Use of muonic atoms in biomedical research

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A review of experimental studies made by scientists in the United States, West Germany, and the Laboratory of Nuclear Problems at the Joint Institute for Nuclear Research, Dubna demonstrates the unique possibilities of using muonic atoms for comparative element analysis, in particular in biology and medicine. The method using muonic atoms has a number of advantages over other nuclear-physics methods and has great possibilities for further development and applications in related fields of science and technology.

INTRODUCTION

The discovery of uranium fission in 1939 initiated a concerted program on a wide front for the exploitation of atomic energy and the theoretical and experimental study of the properties of the atom and the nucleus. Fundamental investigations in nuclear physics led to the creation of a large number of accelerators capable of accelerating particles to different energies, reactors, and the development of highly sensitive means of detection of nuclear processes.

The radiation sources and detectors of the various forms of radiation developed in experimental physics have proved to be remarkably convenient and promising instruments for numerous branches of science and technology.

One of the directions in which nuclear-physics methods are used is in the analysis of the element composition of objects. Initially, there were only a few poorly developed and not widely accessible opportunities for using these methods, but they have now become one of the most powerful means of solving modern scientific–technical analytic problems requiring the determination of extremely low concentrations and mass analyses in industrial production. One of the achievements of the nuclear-physics methods of element analysis is their use in biology and medicine.

Investigation of the chemical composition of a living organism without disturbing its biological functions is very important and greatly enriches investigations in biology and medicine. Numerous investigations have been made[1–5] of the concentration and physiological role of various elements in living organisms. Nevertheless, the understanding of biologists and doctors of the biological significance of many elements and the possibilities of using data on the element composition of biofluids, tissues, individual organs, and the entire human body in diagnostics, healing, and prophylaxis of many diseases is insufficient.[4]

Different methods are used for element analysis *in vivo* or *in vitro*. Initially, the method of radioactive isotopes was used; in it, the process of exchange of material and the information of the structure of the different tissues of an organism was studied on the basis of the rate of incorporation of isotopes in different organs (see Ref. 7, p. 1, and Ref. 23, pp. 299 and 315). This method has severe restrictions and problems in obtaining information about the element composition of an organism. The methods of x-ray densitometry[8] and photon absorptiometry[9,40] gave a more complete picture of the element composition of a living body or its parts, but this was still insufficient since both methods are based on measurement of the penetrability of, for example, bone tissue for electromagnetic radiation and therefore only indirectly characterize the concentration of phosphorous and calcium in the tissue. The methods of activation analysis[4,7,23] and fluorescence analysis[6,23] have great advantages over these methods. In the activation method, neutrons, protons, γ rays, and heavy ions are used as activators.

The activation method achieved a new qualitative level by the use of three-dimensional acquisition of information about the structure and composition of internal organs of the body and the processes taking place in them and the use for this of minicomputers and microcomputers. This new direction is called tomography.[43]

The neutron-activation method is the most common and best studied. The cross sections for capture of neutrons by elements and the decay schemes of excited nuclei after neutron capture have been well studied and are widely available in the literature.[10,11] In some cases, the neutron-activation method can determine the concentration of certain elements with an error between 1.5 and 2.0%. But this method also has its shortcomings. Above all, greater accuracy is achieved at a high price—the radiation dose is several tens and sometimes hundreds and even thousand of rads.[7] Therefore, such investigations, in which high accuracies are needed, are made, as a rule, *in vitro*, while in a number of cases investigations were made on patients in radiation-therapy clinics. All forms of activation and fluorescence *in vivo* investigations using heavy particles and especially electromagnetic radiation suffer from the characteristic difficulty of the impossibility of localizing them in the investigated part of the organism. In the neutron-activation method there is an effect that can strongly influence the error in the measurement; this is the interference effect that can result from a pair of reactions such as $^{27}\mathrm{Al}(n, \gamma)\,^{28}\mathrm{Al}$ and $^{28}\mathrm{Si}(n, p)\,^{28}\mathrm{Al}$. In other cases, the γ lines excited by neutron capture may lie entirely outside the sensitivity region of the Ge (Li) and NaI (Tl) detectors.

1. PHYSICAL BASIS OF THE METHOD OF MUONIC ATOMS

The search for and development of new adequate physical methods of nondestructive *in vivo* element analysis were and remain topical for experimental biology and diagnostic

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medical research. One such new method appeared at the frontier of elementary-particle physics and atomic physics after the discovery of the μ^- meson (the muon) and after numerous experimental and theoretical investigations of the process of the atomic capture of such particles stopped in matter.[13]

The method is based on the capability of a muon, decelerated in matter to low velocities, of going over to a bound state in an atom, replacing an electron in one of the atomic orbits and thereby forming an excited mesic atom. In this process, the muon plays the part of an electron in the atom. It differs from the electron in its mass ($m_\mu \cong 105.7$ MeV) and lifetime (the lifetime of the free muon is $\tau_0 \cong 2.2 \times 10^{-6}$ sec, while for a muon bound in an atom the lifetime τ_1 decreases with increasing atomic number Z).

When the mesic atom is de-excited, the muon goes over in a cascade to less excited levels and radiates characteristic electromagnetic radiation x_μ. This radiation is due to the electromagnetic transitions of the muon from the more highly to the less highly excited levels. The transitions to the ground state form the K series of the spectrum. The main parameters to be determined when they are detected are the energy and intensity of the individual lines of a series as well as of the complete series. Because the muon mass is more than 200 times greater than the electron mass ($m_e = 0.511$ MeV), the energy of the mesic x rays is approximately the same number of times greater than the energy of the ordinary electron x-ray emission of the same element.[14] The energies of the K series of elements in living organisms, except hydrogen, are in the region of energies accessible for measurement by the existing detectors widely used in modern nuclear physics, namely, the scintillation NaI (Tl) spectrometers and semiconductor Ge (Li) and Si (Li) spectrometers. In Table I, we give the basic elements in the human organism, their approximate abundance in the tissues of the organism, and the energies of their mesic x rays. The data on the percentage concentration of elements in human tissues are taken from Ref. 44.

It should be mentioned that the data on the element composition of the human organism given in the literature are rather contradictory.

The K series of the mesic x-ray radiation (Lyman series) is due solely to transitions to the ground state. Therefore, there must be a correspondence between the total number of μ^- mesons captured by the atoms of an element in an irradiated sample and the total number of emitted γ rays of the Lyman series of the same element, i.e., by determining the relative number x_μ of the Lyman series for the different elements in the target, one can determine the relative number of muons captured by the atoms of the same elements.

