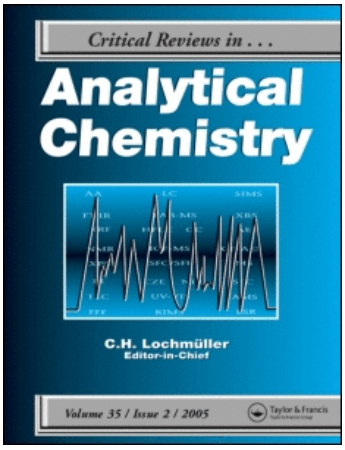

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APPLICATION OF ACTIVATION TECHNIQUES TO BIOLOGICAL ANALYSIS

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I. PRINCIPLES AND MATHEMATICAL FORMULATION

The principle of activation analysis is that elements can be made radioactive by exposure to radiations such as neutrons, protons, or high-energy photons. Two physical processes, prompt and delayed, are associated with activation. For example, in the reaction between thermal neutrons and sodium

$$^{1}n + {}^{23}Na \rightarrow {}^{24}Na + \gamma$$

the gamma rays are emitted within 10^{-12} sec of the absorption of the neutron. Several analytical techniques, such as X-ray fluorescence spectrometry, utilize prompt radiation, but these will not be discussed here. Activation analysis proper is concerned with the delayed event resulting from the decay of the activated nuclide. Thus in the reaction above, sodium-24 is radioactive and decays with a half-life of 15 hr, giving off characteristic beta and gamma radiation and forming stable ^{24}Mg.

The relation between the absolute activity a of a radionuclide Y generated in a mass m of element X by activation in a flux of ϕ activating particles per m^2 sec can be written:

$$a = mNf\phi\,\sigma\,A^{-1}\,(0.5)^{t_d/t_{1/2}}[1 - (0.5)^{t/t_{1/2}}] \qquad (1)$$

where N = Avogadro's number, f = the fractional abundance of parent nuclide ^{A}X in element X, σ = the cross section for the reaction (^{A}X + particle → Y) in m^2 per nucleus, per A = the atomic weight of element X, $t_{1/2}$ = the half-life of Y, t_d = the delay period between activation and counting, and t = the period of exposure to activating particles.

The relation between a and t is shown in Figure 1, from which it may be seen that the activity tends asymptotically to a maximum as the period of activation increases, and little is to be gained by increasing t beyond ten half-lives.

In practice, experimental uncertainties in ϕ, σ, and counter efficiencies mean that Equation 1 is very rarely used for absolute analyses. Note that since the flux ϕ is limited by engineering considerations, the variables under the analyst's control are only t and

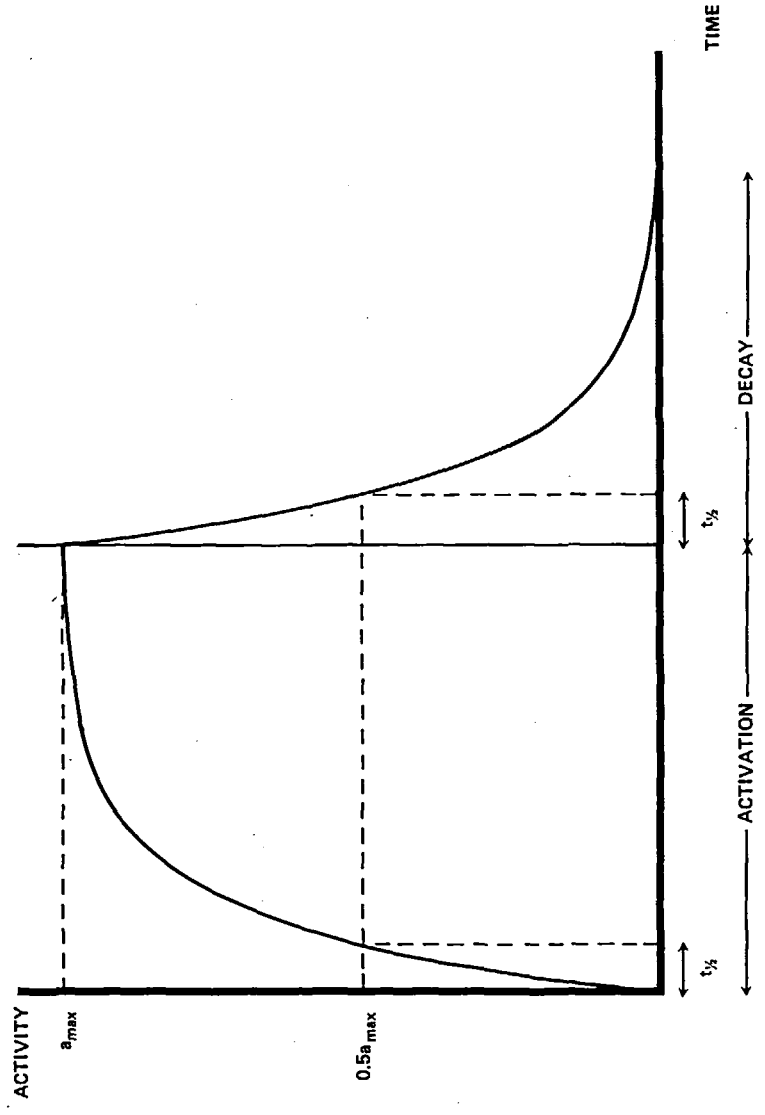

FIGURE 1. Radioactivity plotted against time for activation and decay.

t_d. If a sample (Subscript 1) and a standard (Subscript 2) are activated in the same time flux for identical periods and are then counted in the same counter after identical delay periods,

$$m_1/m_2 = c_1/c_2 \quad (2)$$

where r_1 and r_2 represent the isotopic counting rates, in counts per second, from sample and standard, and are equal to εa_1 and εa_2 respectively, where ε is the efficiency of the counter. Since m_2 (the mass of element X in the standard) is known, the mass of X in the sample can easily be calculated.

It should be realized that Equation 1 applies to all the many nuclear reactions which produce radionuclides in the sample, and that almost all the elements present become radioactive. To minimize interference from other radionuclides, it is common practice to set $t = t_{1/2}$, and to chose a value of t_d which allows short-lived radioactivities to decay away before counting is begun. It should also be appreciated that the fraction of atoms activated in a sample is very small, usually less than one in a million, so that a sample can be activated and allowed to decay many times in nondestructive work.

II. STAGES IN ACTIVATION ANALYSIS

In any real analysis, the stages will be (1) collection of sample, (2) preparation of standards, (3) the activation process, (4) radiochemical separations (which may be unnecessary in purely instrumental work), and (5) determination of radioactivity. These will be briefly discussed in turn.

A. Collection of Sample

This is usually the most difficult part of the analysis, since the potential for contamination is high and some elements may be lost by volatilization. For discussions of the problems involved, see Hamilton et al.[1] and Sansoni and Iyengar.[2] When the highest sensitivity is required, these problems must be minimized by careful selection of sample, dust control, use of clean sampling tools and containers, and temperature control during drying or ashing.

Samples collected in the field are invariably contaminated by soil, sediment, dirt, or epiphytes. Ideal samples are those like fruits or nuts which are enclosed in natural bags and need not be opened until after activation. Small biopsy samples may not be truly representative.[3] The homogenization of samples is discussed by Iyengar and Kasparek.[4]

The rate of dust fall is usually in the range 2 to 30 mg m^{-2} sec^{-1}, and can be substantially reduced by working in plastic dry-boxes or in fume-hoods supplied with filtered air. Lievens et al.[5] give values for Al, As, Br, Cl, Co, Cu, Fe, I, Mn, Na, Sb, and V in dust from their laboratory and show that the rate of fallout is reduced by at least an order of magnitude by installing air filters. Samples carried in cars will be contaminated by exhaust fumes containing bromine (which activates strongly) and lead. Marine plankton collected in the wake of a ship may be contaminated by fragments of paint and fuel residues. Museum specimens are dusty and may have been treated with preservatives containing chlorine, copper, mercury, etc.

Cutting tools are best made from honed silica or metallic titanium, since both materials can be obtained very pure and neither activate strongly. Stainless steels should be avoided since biological fluids attack them. Thus blood and liver samples were shown to be moderately to overwhelmingly contaminated by Co, Cr, Cu, Mn, and Ni when collected with steel syringes or scalpels while contamination by iron and zinc was negligible.[6] Blood samples should not be stabilized by impure preparations such as heparin.[7] Substantial contamination by antimony from red rubber tubing and by zinc

from the zinc stearate used to lubricate plastic surfaces have been noted. Any preconcentration technique, such as those used for elements with short-lived nuclides such as Al[8] or V,[9-11] increases the likelihood of contamination.

Samples for activation rarely weigh more than 1 g, and often eigh much less. When neutrons are the activating radiation, samples may be enclosed in polyethylene, aluminum foil, or sealed silica tubes, but not in glass because the sodium in commercial glasses activates strongly. Polyethylene bags or vials are useful for short irradiations but become brittle after about 1 hr of exposure to fluxes of 10^{18} neutrons/m^2. Even after cleaning they need testing for blanks, which may be highly variable;[12] typical blank values for Au, Cu, Mn, W, and Zn have been reported.[13] Aluminum foil can be used to wrap powders for long irradiations, but it produces large amounts of 2.3-min ^{28}Al and smaller amounts of 15-hr ^{24}Na b (n,γ) and (n,α) reactions, respectively. Silica tubes are used for long activations or where volatile elements such as mercury may be lost during the activation period. Tjioe et al.[14] give the following blank values for cleaned silica vials: Fe—100 to 1000 ng; Br—10 to 100 ng; Cr, Cu, Zn—1 to 10 ng; As, Cd, Co, Mo, Sb, Se—0.1 to 1 ng; Hg—10 to 100 pg; Au—1 to 10 pg. However, Maziere et al.[15] found significant blanks for the following elements from silica tubes used to activate 0.1 m*l* samples of serum: Au, Cd, Ce, Co, Cr, Cs, Hg, Mn, Sb, Sc, and Zn. They attributed these to material volatilized from the vial during the sealing-off process. Exchange of activated atoms between sample and container, a kind of Szilard-Chalmers reaction, has been reviewed by Brune.[16] When charged particles are used for activation, samples should be prepared as thin layers on foils of carbon, formvar,[17] nickel, or tantalum, as penetration is poor.

Apart from contamination problems, elements can be lost by volatilization during freeze- or oven-drying below 100°C, as well as by ashing at higher temperatures.[18-24] Such drying or ashing saves the expense of activating water when reactor space or cyclotron time is required. We are ignorant of the chemical nature of most elements in biological matter, so radiotracer methods of testing losses which involve adding inorganic tracers are suspect. Activation analysis has been used to study the losses from standard reference materials.[20,22] Elements known to be lost at temperatures less than 100°C include Br, Cl, Hg, I, and Os, while As, Au, Cd, Cr, Se, and Te may be lost below 500°C. All the elements known to form bonds with carbon in natural organic compounds are potentially volatile; at present these include As, Br, C, Cl, F, H, Hg, I, O, N, Pb, S, Se, Sn, and Te, and it is quite likely that other elements such as Ag, Au, Cd, Pd, Pt, Si, and V may be added to this list as our knowledge of their environmental chemistry develops. Schramel found substantial losses of tin and antimony during drying and sealing in silica.[25]

B. Preparation of Standards

Standards should be closely similar to samples both in physical form and in elemental composition, in order to minimize errors due to self-shielding during the activation process and self-absorption during counting. The best standards are probably those made by the method of standard additions, using the sample itself as a matrix to absorb weighed drops of standard solutions.[26] Dry, powdered biological materials can be made into discs in a pellet press, which are convenient for instrumental analysis or for absorbing standard solutions.[12,27-29]

The standard solutions should usually contain between 0.1 and 10 μg X per drop, or 3 to 300 μg/m*l*, and should be freshly made up. Other absorbents, more or less free from blanks, are squares of ashless filter paper,[28] discs of cellulose,[29] gelatin,[30] or resin beads.[31,32] Artificial multielement standards have been used by many authors,[33] and standard reference materials are coming into use for this purpose.[34] Pure wires of aluminum, cobalt, or nickel have been used as flux monitors when many samples are

activated under similar conditions.[35,36] Where radiochemical separations are used some workers prefer to leave the activated standard sealed up until the sample is being counted as a precaution against cross contamination.[37]

C. Sources of Activating Particles

The most commonly used activating particles are neutrons, which account for more than 95% of reports on the analysis of biological materials. Nearly all these reports utilize thermal neutrons, which have energies of about 0.025 eV. The (n,γ) reaction is the only type of reaction which does not require a threshold energy, and takes place when thermal neutrons react with almost all known stable nuclides. Most neutron activation analysis (NAA) uses research reactors as sources of neutrons. Such reactors consist of uranium or uranium oxide, more or less enriched in ^{235}U, dispersed in a moderator of graphite, water, or deuterium oxide which slows down the fast neutrons produced in fission to thermal energies. Well-known research reactors include the Triga® and much cheaper Slowpoke models with thermal neutron fluxes of the order of 10^{16} m^{-2} sec^{-1}, but larger reactors may have fluxes up to a hundred times this figure and give correspondingly greater analytical sensitivity. When samples are activated in a reactor, they are exposed to fast neutrons and high-energy photons as well as to thermal neutrons. Pure thermal neutrons, free of fast neutrons and protons, can only be obtained by sacrificing two orders of magnitude of flux, and are rarely needed. Epithermal neutrons are obtained by exposing samples wrapped in cadmium foil to reactor neutrons, and show some advantages when analyzing the following elements: Al, Au, Be, Cd, Cl, Cs, Fe, Hf, Mn, Ni, Rb, S, Sb, Sc, Se, Sr, Ta, Th, U, and V.[38]* Such neutrons reduce the activation of sodium, and are sometimes employed for biological materials such as blood, liver, and muscle.[39-41]

Some reactors, such as Triga®, can be made to go supercritical for a few milliseconds, giving pulses of neutrons with a peak flux of 2×10^{21} m^{-2} s^{-1}. Such pulses are useful for the detection of elements that form very short-lived nuclides such as 0.8 sec^{207m} Pb.

