IDENTIFICATION OF TRACE ELEMENTS IN HUMAN BLOOD BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

IDENTIFICATION OF TRACE ELEMENTS IN HUMAN BLOOD
BY NEUTRON ACTIVATION ANALYSIS

by William W. Freeman

A procedure is presented for the identification of the trace elements in human blood using neutron activation analysis, a radiochemical separation and gamma-ray spectrometry. It is proposed that, using this procedure, the unique character of an individual blood sample may be determined and that its origin may be identified. Data is presented on the trace element content of blood samples from several donors and comparisons of the samples are made.

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By

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A THESIS

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CHAPTER I

INTRODUCTION

The Problem

One of the most frequent, and often one of the most important, pieces of evidence found in the process of investigating a crime is blood. In most crimes of violence such as murder and assault, blood may be found at the crime scene in general, but particularly on the victim's person, on the weapon, and occasionally on body or clothing of the perpetrator of the crime. The individualization of blood is therefore a valuable technique in providing evidence which may associate a suspect with, or eliminate him from, the crime scene.

Nearly all of the chemical and spectroscopic tests for blood that are presently used (such as the Benzidine, Luminol, and Teichmann (crystal) tests) are based on the detection of hemoglobin or its derivatives. Chemical examination of suspected blood stains can determine whether or not the material actually is blood, whether it is human or animal in origin, the blood group to which the person belongs if it is human in origin, and the presence or absence of syphilis in the donor (using whole blood).

The four major blood groups into which blood is presently classified, based on the presence or absence of the A and B agglutinogens, give

only a general classification of the individuals in the human race. Kirk[1] lists the occurrence in the population of the various blood groups, and this shows that almost half of the human race (43%) have type "O" blood, and while only three percent of the people have type "AB" blood, this still yields only a broad distinction. Further distinctions, beyond that of the four major groups, can be made based on the presence or absence of type M and N agglutinogens and by identifying the eight Rh blood types. Under optimum conditions of freshness, quantity, and preservation, by using the various groups and subgroups (types), two hundred and eighty eight kinds of human blood can be identified.[2] In the great majority of blood examinations done in the forensic laboratory, the identification cannot go beyond the determination that the blood belongs to one of the four major groups.

Some useful conclusions can be drawn, concerning the source of blood specimens, using these rather broad groupings and types, such as showing that the blood could not have come from a certain individual. This information is valuable, for example, in eliminating persons as suspects in a crime or in settling paternity problems.

There is no technique presently available, however, whereby blood can be identified as coming from a particular individual. There is no

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[1]Paul L. Kirk, Crime Investigation, Interscience Publishers, New York, 1960, Chapter 13.

[2]C. E. O'Hara, Fundamentals of Criminal Investigation, 2nd ed., Charles Thomas Co., Springfield, Ill., 1970, p. 453.

procedure which would allow the forensic chemist to determine, for example, not only that the suspect in a criminal investigation is one of the 14% of the population that has type "B" blood, but also that the suspect's blood has individual characteristics which set him apart from all others in that 14% grouping.

The late Dr. P. L. Kirk, Professor of Criminalistics at the University of California (Berkeley), discussed in his paper "Some Criminalistics Problems, and Neutron Activation Analysis", the importance of trace elements in establishing, with increased probability, the possible common origin of two evidence samples--whether natural or manufactured materials. He said, "With the great sensitivity of high-flux N.A.A., many trace elements can be quantitatively determined in most materials, even when only tiny specimens are available. This can greatly extend the capabilities of the criminalist in his comparisons of evidence samples.[3] N.A.A. has already been used in the identification of individuals by determining the trace element content of hair samples.[4] Based on this discussion, the following hypothesis is advanced.

Hypothesis

> By means of an extremely accurate analytical technique such as Neutron Activation Analysis (which may be used to examine extremely minute pieces of evidence), it should be possible to individualize blood samples. This identification of blood as coming from a unique source, rather than a broad group, could be made by characterization of the various trace elements which are present in human blood.

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[3] V. P. Guinn and R. H. Pinker, The World-Wide Status of Forensic Activation Analysis, paper presented at the 19th Annual Meeting of the American Academy of Forensic Sciences, Honolulu, Hawaii, February, 1967.