The probability of a muon being captured by a given species of atom and the nature of the distribution of the muons between the different atoms in a chemically complicated sample irradiated by muons have not yet been completely elucidated and are still under investigation by numerous groups of physicists in different scientific centers in the Soviet Union, Europe, the United States, Canada, and Japan. As yet, it can only be asserted confidently that the probability of muon capture by atoms depends strongly on the atomic number Z and on the number of atoms in the sample. The first and simplest theory for the probability of muon capture by a given atom in a chemical compound was proposed by Fermi and Teller in 1947 and is called the Z law,[12] according to which the probability of muon capture by an atom is proportional to its atomic number Z and its concentration. Since its proposal, large deviations from the Z law have been found for different chemical compounds. In addition, it has been found that there is a strong dependence of the structure of the K series of the x_μ spectra for some atoms on the type of chemical bond in the compound containing the given atom,[13] i.e., it has been found that the molecular environment has a strong influence on the mesoatomic cascade. This means that there is a possibility of obtaining at least qualitative information about the molecular state of the substance in which muons are stopped by observing the relative intensities of the K series of the x_μ spectra.

Although a clear relationship has not been established between the probability of muon capture by an atom and the various physicochemical parameters of a material, as a first approximation (more valid, it seems, for a mechanical mixture of atoms or for biological objects) one can use, with an error of a few percent, the expression $\alpha W_i/\Sigma W_i \simeq P_i/\Sigma P_i$, which means that there is approximate equality between the relative intensity $W_i/\Sigma W_i$ of the mesic x-ray spectrum for element i in a mechanical mixture and the relative mass concentration $P_i/\Sigma P_i$ of the element. This fairly simple relation requires experimental investigation in the case of biomedical objects for different tissues and organs of a living organism

TABLE I. The main elements in tissues of the human organism, their percentage concentration, and the energies of the K_α lines of their mesic x-ray radiation.

Element	Atomic number	Mass concentration, %			Energy of K_α lines, keV
		Fat	Muscle	Bone	
H	1	12.21	10.2	3.39	2.29
C	6	76.08	12.3	15.5	75.25
N	7	—	3.5	3.97	102.44
O	8	11.71	72.893	44.1	133.53
Na	11	—	0.08	0.06	250.20
Mg	12	—	0.02	0.21	294.30
P	15	—	0.2	10.2	456.22
S	16	—	0.5	0.31	516.19
K	19	—	0.3	—	712.23
Ca	20	—	0.007	22.2	782.76

in vitro, but for bone tissue, for example, it can evidently be adopted with greater confidence, since the mineral and organic components of bone are in a certain sense an organic compound of hydroxiapatite and collagen.[32]

A shortcoming of the method of muonic atoms is its insensitivity to hydrogen, or rather, the impossibility of detecting its mesic x-ray radiation by the ordinary methods of semiconductor or scintillation spectrometry. However, one can take into account the contribution of muons captured by hydrogen indirectly, since the resulting $\mu^- p$ system has dimensions approximately 100 times smaller than the ordinary hydrogen atom. Because of this, the resulting neutral $\mu^- p$ system can penetrate within the electron shell of any atom with larger Z and approach close to the nucleus, as a result of which the muon can be recaptured by low-lying mesoatomic orbits of this atom, i.e., it can make a certain contribution to the x_μ spectrum of the mesic atom Z. The process of muon recapture from hydrogen by a Z atom is also under investigation, and therefore it is not yet possible to make a quantitative estimate of this process; one can only make a qualitative one.[28,29]

Therefore, at the present time one cannot hope for absolute measurements of the element composition of matter by means of muonic atoms. Many investigations are needed before absolute measurements can be made. However, the relative change in the amount of any element in a sample can be detected with fairly high accuracy using the latest achievements in the high-precision spectrometry of in-beam mesic x-ray radiation.

2. EXPERIMENTS WITH ANIMALS

The idea of using muons for element analysis of living organisms was advanced independently by Professor H. Daniel[15] of West Germany and a group of Soviet physicists (Zinov, Konin, and Mukhin).[16] Soon after this, computer calculations were made at Los Alamos in the United States for a "phantom" human being, this yielding a synthetic spectrum of the mesic x-ray radiation expected from irradiation of a human by a μ^- beam (Fig. 1).[17] The following approximation was adopted: All elements in the human organism emit mesic x rays from a depth of 10 cm of soft living tissue, and the x_μ radiation is detected by a Ge(Li) detector with a sensitive volume of 30 cm^3.

Subsequently, *in vitro* measurements were made of mesic x-ray spectra from individual organs and tissues of animals and human beings.[18–21]

The mesic x-ray spectrum from an ox bone weighing 554 g was measured in Ref. 18. For 3×10^7 stopped muons in the target, the corresponding radiation dose was about 200 mrd.

A Ge(Li) detector with 40-cm^3 sensitive volume was used. The x_μ spectrum published in Ref. 18 clearly reveals the K series of carbon and oxygen and K_α lines of nitrogen, aluminum, phosphorous, and calcium. The obtained mass concentrations of calcium and phosphorous were $10 \pm 2\%$ and $4.5 \pm 1.0\%$, respectively, which are close to certain literature data, 11 and 5%, respectively, but differ appreciably from the data of Ref. 44 (see Table I).

A group of American physicists made extensive *in vitro* investigations of different tissues and organs of different animals by means of muonic atoms using the muon beam of the Los Alamos meson factory.[21] The mesic x-ray spectra were measured for swine muscle and adipose tissue and liver, boving muscle tissue and liver, and canine bone tissue, liver, and peripheral blood in order to illustrate the sensitivity of the method of muonic atoms to differences in the element compositions of the listed samples. For some samples, a chemical analysis was also made by physicochemical methods and the results were compared with the x_μ measurements.

The mesic x rays were detected by a 25-cm^3 Ge(Li) detector. To obtain the intensities of the x_μ lines, corrections were introduced for the detector efficiency, the x_μ self-absorption in the samples, and background radiation. The obtained intensities of the x_μ lines for N, C, and O were normalized to 100%. For the soft tissues, only carbon, nitrogen, and oxygen were studied; for the bones, phosphorous and

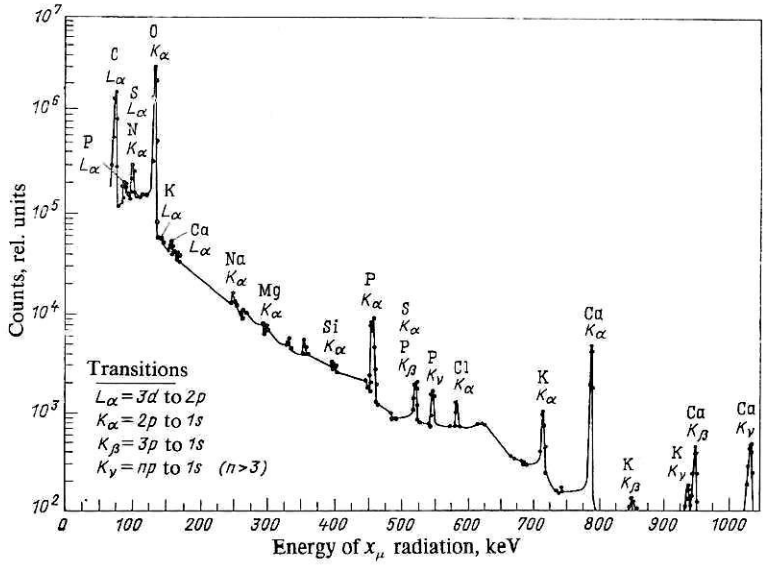

FIG. 1. Synthetic mesic x-ray spectrum of a phantom man calculated on a computer.

calcium as well. For the chemical analysis, up to ten pieces were cut from different parts of each sample; the error of the chemical analysis was less than 0.1%.

