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Activation Analysis in Biological Material

J. TURKSTRA, D. H. RETIEF, P. E. CLEATON-JONES

SUMMARY

The principles of neutron activation analysis are briefly discussed. Advantages and limitations of the technique are considered in detail, and certain applications in the biological field are illustrated.

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The functions of trace elements or micronutrients in biological processes are being investigated by numerous research workers. In former years trace element impurities in most physical, physicochemical and biochemical systems and materials were regarded as being of minor or negligible importance. However, during the past few decades studies in agricultural, archaeological, criminological, medical, biological, industrial and geological fields have stressed the importance of trace element analysis. Investigations of the major, minor and trace element distributions in the earth's crust, in meteorites and in lunar samples, etc., regarding the composition, history and creation of the earth and the whole solar system, have become of particular importance in geochemistry.[1-3] It also became apparent that even minute amounts of certain elements play an important role in the metabolism of all living organisms,[4,5] and in the growth, development and maintenance of health in man. Before the function of various elements in biological systems can fully be explained, or the changes that occur in their distribution in pathological states can be interpreted, their normal distribution must first be known.

These investigations were stimulated by the marked progress in the development of techniques for trace element analysis. A number of sophisticated physical methods such as X-ray fluorescence, emission and atomic absorption spectrometry, spark-source mass spectrometry and nuclear activation techniques have in recent years become available for this purpose.

Of these techniques, it is fairly generally conceded that in most instances nuclear activation analysis affords the most sensitive and versatile technique.[6]

PRINCIPLES

Although the discovery of the principles of activation analysis dates back to the 1930s, the assessment and application of activation analysis as an analytical technique was hampered until, towards the end of the 1950s, high-flux nuclear reactors and high-intensity accelerators became available in certain countries. Since then, many more countries have acquired nuclear reactors, accelerators and other activation devices and sources. Consequently, the use of activation analysis for diverse analytical purposes has increased immensely, as is evident from the extensive literature on the subject.[7]

Several types of nuclear reactions may be used for activation analysis by applying the various types of nuclear irradiations. However, neutron activation is the most widely applied nuclear activation technique for the determination of trace elements in biological material.[8-10] The following discussions are thus restricted exclusively to neutron activation analysis.[11]

When a sample is to be analysed by neutron activation analysis, it is exposed to bombardment by neutrons originating from a neutron source, viz. a reactor or a neutron generator. Neutrons are extremely suitable for activation because they possess no charge and can thus easily reach the positive nucleus of an atom. They have this penetrating ability, even in respect of large samples. Because the neutron density in a nuclear reactor is very high, irradiation in reactors furnishes much higher sensitivities with regard to most elements as compared with other methods of activation. Some of the atoms in the sample will interact with the bombarding particles and may be converted into different isotopes of the same element or isotopes of different elements. In many cases the isotopes produced are unstable (radioactive) and will pass to a more stable nuclear state with a characteristic decay time by way of emission of a β-particle and γ-radiation. This can be represented by the following formula:

$$ {}^{m}_{z}A + {}^{1}_{0}n \rightarrow \gamma + {}^{m+1}_{z}A \xrightarrow{\lambda} {}^{m+1}_{z+1}B $$

or

$$ {}^{m}_{z}A(n,\gamma) {}^{m+1}_{z}A \xrightarrow{\lambda} {}^{m+1}_{z+1}B $$

where ${}^{m}_{z}A$ is a stable isotope with atomic number z and mass number m,

${}^{m+1}_{z}A$ is the radio-isotope which emits beta particles, accompanied mostly by gamma rays,

${}^{m+1}_{z+1}B$ is the stable nuclide formed with atomic number z + 1 and mass number m + 1,

n are the neutrons,

γ is the prompt-gamma radiation which develops as a

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result of the release of the binding energy of the neutron, and

λ is the radioactive disintegration constant characteristic of the radio-isotope under consideration.