[4] R. E. Jervis and A. K. Perkons, "Applications of Radio-Activation Analysis in Forensic Investigations", Journal of Forensic Sciences, I, 4, 449, (1962).

CHAPTER II

THE HISTORY OF NEUTRON ACTIVATION ANALYSIS

Soon after the discovery of artificial radioactivity in 1934, scientists began to use this new technique of nuclear transmutation as a test for minute traces of elements in various materials. Table 1 shows a chronological list of various developments in the field of neutron activation analysis (N.A.A.), as it went from a mere laboratory curiosity, three decades ago, to a widely used analytical tool, today.

TABLE 1

1938	Seaborg and Livingood[1] detected extremely small amounts (6p.p.m.) of gallium in iron samples.
1943	Oak Ridge National Laboratory began to use N.A.A. as an "everyday procedure" for the detection of impurities in metals and alloys.
1947-1948	Equipment became commercially procurable for the detection and measurement of all types of radiation given off by radioactive decay.
1952	The Oak Ridge National Laboratory made an Activation Analysis service available to the public.
1953-1971	Many more research-type reactors were built, both at Atomic Energy Commission (AEC) facilities and at non-AEC labs. More sophisticated and efficient radiation detection equipment became available.
1969-1971	^{252}Californium neutron sources were made available, allowing N.A.A. to become portable, or not dependent on a nuclear reactor site as a source of neutrons.

[1] G. Seaborg and J. Livingood, "Artificial Radioactivity as a Test for Minute Traces of Elements," Journal of the American Chemical Society, 60, 1784 (1938).

CHAPTER III

THE HISTORY OF NEUTRON ACTIVATION ANALYSIS IN THE CRIMINALISTICS FIELD

Within the last ten years, N.A.A., which had already gained acceptance as a precise analytical technique in various industrial and academic applications, has begun to be used as a tool of the forensic scientist. Table 2 shows a list of the recent developments in the forensic science field.

TABLE 2

1959	At the suggestion of the U. S. Atomic Energy Commission, studies were begun, at the Oak Ridge National Laboratory, to test the applicability of N.A.A. in the forensic science field.
1962	Strong evidence was discovered,[1] that Napoleon Bonaparte died of arsenic poisoning (either intentional or accidental). This was determined by N.A.A. of samples of his hair.
1963	N.A.A. was used to detect Barium and Antimony (gunshot residues) on both hands and the right cheek of Lee Harvey Oswald, in the investigation of the assassination of President John F. Kennedy.[2]
1964	The first test case in the U. S. in which the prosecution's case rested almost entirely on N.A.A. occurred.[3] This involved the analysis of paint particles on a tire iron.

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[1] Smith, Forshufvud and Wassen, "Distribution of Arsenic in Napoleon's Hair," Nature, 194, 725 (1962).

[2] J. Lenihan and S. Thomson, Activation Analysis, Academic Press, London, 1965, Chapter 19.

[3] Time, Aug. 7, 1964, p. 58., "Atomic Fingerprints"

TABLE 2 (Cont'd)

1968	a) N.A.A. was first used in a civil suit[4] involving Mercury poisoning of race horses.
	b) N.A.A. results were first presented by the defense in a criminal case;[5] (all previous uses of N.A.A. were by the prosecution).
1970	The Stifel case was decided.[6] Up to the present time, it has set the legal precedent regarding the admissability of N.A.A. results. N.A.A. was used to identify fragments of a package which contained a bomb.

[4] V. Guinn and M. Pro, Transactions of the American Nuclear Society, 12, 506, (1970).

[5] Ibid.

[6] U. S. v. Stifel, No. 19958, U. S. Court of Appeals (6th Circuit), Oct. 29, 1970.

CHAPTER IV

EXAMPLES OF CURRENT INDUSTRIAL AND ACADEMIC USES OF N.A.A.

In recent years, N.A.A. has become an increasingly more useful analytical tool and has found a variety of applications in diverse industrial and academic areas. For example, N.A.A. has been used to detect the amounts of arsenic, copper and mercury in different layers of tooth enamel in a study of means to prevent dental caries. N.A.A. is now being used extensively in the areas of Cosmochemistry and Geochemistry, for example, in the analysis of meteorites[1] for the heavier elements such as indium.