The investigation demonstrated the obvious variation in the amount of C, N, and O in the investigated tissue samples, the relative oxygen concentration increasing in the following progression: adipose tissue, muscle tissue, liver, blood.

Comparison of the relative contribution of the elements C, N, and O in the investigated fiber samples of animals by the two methods did not reveal good agreement between the results, especially for oxygen and carbon. For nitrogen the agreement was relatively good. The difference between the results was attributed not to the error of the x_μ method but to errors due to the preparation of the samples for chemical analysis.

Of course, this is entirely possible, since for the chemical analysis one takes thin slices from the surface of the samples, while the x_μ method "scans" the entire volume. And it is by no means obvious that the mean element concentration of pieces on the surface is the same as the average over the complete volume.

Unfortunately, a chemical analysis of the bone was not made. However, the ratio of the phosphorous and calcium contributions to the x_μ spectra were in reasonable agreement with the results of the chemical analysis in Ref. 22.

Thus, the studies of Refs. 15–21 demonstrated the unique and far-reaching possibilities of the new method, which had already become known as muon diagnosis. And still the most important step was not taken—no experiment was made with a living organism *in vivo*.

The first such experiments were begun at the Laboratory of Nuclear Problems of the Joint Institute for Nuclear Research in 1973 under the direction of Dr. V. S. Evseev in the framework of a special biological experiment in collaboration with the Institute of Medical and Biological Problems. The first experiments were made on animals—white rats of the Wistar line.[31] Each rat weighed 180–200 g.

The method used to measure the mesic x-ray spectra was fairly well developed and was as follows: High-energy μ^- mesons obtained from the 680-MeV synchrocyclotron of the Laboratory of Nuclear Problems were transported by means of the meson tract[37] to the experimental area and were decelerated in a block of polystyrene to an energy at which they were stopped in the complete body of the animal (Fig. 2). The time of stopping of a muon was determined by the

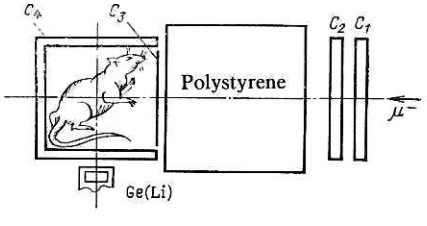

FIG. 2. Arrangement of apparatus in the beam (seen from above). C_1–C_4 are scintillators; the dimensions of C_1 and C_2 are $100\times100\times10$ mm; of C_3, $80\times80\times1$ mm; C_4 in the direction perpendicular to the plane of the drawing measures 120 mm, and in the plane of the drawing $100\times80\times100$ mm with thickness 6 mm.

system of coincidence scintillation counters $C_1C_2C_3\,\overline{C_4}$. The method of measuring the mesic x-ray spectra is described in detail in Refs. 25 and 47. The rat was placed in a specially prepared foam polystyrene box with air openings. Forced ventilation ensured constant circulation of air around and within the box. The size of the muon beam ensured uniform irradiation of the entire body of the animal. To increase the counting rate, the detector was placed in the immediate proximity of the investigated object (Fig. 2).

To achieve a more uniform "scanning" of all parts of the organism, the position of the object relative to the detector was varied periodically. Naturally, the inner part of the animal's body was "scanned" by the detector less effectively. The mesic x rays from the target were detected by a semiconductor Ge(Li) spectrometer with 41-cm^3 sensitive volume. The analog and time pulses obtained from the Ge(Li) detector were sent, respectively, to a pulse-height converter and a regime of fast coincidences with the signal of μ^- stopping in the target. The electronic blocks for the time channel of the Ge(Li) detector were developed in the Laboratory of Nuclear Problems specially for time measurements[24] and made it possible to obtain a time resolution for fast coincidences of $T_\gamma - T_{\mu-\text{stop}} \sim 12$–15 nsec, which made it possible to identify reliably the mesic x rays on the background of the scattered radiation from the accelerator at the position of the experimental area. The mesic x-ray signals selected in this manner were sorted in accordance with their time of arrival at the γ detector by means of an on-line minicomputer HP-2116C and were stored on magnetic disks and magnetic tape. With this regime, it was possible to have a constant visual control of the experiment on a graphical display.[26] One of the working spectra obtained by the irradiation of the rat is shown in Fig. 3.

In the group of lines corresponding to mesic atoms heavier than the oxygen atom one can see the strongest K_α lines corresponding to the atomic transitions $2p\rightarrow1s$. The low-energy part of the x_μ spectra was investigated by means of a Ge(Li) detector with a sensitive volume of 2.4 cm^3 (Fig. 4), muons being stopped in nine mice exposed to the meson beam under identical experimental conditions.

The mesic x-ray spectra were analyzed using a CDC-6500 computer by means of the SAMPO program[27] adapted at the Joint Institute. The number of pulses below each peak corresponded to the relative contribution of the given element to the investigated object. A correction was introduced for the dependence of the detector efficiency on the energy of the mesic x rays and for the contribution of higher transitions to the K series of each element. These lines were taken into account using both literature data[18] and the results of our measurements of the mesic x-ray spectra for a number of compounds found in living organisms. For oxygen, carbon, and nitrogen, for which all lines of the K series were observed, the sums of the measured intensities of all lines of the K series were taken.

In connection with man's conquest of space, investigation of the mineral saturation of bone acquired particular topicality.[9,33] Our ideas about functional biochemistry of bone tissue have not yet been sufficiently developed because of the absence of adequate methods of investigation. And

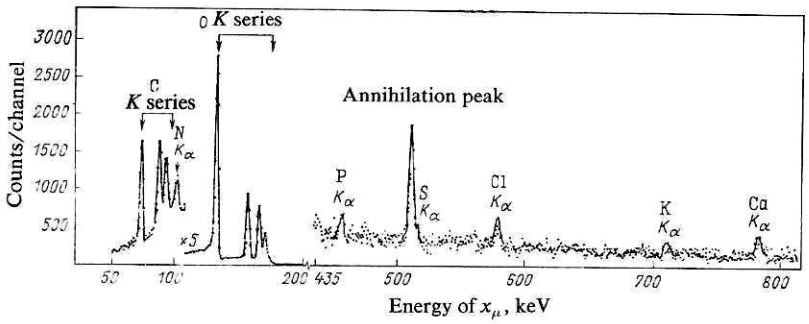

FIG. 3. Spectrum of μ-mesic x-ray radiation from the organism of the rat. Along the abscissa, the x_μ energy; along the ordinate, the number of pulses per energy interval.

since the method using muonic atoms is best adapted to the measurement of a relative change in the concentration of elements in a sample, it appeared promising to use this method in the following biological experiment made at the Laboratory of Nuclear Problems—an investigation of the influence of space flight (hypokinesia) on the element concentration of living organisms and, in particular, on the calcium concentration in an animal skeleton. For this, the mesic x-ray spectra were measured for three Wistar rats that had undergone a three-week space flight in the artificial satellites Kosmos-605 and Kosmos-690. The rats were exposed to μ^- beams before the flight, immediately after the flight, and a month after landing.[31] The method of measurement was similar to that described above, but to achieve a more effective detection of the mesic x rays of heavy elements (from phosphorous to calcium) a Ge(Li) detector with a 55-cm^3 sensitive volume was used. It was specially prepared for this purpose at the Riga Scientific-Research Institute of Radioisotope Instrument Construction.[34] The results of the measurements are given in Tables II and III.