Most commercial neutron generators are nearly point sources of MeV neutrons, which are produced by bombarding a zirconium tritide target with 0.1 to 0.5 MeV deuterons, when the following nuclear reaction takes place:

$$^{2}H + {}^{3}H \rightarrow {}^{4}He + {}^{1}n$$

The number of neutrons produced is in the range 10^{10}– 6×10^{13} s^{-1}, but the flux is somewhat erratic as the target burns away, and may not be spherically symmetrical around the target. Fast neutrons mostly have lower cross sections for nuclear reactions than do thermal neutrons. Fast NAA has found limited applications in biology, but shows some promise for in vivo analysis and for determinations of oxygen and nitrogen by the nuclear reactions:[42-48]

$$^{16}O + {}^{1}n \rightarrow {}^{16}N + {}^{1}p$$

$$^{14}N + {}^{1}n \rightarrow {}^{13}N + 2\,{}^{1}n$$

^{16}N and ^{13}N have half-lives of 7.1 sec and 10 min, respectively, and this is one of the very few direct methods of determining oxygen.

For teaching purposes and where lesser sensitivity is adequate, activation may be

* Nickel is normally determined by the (n,p) reaction yielding ^{58}Co, rather than by the (n,γ) reaction, after activation in reactors.

carried out with isotopic neutron sources such as ^{252}Cf or beryllium plus americium 241, antimony-124, plutonium-238, or radium-226. The sources are usually kept in water or paraffin wax in order to show down the fast neutrons from the source. Isotopic sources have a very stable flux and negligible running costs, and their main use in biology has been for in vivo work.[49-55] A neutron multiplier, consisting of a ^{252}Cf source in a subcritical assembly of ^{235}U, can give fluxes of 2×10^{12} m^{-2} sec^{-1}; one such device has proved useful in rapid, precise determinations of halogens in organic chemicals.[56]

Charged particles such as protons, deuterons, and helium-3 ions have been used to analyze biological materials by a small number of laboratories with access to cyclotrons. Most of the applications use prompt radiation. Charged particles penetrate poorly into biological and other materials. Their cross sections for nuclear reactions are high, but interference from other such reactions is much commoner than in NAA. Recent applications have mostly determined light elements from the first row of the periodic table (B, C, N, and O),[57-60] which are not activated by thermal neutrons, but one paper reports measurements of As, Ca, Cu, Fe, Mo, Pb, Sr, Ti, Zn, and Zr.[61] Helium-3 activation has been used to determine lead in teeth,[62,63] and could be used for determination of Mg and Zr.[61]

Cross sections for photon activation are lower than for other particles, and threshold energies are higher. Apart from two nuclides, ^{2}H and ^{9}Be, threshold energies range from 7.5 MeV for uranium to about 20 MeV for light nuclei. The cross sections for the (γ,n) reactions are greatest around 15 MeV for heavy nuclei and around 25 MeV for light nuclei. There are no problems of self-shielding or penetration in the sample, but few laboratories have access to a high-energy photon generator. A minor disadvantage is that most of the radionuclides produced are positron emitters, and all give rise to a large peak at 0.51 MeV in their gamma spectra. Photon activation has advantages over NAA for determining Nb, Ni, Rh, Ti, and Zr, but there is little demand for determinations of these elements in biological materials. It has been used to determine I^{64}, $^{18}O^{65}$, Pb^{66}, and Se^{67}, and an increasing number of multielement studies are being published.[29,68-74]

1. Physical Conditions During Activation

Biological samples consist mostly of C, H, N, and O, none of which absorbs neutrons strongly, so self-shielding problems are less than with other matrices. Energy deposition during activation is usually of the order of 10 to 100 mW/g and rarely gives rise to any problems, except perhaps in determining mercury. Absorption of charged particles is much more serious, as energy deposition in the thin sample can be as much as a kilowatt, so the sample must be cooled during activation.

Whether or not samples are rotated during activation depends on flux homogeneity, which in turn depends on the physical size of the reactor core-tube or particle source. In large reactors, the thermal neutron flux may vary by less than 1%/cm, but much larger variations occur with neutron generators and sources of charged particles and photons.

It is often desirable to activate samples in pneumatic tube systems so that they can be returned to the laboratory rapidly. Such tubes are known as rabbit tubes, and the plastic containers forced down them by compressed air are called rabbits. Rabbits can usually be retrieved within 1 sec of activation, so that it is difficult to study radionuclides with half-lives much shorter than this; for example, Heydorn et al. have determined ^{8}Li, with a half-life of 0.85 sec, using a fast rabbit.[75] A greater delay usually arises from the need to open rabbits and transfer their contents to fresh vials for counting and/or radiochemistry.

2. Chemical Events During Activation

Biological material maintains its form and appearance during short activation periods, but slowly becomes discolored, turns brittle, and falls to powder when activation is prolonged. Chemical decomposition by ionizing radiation is a complex process, probably initiated by hydroxyl radicals and hydrated electrons. It includes the decomposition of water to gaseous products, which may generate a pressure inside sealed silica tubes during activation. Such tubes should therefore be opened with care, preferably under water. Another feature of activation is the discoloration produced in silica vials from the induced point defects.

D. Radiochemical Separations Following Activation

1. Ashing

Dry biological material can be dissolved or wet-ashed in a few minutes by boiling it first with nitric acid and then adding boiling perchloric acid; the danger of explosion is small with experienced chemists, and is negligible if a few milliliters of sulfuric acid is present and the vessel is never allowed to boil dry.[24] If volatile elements such as Hg or Os are sought, the ashing should be carried out under reflux in a Bethge apparatus. An alternative wet-ashing mixture is sulfuric acid and 30% hydrogen peroxide. In the presence of sulfuric acid, sulfates of Ba, Ca, Pb, and Sr tend to precipitate on dilution. Other methods of ashing avoiding the use of sulfuric acid include burning in oxygen by the Schöniger technique,[20] wet-ashing with hydrogen peroxide in the presence of Fe(II),[76] gas-phase oxidation by hydrogen nitrate,[77] attack by nitric acid at 350°C in a teflon-lined bomb,[18] and decomposition by a 20-fold excess of molten 1:1 sodium nitrate/potassium nitrate.[78]

Unfortunately, most methods of wet-ashing fail to dissolve silica, and the small residue left behind, although hardly visible, can retain substantial amounts of Al[79], Cr[80], and probably other elements such as Hf, Nb, Sc, Ta, Y, Zr, and rare earths. These elements could be brought into solution by treatment with hydrofluoric acid or by fusion with molten sodium peroxide.

2. Separations

Chemical separations of nano- or picogram amounts of an element are difficult, because the amounts involved are invisibly small and can be lost by adsorption during the chemical manipulations. In order to prevent such losses and to correct for nonquantitative separations, it is conventional to add a weighable amount of each element sought to the activated sample before ashing it. The inactive additions are called carriers, and may include elements which need to be removed as well as those specifically sought. At the end of the separation, the chemical yield of carrier plus tracer is determined by gravimetry, spectrophotometry,[81] or reactivation.[82,83] Any losses in separating sample or standard can then be allowed for. Occasionally chemical yields are measured by adding a long-lived radiotracer which can be counted in the presence of the radionuclide produced by activation.

Separations for individual elements are described in numerous papers and a few compilations.[84-86] They are mostly based on solvent extraction or precipitation (especially for scavenging impure solutions), or on ion-exchange for closely related elements such as the rare earths. Problems arise when short-lived nuclides have to be separated, but fast separation techniques are available,[87] and multistage separations have been used for vanadium-52, with a half-life of 225 sec.[37,88,89]

Most biological materials contain so much sodium that ^{24}Na dominates the gamma spectrum for the first week after activation. After this, peaks from ^{82}Br may become prominent. Several separation schemes have been proposed to remove most of this sodium-24, which has a high gamma energy and interferes with the determination of

many other radionuclides. The most commonly used method absorbs the sodium from 7 *M* hydrochloric acid onto a column of hydrated antimony(V) oxide.[90,91] A column of sodium chloride or bromide will remove sodium from a solution of biological material in 10% v/v perchloric acid in acetone.[92-99] Other columns have been suggested to remove ^{42}K, ^{24}Na, and ^{32}P.[95-98]

A number of authors have exercised their ingenuity in designing more or less automatic separation devices. These separate the activated nuclides into groups rather than into radiochemically pure elements; the fractions are then counted using gamma spectrometry to distinguish individual nuclides. Most devices follow Samsahl's lead in first distilling off volatile elements and then pumping the wet-ashed material through a series of columns containing absorbents or ion-exchange resins,[99] but others use solvent extraction or precipitation. Table 1 summarizes the capabilities of some of these multielement separation schemes. Commercial separators have not been successful, probably because of the great power of other competing techniques, including instrumental NAA, which are faster or more convenient in other ways.

E. Determination of Radioactivity

When an element as been separated from an activated sample in a radiochemically pure state, it can be counted using a simple, cheap beta or gamma counter. Its radiochemical purity can be tested by comparing its decay curve with that of a standard activated with it, or by recording its gamma spectrum. In many cases the gamma spectrum of the intact activated sample, or of radiochemically impure fractions separated from it, can be used for analysis. When no radiochemical separation is used, the technique is called instrumental activation analysis or IAA.

1. Simple Detectors

Simple beta detectors have been fully monographed.[115,116] End-window Geiger counters are the cheapest, but become saturated at count rates exceeding 2000 sec^{-1}. Anthracene scintillation counters are equally convenient and can be used at count rates up to 10^6 s^{-1} because of their small dead time. Both these counters require flat, disc-like sources of effectively infinite thickness, which in practice is of the order of 1 mm. For betas with energies less than 1 MeV, the count rate becomes increasingly imprecise because of imperfections in the source, and the precision of counting O.1 MeV betas with these counters is about ±10%. Their efficiencies lie in the range 0.1 to 0.3.[117] For low-energy betas, one can employ windowless proportional counters with methane flowing through them, or liquid scintillation counting.[118,119] Cerenkov counting has been used for a few energetic beta-emitters, for which its efficiency is high.[119-121]

For simple gamma counting, scintillation detectors made of sodium iodide doped with thallium are standard items.[118] These detectors have a density of 3.67 g/cm^3, energy resolution of 7 to 9%, and give an almost linear relation between signal pulse and gamma energy. The counting efficiency depends on the size and shape of the detector and the energy of the gamma photons. Well-type counters have the highest efficiency, but somewhat poorer resolution than cylindrical detectors. Efficiencies of well-type counters range from 0.1 at 1 MeV to nearly 1 at 0.1 MeV.[122] The source can be liquid or solid provided that both sample and standard are of similar size and shape.

2. Gamma Spectrometry

There are several useful tabulations of gamma ray energies.[123,124] Sodium iodide detectors are still used because of their robustness and good efficiencies. Because of their poor energy resolution, they do not require multichannel analyzers with more than 256 channels, but they are of little use for direct IAA of most activated biological material for 2 to 3 weeks after activation, by which time ^{24}Na, ^{82}Br, and many other nuclides

Table 1

MULTI-ELEMENT SEPARATION SCHEMES USED FOR ACTIVATED BIOLOGICAL MATERIAL

Solvent	Number of elements	Number of fractions	Technique[a]	Overall speed	Remarks	Ref.
1.8 M H_2SO_4	20—40	7—12	D + C	*5—10 min*	*Fast, much used*	*25, 99, 100*
9 M HCl	10—14	6	C	ca. 3 hr	NaI γ-spectra	101
8 M HCl	27	6	D + C	ca. 6 hr	Delayed counts	102
H_2SO_4	45	12—25	D + C + P	6— 7 hr		103
6 M HCl	42 + RE[b]	12—15	C	ca. 1 hr	No carriers	104
HNO_3/HF	21—30	18	D + C	ca. 12 hr		105
HCl	18	5	D + C	5 hr		106, 107
0.1 N H_3PO_4	24—40	8	C	6 hr		108
6 M HCl	22	17	D + C	5 hr		109
6 M HCl	25	15	C	3 hr	Volatiles lost	110
8 M HCl	14	10	D + C	1.7 hr	t_d[c] = 3 days	111, 112
2 M $HClO_4$	25	6	S	1.5 hr	Used for glass	113
6 M HCl	11	3	D + S + P	1 hr	Poor yields	83
pH 2	9	5	S	2 hr	Na not removed	114
	13 + RE[b]	8	P	ca. 2 hr		82

[a] C, column absorption; D, distillation; P, precipitation; S, solvent extraction.

[b] RE, rare earths.

[c] t_d, delay period.

have decayed. If sodium and bromine have been efficiently separated, these detectors are useful for resolving the gamma energies from the residue or from other radiochemical fractions.