In the plastics industry, N.A.A. is used to detect the amounts of the polymerization catalysts[2] (in many cases, Ziegler's catalyst, containing Titanium and Aluminum) which are carried over into the final product.

Today, N.A.A. is being used in the field of enviornmental studies, for example, to detect trace amounts of poisonous metals such as mercury and arsenic in water supplies as well as in fish and wild life. This writer has recently used N.A.A. to determine quantitatively the amount of mercury in various species of fish taken from waters in the Great Lakes area.

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[1]E. M. Burbidge, et al., "Synthesis of the Elements in Stars," Rev. Mod. Phys., 29, 547, (1957).

[2]Lenihan and Thomson, Chapter 20, p. 130.

CHAPTER V

PRINCIPLES OF NEUTRON ACTIVATION ANALYSIS

Neutron Interactions

Neutron Activation Analysis (N.A.A.) is essentially a method of making qualitative and quantitative elementary analysis by means of nuclear transmutations. Upon exposure to the nuclear particles produced by a nuclear reactor, particle accelerator, or other source, some of the atoms of the target material are converted, by interaction with the bombarding particles, into different isotopes of the same element or into isotopes of different elements, depending on the natures of the bombarding particles and bombarded material.

The bombarding particles can be neutrons, protons, deuterons or even high energy gamma photons. The target atoms, which, as a result of this bombardment, undergo transmutations and become radioactive, have discreet radiation properties which can serve both to identify and measure the quantity of an element in a sample. This radiation may be emitted either during the instant of bombardment or in the course of radioactive decay.

The nuclear process chart[1] (Figure 1) can be used to predict which isotope will result from a given nuclear transmutation. For example, a

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[1] R. Wainerdi and N. Du Beau, "Nuclear Activation Analysis," Science, 139, 1027, (1963).

target nucleus of mass M (square 6) will be converted to the next heavier isotope of the same element (square 7) upon capture of a neutron. Also, a nuclear process which causes an alpha particle to be given off by the target nucleus will cause that nucleus to have an isotopic mass of (M-4) and all of the nuclear properties of the isotope in square 1.