The errors in the tables are statistical, including for all elements except C and O the errors due to allowance for the high lines of the K series. The errors taking into account the detector efficiency are not included, since we are interested only in the relative changes in the element concentrations.

It can be seen from Tables II and III that to within the statistical errors the chemical compositions of the animals before and after the flight do not differ, except for carbon, for which there was a clear increase in its concentration after the flight. The reasons for this increase are as yet unclear. The small decrease in the concentrations of the sodium and the chlorine for the rat that flew in Kosmos-605 may be due to a change in the food ration it was given after the flight had ended.

Our data show that as a result of the space flight there was not a significant loss of heavy elements from the bodies of the animals but, probably, a redistribution of them between the tissues or organs of the body. Information of this kind is also found in the results of Ref. 35. It therefore becomes obvious that a change in the mineral saturation of

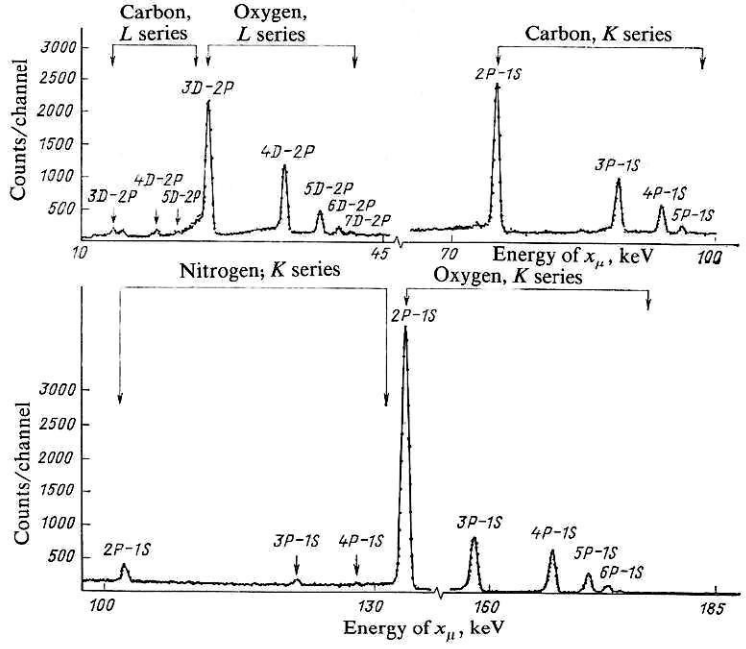

FIG. 4. Spectrum of the low-energy part of the mesic x-ray radiation from the organism of nine mice, measured by means of a Ge(Li) detector with 2.4-cm^3 sensitive volume.

TABLE II. Relative amounts of elements in rats (No. 1 is the laboratory control, No. 2 the rat in the satellite Kosmos-605).

Element	Relative amounts of elements, %		
	№ 1	№ 2	
		one day after end of flight	30 days after end of flight
C		15,7±0,5	19,7±0,5
N		2,7±0,6	3,0±0,6
O	80,0±1,9	78,7±1,8	75,0±1,8
Na	0,17±0,01	0,18±0,01	0,123±0,011
Mg	0,03±0,01	0,036±0,012	0,032±0,014
P	0,47±0,02	0,48±0,01	0,492±0,015
S	0,28±0,02	0,32±0,01	0,031±0,013
Cl	0,17±0,01	0,16±0,02	0,110±0,012
K	0,30±0,02	0,29±0,03	0,250±0,014
Ca	0,99±0,07	1,07±0,07	0,954±0,028

bone tissue is to be observed in individual sections of the skeleton and not in the complete organism. In addition, in connection with further increase in the duration of human space flights and the discovery of appreciable deviations in the activity of the entire organism and its individual organs[33,39] it is obviously necessary to develop the most realistic estimates for prophylaxis and therapy in the case of breakdown of the water–salt exchange. Such an estimate can be made by simulating the factors in space flight, in particular during prolonged antiorthostatic hypokinesis.

3. INVESTIGATIONS WITH HUMAN BEINGS

In the framework of the collaboration between the Joint Institute for Nuclear Research and the Institute of Medical and Biological Problems of the Ministry of Public Health of the USSR, an experiment was planned and carried out with the aim of developing a method of mesochemical analysis of the mineral composition of human bone fiber after a period under extremal conditions.[38] The investigation was made on 18 healthy men in the age range 32–37 years. For 182 days they all remained under conditions of a rigorous bed regime (antiorthostatic hypokinesis). To simulate the conditions of weightlessness, the beds were set up with a small slope (of about 4°) toward the head. The investigated subjects were divided into three groups. The first group was a control, and was kept under the conditions of the bed regime without prophylactic measures. The men in the second group carried out a set of prophylactic measures. This included physical training (two hours per day with an energy expenditure of 300–350 kcal/h), myoelectrostimulation, and, before the end of the bed regime, training with application of negative pressure to the lower part of the body and water–salt additives in the rations. The prophylactic measures for the third group consisted of physical exertions whose extent did not exceed 35% of the physical load of the second group. Measurements of the mesic x-ray spectra were made on heel bone fiber four days before the start of the hypokinesis regime and four days after it ended.

The choice of heel bone as the object of investigation was dictated by the homogeneity of its structure, the accessibility for measurements, and also the possibility of reliable identification of muon stoppings in the heel bone with elimination of the contribution from adjacent soft tissues; it resulted automatically in the localization of the stoppings in the cancellous (spongy) part of the heel bone.

Some special preparatory work was done before the measurements. First, to accommodate the subject and the electronics together with the Ge(Li) detector a metal box was made with double steel walls, the space between them being filled with water to reduce the background from the accelerator; the placing of the electronics within the metal box greatly reduced the electromagnetic inductions; this facilitated the operation of the sensitive electronics of the semiconductor detector, which increased the reliability of the

TABLE III. Changes in the amounts of elements in rats that flew in the satellites Kosmos-605 and Kosmos-690.