The qualitative analysis of ${}^{m}_{z}A$ can be done from the half-life ($T_{\frac{1}{2}}$) of the radio-isotope, ${}^{m+1}_{z}A$, which represents the time during which the number of radioactive atoms decreases to half the number originally present, and from the γ-rays characteristic of the emitting nucleus. It has to be kept in mind that the half-life is a function of the disintegration constant. For quantitative analysis the comparative procedure is applied in most work on neutron activation analysis. In this method a known quantity of an element to be determined in a sample is irradiated simultaneously with the sample, under essentially the same experimental conditions. From a comparison of the relative intensities of the activity resulting from the element in the sample with that arising from the element in the reference standard, the concentration of the element in the sample can be calculated according to the following formula:

$$\frac{A_s}{A_{st}} = \frac{W_s}{W_{st}}$$

where A_s is the activity of the element in the sample,

A_{st} is the activity of the element in the reference standard,

W_s is the weight of the element in the sample, and

W_{st} is the weight of the element in the reference standard.

In neutron activation analysis, samples and reference standards are usually sealed in pure and cleaned quartz or polyethylene containers. This is possible by virtue of the fact that neutrons are uncharged particles and thus usually afford penetrating and homogeneous irradiation. In the case of a sample, the weight required for irradiation depends on the elements and their concentrations present in the sample. Because of large differences in isotopic thermal neutron cross-sections (i.e. the tendency to capture neutrons) of the elements, and large variations in the half-lives of the radio-isotopes produced, different irradiation times have to be used to achieve the highest sensitivity for each element or group of elements present in the sample. In general, a short irradiation time is sufficient to produce only short-lived radio-isotope activities. On the other hand, a relatively long irradiation time produces long-lived radio-isotopes which predominate after a long decay time.

In studies involving mixtures of synthetic radio-isotopes such as encountered in neutron activation analysis, radiochemical separations were first applied to isolate the pure radionuclides. Despite significant improvements and innovations regarding radiation detectors in subsequent years, this remained the position until fairly recently.[12] Initially, Geiger-Muller or proportional counters, for the most part, were used for measuring β-activities; the proportional counter was occasionally also used to count soft γ-rays. With the advent of the NaI thallium-activated scintillation detectors and the development of multichannel pulse-height analysers (γ-spectrometers) around 1950, it became possible for the first time to record, conveniently and instrumentally, a complete γ-spectrum. The NaI(Tl) detectors are able to handle appreciably higher counting rates and are also far more capable than the previous detectors of differentiating between different types of radiation. For the radionuclides encountered in neutron activation analysis, the counting of γ-rays has important and generally conceded benefits, as compared with β-counting and α-counting, such as negligible absorption of γ-rays by samples and sample containers, except at very low energies. This is a very important aspect because, in many instances, samples can be counted in their containers after irradiation, unless interfering activities from the containers are detected. A second advantage is the characteristic and mono-energetic nature of γ-rays. This is of special significance for spectrometric purposes using counting systems capable of energy discrimination. Despite the inherently high sensitivity of thermal neutron activation analysis for most elements of the periodic table, as well as other distinct advantages of the technique, the method had one serious drawback. This militated against its selection as a method of choice, in many instances, when compared with other analytical techniques, especially emission and atomic absorption spectrometry. The disadvantage was that rather tedious radiochemical separations were necessary for the determination of one or more components of irradiated samples, unless resulting γ-scintillation spectra were rather uncomplicated, or unless favourable half-life differences of the induced activities could be exploited in the absence of additional γ-spectra interferences. Non-destructive and multi-element analyses by instrumental activation analysis were thus confined to a small number of particular types of analyses and samples. However, various investigators attempted to increase the scope of instrumental activation analysis by applying special detection systems and selective activation procedures, and by unravelling complex scintillation γ-spectra by computer manoeuvring.

Regarding the problems mentioned above for instrumental neutron activation analysis which result from the complexity of scintillation γ-spectra, significant progress has been made in obviating these since the advent of solid-state germanium and silicon detectors for γ-spectrometry.[12] In particular, Ge(Li) diodes have, since 1964, aroused considerable interest on account of their excellent resolving power. The Ge(Li) detector permits studies of complex radioactive mixtures in much greater detail than was previously possible with the conventional NaI(Tl) scintillation γ-spectrometers. Furthermore, the possibilities of measuring directly fully, resolved and narrow photopeaks, even in a very complex γ-spectrum with a multitude of photopeaks, to an appreciably higher degree of accuracy, are indeed attractive. For qualitative identification of components of samples by activation, the value

of high-resolution γ-spectrometry is obviously equally significant. Its potential as a quantitative analytical tool, however, is particularly illustrated in non-destructive multi-element analysis. In many cases, complicated and time-consuming dissolution and chemical separation procedures of samples can be completely eliminated. The rapid procuring of activation analysis data from Ge(Li) detector γ-spectrometers has placed considerable emphasis on the need for computerised photopeak identification and photopeak area analysis. Several programmes already available from the literature can be applied in non-destructive multi-element analysis.[13]