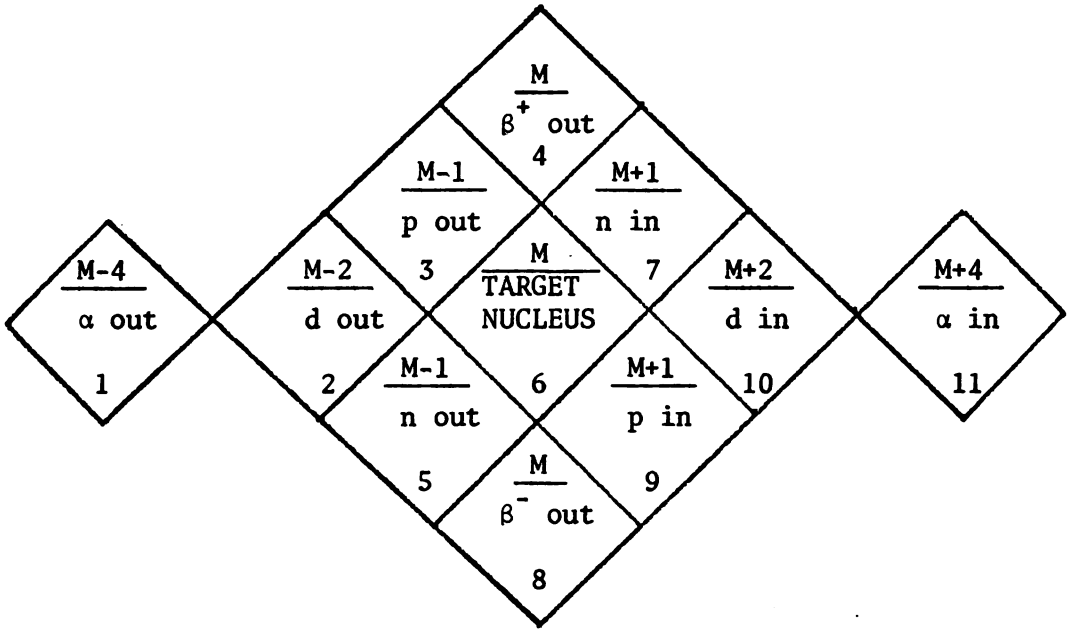

FIGURE 1

Nuclear Process Chart

Neutrons are the most widely used bombarding particles in activation analysis work, since, for charged particles, there is always a threshold energy which must be overcome before activation takes place, and, with the exception of some of the light elements, most elements have low capture cross-sections for the charged particles. In the case of the high energy photons, the threshold energies are even higher and the capture cross-sections are even lower. The attention here, therefore, will be devoted to neutron bombardment and the resultant reactions.

Since neutrons are neutral particles, they are not inhibited by a threshold energy which must be overcome before nuclear interaction can take place. Also, most nuclei have reasonably high capture cross sections for thermal (slow) neutrons.

The production of radioactive nuclides is given by

$$\frac{dN'}{dt_1} = \sigma N \Phi \qquad (1)$$

where

N' = Number of product nuclei due to neutron absorption

N = Number of parent atoms of a particular atomic and mass number in the sample

σ = Cross-section for the production of radioisotope N' in units of cm^2.

Φ = Neutron flux in units of neutrons per cm^2 per second (n/cm^2-sec).

t_1 = Irradiation time

If the product is radioactive with a half life, $T_{1/2}$, the disintegration rate at any time is

$$\frac{dN'}{dt} = -\lambda N' = \frac{-0.693}{T_{1/2}} N' \qquad (2)$$

Upon combination and solution, equations (1) and (2) give

$$N' = \frac{\sigma N \Phi}{\lambda} (1-e^{-\lambda t_1}) \qquad (3)$$

The amount of activity, A_t, in units of disintegrations per second, exhibited by the atoms N' produced up to a time t_1 is given by the expression

$$A_t = \lambda N' = \Phi \sigma N (1-e^{-\lambda t_1}) = A_\infty (1-e^{\frac{-0.693 t_1}{T_{1/2}}}) \qquad (4)$$

where the product $\Phi\sigma N$ in equation (4) is the saturation activity, A_∞, or theoretically, the saturation produced by an infinitely long irradiation. The factor within the parenthesis in equation (4) is termed the "saturation factor," S, which varies between zero and one.

The rate of decay of the product radioisotope will be proportional to the number of radioactive atoms present. At the beginning of an irradiation, there are no (or extremely few) radioactive atoms present and the rate of decay is insignificant compared to the rate of production and the amount of activity will increase linearly with exposure time. While the population of radioactive atoms is increasing, some are already beginning to decay, and as the decay rate becomes greater, the net production rate begins to decrease. A point is finally reached at which the rates of production and decay are equal, and no greater radioactivity will be produced upon further irradiation. This is the saturation point or limit.

The common reactions for the production of radioisotopes by neutron activation are listed in Table 3,[2] where A=mass number, Z=atomic number, n=neutron, p=proton, α=alpha particle, and γ=gamma ray.

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[2] O. J. Hahn, Application of Neutron Activation Analysis in Criminal Investigations, Bulletin 89, College of Engineering, University of Kentucky, Lexington, Kentucky, 1969.

TABLE 3

COMMON REACTIONS FOR PRODUCTION OF RADIOISOTOPES

Reaction	Notation	Predominate Energy of Neutrons
$^{A}_{Z}+n \rightarrow {}^{A+1}_{Z+\gamma}$	$^{A}_{Z}(n,\gamma)^{A+1}_{Z}$	Thermal (0.025 electron volts at 20°C)
$^{A}_{Z}+n \rightarrow {}^{A^*}_{Z+n}$	$^{A}_{Z}(n,n')^{A^*}_{Z}$	Fast 1-3 MeV *
$^{A}_{Z}+n \rightarrow {}^{A}_{(Z-1)+p}$	$^{A}_{Z}(n,p)^{A}_{(Z-1)}$	Fast 1-3 MeV *
$^{A}_{Z}+n \rightarrow {}^{A-3}_{(Z-2)+\alpha}$	$^{A}_{Z}(n,\alpha)^{A-3}_{(Z-2)}$	Fast 10-20 MeV *
$^{A}_{Z}+n \rightarrow {}^{A-1}_{Z+2n}$	$^{A}_{Z}(n,2n)^{A-1}_{Z}$	Fast 10-20 MeV *

*Approximate threshold energies in Million electron volts below which the reactions are not possible.