Element	Ratio of amount of the element after the flight to the amount before the flight		
	Kosmos-605	Kosmos-690	
	Rat No. 1	Rat No. 2	Rat No. 3
C	1,26±0,05	1,11±0,03	1,13±0,03
N	1,11±0,29	1,03±0,02	1,02±0,02
O	0,95±0,03	0,98±0,03	0,98±0,02
Na	0,70±0,07	1,06±0,08	1,10±0,35
Mg	0,90±0,52	1,2±0,6	—
P	1,02±0,04	1,02±0,05	0,98±0,08
S	0,97±0,05	1,14±0,09	1,18±0,14
Cl	0,69±0,17	0,94±0,13	1,0±0,3
K	0,86±0,10	0,97±0,12	1,07±0,17
Ca	0,89±0,06	1,08±0,10	0,85±0,07

results. There was a supply of fresh air, and the subject and the position of his leg, which was fixed in the meson beam, were observed continuously by means of television cameras. The background from the accelerator at the position of the subject according to the measurements of the radiation monitors was ~7.0 mrem during 3 h of operation of the accelerator (the time during which the subject was exposed to the beam), i.e., approximately 0.03 of the maximally allowed quarterly dose for the complete organism for nonprofessionals. A special beam of muons[37] with an energy of about 30 MeV was formed in the meson channel and, passing through an opening in the wall of the box, arrived at the device that ensured stopping of the muons in the central part of the heel bone of the left foot of the man (Fig. 5). An estimate of the absorbed dose in the heel bone due to the total number of stopped μ^- mesons at a rate of real stoppings of about 10^3 μ^-/sec during the 3 h of measurements was not more than 0.25 mrem, which is about 0.07 of the maximally allowed quarterly local dose in the heel bone for nonprofessionals.

The muons, which passed through two monitor scintillation counters 1 and 2, through a collimator 3 with a lining 4, a block of polystyrene of variable thickness 5, where they lost energy, and a thin counter 6, were stopped in the region 7 of the heel bone 8. Since the spread in the range of 30-MeV muons is about 2 g/cm^2,[37] and the thickness of the heel bone along the beam is about 4.5–5.0 g/cm^2, more than 95% of all the muon stoppings occurred in the cancellous, inner part of the heel bone (Fig. 6).

To increase the stability and reliability of the operation of the apparatus, the analog and logic parts of the spectrometric electronics were arranged in a CAMAC standard, which made it possible to control the experiment and monitor its progress by means of an HP-2116C computer.[36,47] Figure 7 shows a typical spectrum of the mesic x rays from the heel bone of a man during a 3-h exposure in a muon beam, obtained by means of a Ge(Li) detector with a 55-cm^3 sensitive volume. The time resolution $T_\gamma + T_{\mu\text{-stop}}$ of coincidences was about 15–20 nsec, and the energy resolution of the spectrometric channel was 2.5–2.6 keV at the ^{60}Co line.

The experiment was augmented by measurements of the mesic x-ray spectra from the heel bone of four of the experimentalists (whom we shall call volunteers, in contrast to the subjects). Measurements made with the same person with an interval of six months, repeated measurements every day to check the influence on the result of the accuracy of stopping over a finite length in the beam, and measurements

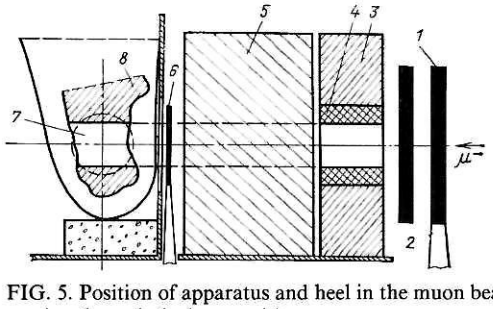

FIG. 5. Position of apparatus and heel in the muon beam (vertical plane passing through the beam axis).

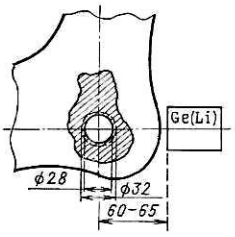

FIG. 6. Relative position of heel and Ge(Li) detector (vertical plane perpendicular to the beam axis).

with a larger diameter of the collimator and nonoptimal (within reasonable limits) thicknesses of the block (block 5 in Fig. 5)—all these measurements indicated the absence of appreciable systematic errors exceeding the statistical error determined by the number of recorded pulses below the peaks of the mesic x-ray lines and background.

Altogether, 41 measurements of mesic x-ray spectra were made: 11 were control measurements, and 24 were measurements on the 12 subjects four days before the experiment and four days after it; for six of the subjects (three in each of the second and third groups) the spectra were measured for technical reasons only after the state of hypokinesis. Since the contribution of each element to the heel bone was determined by means of the expression $W_i/\Sigma W_i$ for all the subjects under absolutely identical conditions, it is entirely reasonable to consider the individual deviations of the contributions of a particular element to the bone of each of the subjects. The results of the measurements showed that the individual spread in the concentrations of the light elements C, N, and O in the heel bones of the majority of the subjects only slightly exceeded the mean statistical error of the measurements, which indicates that they have more or less the same concentrations in the bone (Fig. 8).

A different picture was obtained for the heavy elements of the mineral component of the bone—phosphorous and calcium. The individual spread of their concentration $W_i/$

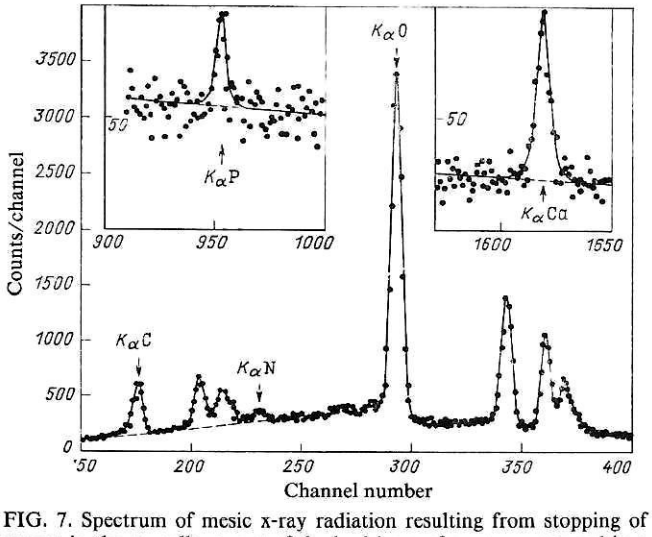

FIG. 7. Spectrum of mesic x-ray radiation resulting from stopping of muons in the cancellous part of the heel bone of a man, measured in a three-hour exposure.

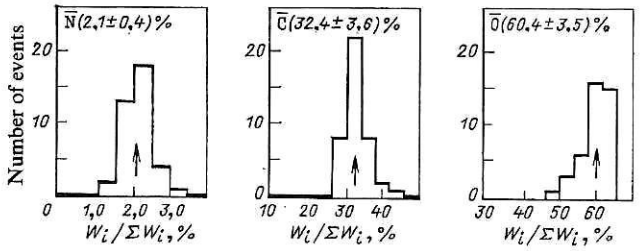

FIG. 8. Distribution of the relative contribution of the mesic x-ray radiation of nitrogen, carbon, and oxygen. In the brackets, the mean values and standard deviations obtained by analysis of the distributions.