Most IAA work uses Ge(Li) semiconductor detectors, and a large literature has developed around these.[125] The detectors are expensive and need to be kept permanently at liquid nitrogen temperatures. Their density is 5.47 g/cm^3, but since they are usually smaller than NaI detectors, their efficiency is lower, though for equal detector volumes the Ge(Li) detector should have higher counting efficiency. The energy resolution of new detectors is about 0.2%, which is far better than the NaI detectors, but their life is only a few years. However, it means that they need expensive multichannel analyzers with about 4096 channels to make use of this high resolution, which in turn makes it useful to have a small computer to handle the large amount of data generated.[126] Using computers, the equipment can be made to compare the areas of specified peaks in the gamma spectra of samples and standards and to compute the sample composition from these data. Technical problems arise from samples with very high count rates, especially from ^{24}Na (and ^{32}P bremsstrahlung) in the case of biological material. Hence one-step chemical separations to remove these interferences are often used. As usual, sample and standard must be presented to the counter in the same shape and volume.

3. Miscellaneous Counting Techniques

Natural uranium can be determined by counting the delayed neutrons from short-lived fission products for a few seconds after a short activation. The method is sensitive to about 1 μg U if a reactor with a fast rabbit and BF_3 neutron counters are available. There are few interferences, but since ^{235}U is actually determined, the method cannot be used for samples whose isotopic composition is unknown. It has been used to measure U in urine[127-129] and rat lung.[130] Uranium has also been determined by counting fission tracks produced in polycarbonate resins after etching, which is really a prompt technique.[131-133]

III. CHARACTERISTICS OF ACTIVATION ANALYSIS

In this section the characteristic properties of the technique will be summarized from the point of view of a potential user. Such characteristics include nature of sample, range of elements determinable, sensitivity, selectivity, interferences, accuracy, precision, recovery of sample, costs, and hazards. Both advantages and disadvantages of the method will be pointed out and compared with other techniques where this is relevant.

A. Nature of Sample

Any type of sample can be activated, but gaseous samples are rarely examined and liquids or wet tissues are best dried (or lyophilized) to save space. Sample masses are usually 0.1 to 1 g dry matter if this is representative, but can be much less, e.g., 0.1 to 1 mg of hair or metalloprotein.

B. Range of Elements Determinable

Table 2 compares the current needs for elemental analysis in various branches of biology with the capabilities of the technique, which we can summarize as follows (@, half-life less than 10 min):

NAA adequate: Ag, Al@, As, Au, Ba, Br, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Eu, F@, Fe, Hf, Hg, I, K, La, Li@, Lu, Mg@, Mn, Mo, N@, Na, Nd, Ni, O@, P, Pb@, Rb, S@, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti@, U, V@, W, Yb, Zn (plus Ir, Pd, Pt, Re, Ru, Ta, Te, Tl in surgical implant work)

Table 2
CURRENT NEEDS FOR ELEMENTAL ANALYSIS IN BIOLOGICAL SCIENCES

X	Biochemistry	Botany	Agriculture	Nutrition	Clinical chemistry	Inorganic drugs	Metal implants	Toxicology	Remarks
Ag							+		NAA works
Al		+				+	+	+	$t_{1/2}$ = 2.3 min
As				+		+		+	NAA works
Au						+	+		NAA works
B		+	+						NNA fails
Ba		+		+		+		+	NAA works
Be								+	NAA fails
Bi						+			NAA fails
Br	+					+		+	NAA works
C	+	+	+	+	+				NAA fails
Ca	+	+	+	+	+				NAA works
Cd							+	+	NAA works
Cl	+	+		+	+				NAA works
Co	+	+	+	+			+		NAA works
Cr	+			+			+		NAA works
Cu	+	+	+	+	+		+		NAA works
F				+				+	$t_{1/2}$ = 11s
Fe	+	+	+	+	+		+		NAA works
H	+	+	+	+	+				NAA fails
Hg							+	+	NAA works
I	+	+		+	+				NAA works
Ir							+		NAA works
K	+	+	+	+	+				NAA works
Li						+			$t_{1/2}$ = 0.8s
Mg	+	+	+	+	+				$t_{1/2}$ = 10 min
Mn	+	+	+	+	+		+		NAA works
Mo	+	+	+	+	+		+		NAA works
N	+	+	+	+	+				Fast NAA
Na	+	+	+	+	+				NAA works
Nb							+		$t_{1/2}$ = 6.3 min
Ni	+			+			+		Fast NAA

O	+	+	+	+	+				Fast NAA
P	+	+	+	+	+				NAA/fast
									NAA
Pb								+	γAA works
Pd							+		NAA works
Pt						+	+		NAA works
Re							+		NAA works
Rh						+	+		NAA works
Ru							+		NAA works
S	+	+	+	+	+				$t_{1/2}$ = 5 min
Sb						+	+	+	NAA works
Se	+	+	+	+				+	NAA works
Si		+	+					+	NAA works
Sn				+		+	+	+	NAA works
Sr				+					NAA works
Ta							+		NAA works
Te								+	NAA works
Ti							+		$t_{1/2}$ = 5.8 min
Tl								+	NAA works
U								+	NAA works
V	+	+		+					$t_{1/2}$ = 3.8 min
W							+		NAA works
Zn	+	+	+	+	+	+	+		NAA works

Note: Metal implants include both surgical implants and tooth fillings.

Sensitivity of NAA inadequate in normal tissues: Be@, Bi@, Er, Ga, Gd, Ge, Ho, In, Ir, Nb@, Pd, Pr, Pt, Re, Rh@, Ru, Ta, Te, Tl, Tm, Y, Zr

NAA unsuitable: B, C, H

Note first that IAA techniques are able to determine 20 to 45 elements in a single sample but need two different activation periods and 3 to 4 different delay periods before counting, the longest of which may be 30 days.[125] Using radiochemistry, NAA can determine 45 or more elements with greater speed, but considerably more skill is required. Secondly, AA gives no information on the valency or chemical state of the element; in this respect it resembles atomic absorption spectrometry, emission spectrometry, and spark source mass spectrometry. Thirdly, the technique determines isotopes, so the sample and standard are assumed to have identical isotopic composition; this assumption can be in error for Li, Pb, Sr, or U.

C. Speed

A single determination usually takes several hours, while multielement INAA may take 15 to 30 days. The time period required per analysis may be split into four distinct sections as follows:

1. Activation period, usually the half-life or 12 hr, whichever is the shorter
2. Separation period, usually 1 to 6 hr, but for single elements can be as little as 5 min.
3. Delay period, usually 5 to 10 days to allow ^{24}Na to decay or 20 to 30 days to allow ^{82}Br to decay as well; needed in IAA but not otherwise
4. Counting period, may be as short as 10 sec or as long as 2 hr after long delay periods

The long delays between obtaining a sample and finding its composition mean that activation analysis cannot compete with other analytical techniques in some fields, such as clinical chemistry. This defect has stimulated research on elements which produce radionuclides whose half-lives are measured in minutes including Ag, Al, Br, Ca, Cl, Co, Cr, Cu, I, In, Mg, Mo, Nb, Rb, Rh, S, Sb, Ti, Tl, V, and W, and on fast radiochemical separations (Section II.D.2). Even faster analyses may be made for the limited number of elements giving rise to radionuclides whose half-lives are of the order of seconds, so that radiochemistry becomes difficult and IAA is mandatory. The latter class of elements include Ag, Ba, Cl, Dy, Er, F, Ge, Hf, Li, N, Pb, Pd, Rh, Sc, Se, W, and Yb. Using carefully controlled activation and counting periods and repeatedly activating and counting each sample (cyclic NAA), it has proved possible to perform rapid determinations of some of these elements such as Li^{75}, Br, Cl, Pb, and Se.[134] Recent work on this topic may be found in papers by Spyrou and Kerr[135] and others.[136,137]

D. Sensitivity

Absolute sensitivities depend on many factors such as the available flux of activating particles, the activation and delay periods, the cross section for the reaction, the counting efficiency, and the definition chosen for sensitivity. Unless conditions are precisely defined, they cannot easily be compared by different investigators, and the orders of magnitude given in Table 3 reflect the uncertainty of any list of calculated sensitivities. Practical sensitivities may be worse than expected because of poor radiochemistry, poor counting efficiency, or matrix interference in IAA. Guinn et al. have shown how detection limits may be predicted when using IAA.[138]

Table 3
CALCULATED SENSITIVITIES FOR NAA AND EXPERIMENTAL SENSITIVITIES FOR γAA

Sensitivity for NAA[a]		Sensitivity for γAA[b]	
10—100 fg	Al, Dy, Eu, In, Mn	10—100 ng	As, Cd, Cl, Cs, Hg, In, Mo, Pb, Sb, Sr, Ti, Zn, Zr
0.1—1 pg	Hf, Ho, Ir, La, Re, Rh, Sm, V	0.1—1 μg	Ag, Ba, Bi, Cr, Fe, I, Na, Ni, Rb, Sc, Se, Te, Tl
1—10 pg	Al, As, Ba, Br, Co, Cu, Er, Ga, Hg, I, In, Na, Pd, Pr, Sb, Sc, U, W, Yb	1—10 μg	Ca, Co, Mg, Mn, Sn, V
10—100 pg	Cd, Ce, Cl, Cs, Gd, Ge, Mo, Nd, Os, Pt, Ru, Sn, Ta, Tb, Th, Tm	10—100 μg	Br
0.1—1 ng	Ag, Ca, Cr, Mg, Ni, Rb, Se, Te, Ti, Zn	0.1—1 mg	
1—10 ng	Zr	>1 mg	K
10—100 ng	Fe, S		

[a] Assuming a flux of 10^{18} thermal neutrons/m^2s; activation period = $10t_{1/2}$ or 100 hr, whichever is shorter; zero delay period.[139]

[b] Measured in a flux of 2×10^{17} photons/m^2s; Eγ = 15 to 44 MeV; activation period = 1 hr.[68,69]

Some experimental sensitivities for photon activation are also given in Table 3,[68,69] and illustrate the superior sensitivity of NAA for most elements apart from Pb, Ti, and Zr. Sensitivities of NAA compare favorably with those obtainable by atomic absorption spectrometry, emission spectrometry, and spark source mass spectrometry, and are much better than those reported for spectrophotometry and X-ray fluorescence.

E. Selectivity

If radiochemical separations are used, the selectivity can be very good, with decontamination factors of more than a millionfold from other elements. Such high selectivities imply skill both in the choice and execution of the separation scheme. IAA using Ge(Li) detectors is also highly selective. Although a few peaks may overlap in the gamma spectra produced, it is often possible to resolve these by counting repeatedly to see how they decay. IAA using sodium iodide detectors has less favorable selectivity, although unresolved peaks may be resolved by the technique of spectrum stripping.[116,126]

F. Interferences

A common source of error arises because sample and standard are not subjected to the same flux of activating particles. This can be partially corrected by rotating the can containing sample and standard during the activation. However, if the absorption of activating particles by the matrices of sample and standards differ, the inner parts of each will be activated in different fluxes, an effect known as self-shielding. Biological matrices are poor absorbers of both neutrons and gamma rays, but good absorbers of charged particles. The effect of self-shielding needs careful consideration and design of standards, especially for charged particle activation but also for neutron activation of thick samples, as for in vivo work.

Other interferences can arise when sample and standard differ in isotopic composition, which can occur for natural samples of B, C, Li, N, O, Pb, S, Sr, and U (such

differences are usually small enough to be neglected) or when the radionuclide produced can arise by more than one nuclear reaction. In NAA such interfrences can arise when determining an element of atomic number Z in the presence of a larger excess of an element of atomic number (Z + 1) or (Z + 2). For example, manganese-56 can be made in three ways:

1. $^{55}Mn\ (n,\gamma)\ ^{56}Mn$
2. $^{56}Fe\ (n,p)\ ^{56}Mn$
3. $^{59}Co\ (n,\alpha)\ ^{56}Mn$

Although the first reaction has by far the largest cross section, corrections must be made when trying to determine manganese in tissues rich in iron (liver, erythrocytes) or cobalt (Vitamin B_{12}). Similar corrections need to be made when determining chromium in iron-rich tissues.[140]

Uranium can give rise to radionuclides of all the elements between zinc and gadolinium by neutron-induced fission. It is rarely present in biological samples at high enough concentrations to interfere seriously with other analyses, except perhaps in the determination of molybdenum.

Still other interferences can arise when the radionuclide has not been equilibrated with its carrier, or when the radiochemical separation is badly carried out (such errors can be checked by preliminary tracer work) as well as when the counting efficiency differs for sample and standard. This may arise when comparing beta counts from precipitates of very different masses, or when peaks in the gamma spectra are inadequately resolved during IAA.

Further, although NAA avoids the problems of reagent blanks which plague other methods of trace analysis, container blanks can occur (Section II.A). The matrix used for absorbing the standard must be tested for blanks. Large errors can be introduced from any reagents used to fractionate samples before activation.

G. Accuracy

There are built-in checks which can be used to test the accuracy of activation analysis. In radiochemical NAA, the half-lives and gamma energies of nuclides separated from sample and standard can be compared to confirm their identity. In IAA, the half-lives of assigned peaks in the gamma spectrum can be compared in the same way.

