important advantages for specific biomedical applications. In particular, its ability to determine elemental concentrations without destroying the sample makes it valuable in complex studies involving multiple analytical approaches. Moreover, as a nuclear physics-based technique, NAA offers the unique possibility of determining chemical elements noninvasively in vivo in tissues, organs, specific body regions, and, under appropriate conditions, the whole body. These advantages support the continued use of NAA as a valuable analytical technique in biomedical and medical research.

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V. Z., I. Z. and M. F.: Conceptualization, Methodology, Literature search; Writing, Reviewing and Editing.

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