THE ADVANTAGES OF ACTIVATION ANALYSIS

Activation analysis is the most sensitive analytical technique available for many elements. It is a relatively rapid method and, for almost 70 elements, its detection limit is less than 10^{-3} μg, with a precision and accuracy of the order of 2 - 5 %. The potential of instrumental neutron activation analysis using a Ge(Li) detector as a quantitative multi-element analytical tool, has already been demonstrated by many investigators, even for very short-lived radio-isotopes. In addition, if chemical separations have to be carried out, an experienced operator can analyse a number of samples daily. By using automated radiochemical group-separation, many more elements can be analysed at a higher speed.

Contamination with analytical materials after irradiation has no detrimental effect, because the concentration of the element in the sample is determined only by its radioactivity.

THE LIMITATIONS OF ACTIVATION ANALYSIS

Like any other analytical method, activation analysis has its limitations. The half-life of some of the radionuclides formed is so short that it is difficult to record the activity after removal from the reactor. Elements in this category include He, Li and B, which have half-lives measured in seconds, or even less. The long-lived radio-isotopes, such as Be and C, also present certain difficulties; their low radioactivity makes the accurate determination of their activity difficult due to technical problems.

The activation cross-section of some elements for thermal neutrons, e.g. H and Pb, are so low that irradiation with high-energy neutrons or even charged particles may give more favourable results.

As heat is produced in a nuclear reactor and by the neutron reaction, the sample will decompose unless it is stable at the particular temperature to which it is exposed. In addition, structural damage and decomposition of a sample, especially of biological material, may occur if it is exposed to neutrons and gamma-rays in a nuclear reactor. This decomposition may produce gas, the pressure of which can break even the sealed containers.

Another disadvantage is that laboratories outside nuclear establishments may not have irradiation facilities at their disposal. Neutron generators and solid neutron sources, on the other hand, can now more readily be afforded, but these sources find only restricted analytical applications, and their equivalent neutron fluxes are still, in most cases, inadequate for use as thermal activation facilities.

Other limiting factors which may influence the activation analysis method are the inhomogeneous reactor-flux distribution on sample and reference standard in any reactor position, and the neutron self-shielding of elements with relatively large absorption cross-sections, as well as the various types of interference reactions which depend on the composition of the sample and the nuclear properties of its constituents. However, by taking suitable precautions, these factors can be minimised.

THE APPLICATION OF ACTIVATION ANALYSIS

The inherent high sensitivity of neutron activation for many elements makes this method extremely suitable for the determination of traces of these elements.

Since 1967 the scope of instrumental multi-element analysis has also been demonstrated by the Activation Analysis Group of the Atomic Energy Board at Pelindaba with regard to a diversity of sample matrices in the geological[14,15] and biological sciences. In the biological field, various elements in normal human enamel,[16] dentine[16] and dental calculus[17,18] have been determined by instrumental analysis (Table I). A typical γ-spectrum of an enamel sample is shown in Fig. 1. Selenium, which is also regarded as an essential trace element for the normal growth, development and maintenance of the health of man, could not be analysed in this way. For this reason a fast radiochemical method was applied to determine selenium in normal human enamel.[19] Its average content in 10 composite enamel samples was 0.08 ± 0.03 ppm.

The technique was also applied to determine as many elements as possible in human hair from Transkeian Blacks (from patients with oesophageal cancer and from healthy persons) in the hope that a concentration variation would be found. Preliminary results (Table II) show that there was a statistical difference using a multivariant stepwise discrimination technique. A similar difference, though not as yet fully substantiated, has been obtained from hair from kwashiorkor patients in Zululand (Table III).[20] Fig. 2 shows a γ-spectrum obtained after 3 days of irradiation and 4 days of decay of a hair sample from a patient with oesophageal cancer. Further work is in progress.