In practice, systematic errors can occur in NAA as in other techniques, and a study of a large number of analyses of a biological reference material (kale) has shown that NAA is no better and no worse than spectrophotometry and atomic absorption spectrometry in this respect.[141] The same study revealed a systematic error by several laboratories in the determination of potassium by IAA using sodium iodide detectors, which does not occur when the more selective Ge(Li) detectors are used. Major discrepancies still occur between different laboratories studying the same sample. As far as biological materials are concerned, these have been noted especially for Ag, Au, Co, Cr,[140] Hg,[142] Mn, Mo, Sb, Se, Th, U, and V,[143] but not for Br, Ca, Cu, Cs, Fe, K, P, Rb, and Zn.[144]

H. Precision

The precision of NAA is usually comparable with that of atomic absorption spectrometry or spectrophotometry but is significantly worse for Mn and Mo in kale.[141] For this material, out of 330 determinations of individual elements, 58% had a standard deviation within ±10% of the mean, and 78% had a standard deviation within ±20% of the mean. Much better precision can be obtained by careful attention to the sources of error mentioned in Section III. F. Examples may be found in the excellent

work of Heydorn and co-workers[145,146] on the determination of As, Mn, and Se in blood. Precisions of less than 1% in the determination of halogens in organic halides have been obtained with a ^{252}Cf neutron source.[56]

The precision of activation analysis is sometimes taken to be identical with the precision of counting, since this is readily calculable from the square root of the total number of counts obtained. Since factors such as sample homogeneity, flux variations, and self-shielding or self-absorption also affect the overall precision, the latter should be determined by replication.

I. Destruction of Sample

In many applications of IAA and in all cases of radiochemical NAA, the sample is destroyed during the analysis. In forensic work the sample may need to be recovered as evidence. It is particularly easy to recover samples after very short activation periods. These are used in in vivo work, where relatively few elements can be determined without exceeding radiation dose limits to the patient, who is normally recovered intact (Section V.H.2).

J. Costs

The costs of radiochemical NAA are roughly comparable with those of spectrophotometry, as in each case the main items are 1 to 2 hr of an analyst's time plus the use of an instrument costing about $1000. The costs of activation itself are usually about $20 per can, where a can will accommodate 20 to 100 samples.

The costs of IAA are different, as they involve activation costs, a technicians time, and the use of counters costing about $30,000. These costs are roughly equivalent to the costs of atomic absorption or plasma emission spectrometry. Sophisticated mechanical systems used to activate, decan, and count samples automatically and computers to analyze gamma spectra and compute data from them will add further costs.

The capital costs of sources of activating particles range from about $200 for a small, homemade, californium-252 source with no running costs, to $5 million for a high-flux research reactor. The latter could have running costs of $500,000 per year. Cyclotrons (sources of charged particles) and linacs (sources of high-energy photons) cost at least $200,000 and have high running costs. The availability rather than the cost of these sources has been the more important factor in the historical development of the technique.

K. Hazards

The hazards of handling activated biological materials are rarely greater than those encountered in other work with radioactive tracers. Sometimes the first stages of a radiochemical separation may have to be carried out behind 5 to 10 cm of lead, but an ordinary laboratory can easily be adapted for such work. Radiation hazards usually arise when biological material contains much sodium, as ^{24}Na emits gamma rays with great penetrating power. Most biological material contains between 0.1 and 4% sodium in its dry matter, with the highest concentrations in marine algae and dried sera. Each milligram of sodium activated for 15 hr in a neutron flux of 10^{16} m^{-2} s^{-1} gives rise to about 7 MBq* of ^{24}Na, from which the hazard of any proposed activation may be estimated.

Opening rabbits after short activations requires careful monitoring to avoid excess radiation reaching the operator's hands and eyes, as well as good ventilation to avoid inhalation of active dusts or gases such as ^{41}Ar or $^{16}N_2$.

* 1 Bq (Becquerel) = one atomic disintegration per second.

IV. BRIEF HISTORY OF ACTIVATION ANALYSIS IN BIOLOGY

A. Developmental Stages

The development of the technique, which was introduced by von Hevesy and Levi,[147] can be arbitrarily divided into three stages.[139] From 1936 until 1948 activation analysis was merely a scientific curiosity, as sources of activating partcles were limited and counting techniques were primitive. During this stage its biological applications were restricted to a few unclassified reports on the analysis of mammalian tissues.[148-151]

The second stage, from 1949 until 1959, saw important technical innovations in the production of research reactors, fast neutron generators and cyclotrons, as well as the introduction of sodium iodide detectors, scintillation counting, and the associated multichannel analyzers. Most of the applications described during this period were by nuclear scientists with an interest in biology, rather than by biologists interested in novel analytical methods. About a hundred research papers appeared during this stage, including the first reports on photon activation,[152] early gamma spectra of blood,[153] determinations of uranium in live rats,[154] and activation of chromatograms for locating biochemicals.[155]

The third, or current, stage includes many applications by experienced groups who are usually attached to particular reactor centers, together with a large number of papers by nonnuclear scientists. The rate of publication is 50 to 100 papers per year with peaks when the reports of large conferences appear, as in 1966, 1967, 1969, 1972, 1973, and 1979. Few radically new developments have appeared, and the technique has reached a somewhat prolix maturity. Important technical advances during this stage include the introduction of automatic multiple element separation devices,[99,100] the rise of Ge(Li) detectors, the use of pulsed reactors, systematic study of photon activation, and the introduction of in vivo analysis of human subjects. At the same time other techniques of elemental analysis, notably atomic absorption spectrometry but also anodic stripping voltammetry, plasma emission spectrometry, spark source mass spectrometry, and X-ray fluorescence spectrometry have made great strides and have become increasingly competitive.

B. Bibliographies and Books

Lutz et al.[156] give a comprehensive bibliography up to the early 1970s and Silver[157] refers to some later works. A journal of Neutron Activation Abstracts is produced quarterly by PRM Science and Technology Agency, Finchley, London. Early books[84,85] on activation devote chapters to biological applications and a number of other books have appeared in the last 10 years.[158-164] Nargolwalla and Przbylowicz[165] deals exclusively with neutron generators and fast NAA. Reports of recent conferences have been issued by the International Atomic Energy Agency (IAEA) in Vienna[166-170] and the U.S. National Bureau of Standards (NBS).[171] NAA in Japan has been reviewed by Kozaka.[172] Other recent reviews are those of Cornelis et al.,[173] Gilmore and Newton,[174] and Kucera[175] on multielement analysis of biological samples. The last comprehensive review of activation analysis in biology appears to be that of Leddicotte,[176] which covers the literature up to about 1970. The present review will deal largely with work published from 1970 onwards. Figure 2 illustrates the rate of growth of literature on biological applications.

C. Elements Most Often Determined

Table 4 summarizes the relative popularity of elemental analyses by activation analyses (including prompt techniques) for two periods, namely, pre-1972[85] and for 1974 to 1978 inclusive (data from *Chemical Abstracts*). Relative popularities have not changed very much but the number of analyses per year has greatly increased with the

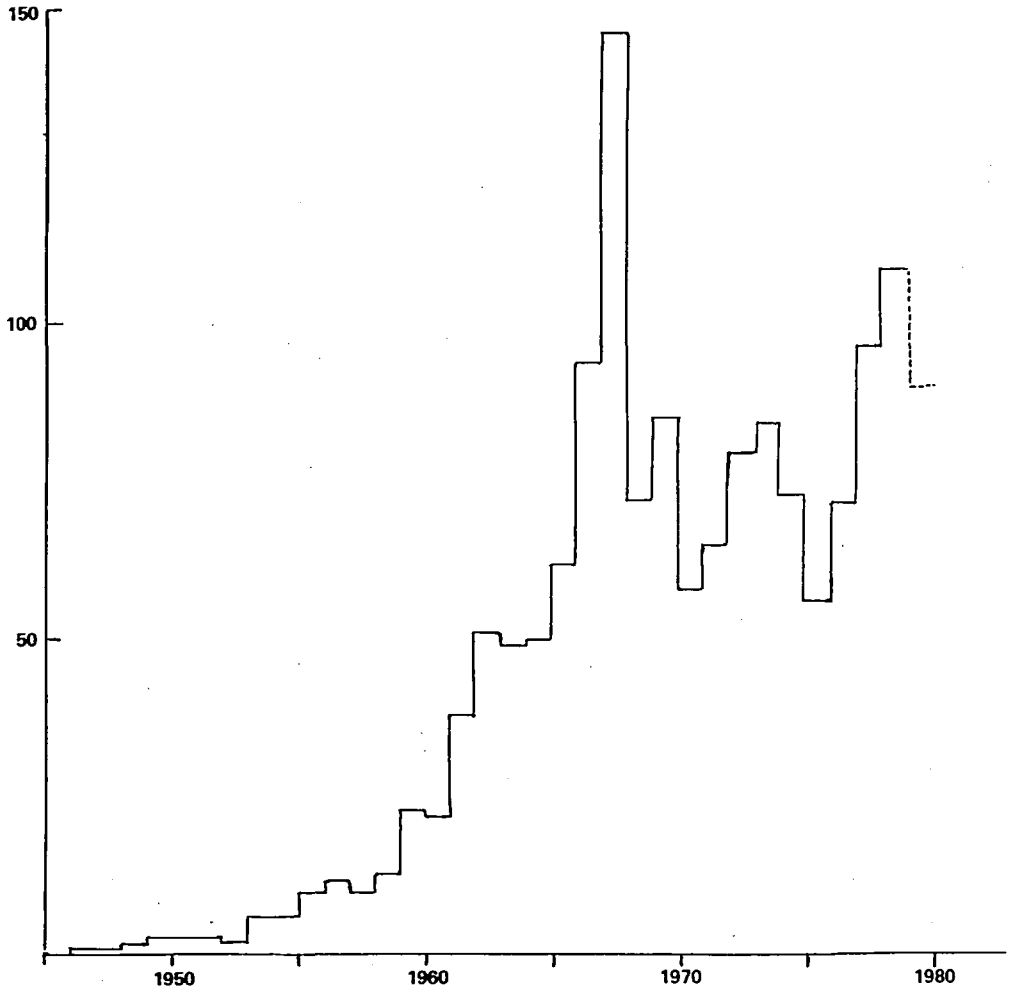

FIGURE 2. The number of papers per year on activation analysis in the life sciences; 1978—1979 data are incomplete. Vertical axis: number of papers per year; horizontal axis: year.

widespread adoption of multielement IAA. It may surprise some biologists to see the large number of determinatons of such elements as Ag, Au, Br, Cs, La, Rb, Sb, and Sc, since most of these are of no special interest to them at present. It so happens that these elements activate well and produce well-resolved paks on gamma spectra, so they are frequently determined along with other elements of more direct interest.

In view of the fact that oxygen comes second in abundance only to carbon in most biological samples and that oxygen in metals is routinely determined by fast NAA,[165] it is surprising how rarely its determination is attempted. This is probably due to conservatism on the part of chemists and biologists who have been brought up to believe that oxygen can only be determined by difference after using classical methods of assaying C, H, N, P, S, and ash. There is a big demand for reliable analyses of nitrogen and sulfur in biological materials which NAA cannot be said to have satisfied. The work of Gerard and Pietruszewski[56] shows that halogens can be determined rapidly and precisely by routine NAA. Their technique could readily be applied to both pesticide work and the extraordinary range of organic halides reported from the marine red alga *Asparagopsis.*[177]

Table 4
RELATIVE POPULARITY OF ELEMENTAL ANALYSES IN BIOLOGICAL MATERIALS BY NAA, ETC.

Number of papers	Pre-1972[85]	1974—1978
0	B, Be, Bi, H, Li, Lu, Nb, Os, Pb, Rh, Tl	Be, Bi, Ge, H, Re, Tl
1—3	Gd, Ge, Pd, Y	Ga, In, Ir, Li, Nb, O, Os, Pd, Pr, Pt, Rh, Ru, Te, Y
4—8	Er, Ho, In, Ir, Nd, Pr, Pt, Re, Ru, Ta, Tb, Te, Th, Tm, Yb, Zr	B, C, Dy, Er, Gd, Ho, Lu, Nd, S, Si, Ta, Ti, Tm, Yb, Zr
9—15	C, Ce, Cs, Dy, Eu, Ga, Hf, Si, Sm, Sn	F, Hf, N, Pb, Sn, Tb, Th, U, W
16—30	F, La, N, Ni, Ti, U, W	Al, Ba, Ce, Eu, I, Mg, Ni, P, Sm, Sr, V
31—60	Ag, Al, Ba, Ca, Cd, Cr, K, Mg, Mo, O, Rb, S, Sc, Se, V	Ag, Au, Cd, Cl, Cr, Cs, K, La, Mn, Mo, Rb, Sc
>60	As, Au, Br, Cl, Co, Cu, Fe, Hg, I, Mn, Na, P, Sb, Sr, Zn	As, Br, Ca, Co, Cu, Fe, Hg, Na, Sb, Se, Zn

V. APPLICATIONS IN BIOLOGY SINCE 1970

A. Reference Materials

With the realization that techniques of elemental analysis are often imperfect and many conflicting results continue to appear in published work has come an increasing demand for reference materials (RMs) and standard reference materials (SRMs). RMs need to be stable, homogenous biological materials available in large quantities; SRMs have the contents of certain elements specified within given limits of error. It is fast becoming customary to report values obtained by analysis of SRMs whenever a new technique of elemental analysis is published. In this way other analysts can judge the accuracy of the technique.