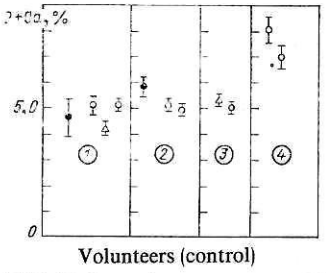

FIG. 10. Control measurements of the mineral component for the experimentalists (volunteers): black circles, 1976; open circles, 1977; open triangles, 1977 (nonstandard conditions).

ΣW_i was fairly large, and the differences reached 2–2.5 times. The total P + Ca contributions differed as much from individual to individual. In contrast, the ratio of the contribution of these elements exhibited a high constancy (within the errors) (Fig. 9). Figure 10 shows the results of the control measurements of the concentration of the mineral component (P + Ca) in the heel bones of the volunteers. A fairly good reproducibility of the measurements can be observed. Volunteer No. 4 had a higher concentration of the mineral component.

The results of the investigation of the influence of a state of immobility (hypokinesis) on the concentration of the mineral component in the human heel bone were as follows: For the subjects of the first control group, the largest range of variation of the total concentration of phosphorous and calcium is observed—from an increase by 25% to a decrease by a factor 2 (Fig. 11). For groups II and III the variations were much less. For six of the subjects (three in each of the groups II and III) the spectra were measured only after the state of hypokinesis for technical reasons.

The mean values of the variations of P + Ca over the three groups (respectively, − 12, 0, and − 12%) demonstrate a certain tendency to a reaction to the prophylactic measures. We recall that group I underwent the bed regime without prophylaxis, while group III had only 35% of the load of the second group; thus, a certain correlation can be observed. But for reliable conclusions about the existence of a reaction of the subjects to the prophylactic measures we require an experiment on a larger number of subjects and with a more prolonged exposure. It is not impossible that the changes in the mineral concentration are due less to the differences in the conditions of the experiment than to the much greater individual reaction of an organism to the hypokinesis state. The latter possiblility is confirmed by the presence of a correlation between the extent of the variation of the mineral concentration and its original level (Fig. 12).

It can be seen from Fig. 12 that the maximal decrease in the P + Ca concentration is observed for the subjects with maximal initial mineral mass of the bone, and vice versa. It is likely that as a result of prolonged antiorthostatic hypokinesis there is an equalization of the mineral concentration of the cancellous (spongy) bone of people for whom a large difference in the phosphorous and calcium concentrations characterizes their normal state.

In Ref. 39, an attempt was made to prevent demineralization of bone tissue during the prolonged bed rest of 90 healthy young people, and the extent of mineral loss was measured by photon absorpsiometry and investigation of excreta. The conclusion was unambiguous—physical exercises did not prevent mineral loss by the bone tissue; much more effective was the addition of phosphates and calcium to the food ration.

In another study by American scientists,[40] in which there were 15 subjects, an investigation was made of the mineral loss by the heel bone (and also the radius and ulna, in which no mineral loss at all was observed), also by a combination of two methods, namely, measurement of the loss of minerals by the organism in the excreta and measurement of the total change in the mineral concentration by photon absorpsiometry [measurement of the absorption in the heel of 27.5-keV ^{125}I x rays by means of an NaI(Tl) detector].

This study gave a weak but opposite result: For people with high initial mineralization of the heel bone, a smaller loss of the mineral component as a result of the bed-rest regime was observed. However, it should be mentioned that this conclusion was largely based on the investigations of the elimination of mineral salts in the excreta. Therefore, if there is a redistribution of the mineral salts into the tissue surrounding a bone including the cortical layer, the method of

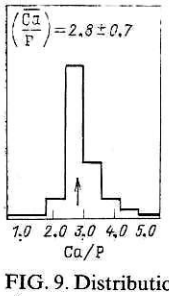

FIG. 9. Distribution of the ratio of the calcium concentration to the phosphorous concentration.

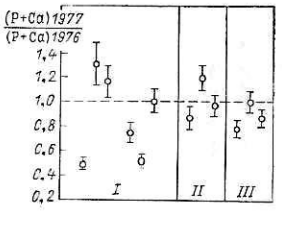

FIG. 11. Change in the total concentration of phosphorous and calcium for the subjects of different groups.

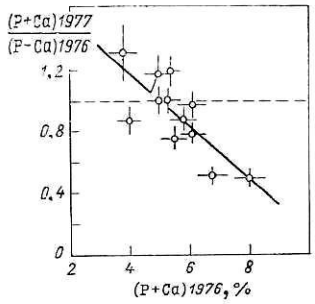

FIG. 12. Correlation between the degree of variation of the mineral concentration (along the ordinate) and its original level (along the abscissa).

x-ray absorption is incapable of distinguishing precisely where the calcium concentration increases and decreases, i.e., by this method one can investigate the composition of the entire heel, including the cortical layer of the bone and the soft muscle tissue, whereas the muon method determines the composition of the predominantly cancellous part of the heel bone. Therefore, these two methods should evidently be regarded as complementing each other. From this point of view, one can understand how the study of Ref. 40 found a greater individual spread in the mineral concentration in the normal state (by up to 5 times) and a converse weaker dependence in the case of prolonged hypokinesis on the initial mineral mass of the entire heel bone. It is therefore also possible that this experiment did not establish any correlation between the change in the concentration of the mineral component of the bone and the various prophylactic measures taken to reduce the influence of hypokinesis. It is not impossible that the mineral concentrations of the cancellous part of the bone and its cortical layer change differently under extremal conditions (see, for example, Ref. 41). For a more detailed study of the redistribution of calcium and phosphorous between different parts of the bone it is evidently expedient to combine these two methods.

4. ABSOLUTE CALIBRATION OF MUON DIAGNOSIS

As we have shown above, by muon diagnosis one can obtain information about only the relative change in the amount of elements in the organic or mineral components of a living organism resulting from functional changes in activity, which may be due to either pathological processes or extremal conditions (for example, weightlessness). To know the actual mass contribution of any element in an investigated living object, it is necessary to know calibration coefficients obtained by comparing the data of muon diagnosis with some other method that determines the absolute concentration of the element. For this, a measurement was made of the mesic x-ray spectrum of a sample of cancellous bone taken from the thigh bone of an effectively healthy man of 45 years who died by accident, after which an analysis was made of the same bone by well-developed physicochemical methods.[42] The mass of the sample was about 150 g; it was fixed in a 2% solution of formalin.

The mesic x-ray measurement was similar to the one described above.[36] For the physicochemical analysis, which was carried out at the Department of Pathological Physiology at the N. A. Semashko Labor Red Flag Order Medical Stomatological Institute, five fragments were taken from different sections of the sample. The material was weighed, dried to a constant mass in a thermostat at temperature 80–100 °C, and calcined in a muffle furnace at 700 °C for 7 h. Each fragment of bone was pulverized in an agate mortar, and three portions were prepared from the ashes of each fragment. The ashes were dissolved in chemically pure hydrochloric acid; the concentrations of calcium and magnesium were determined in solution using an atomic absorpsiometer; the concentrations of sodium and potassium were determined using a flame photometer; and of phosphorous, using a spectrophotometer. Then the amounts of these elements by mass in 100 g of dry tissue were calculated. The results are given in Table IV.