Fast instrumental analysis was used to determine selenium in liver samples obtained from the roan and the sable antelope. During capture operations, 60% of these animals die and, for gravid females, this figure is even higher. One of the causes of this acute form of stress may be a deficiency of the selenium-vitamin E complex. The content of 15 liver samples varied between 0.6 and 1,3 ppm of selenium, depending on the place of origin of the animals and on the season in which they were trapped.

To date, the abovementioned analyses are the most important investigations in the field of biology initiated

TABLE I. CONCENTRATION OF VARIOUS ELEMENTS IN NORMAL HUMAN ENAMEL, DENTINE AND DENTAL CALCULUS

Element	Enamel mean concentration ± SD*	Dentine mean concentration ± SD*	Dental calculus mean concentration ± SD*
	(%)	(%)	(%)
Mg	0,3 ± 0,01	0,9 ± 0,03	0,5 ± 0,04
Na	0,7 ± 0,01	0,6 ± 0,03	0,4 ± 0,03
Cl	0,3 ± 0,01	0,4 ± 0,003	0,09 ± 0,01
Al	0,009 ± 0,0004	0,007 ± 0,002	0,01 ± 0,001
Ca	37 ± 0,6	26 ± 1,5	28 ± 2
	(ppm)	(ppm)	(ppm)
Cr	1 ± 0,5	2 ± 0,8	—
Ba	125 ± 24	129 ± 55	—
Sb	1 ± 0,7	0,7 ± 0,4	0,7 ± 0,1
Ag	0,6 ± 0,3	2 ± 0,8	0,2 ± 0,6
Zn	263 ± 15	173 ± 11	174 ± 7
Co	0,1 ± 0,1	1 ± 0,3	0,08 ± 0,01
Fe	118 ± 72	93 ± 35	54 ± 7
	(ppm)	(ppm)	(ppm)
Sr	111 ± 10	94 ± 11	—
	(ppm)	(ppm)	(ppm)
Au	0,1 ± 0,07	0,07 ± 0,04	—
Br	34 ± 6,	114 ± 3	—
Mn	0,6 ± 0,04	0,6 ± 0,05	—