The RM kale produced by Bowen[178] has been widely used in this way.[141,179-181,288] Reasonably precise figures are available for the following elements: Al, As, Au, B, Ba, Br, Ca, Cd, Cl, Co, Cr, Cs, Cu, Fe, Hg, K, La, Mg, Mn, Mo, N, Na, Ni, O, P, Pb, Rb, S, Sb, Sc, Se, Sn, Sr, V, W, and Zn, together with single or less precise values for Ag, Ce, Eu, Ga, I, In, Li, Lu, Pd, Ru, Sm, Th, Ti, U, and Zr. The advent of this RM was soon followed by the first, and most used, SRMs from the NBS: orchard leaves and bovine liver.[182] The former was evidently collected from orchards where arsenate sprays had been used, as it contains unusually large amounts of antimony and arsenic, whose gamma spectrum can interfere with the spectra from other elements[183]. These well known SRMs have certified values for As, B, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, N, Na, Ni, P, Rb, Sb, Se, Sr, U, and Zn, and many other elements have been determined in them; an incomplete list of papers covering individual elements is given in Table 5. Note that the original certified arsenic content of orchard leaves was an overestimate, corrected by the work of Damsgaard and Heydorn.[184]

The National Bureau of Standards is continuing to issue new biological SRMs, with relatively few elements certified in them. The certified values can be obtained from the suppliers, and some references to NAA values are given here. The SRMs include

Table 5
REFERENCES TO DETERMINATIONS OF ELEMENTS IN THREE RMs BY ACTIVATION ANALYSIS

X	Kale	Orchard leaves[a]	Bovine liver[a]
Ag	5, 179, 230—232, 247	231, 278	5, 106, 297—299, 305
Al	79, 181, 220, 230, 231, 233	61, 102, 230, 231, 233, 279, 292	61, 220, 233, 299, 304
As	5, 83, 112, 179, 183, 220, 232, 234—241	C (see also 184)	C
Au	112, 179, 206, 232, 239, 240, 242	102, 189, 206, 240, 278, 280	112, 280, 298, 299
B		C, 281	
Ba	231, 237, 239, 242	61, 68, 102, 189, 206, 231, 279, 292	108, 220, 283, 299
Br	5, 112, 179, 205, 206, 220, 230—233, 239, 242, 243	61, 102, 134, 183, 189, 205, 206, 230, 231, 282, 284	5, 61, 108, 112, 206, 220, 283, 297, 300, 304
Ca	27, 83, 181, 201, 220, 230, 231, 242—244	C	C
Cd	5, 112, 121, 186, 202, 206, 236—238, 242, 245, 247	C	C
Ce	242, 247	102, 187, 206, 279	108, 298
Cl	5, 27, 201, 220, 230, 231, 236, 243, 244	27, 61, 102, 109, 134, 230, 231, 236, 282	5, 27, 61, 109, 134, 220, 236, 283, 298—300, 304, 305
Co	5, 29, 71, 83, 112, 179, 181, 183, 201, 206, 220, 231, 232, 236, 237, 239, 243, 246	29, 61, 71, 102, 109, 183, 189, 206, 236, 271, 279, 280, 283, 285—287, 292	5, 25, 61, 71, 82, 83, 106, 108, 109, 112, 183, 206, 220, 236, 237, 246, 280, 283, 285, 297, 298, 301, 305
Cr	5, 29, 70, 112, 140, 181, 183, 206, 217, 231, 236, 237, 239, 242, 243, 248, 249	29, 61, 71, 102, 109, 140, 183, 189, 206, 236, 271, 279, 283, 285—287, 288—290	C
Cs	5, 181, 206, 220, 239, 242, 243	109, 183, 189, 279	5, 106, 108, 206, 220, 297—299, 305
Cu	5, 71, 112, 179, 185, 201, 202, 221, 230, 232, 236, 237, 239, 250—254	C	C
Dy		187	108
Eu	179, 183, 239, 240	102, 183, 187, 279, 280	108, 220, 280
F	255	255, 282	
Fe	5, 27, 71, 112, 121, 179, 181, 201, 206, 218, 220, 231, 232, 236, 237, 239, 242, 243	C	C
Ga		102, 109, 280, 291	82, 280, 291
Gd		187	
Hf		279	
Hg	5, 112, 121, 179, 183, 220, 221, 231, 232, 237, 238, 242, 243, 257—261, 292	C	C
I	5	14, 64, 282	5, 14, 298, 299

Table 5 (continued)
REFERENCES TO DETERMINATION OF ELEMENTS IN THREE RMs BY ACTIVATION ANALYSIS

X	Kale	Orchard leaves[a]	Bovine liver[a]
In	237, 253	253, 280	253, 280
Ir		278	
K	5, 27, 181, 201, 206, 220, 230—232, 236, 243, 244	C	C
La	5, 179, 181, 206, 220, 230, 239, 242	61, 102, 183, 187, 189, 206, 212, 230, 279, 280	5, 82, 108, 206, 220, 237, 280, 298, 301
Li	75	75	
Lu	220	187	
Mg	5, 27, 121, 181, 201, 220, 230, 231, 236, 237, 244, 262	C	C
Mn	2, 27, 71, 145, 181, 185, 220, 230—232, 235—237, 239, 242, 243, 250, 253, 254	C	C
Mo	5, 83, 112, 183, 201—204, 206, 220, 232, 236, 237, 246, 252, 256, 263, 264, 267	61, 109, 206, 264, 293	5, 61, 108, 109, 112, 206, 220, 236, 237, 256, 264, 293, 298, 299, 301
N	244	C	C
Na	5, 27, 181, 185, 201, 206, 220, 230—232, 236, 243	C	C
Nd		187	108
Ni	29, 71, 112, 121, 220, 237, 246	C	29, 71, 112, 121, 220, 246, 285, 299
P	232, 244	C	C
Pb	29, 66, 265, 266	C	66
Pd	268		
Pr			108
Pt		278, 294	
Rb	5, 27, 112, 181, 201, 206, 220, 231, 237, 239, 242, 243	C	C
Re	237		
Ru	269	295	
S	230, 232		
Sb	5, 12, 112, 179, 183, 201, 206, 219—221, 234, 238, 239, 242, 243, 247	C	5, 61, 82, 106, 112, 183, 206, 220, 280, 283, 287, 297—299, 305
Sc	5, 121, 179, 181, 201, 206, 220, 232, 240, 243	68, 102, 183, 187, 189, 206, 279	5, 82, 83, 106, 108, 206, 220, 283, 297—299
Se	5, 83, 112, 179, 183, 220, 232, 235—237, 241, 242, 270—272, 292	C	C
Si	121, 244	109, 121	
Sm	206, 237, 242	102, 187, 189, 206, 212	82, 108, 206, 237

Table 5 (continued)
REFERENCES TO DETERMINATION OF ELEMENTS IN THREE RMs BY ACTIVATION ANALYSIS

X	Kale	Orchard leaves*	Bovine liver*
Sn	183, 195, 198, 206, 273—275	195, 198, 275	198
Sr	27, 181, 220, 230, 237, 242	C	108, 280
Ta		189, 279	
Tb		187, 279	
Th		206, 279	
Ti		61, 73, 279	61
Tl		73	
Tm		187	
U		C	302, 303
V	5, 10, 88, 181, 201, 220, 230, 231, 237, 276	11, 61, 88, 212, 230, 231, 276, 286, 296	5, 10, 61, 88, 237, 299
W	237, 277	231, 280	83, 108, 237, 280, 298
Yb	220, 237	187, 279	108, 220
Zn	5, 27, 29, 71, 83, 112, 179, 181, 183, 185, 186, 201, 202, 206, 217, 220, 231, 232, 236, 239, 242, 243, 246, 252—254	C	C
Zr	179, 239	61, 68, 73, 279	61

Note: In addition to giving data on the elemental composition of RMs, many of the references give useful information on methodology and detection limits for the elements in biological material.

* C, certified by NBS.

pine needles, spinach leaves, and tomato leaves,[64,88,109,185-189] wheat and rice flour,[190] tuna fish,[191] and brewer's yeast.[22,192] Another useful series of SRMs has originated from the IAEA, e.g., wheat flour,[83,88,193-198] potato flour,[112,140,199-201] cow bone,[112,140,196,199,201] animal blood,[14,195-198,202-205] animal muscle,[140,187,188,198,206] and cow milk powder.[83,199,200,207] The IAEA marine station at Monaco has provided SRM oyster flesh,[64,75,88,112,208-210] algae and copepods,[88,211] and fish serum.[187,197] Japanese workers have produced several new RMs,[212-214] and other RMs include tobacco[27,186,215-221] and various commercial sera.[222,223] The homogenity of some RMs has been tested by NAA.[178,224]

B. Biochemical Applications

A few authors have used NAA to analyze subcellular particles.[193,225-229] For example, it has been shown that Mg^{2+} is the major ion present in the thylakoid membrane of chloroplasts from *Pisum*, while K^+ is the major ion in the stroma.[229] Such experiments are technically difficult because major losses of ions can occur in the isolation process, especially if this is carried out in aqueous solutions.

Although the major elements C, N, and O can be determined in biochemical substances by photon or charged particle activation,[59-60, 306] virtually all such analyses are carried out by combustion and separation of the products by gas chromatography.

Table 6
SOME METALLOPROTEINS ANALYZED BY NAA

Name of protein	Source	Molar mass/1000	Analytical remarks	Ref.
Benzylamine oxidase	Pig plasma	195	3 Cu/molecule ?	308
Stellacyanin	Plant		0.89 μg Cu/10 μl sample	309
o-Diphenol oxidase	*Ipomoea*	23.5	0.27% Cu	310
Leucine aminopeptidase	Cow eye		8—12 Zn/molecule	311
			Mn and Zn found	312
Amine dehydrogenase	*Pseudomonas*	133	<0.27 Cu/molecule	313
Deoxynucleotide transferase	Mammal		10 ppm Ca, 6 ppm Mg	121
Ascorbate oxidase	*Cucurbita*	140	8 Cu/molecule	307
Alkaline phosphatase	Cow	140	(4 Zn + 0.8 Cu + 0.8 Fe + 1 P)/ molecule	307
	Escherichia	80	(4 Zn + 0.8 Cu + 0.7 Fe + 0.7 P)/ molecule	307
Xanthine oxidase	Milk	275	(8 Fe + 2 Mo + 4 P)/molecule	307
Metallothionein	Rat liver	10	3.1% Cd, 1.3% Zn, 0.2% Cu	307

The latter method is fast and precise, but does not determine oxygen directly, as fast NAA can.

Among the trace elements, Ca, Cd, Cl, Co, Cu, Fe, I, Mn, Mo, Ni, Se, and Zn all occur as metalloproteins or metalloenzymes, and can be determined by NAA. The main problem is that of isolating the protein in a pure state. Some examples of analyses of more or less pure proteins by NAA are given in Table 6, and should be noted that many elemental impurities have been observed in some of the samples.[307] Since these determinations can be made with very small masses of protein, this type of application is expected to increase.

Other workers have looked at the elements in protein fractions from serum, after separation by gel electrophoresis or other methods.[314-318] Protein-bound iodine has long been of medical interest and is readily determined by NAA.[319] Serum copper has been shown to be associated with ceruloplasm[315] and vanadium with transferrin.[320] Similar work with liver proteins has demonstrated the association of Cd, Cu, Hg, Mo, Se, and Zn with proteins in specific molar mass ranges.[321,792,793] Many elements have been determined in collagen[322] and in nucleic acids.[307, 322-324,794] Cr, Cu, Fe, and Zn appear to be the commonest elements in the latter, but have not been shown to occur in any fixed stoichiometric ratio. Biochemicals containing activatable elements have been analyzed by NAA, e.g., Co for vitamin B_{12},[325-326] I for labeled insulin,[327] S for polysaccharide sulphates,[328,329] Se for selenoaminoacids,[330] and especially P for organic phosphates and nucleotides.[120,155,331-333] In the 1960 activation analysis was sometimes used as a means of locating and analyzing substances on chromatograms, especially those containing activatable elements such as Br, Co, Hg, I, P, S, Se, and V. High blanks from the chromatographic matrix and the long delay needed to allow the inevitable ^{24}Na impurity to decay have prevented this technique from finding general acceptance.

C. Microbiological Applications

NAA has been relatively little used by microbiologists, though it could be useful in analyzing nutrient media and testing the essentiality of certain elements. Recent work

Table 7
ELEMENTS DETERMINED BY NAA IN BACTERIA AND FUNGI

Elements determined	Organism	Ref.
Mn, Na	*Bacillus megatherium*	334
As, Cd, Co, Cr, Cu, Fe, Mn, Se, Zn	A bacterium	335
Al, Co, Fe, K, Mg, Mn, Na, Rb, Sb, Zn	*Mycobacterium* sp.,	336
Se	*Torula* (yeast)	337
Al, Br, Ce, Cl, Co, Cr, Cu, Fe, Gd, Hf, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Th, Ti, U, V, Zn	*Saccharomyces*? (yeast)	192
Ag, Co, Cr, Cs, Fe, Rb, Sc, Th, Zn	Various macrofungi	338
As, Br, Cd, Cu, Hg, I, Mn, Se, V, Zn	Various macrofungi	339
Cd	Various macrofungi	340
Cd, Cu, Zn	Various macrofungi	186
Se	*Boletus* (fungus)	795
V	*Amanita muscaria* (fungus)	88

is summarized in Table 7. Of particular interest is the discovery of accumulator species among the fungi for Br, Cd, Mn, Se, V, and Zn. Further work is required to investigate compounds such as amavanadine from *Amanita muscaria,* for which NAA would be useful. Another field where NAA might usefully be employed is in microbial methylation,[341] which gives rise to more or less volatile derivatives of As, Hg, I, Pb, Se, Sn, and Te and probably other elements.