The results for the same elements obtained by the mesic x-ray method are given in Table V in the form of the mean values for five fragments (rms errors).

It can be seen from Table V that for Na and P the discrepancy is a factor of about two, while for Ca the results agree to within the statistical error. For the sum P + Ca the results of the two methods also agree to within a 7% error. The values of α given in Table V are the correction coefficients by which one must multiply the relative intensity of the mesic x-ray spectrum of the given element to obtain its mass concentration.

Of course, we have thereby merely demonstrated the possibility of absolute calibration of the mesic x-ray method. To obtain the true coefficients, extensive experimental investigations will be necessary.

CONCLUSIONS

In this review we have considered the varied investigations that have demonstrated the unique possibilities of using muonic atoms and their mesic x-ray radiation for element analysis in biomedical research and to establish the relative change in the contribution of any element in a sample of living tissue.

TABLE IV. Amounts of the elements in 100 g of dry bond tissue determined by physicochemical methods.

Number of sample	Sodium, mg	Potassium, mg	Calcium, g	Magnesium, mg	Phosphorous, g
1	133,2	8,1	9,1	130,5	4,2
2	132,7	7,9	9,2	128,5	4,2
3	134,7	10,3	8,9	127,3	4,0
4	140,3	6,5	8,9	116,7	4,2
5	130,0	6,9	9,0	122,3	4,2

TABLE V. Comparison of the mass concentration of elements, %, measured by physicochemical methods, $(P_i/\Sigma P_i)_{exp}$, and relative intensity of the mesic x-ray K series of the same elements, $(W_i/\Sigma W_i)_{exp}$, in dry bone tissue.

Element	$(P_i/\Sigma P_i)_{exp}$	$(W_i/\Sigma W_i)_{exp}$	α
Na	0,134±0,004	0,29±0,04	0,46±0,07
P	4,16±0,09	2,39±0,12	1,74±0,10
Ca	9,03±0,13	10,05±0,77	0,90±0,08
P+Ca	13,90±0,07	12,44±0,78	1,12±0,07

An undoubted advantage of the muon method is the possibility of investigating the concentration of an element in a living tissue without surgical intervention. And this *in vivo* nondestructive method of element analysis has an overwhelming advantage over the other nuclear-physical methods hitherto used on two main scores: a) the possibility of achieving three-dimensional localization of the muon beam in almost any region of the organism; b) the high sensitivity of the method at a very low radiation dose. In the studies we have reviewed, the sensitivity of the method was not better than 10^{-4} with respect to the mass. By using more intense muon beams and semiconductor detectors with larger sensitive volume, and also more up-to-date instruments one could achieve a sensitivity of 10^{-5}–10^{-6}. Moreover, muons are equally sensitive to all atoms, while, for example, neutrons are not to all.

By the muon method one can determine the concentration of any element, irrespective of the chemical compound in which it occurs. Moreover, the analysis can be carried out simultaneously for all elements of the periodic table.

The method opens up great prospects. As examples, we may mention the following:

1. In the review of Ref. 45, Barbolin showed that in different forms of cancerous tumors there is a change in the concentration in the organism of elements such as Ca, P, K, Na, and Fe. Using muonic atoms and comparing healthy and diseased tissue, one can develop an effective clinical diagnosis of tumor formation. Because of the high sensitivity of the method, one can recognize pathological changes in an early stage, this being possible, moreover, at greater depths of the tissue, owing to the absence of the matrix effect inherent in the ordinary x-ray fluorescence method. The process of recovery can be followed as effectively.

2. Using the dependence of the structure of the mesic x-ray radiation on the chemical state of the element and its molecular environment, and "calibrating" by means of pure chemical compounds and elements, one can observe the change in the chemical state of the element during some physiological process in a living organism or in a biological object.

3. In connection with the extension in recent years of the method of neutron-capture therapy of malignant growths, it has been found that there is more rapid accumulation of boron in tumors than in healthy tissue.[46] This discovery opens up the possibility of cancer therapy by means of neutrons at low radiation doses of healthy tissues by virtue of the large cross section for neutron capture by boron nuclei. But to determine the optimal regime of accumulation of boron and the choice of the tumor irradiation needed in such an approach, a method is needed for "express" measurement of the amount of boron in the tumor, the dynamics of its accumulation, and determination of the saturation regime. Obviously, the method of muonic atoms is the most adequate for this problem.

A limitation of the method is the absence of high-intensity meson beams, which affects the sensitivity of the method.

Another shortcoming is the impossibility of detecting hydrogen. Therefore, this is not a unique comprehensive method for investigating different functional changes in a living organism and must be augmented by other methods of analysis.

The further development and extension of the fields of application of the method require a further improvement of it, modern electronic devices, wide use of computers, and a significant increase in the intensity of the meson beams.

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[1]A. O. Voĭnar, Biologickeskaya rol' mikroélementov v organizme zhivotnykh i cheloveka (Biological Role of Trace Elements in the Animal and Human Organism), Sovetskaya Nauka, Moscow (1953).
[2]A. P. Skoblin and A. M. Belous, Mikroélementy v kostnoĭ tkani (Trace Elements in Bone Tissue), Meditsina, Moscow (1968).
[3]M. G. Kolomiĭtseva and R. D. Gabovich, Mikroélementy v meditsine (Trace Elements in Medicine), Meditsina, Moscow (1970).
[4]Trudy II Soveshchaniya po ispol'zovaniyu novykh yaderno-fizicheskikh metodov dlya resheniya nauchno-tekhnicheskikh i narodno-khozyaĭstvennykh zadach (Proc. of the Second Symposium on the Use of New Nuclear-Physics Methods for Solving Scientific–Technical and Economic Problems), JINR, Dubna (1975).
[5]Trudy II Vsesoyuznogo simpoziuma po metodam opredeleniya mikroélementov v prirodnykh ob'ektakh (Proc. of the Second All-Union Symposium on Methods of Determining Trace Elements in Natural Objects), Samarkand University (1973).
[6]H. A. Van Rinsvelt *et al.*, Nucl. Instrum. Methods **142**, 171 (1977); **149**, 489 (1978).
[7]In Vivo Neutron Activation Analysis. Proc. of a Panel, Vienna, 17–21 April (1972).
[8]E. Ackerman, Biophysical Science, Englewood Cliffs, N. J. (1962) [Russian translation published by Mir, Moscow (1964)].
[9]P. Rambaut *et al.*, Aerosp. Med. **43**, 646 (1973); D. M. Smith *et al.*, "The measurement of rates of mineral loss aging," J. Lab. Clin. Med. **87**, 5 (1976).
[10]Yu. Ya. Stavisskiĭ *et al.*, Radiatsionnyĭ zakhavat bystrykh neĭtronov (Radiative Capture of Fast Neutrons), Atomizdat, Moscow (1970).
[11]B. S. Dzhelepov and L. K. Peker, Skhemy raspada radioaktivnykh yader (Decay Schemes of Radioactive Nuclei), Nauka, Moscow–Leningrad (1966).
[12]E. Fermi and E. Teller, Phys. Rev. **72**, 399 (1947).
[13]Mezony v veshchestve. Trudy Mezhdunar. simp. po problemam mezonnoĭ khimii i mezomolekulyarnykh protessov v veshchestve (Mesons