* Standard deviation

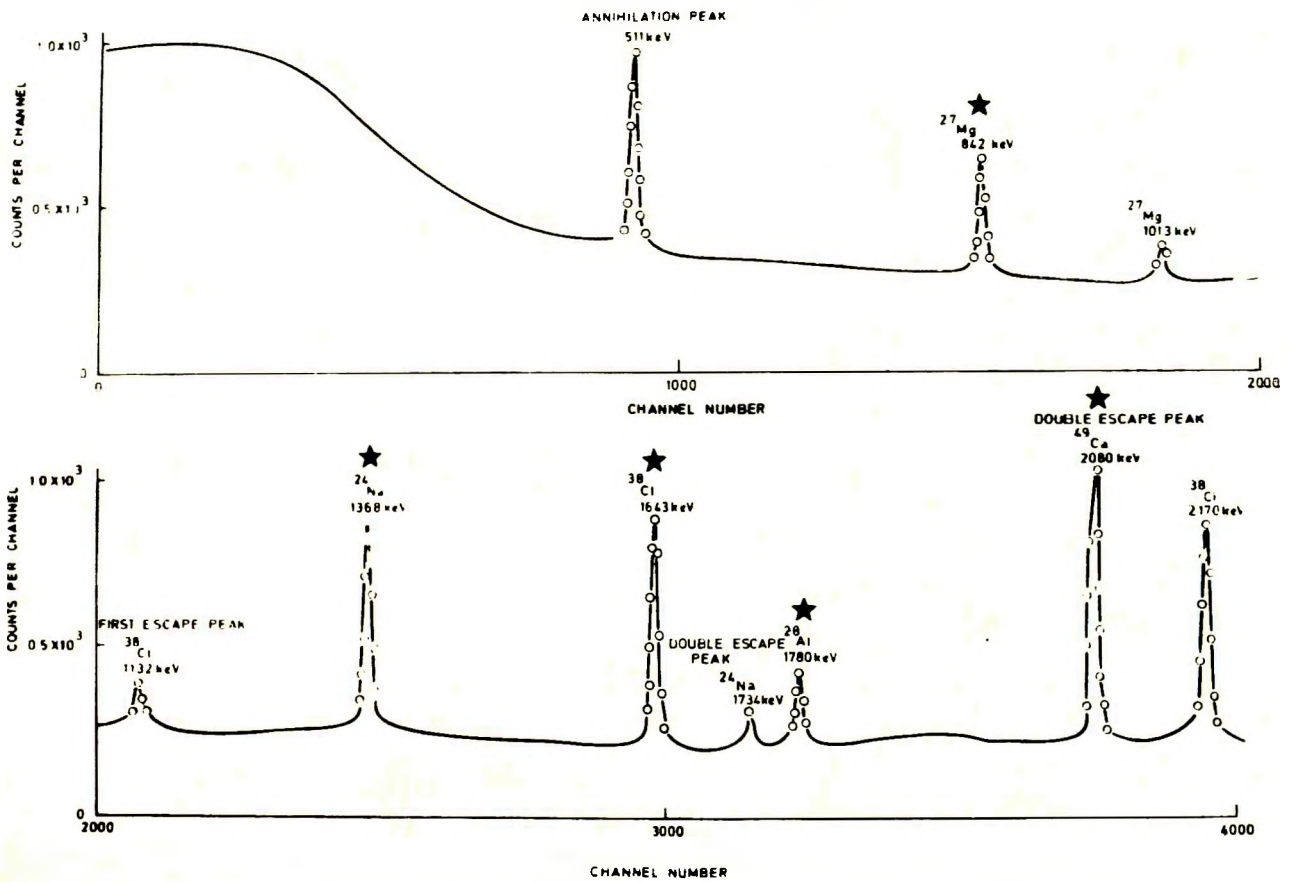


Fig. 1. Gamma spectrum, 15 seconds after irradiation, of an enamel sample irradiated for 4 minutes.

TABLE II. CONCENTRATIONS OF ELEMENTS IN BANTU HAIR SAMPLES FROM TRANSKEIAN BLACKS

	Hair						
Element	H* (ppm)	I* (ppm)	J* (ppm)	K- (ppm)	L- (ppm)	M- (ppm)	N- (ppm)
Ag	2,7	0,8	0,8	0,5	0,2	0,5	w
Al	2 150	320	350	250	660	390	730
As	w	w	w	w	w	w	w
Au	0,5	0,3	0,4	0,4	0,7	0,5	0,5
Ba	100	w	8	w	38	a	34
Br	14 560	4 990	2 370	w	w	w	w
Ca	220	160	700	1 250	170	270	280
Ce	w	w	w	w	w	w	w
Cl	5 550	6 240	1 010	2 280	13 430	13 760	4 410
Co	0,5	0,3	0,3	0,3	0,4	0,3	0,4
Cr	15	6	11	36	34	32	29
Cs	w	w	w	w	w	w	w
Fe	1 870	500	540	700	1 630	1 550	1 360
Hf	2	2	1	1	4	w	w
I	96	w	130	w	w	w	w
K	1 940	2 600	380	w	1 420	2 570	630
La	1,5	0,4	0,3	0,3	0,5	0,8	0,8
Mg	720	420	440	480	1 450	480	700
Mn	23	4°	7	15	20	11	18
Na	2 560	1 860	700	2 230	4 910	5 270	1 630
Rb	7	a	w	a	a	3	2
Sb	5	6	5	1	8	14	20
Sc	0,2	0,1	0,1	0,1	0,2	0,1	0,1
Se	0,5	0,9	1	0,8	1,2	0,9	0,8
Sm	w	w	w	w	w	a	w
V	2	0,8°	0,9	1	1	0,7°	2
Zn	w	w	w	w	w	w	w

x = cancer patients.
– = healthy persons.
a = not detectable.
w = detectable
o = only one value

TABLE III. CONCENTRATIONS OF ELEMENTS IN KWASHIORKOR PATIENTS IN ZULULAND

Elements	On admission (ppm)	On discharge (ppm)
Ba	4,2	3,6
Mo*	2,8	4,5
Cr	3,2	7,4
Se	2,5	1,7
Cs	0,34	0,4
Cd	16	11
Fe	30	30
Zn	125	92
Co	0,4	0,7
Sb	19	22
Cu*	5	8

* Determined by atomic absorption

at Pelindaba. They demonstrate the possibility of determining micronutrients of vital importance in living organisms. Furthermore, automated rapid radiochemical group-separation schemes are available which can be applied to most biological material in the analysis of at least the essential elements present.