D. Plant Science Applications

Agricultural applications have been reviewed,[342,343] and many are listed in Table 8. There is not space here to discuss the numerous applications in soil science. In agriculture, NAA is not known to be used for routine measurements, as atomic absorption spectrometry, spectrophotometry, and volumetry are adequate for the tasks required. It may be that in some countries the determination of nitrogen by the fast neutron reaction ^{14}N (n,2n) ^{13}N is actually used routinely, as it is faster than the Kjeldahl method. Several workers have described equipment for this determination, for example, using 1 to 2 kg plant material activated by a laboratory neutron source.[244,344-349] Other major elements that could be determined in herbage in this way include Al,[350] Ca,[244,345] Cl,[244,350] K,[244,272,345] Mg,[244,272] Mn,[350], Na,[350] P,[244,272,343,345] and Si.[244,272,343] Boron, though essential to plants, cannot be measured by NAA.

There have been numerous determinations of elements in cereal grasses such as barley (*Hordeum*),[259,351-353] maize (*Zea*),[189,354,394] rice (*Oryza*),[259,355-358] and wheat (*Triticum*).[259,353,359-361] For example, NAA has been used to show that cereal grains are no more polluted with mercury today than they were in the 1930s.[259] Wheat and barley have been used as pollution indicators in Norway,[353] but are not as good as lower

Table 8
REFERENCES TO ELEMENTAL ANALYSES BY ACTIVATION ANALYSIS IN PLANT SCIENCE

X	Agricultural plants	Cereal grasses	Tobacco	Woody plants	Wild herbs
Ag		358, 388	361, 363, 366	231, 338, 378, 379, 389	
Al	350, 800—802	231,354, 357, 358		379,389	390
As	41, 350, 355, 391, 800	189, 205, 352, 353, 355, 357, 358, 388	69, 239, 362, 363, 367	373	240, 241, 372, 376, 390, 796
Au	802	189, 388, 394	239, 361, 362, 366, 378	374, 375, 379	240, 370, 373, 374, 378, 392—394
B	395				
Ba	396, 800, 802	189, 354	239, 361, 363, 367	231, 380	390
Br	217, 355, 391, 397—399, 800—802	189, 205, 354—358, 388, 400	69, 239, 361, 363—367	231, 380, 389	370, 390
Ca	345, 402, 801, 802	189, 244, 354, 357, 358	69	231, 372, 379, 380, 403, 404	370, 390
Cd	41, 286, 355	286, 352, 353, 357	186		405
Ce	800, 802		361, 366	406	390
Cl	244, 350, 399, 801—802	244, 354, 357, 358	69, 365	231, 379, 380, 389	370, 371, 390
Co	286, 800, 802	189, 353, 354, 357, 358, 388	239, 361, 363, 366	231, 338, 377—379	370, 371, 390
Cr	286, 800, 802	189, 352, 354, 356—358, 388	239, 361, 363, 366	231, 338, 377—379	370, 371, 390
Cs	396, 800, 802	189, 354, 357, 388	239, 361, 363, 366, 367	377	370, 371, 390
Cu	355, 397, 402, 407	353, 355—358	186, 239	379, 380, 389	370, 372
Dy				389, 406	
Er	800			406	
Eu	800, 802	388	239, 361, 363, 366	389, 406	390
F				403	
Fe	402, 800—802	189, 353, 354, 357—358, 388	69, 239, 361, 363, 366	231, 338, 377, 379, 404	370, 371, 390
Gd	800			406	
Hf	800, 802		361, 363, 366		390
Hg	355, 391, 408, 801, 802	257, 352—355, 357, 358, 361, 388, 409	361, 363, 366, 367	231, 378, 379	

Ho	800			406	
I	41, 217, 410, 801, 802	400	69	380	
Ir			363		
K	189, 272, 345, 355, 397, 402, 800—802	244, 354—358	69, 361, 365—367	231, 379, 380, 389, 403	370, 371, 390
La	800, 802	189, 356, 358, 388	239, 361, 363, 366, 367	378, 379, 389	370
Lu	800, 802			406	
Mg	41, 272, 801—802	244, 354, 357, 358, 411	69	231, 380, 389	370, 390
Mn	286, 350, 355, 397, 402, 407, 800, 802	189, 354—358, 411	69, 239, 361, 366	231, 379, 380, 383, 389	370, 371, 390
Mo	801, 802	353, 357, 358			
N	244, 344—349				
Na	350, 355, 397, 800, 802	189, 354—358	69, 361, 365, 366	231, 379, 380, 389	370, 371, 390
Nd	800			406	
Ni	800		69	377	371
P	244, 272, 345				
Pb		352	69		
Pr	800			406	
Rb	396, 800, 802	189, 354, 357, 358	69, 239, 361, 363, 366	231, 338, 378, 379, 412	370, 371, 390
S	802			380	
Sb	350, 800—802	189, 219, 354, 357, 358, 388	69, 239, 361, 366	378, 379	370, 372, 390
Sc	800—802	189, 354, 357, 358, 388	239, 361, 363, 366, 367	338, 379	370, 390
Se	800	195, 352, 353, 388, 413, 414	361, 363, 366		241, 371, 372, 390, 415, 795
Si	41, 244, 272, 802	799			
Sm	800, 802	189, 358	239, 357, 361, 366	406	390
Sn	41, 802				241
Sr	41, 800, 802		69, 239, 363	380, 404	
Ta	800—802	189			
Tb	800		366	406	
Th	800, 802		361, 366, 367	338	798
Ti	801, 802			404	
Tl			363		
Tm	800			406	
U	395, 800, 802			188	797, 798
V	286, 801—802	352, 358		231	390

Table 8 (continued)
REFERENCES TO ELEMENTAL ANALYSES BY ACTIVATION ANALYSIS IN PLANT SCIENCE

X	Agricultural plants	Cereal grasses	Tobacco	Woody plants	Wild herbs
W	801, 802			231	
Yb	800—802			406	
Zn	286, 397, 402, 800, 802	189, 353, 354, 356—358, 388	69, 186, 239, 361, 363, 366	222, 231, 338, 379	370, 371, 390
Zr			69, 239	404	

plants in this respect. Many workers have measured the elemental content of drug plants, especially tobacco (*Nicotiana*),[69,186,239,361-367] hemp (*Cannabis*),[368,369] and opium (*Papaver*).[368] Here the interest is usually in establishing the provenance of different brands. Tobacco, like kale, is rich in most of the trace elements while the leaves of grasses are not.

Interest in the elemental composition of native plants has included plants with pharmacological properties such as *Astragalus alopecuroides*,[370] and both *Helleborus cyclophyllus* and *Psoralea* sp. appear to have rather high antimony contents.[371,372] Plants have been used for biogeochemical prospecting for gold,[240,241,373-376] thorium, and uranium,[798] and for other elements on serpentine in Spain using the olive (*Olea europaea*) as a test plant.[377] Pollution by arsenic near a gold mine in Ghana[796] and by uranium near a phosphate factory[797] have been investigated. Mosses and lichens collected from sites all over Norway clearly showed that the south of that country was more polluted than the north.[378] Attempts to study the history of environmental pollution by analyzing dated tree rings various elements seem to show that most elements are rather mobile in old wood,[231,378-380] so that the method is of doubtful validity. Bark has also been analyzed;[381] the results are of some interest as bark is an important substrate for mosses and lichens. Many elements (As, Br, Co, Cr, Cs, Eu, Fe, La, Rb, Sb, Sc, and Zn) have been determined in aquatic plants from Italy,[382] and the behavior of manganese in a wetland ecosystem has been investigated using NAA.[383] Some interesting academic studies have been made of the content of Co, Fe, Na, P, Rb, Sc, and Zn in pollen and pollen cell walls.[384-387]

E. Marine Biological Applications

The elements that have been determined in marine biota by activation are listed in Table 9. The analysis of plankton has been reported by several workers.[222,416-420,803] The interpretation of these analyses is difficult, as the material is heterogeneous and very liable to contamination by particulate debris, but coastal plankton appear to scavenge many elements.[420]

Marine algae have also been investigated by a number of workers, and it has been confirmed that some species accumulate As,[421] Ba,[422] Br,[355] I, and Sr.[422] The bromo- and iodo-compounds formed appear to be natural antibiotics. Koda[423] has succeeded in measuring ruthenium in an alga, which does not seem to have been done for the plants in the Irish Sea where ^{106}Ru is discharged with other fission products.

Miscellaneous marine invertebrates have been studied. The occurrence of vanadium in ascidians is well known, and the eggs have been investigated.[424] The accumulation of tantalum by ascidians has been queried,[425] but there is no doubt that many marine invertebrates accumulate toxic element such as As, Cd, Hg, and Se (together with Cu and Zn) from polluted estuaries and coastal waters. Bryan[426] gives an excellent review of such pollution work. Multielement studies will no doubt increase, for example, the determination of 26 elements in North Sea mussels (*Mytilus*) by Karbe et al.[427]

The majority of marine studies have concerned themselves with the analysis of fish. During the last 15 years the great "mercury in fish" scare developed and declined.[426] Fish naturally concentrate mercury from sea water. Normal concentrations in fish tissue are a few micrograms per kilogram, but some large fish may contain as much as 1 mg/kg, which is near the maximum permitted level for human diets. Analysis of preserved specimens has shown that this concentration is not a result of recent environmental contamination.[428] NAA has been useful in establishing the baseline concentrations of mercury in biota, but other techniques are needed to measure the relative amounts of inorganic and methyl mercury present. Rather surprisingly, livers taken from Dover sole (*Microstomus*) in polluted and unpolluted waters do not differ significantly in elemental content.[804] The occurrence and localization of other toxic elements

Table 9
REFERENCES TO ELEMENTS DETERMINED BY NAA IN MARINE BIOTA

X	Plankton	Algae	Invertebrate animals	Fish
Ag	419	338, 422	419, 427, 431, 432	419, 432, 804
Al				354
As	420	238, 355, 421, 422, 433	238, 355, 427, 433—435	355, 429, 433, 435—439, 804
Au		422, 433	427, 433	433
Ba		422, 440	427, 441	354, 430
Br		355, 422	355, 427, 441	354, 355
Ca			427	354, 430, 439, 445
Cd	420	238, 355	238, 355, 427, 435	238, 354, 355, 435—439, 446, 804
Cl			441	354, 439, 445
Co	222, 420	222, 338, 421, 422	222, 427, 431, 432	222, 354, 432, 447, 804
Cr	222, 419, 420	222, 338, 422	222, 419, 427, 431, 432	222, 354, 419, 432
Cs	222	222, 338, 422, 442	222, 427	354, 430, 447, 448
Cu	420	355, 421	355, 441	355, 436, 804
Dy				443
Eu	803		427, 449	354, 443
Fe	222, 420	222, 338, 422	222, 427, 431, 450	222, 354, 439, 804
Hf		355, 422	355, 427	354, 355
Hg	417, 420	238, 408, 417, 422	238, 408, 409, 427, 431, 434, 435, 451—454	238, 259, 354, 408, 435—438, 446, 453—458, 804
Ho	803			443
I			441	
K		355	355	354, 430, 439, 445
La	803	422		354, 443
Mg				354, 439
Mn	222, 420	222, 355	222, 355, 459	222, 354, 355, 439
Mo	416	416, 421, 433		354, 416, 804
Na		355, 422	355, 459	354, 355, 439, 445
Nd	803	422		
Ni			427, 432	432, 439
P				445
P				445
Pb				430, 439
Pr	803			443
Rb		338, 422, 442	427, 431, 432	354, 430, 432
Re		433, 444	433	
Ru		423		
Sb	222, 420	222, 238, 421, 422	219, 222, 238, 431, 432, 435	222, 238, 354, 432, 435, 436, 439, 804
Sc		338, 422	427, 431, 450	354
Se	418, 420	421—422	427, 431, 432	354, 429, 432, 457
Sm	803		450	443
Sn			274, 427, 460	354, 460
Sr		422, 440	427	430
Ta			425, 427	
Tb	803		427	
Th		338, 422	427	354
U		422		
V		433	11, 424, 433	354
W		433	433	
Yb	803	422	427	354
Zn	222, 419, 420	222, 238, 338, 421, 422	222, 238, 419, 427, 431, 432, 435	222, 354, 419, 432, 435, 436, 439, 447, 457, 804
Zr		422	427	439

such as As, Cd, Pb, Sb, and Se have also received attention. Unidentified arsenic compounds have been found in fish oils by Lunde.[429] The work of Patterson and Settle[430] is notable for the care taken to eliminate contamination in order to establish baseline concentrations of lead and other elements in fish.

NAA has great advantages when attempting to determine some of the rarer trace elements in marine biota, such as Au, Ba, Cs, Hf, Re, Ru, Sb, Sc, Ta, Th, U, W, and rare earths. Many of these have only been assayed using this method.

Freshwater Biological Applications — Only a few papers have been seen on the analysis of freshwater fish[354, 355, 436, 461-463] and invertebrates.[461,463] Mercury concentrations in freshwater fish can rise to unacceptable levels near mercury mines or near factories discharging the elements.