in Matter. Proc. of the Intern. Symposium on Problems of Meson Chemistry and Mesomolecular Processes in Matter), D1,2,14-10908, JINR, Dubna (1977).
[14]A. O. Vaĭsenberg, Myu-mezon (The μ Meson), Nauka, Moscow (1964).
[15]H. Daniel, Nucl. Med. **8**, 311 (1969).
[16]V. G. Zinon, A. D. Konin, and A. I. Mukhin, Avt. svid-vo SSSR No. 333452 ot 12 maya 1970 (USSR Inventor's Certificate No. 333452 of May 12, 1970), Byul. OIPOTZ, No. 11, 169 (1972); R14-6407, JINR, Dubna (1972).
[17]L. Rosen, in: Trudy IV Mezhdunar. konf. po fizike vysokikh énergiĭ i strukture yadra (Proc. of the Fourth Intern. Conf. on High Energy Physics and Nuclear Structure), D1-6349, Dubna (1972), p. 589.
[18]M. L. Taylor *et al.*, Radiat. Res. **54**, 335 (1973).
[19]H. Daniel *et al.*, Biomed. Tech. **13**, 222 (1973).
[20]H. Daniel *et al.*, Phys. Med. Biol. **20**, 1035 (1975).
[21]R. L. Huston *et al.*, Radiat. Phys. **120**, 193 (1976).
[22]H. Woodward, Health Phys. **8**, 513 (1962).
[23]Trudy III Soveshchaniya po ispol'zovaniyu novykh yaderno-fizicheskikh metodov dlya resheniya nauchno-tekhnicheskikh i narodnokhozyaĭstvennykh zadach (Proc. of the Third Symposium on the Use of New Nuclear-Physics Methods for Solving Scientific–Technical and Economic Problems), JINR, Dubna (1978).
[24]Yu. K. Akimov *et al.*, Nucl. Instrum. Methods **104**, 581 (1972).
[25]K. Andert *et al.*, Soobshchenie (Communication) R15-10373, JINR, Dubna (1977).
[26]M. Gonusek and V. D. Fromm, Soobshchenie (Communication) 10-10007, JINR, Dubna (1976).
[27]J. T. Routti, Report UCRL-19452, University of California (1969; J. T. Routti and S. G. Prussin, Nucl. Instrum. Methods **72**, 125 (1969).
[28]S. S. Gershteĭn *et al.*, Usp. Fiz. Nauk **97**, 3 (1969) [Sov. Phys. Usp. **12**, 1 (1969)].
[29]V. G. Kirillov-Ugryumov *et al.*, Atomy i mezony (Atoms and Mesons), Moscow (1980).
[30]H. Daniel, in: Mesons in Matter, 10908, JINR, Dubna (1977), p. 88; V. I. Petrukhin *et al.*, Zh. Eksp. Teor. Fiz. **70**, 1145 (1976) [Sov. Phys. JETP **43**, 595 (1976)]; P. Vogel *et al.*, Phys. Lett. **70B**, 39 (1977); H. Schneuwly *et al.*, Phys. Lett. **66A**, 188 (1978); H. Schneuwly *et al.*, Nucl. Phys. **A312**, 419 (1978); R. A. Naumann and H. Daniel, Z. Phys. **A291**, 33 (1979).
[31]R.-D Arl't *et al.*, Soobshchenie (Communication) 18-11844, JINR, Dubna (1978).
[32]M. Glimcher, in: Sovremennye problemy bviofiiziki (Modern Problems of Biophysics: Russian translations), Izd. Insostr. Lit., Moscow (1961), p. 94.
[33]E. N. Biryukov and I. G. Krasnykh, Kosm. Biol. Aviakosmicheskaya Med. **4**, No. 6, 42 (1970); I. G. Krasnykh, Kosm. Biol. Aviakosmicheskaya Med. **8**, No. 1, 68 (1974); G. Whedon *et al.*, in: Proc. of the Skylab Life Sciences Symposium, Vol. 1 (1974), p. 353; J. Vogel and P. Rambaut, in: Skylab l/2 Preliminary Biomed. Report, NASA (1973), p. 199; P. Karjalainen, Ann. Clin. Res. **5**, 231 (1973).
[34]A. E. Bainfatov *et al.*, Prib. Tekh. Eksp. **1**, 50 (1977).
[35]G. D. Whedon *et al.*, "Mineral and nitrogen balance study observations," Preprint, ASM, AMA (1974), p. 210; J. Vogel and M. Whittle, "Bone mineral measurements of the second Skylab," Preprint, ASM, AMA (1974), p. 211.
[36]Yu. K. Akimov *et al.*, Preprints 13-12021, 13-12022 [in Russian], JINR, Dubna (1978); Yu. K. Akimov, Nucl. Instrum. Methods **165** (1979); Part I: Electronics, p. 385; Part II: Programming Techniques, p. 387.
[37]A. V. Dem'yanov and V. S. Roganov, Preprint 1-4026 [in Russian], JINR, Dubna (1968).
[38]V. S. Evseev *et al.*, Soobshchenie (Communication) 18-12286, JINR, Dubna (1979).
[39]V. Schneider, "Attempts to prevent bone mineral loss during prolonged bedrest," Eighth Meeting of the Joint US–USSR Working Group on Space Biology and Medicine, Washington, D. C. (1977).
[40]J. D. Vogel and M. W. Whittle, in: Proc. of the Skylab Life Sciences Symposium, Aug. 27–28, Vol. 1, p. 387.
[41]A. P. Volozhin *et al.*, Patol. Fiziol. Eksp. Ter. No. 2, 31 (1979); A. P. Volozhin *et al.*, Kosm. Biol. Med. **3**, 10 (1972).
[42]A. P. Volozhin *et al.*, Soobshchenie (Communication) R15-80-210, JINR, Dubna (1980).
[43]K. M. Hanson, Working Conf. on Comp. Aided Tomography and Ultrasonics in Medicine, LA-UR-78-1827 (1978); LA-7107-MS (1978).
[44]D. R. White, Phys. Med. Biol. **22**, 219 (1977).
[45]V. I. Barbolin, Med. Radiol. No. 1, 65 (1981).
[46]R. G. Zamenhof et al., Med. Phys. **2**, 47 (1975).
[47]B. M. Sabirov *et al.*, in: Proc. of the Tenth Symposium on Selected Topics of the Interaction of Fast Neutrons and High Ions with Atomic Nuclei, Gaussing, GDR, November (1980), p. 194.

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