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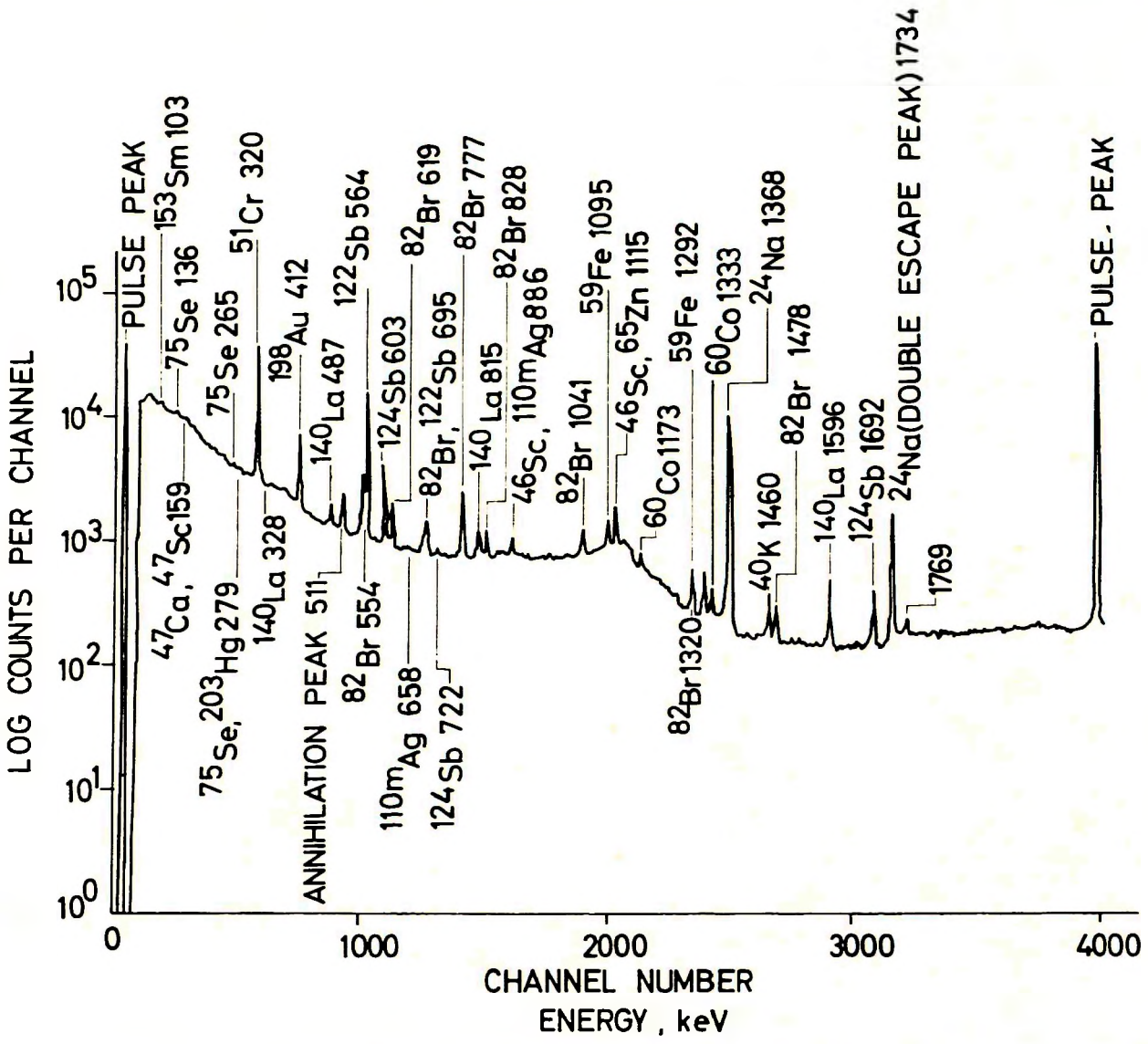


Fig. 2. Gamma spectrum, 4 days after irradiation, of a hair sample irradiated for 3 days.

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