F. Entomological Applications

Very few applications have been published, and the elemental content of insects is poorly known. Mayflies from polluted streams have been analyzed for toxic elements (As, Cd, Cu, Fe, Hg, Mo, V, and Zn).[464] Because of concern about releasing radioactive insects in the environment, entomologists have used stable As,[465] Au,[466] Ce,[467] and Ir[468] to label batches of insects. When the insects are recaptured, they are activated and are readily distinguished from unlabeled specimens by simple counters. There are significant differences in the amounts of Zn (and V) in cysts of the eelworm *Heterodera* collected in spring and autumn, and zinc has been suggested as a possible hatching factor.[805]

G. Vertebrate Applications (Excluding Man)

Numerous studies have been made of animal tissues, as shown in Table 10. In the classic work of Tobias and Dunn,[150] stable gold was injected into a mouse and the tissues were subsequently analyzed by activation with neutrons. Similar studies are still being made, e.g., for europium in rats,[469], lutecium in sheep,[806] mercury in dogs,[470] and platinum[471] and uranium[132] in rats. Rats have been made to breathe welding fumes and their lungs and respiratory tracts then analyzed for Co, Cr, Fe, Sb, and Zn.[472,473] Relatively few workers have studied animal bone[474-477] or blood,[476,478-483] although reference materials are available for these tissues. Analyses of milk (mostly from cows)[396,401,484-489] have been reviewed.[490] In one of a few studies of native wild mammals, the livers of various African species have been examined for 15 elements by IAA; several of the elements show marked seasonal fluctuations which require confirmation.[491] The wool from Norwegian sheep has been tested for 14 elements as a possible indicator of air pollution in different parts of the country.[492] Bird feathers have been examined for possible gunshot residues including elements such as Ba, Cu, Pb, S, and Sb.[493]

In vivo analysis techniques are often tested using whole mice or rats, and the following elements can be determined without exposing the animals to a lethal dose of radiation: Al, Ca, Cl, K, Mg, Mn, N, Na, P, and U.[154,494-501] At present these analyses are of academic interest only. For larger animals, problems arise in constructing artificial standards of the same shape and composition. It would be useful to be able to measure iodine by activating the thyroid gland in vivo and this can be done, though for accurate results it is desirable to use ^{129}I as an internal standard.[502]

The use of NAA in mammalian metabolism has been briefly reviewed.[503] Rat diets have been analyzed for 33 elements by IAA,[307] as well as for nitrogen, phosphorus, and selenium.[67,504,505]. Commercial animal feeds have been analyzed for toxic elements.[527] Methods for analyzing animal urine have been described.[482,486] A comprehensive study has been made of 14 elements in cattle food and feces using NAA together

Table 10
REFERENCES TO NAA FOR ELEMENTS IN ANIMAL TISSUES

Element	Rat	Mouse	Pig	Cow	Bird	Other mammals[a]
Ag	307, 510, 511	513	107	514		R 33
Al	510		354	79,485		R 33
As	312, 510, 515	513	107	41,488, 489, 516	436, 517	R 33, 307
Au	307, 510, 515	150	481	507,514		R 33
Ba			354	396	493	R 33
Br	510—1,5, 518	513	354, 481, 519	485, 514, 520		R 33
Ca	474, 479, 482, 495, 497—499, 501, 510	500	481	485, 514		R 33
Cd	521		107	41, 489 514, 516, 508, 514	436	R 33
Ce				508, 514		R 33
Cl	474, 495, 510		354	485, 514, 520		R 33
Co	307, 472, 473, 478, 479, 483, 510—512, 515, 518, 521	513	107, 354, 481	208, 485, 488, 489, 514, 520		R 33
Cr	307, 472—473, 510—512, 518	513	107, 354, 481	485, 488, 514, 520		R 33
Cs	307, 512, 518, 521	513	107, 354, 481	396, 485, 514		R 33
Cu	307, 312, 478, 479, 483, 510, 522, 523		107	488, 489, 514, 516, 520	436, 493	R 33
Eu	469		354			R 33
Fe	307, 472, 473, 478, 479, 510, 511, 518, 521	513, 524	107, 354, 481	485, 489, 514, 520	525	R 33
Ga						R 33
Hf			354			
Hg	307, 510, 515, 521	107	408, 489		436	R 33, D 470
I	64	526		41, 486, 514		R 33, M 486
K	482, 483, 510		354	485, 514, 520		R 33
La		513	107, 354	514		R 33
Lu			354			
Mg	474, 482, 497		354	41, 485		R 33

Mn	477, 479, 483, 510, 515, 521, 522, 524		354	489, 514, 520		R 33
Mo	479		107, 354, 293			
N	495, 498					
Na	479, 482, 498, 499, 510, 511, 518		354, 481	484, 485, 514, 520		R 33
Ni	510					
P	307, 482, 495, 498	494		514		
Pb				401	493	
Pt	471					
Rb	307, 479, 483, 510, 518, 521	524	107, 354, 481	396, 485, 488, 514, 520		R 33
S					493	
Sb	307, 472, 510—512	513	107, 354	489, 514, 520	436, 493	R 33
Sc	307, 481, 510, 511	513	107, 354	514		R 33
Se	307, 480, 483, 510, 515, 521, 528	513, 530	107, 354, 476	488, 489, 514	293	R 33
Si	510					R 33
Sm						R 33
Sn			107	489, 516		R 33
Sr	477, 510			41		R 33
Th			354	475		
U	130, 132, 478, 516, 531					
V	296, 510		354	487, 489		R 33
W				514		
Y						R 33
Yb			354			R 33
Zn	307, 312, 472, 473, 478, 479, 483, 510, 511, 518, 521—523	513, 524	107, 354, 481	485, 488, 489, 514, 516, 520	436	R 33
Zr	510					

Note: Bird tissues include eggs and feathers.

D, dog; M, monkey; R, rabbit.

with other techniques for a few extra elements.[506] Both stable gold[507] and cerium[508] have been used for labeling fodder in digestibility studies with ruminants, as they are readily activated in feces, etc., and other rare earth elements have been suggested for similar work.[509] The heavy isotope ^{18}O has been employed to investigate liquid exchange in rats. This isotope can be determined by photon activation, but is too expensive for extension of the technique to large mammals.[65]

H. Applications to Man

1. Human Tissues

So many papers have appeared on this topic that only an outline can be attempted here. All the elements known to be essential to man (Ca, Cl, Co, Cr, Cu, Fe, I, K, Mg, Mn, Mo, Na, P, S, Se, and Zn), as well as those for which the evidence is recent or unconfirmed (As, F, Ni, Si, Sn, and V), can and have been determined by NAA.[673] References to recent applications to human soft tissues, blood, bone, and hair are listed in Table 11 and 12. Attempts to calculate normal rangers of composition and mean values have been made in the extensive tabulation of data by Iyengar et al.[144] An empirical generalization is that biologically essential elements tend to be normally distributed in human tissues. Another is that different human races and populations have tissues which do not differ significantly in elemental content, though exceptions have been found, e.g., for As in blood,[674] Se in blood,[675] and for several elements in hair. NAA has been valuable in accumulating the data needed to establish these generalizations. However, numerous inconsistencies can be found in the literature, some of which may well be due to analytical errors.[144,677]

In applications to human blood, plasma, serum, or erythrocytes, NAA appears highly competitive for determining Au, Br, Co, Cr, I, Mn, and Se, and rather less so for Hg, Mo, and V. In clinical practice, the majority of determinations are made by atomic absorption spectrometry or other methods suitable for automatic analyzers, but NAA is sometimes used routinely for determinations of iodine. Two important findings are the demonstration that blood samples can be seriously contaminated with metals from hypodermic needles[676] (see the useful review by Cornelis et al.[173]) and that normal concentrations of several elements in serum vary markedly according to the time elapsed since the last intake of food.[590,598] Although neither of these discoveries are unexpected, they do not seem to be generally known and it will probably be many years before papers describing the composition of contaminated blood disappear from the literature. Other body fluids which have been examined by NAA include milk,[487-489,677,678] pancreatic juice,[679] and saliva.[74,680,681]

Applications to soft tissues have been mainly to muscle and heart, which are poor in trace elements, or to liver and kidney, which are somewhat richer.[673] Liver is somewhat enriched in the metals Cu, Fe, and Mo, which are of great catalytic importance in biochemistry, while the kidney can be enriched in the toxic metals Cd and Hg, which it retains.[557,558,578] Some tissues, notably kidney, change in elemental composition with age.[144,539] Among the less common tissues which have been studied by NAA may be mentioned arteries,[542] brain fractions,[535,573,576] eyes,[540,551,563,564] and the pineal gland.[565]

Relatively few applications have been made to bone and tooth. Bone is strongly enriched in Ba, Pb, and Sr with respect to other tissues, as these elements can partially replace calcium in bone mineral.[673]There has been some interest in comparing the composition of ancient and modern bones to see what effects industrialization has had.[398,653,656] Milk teeth from children have been suggested as a possible indicator of exposure to metals, but teeth are likely to be contaminated from alloys used in filling them.[648,667,683]

Applications to hair have been reviewed in a book by Valkovic[682] and a paper by Katz;[689] the forensic applications have been discussed by Ziegelmann.[690] Hair is easily

Table 11
REFERENCES TO ELEMENTS DETERMINED BY NAA IN HUMAN SOFT TISSUES AND BLOOD OR BLOOD FRACTIONS

X	Soft tissues	Blood/plasma, etc.
Ag	5, 6, 107, 232, 242, 534—536	32, 297, 299, 536, 590—594
Al	233, 354, 536—541	299, 536
As	5, 40, 107, 232, 242, 516, 542, 545, 546, 813	146, 299, 595, 674
Au	232, 242, 536, 542, 546—550	299, 536, 593, 594, 685
Ba	242, 354, 542	32, 299
Br	5, 40, 232, 242, 354, 536, 540, 542, 544, 548, 549, 551, 552, 807	297, 299, 533, 536, 590, 596—598, 809
Ca	242, 354, 544, 549, 554, 555, 807	299, 599
Cd	5, 40, 107, 242, 245, 300, 354, 516, 541, 542, 546, 548, 549, 553, 556—559	553, 593, 594
Ce	242, 542, 544	
Cl	5, 300, 354, 534, 536, 555, 559—561, 807	299, 536, 809
Co	3, 5, 6, 107, 232, 242, 354, 534—536, 539, 542, 544, 546, 549, 550, 562—567, 807	32, 297, 299, 536, 590—594, 686, 809
Cr	5, 6, 107, 232, 242, 300, 354, 534—536, 539, 546, 548, 549, 553, 559, 564	32, 297, 536, 553, 590, 591, 593, 594, 600, 686, 809
Cs	107, 242, 354, 424, 534—536, 542, 546, 548—550, 556	32, 297, 299, 533, 536, 591—594, 598, 809
Cu	5, 40, 107, 232, 242, 252, 300, 516, 540—542, 544, 546, 547, 549, 553, 559, 563, 568—573, 808	299, 593, 601—603, 785
Eu	242, 354, 539	
Fe	3, 5, 6, 40, 107, 232, 242, 300, 354, 534—536, 539, 542, 544, 546, 549, 550, 563—566, 571, 574, 575, 807	32, 297, 299, 536, 553, 590—593, 599, 809
Hf	354, 536	536, 809
Hg	5, 107, 232, 292, 354, 542, 546, 548, 550, 558, 576—578, 807	32, 257, 299, 592, 604
I	5, 526, 551, 579—582	299, 319, 605—609
K	5, 232, 300, 354, 536, 549, 551, 553, 560, 561, 572, 807	299, 536, 536, 593, 599
La	5, 107, 242, 354, 542, 544, 546, 548, 549	809
Lu	354	
Mg	5, 354, 536	299, 536
Mn	5, 40, 232, 300, 354, 540, 541, 543, 546, 549, 555, 559, 568—570, 572, 807, 808	145, 146, 153, 299, 595, 603, 610, 611
Mo	5, 40, 107, 232, 242, 252, 354, 542, 544, 546, 548, 549	203, 299, 593, 594, 612
N	551	
Na	5, 40, 232, 300, 354, 536, 544, 549—551, 553, 555, 559—561, 572	297, 299, 536, 598, 809
Ni	6, 536, 564	
P	3, 232, 534, 546, 551, 555, 561	

Table 11 (continued)
REFERENCES TO ELEMENTS DETERMINED BY NAA IN HUMAN SOFT TISSUES AND BLOOD OR BLOOD FRACTIONS

X	Soft tissues	Blood/plasma, etc.
Pb	541	
Rb	5, 107, 242, 354, 534—536, 539, 542, 544, 546, 548—550, 553, 563—565, 567, 575, 807	32, 297, 299, 533, 536, 553, 591—594, 598
S	232, 551, 564	
Sb	3, 5, 6, 107, 242, 354, 534, 536, 539, 542, 544, 544, 546, 548, 549, 566, 574, 584	32, 297, 299, 536, 590—594
Sc	5, 107, 232, 354, 534—536, 539, 544, 546, 548, 563, 574	32, 297, 299, 536, 591, 592, 809
Se	3, 5, 40, 107, 232, 242, 292, 354, 534—536, 539, 542—544, 546, 548—550, 553, 564—567, 575, 585—588, 807	32, 146, 297, 299, 533, 536, 553, 590—594, 595, 598, 600, 613, 809
Si	589	
Sm	242, 542, 548	
Sn	6, 107, 275, 516, 544, 546	
Sr	242, 354	735
Ta	6, 562	809
Th	354, 536	536, 614
U	536	128, 131, 536, 614
V	5, 296, 354, 487, 536, 540	10, 37, 299, 487, 536
W	277, 542, 548, 562	
Yb	354	
Zn	3, 5, 6, 40, 107, 232, 242, 245, 252, 300, 354, 516, 534—536, 539, 541, 542, 544, 546, 549, 550, 553, 558, 559, 563—572, 574, 575, 684, 807, 808	32, 297, 299, 533, 536, 553, 590—593, 598, 599, 601, 603, 785, 809

Table 12
REFERENCES TO ELEMENTS DETERMINED BY NAA IN HAIR (AND NAIL) OR BONE (AND TOOTH)

Element	Hair and nail	Bone and tooth
Ag	320, 550, 615—626, 741	648
Al	230, 617—619, 624, 625, 629—633	8, 233, 648—653
As	545, 616—619, 621—622, 624—626, 628, 630, 634—639, 647	634
Au	550, 615—622, 624, 625, 630, 633, 635, 636, 639—641, 687, 688	654
Ba	617, 619, 627	396, 648, 655, 656
Br	230, 616—619, 621—622, 624—627, 632, 633, 637, 639, 642, 688	654, 655
Ca	230, 617, 624—626, 630—632, 637, 687, 688	561, 648, 650, 654, 655, 657—661
Cd	621, 622, 624, 637, 638	
Ce	619, 621, 625, 633	655
Cl	230, 617—621, 624—627, 629, 631—633, 642, 687, 688	648, 654

Table 12 (continued)
REFERENCES TO ELEMENTS DETERMINED BY NAA IN HAIR (AND NAIL) OR BONE (AND TOOTH)

Element	Hair and nail	Bone and tooth
Co	550, 615, 618, 619, 621—626, 633, 637, 639, 687, 741	648, 655
Cr	550, 615, 618, 619, 621—625, 630, 633, 639, 687, 688, 741	655
Cs	619, 623, 741	396
Cu	230, 617—621, 624—626, 630—632, 635, 636, 639, 640, 642, 643, 688	662
Dy	619	
Eu	619, 624, 741	655
F	620	70, 583, 650, 655, 659, 663—665, 811
Fe	615, 617—619, 621—626, 633, 637, 639, 687, 741	648, 651, 655, 666
Hf		655
Hg	550, 604, 615, 616, 618, 619, 622—627, 630, 633, 636—639, 641, 644, 687, 688, 741, 810	648, 655, 667
I	617—621, 624—626, 630-632, 688	
Ir	616	
K	230, 617, 619, 621, 264, 625, 632, 633, 639	654, 655, 658, 668
La	230, 616, 618, 619, 621, 622, 624, 625, 633, 639	654, 655
Mg	230, 617, 619, 624—627, 629, 631, 632	650, 654
Mn	230, 616—621, 624—627, 629—633, 635, 636, 640, 642, 687, 688	650, 654, 655
Mo	625	
N		70, 661, 670, 811
Na	230, 550, 616—619, 621, 624—626, 629, 631—633, 635, 637, 639, 640, 642, 645, 687, 688	648, 650, 654, 655, 657—659, 668
Ni	622, 623, 627, 637, 646, 741	
P		70, 561, 649, 654, 657, 658, 660, 661, 811
Pb	637, 638	62, 63, 266, 655
Rb	619, 621, 623, 741	396
Rh	619	
S	230, 617, 619, 624—626, 631, 632	
Sb	550, 615, 616, 618, 619, 621—625, 630, 633, 636—639, 687, 688, 741	648, 654, 655
Sc	550, 618, 621, 623—626, 633, 741	655
Se	615—626, 630, 637, 639, 687, 741, 795, 796	655
Si		651
Sm	619, 624, 625	655, 669
Sn	273, 625	
Sr	230, 616, 617, 619, 627	654—656
Ta		655
Tb		655, 669
Th	622	654, 655
Ti	617, 624—626	

Table 12 (continued)
REFERENCES TO ELEMENTS DETERMINED BY NAA IN HAIR (AND NAIL) OR BONE (AND TOOTH)

Element	Hair and nail	Bone and tooth
U	619, 622, 625	671, 672
V	230, 487, 617, 619, 624—627, 631, 633	487, 650
W	619	
Yb		669
Zn	550, 615—626, 630, 632, 633, 635—641, 687, 688, 741	648, 654, 655, 662
Zr	637	

contaminated both by external agents such as dusts, dyes, and washing solutions, as well as by food or drugs taken internally.[636] No two authors agree on the best method of washing hair to remove external contamination without greatly changing the content of mobile elements such as Cl and Na.[689,691,692] Different hairs from the same head are not homogeneous with respect to elemental content, both spatially and temporally.[636] Using NAA, a single hair is adequate not only for the determination of many elements, but for studying the variation in concentration along its axis.[615,688] NAA has helped to show that hair is unusually rich in Ag, As, Au, Br, Hg, I, S, and Sb, but poor in Na, K, and Rb compared with other tissues.[144] In some populations, significant differences between the sexes have been noticed for Au,[641,688] Hg,[641] Mn,[687] and Ni[646] in hair. There are suggestions of population or racial differences for Br, Ca, Cl, Cr, Fe, I, Mn, and Sb,[144,168] but some of these may be due to analytical errors. Some of the variation between populations may be due to environmental pollutants (As, Br, Cr, Ni, Pb, Sb, and V), natural dust fallout (Al, Fe, Mn, Sc, and Ti) or differences in washing procedures (Br, Cl, I, K, and Na). Comparisons of the hair from rural and urban populations has been carried out in Canada,[637,638] Hong Kong,[619] Germany,[620] and Japan.[625,626]

2. In Vivo Analysis of Man

A considerable literature has grown up in this field, including an IAEA panel meeting[693] and many reviews.[694-699] The technique has to be nondestructive, and a major problem is that of limiting the radiation dose to the subject to 1 rem or less. Sometimes the activation can be restricted to parts of the body, such as the hand or thyroid. The elements determined are Br, Ca, Cl, I, K, and Na by thermal NAA, together with Fe, O, N, and P by fast NAA. Elements determined by prompt techniques (Cd, Cu, and H) will not be considered here, nor will irradiation devices, whole body counters, and the construction of calibration phantoms. Such phantoms are the standards, and their adequacy determines the accuracy of the technique.

Total body bromine can be determined by thermal NAA,[700] with or without the use of the occupancy principle.[701] Calcium was first determined by Chamberlain et al.[702] and Palmer et al.,[703] and subsequently by many others.[45,48-52,54,55,699,704-720] Calcium-49 is the radionuclide usually measured, but a side reaction gives argon-37, which can be measured in the respired air,[44,721] first determined by Anderson et al.[722] and afterwards by others.[49,718,723-725] Iron has been determined in liver by fast NAA.[42] In vivo iodine measurements are usually made on the thyroid,[53,581,704,726,727] but as with animals there is a problem in providing an internal standards.[502] Total body potassium is usually measured by direct counting of natural ^{40}K, but it can also be determined by activation to ^{42}K.[728,729] Total body sodium was first determined by Anderson et al.[722] and subsequently by others.[49,718,723,725,731,732] Nitrogen can be determined by the (n,2n) reaction

to short-lived ^{13}N,[46,718,721,729] but there are potential errors in this technique.[730] Total body oxygen can be measured by the (n,p) reaction,[47,48] and total body phosphorus by the (n,α) reaction to ^{28}Al.[45,48,52,716,718,723] Photon activation is said to be able to detect Ca, Cl, Cr, Fe, Mg, Ni, Pb, Sn, Sr, and Z in vivo.[733] Apart from calcium, most of these determinations are mainly of academic interest at present.

3. Balance Studies

An important part of human physiology is to estimate inputs and outputs for normal subjects. Inputs include food, water, and respired air, while outputs are urine, feces, sweat, etc. A classic application of NAA in elemental balance studies is the work of Harrison and co-workers[734,735] on barium and strontium. Since then there have been studies of Br,[552] Hg,[454] Mn,[736] Rb,[747] Se,[737,795] and V[487,752] in European diets, and multielement studies of diets in Germany,[738] Italy,[739-741] Korea,[742] and the U.S.[200] Hospital diets have been examined for numerous elements by Wester and co-workers[743-745] and also by Mazière et al.[746] Many workers have determined elements in foods by NAA,[527,748-749] and this subject has been reviewed.[750] Table 13 lists the elements that have been determined in diets by NAA. There are gaps for a few important elements such as F, I, and Sn.

Urine is a difficult substrate to analyze by any technique, and it often has a high salt content. However, Cornelis et al.[753] have been able to determine As, Br, Ca, Cl, Co, Cr, Cs, Cu, I, K, Mn, Na, Rb, Se, and Zn in urine by a combination of IAA and radiochemical NAA, and review the problems involved. Other papers on this topic are given in Table 13.

4. Stable Isotope Labels in Human Physiology

Where radioactive tracers are a particular hazard, tracing can be carried out with stable isotopes. These can later be activated in small samples of blood or excreta. Examples of such studies include calcium absorption by newborn infants using enriched ^{46}Ca,[763] and the metabolism of bromine,[764] chromium, and iron[765] by pregnant women or nursing mothers.

5. Toxicity Studies

One of the earliest toxicological applications of NAA was the determination of arsenic in hair in a poisoning case.[766] NAA has also been used to look at various elements in tissues from post mortems as follows: As;[634] Br, Cr, and Cu;[767] Hg;[644] elemental P_4 and phosphides;[768] and Tl.[769] The subject has been reviewed by Smith,[770] and another review concerns the toxicity of mercury to dentists.[771] Several studies of elevated metal concentrations in populations of industrial workers have been reported, e.g., high arsenic near Yellowknife, Canada,[772] and in Ghana;[796] high mercury near Idrija, Yugoslavia;[773] and high tungsten, etc. in diseased lungs of factory workers.[546,774] NAA has also been used in investigations of nickel dermatitis,[646] argyrosis,[775] and metallosis due to surgical implants.[776-779] It has been found useful for determining arsenic in biopsy samples from a patient undergoing therapy for poisoning.[813]

6. Diagnostic Medicine

Since we do not know how a healthy cell regulates its elemental composition, we cannot predict what changes are likely to occur in diseased cells. Although the normal ranges of composition for different tissues are inadequately known, the indications are that most tissues are fairly well buffered against external changes. It also appears that trace element deficiencies in man are rare, and that biochemical and enzymatic changes will probably prove to be more sensitive indicators than are total metal contents in diagnosing abnormal conditions. Nevertheless, the following changes in ele-

Table 13
REFERENCES TO ELEMENTS DETERMINED BY NAA IN HUMAN DIETS AND URINE

Element	In diet	In urine
Ag	738, 740, 743—745	740
Al	200	652
As	738, 742—745	752, 753
Au	738, 743—745	
Ba	734, 738	734
Br	552, 738, 742—745	753, 755
Ca	200, 738, 744	753
Cd	738, 743—745	
Ce	743	
Cl	200, 738	753
Co	200, 738, 739, 743—745	740, 753
Cr	600, 738, 739, 744, 745	740, 753, 754
Cs	738, 739, 743—745	740, 753, 754
Cu	200, 738, 742—746	753
F		757
Fe	200, 738, 739, 743—745	740
Hg	200, 454, 710, 738, 739, 742—745	260, 740, 754, 758, 759
I		753, 755, 756, 760, 761
K	200, 738, 742	753
La	738, 743, 745	
Li		75
Mg	200	
Mn	200, 736, 738, 742, 746	753
Mo	738, 743—745	
Na	200, 738	753
Ni	739	740, 754
P	744	
Rb	738, 739, 743—745, 747	740, 747, 753
Sb	738, 739, 743—745	740
Sc	738, 739, 743—745	740
Se	200, 600, 737—739, 743—746, 795	740, 753, 754
Sm	738, 743, 745	
Sr	734, 735, 751	734, 735
Th		129, 614
U	671	614, 762
V	487, 752	487
W	738, 743—745	
Zn	200, 738, 739, 743—745	550, 740, 753

mental concentrations, discovered or confirmed by NAA data, may have some diagnostic value.

Whole body calcium is depressed in osteoporosis[714,719] but not in cases of cirrhosis.[715] Copper is abnormally high in the livers of patients with Wilson's disease,[780-782] but not in their muscles.[568,570] Copper is somewhat high in the serum and skin of sufferers from psoriasis,[784] and high copper in nail is a possible method of diagnosing cystic fibrosis in infants.[643] Copper and manganese appear to be high in skin tumors.[808] Serum proteins containing iodine have long been used in the diagnosis of thyroid disorders.[319,605] Serum manganese rises during hepatitis and falls on recovery.[611] Manganese and other elements are high in the hair of children suffering from protein defi-

ciency.[642] Sodium is enriched in hair and nail in cystic fibrosis,[632,645,783] but other methods of diagnosis are available for this disease. Plasma zinc is low in the rare skin condition *Acrodermatitis enteropathica*, which can be cured by supplementing the diet with zinc sulfate.[785] Zinc is high in lenses of patients with cataract,[575] and low in pancreatic juice in sufferers from pancreatitis.[679]

Preliminary investigations by NAA of trace element contents in the following diseases has failed to show any outstanding differences between tissues of patients and healthy controls: cancer,[534,633,809] dental caries,[176] cirrhosis,[571,574] heart disease,[170,542,549] hypertension,[288] muscular dystrophy,[176] nephropathy[553] (including useful data on the composition of kidney stones[787-791]), sickle cell anemia,[631] and wound healing.[786]

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