The first reaction is most predominant with thermal neutrons. A nucleus with mass number A and atomic number Z is transformed to a radioisotope with mass number A+1; the excess energy is given off as a gamma ray. The remaining reactions in the table, such as inelastic scattering (second equation) and the neutron-proton reaction (third equation) generally take place only with high energy neutrons.

Detection of Radioactive Emissions

Various instruments such as Geiger-Mueller counters, scintillation counters, and semiconductor detectors, are commercially available for the detection of charged particles and/or gamma rays. In activation analysis using thermal (slow) neutrons, β^- particles, and particularly γ-rays are the types of radioactive emissions usually investigated.

Beta particles (β^-) are not monoenergetic but exhibit a continuous energy distribution from zero to a maximum value which is characteristic of the emitting nuclide.[3] Most samples will, upon irradiation, contain more than one β^- emitter, each with its own continuous spectrum, and a chemical separation of the nuclides must be made before measurements can be undertaken.

Almost all activated nuclides emit characteristic monoenergetic γ-rays. The qualitative and quantitative identification of the various elements present in the material being analyzed can be determined from the total γ-ray spectrum.

Interactions of Gamma Rays With Matter

When a γ-ray quantum is incident on a material, its energy can be transferred in any or all of three ways.[4] Photoelectric absorption involves the ejection of one of the orbital electrons from the atom. The outer electrons are more easily removed than those closer to the nucleus. The γ-ray actually imparts all of its energy to the orbital electron, which, upon ejection, has a kinetic energy equal to that of the original γ-ray minus the binding energy of the electron. This is the major mode of interaction for low energy γ-rays.

Compton scattering, or the Compton effect, is the transfer of only part of the energy of a γ-ray to an electron. The γ-photon is deflected

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[3] W. S. Lyon, Guide to Activation Analysis, Van Nostrand Co., Inc., New York, 1964, Chapter 6.

[4] Lenihan & Thomson, Chapter 3.

or scattered as though an elastic collision has taken place. The photon moves at an angle, dependent on the particular collision involved, with an energy less than that of the original γ-ray. The Compton electron is also scattered at an angle, with a given energy value.

The third possible interaction is pair production. In this case the γ-ray vanishes completely and its energy appears in the form of β^+-and β^--particles. The threshold for this phenomenon is at 1.02 MeV, since the rest mass energy of each particle is 0.51 MeV.[5] The probability for pair production increases with energy. The reverse effect, electron-positron annihilation, is also possible. In this case, the two particles collide and their energy and mass are converted into electromagnetic radiation. Instead of one quantum being formed, two quanta, each moving in opposite directions, are formed, and each has an energy of 0.51 MeV.

Scintillation Counting

Phosphors are solid or liquid materials which have a high fluorescent efficiency and high absorption for γ-radiation. When the reactions described above occur in a phosphor material (such as the inorganic solid phosphor, NaI(Tl), sodium iodide doped with Thallium), the processes of de-excitation and recombination convert the absorbed energy into light pulses or "scintillations."[6] The phosphor has a photocathode, or photomultiplier tube on one side of it so that electrons will be emitted when light pulses strike its face. These electrons are multiplied by

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[5]Lenihan & Thomson, Chapter 3.

[6]J. Bowen and D. Gibbons, Radioactive Analysis, Oxford University Press, New York, 1963.

secondary emission from a series of amplifying tubes or transistors, eventually resulting in a millivolt signal which is sent to the counting and discriminating equipment.

The Multichannel Analyzer

The analysis and distribution of these signals (pulses) are carried out by a multichannel analyzer which may have, for example, from 512 to 4096 channels. In the modern, so-called computer type multichannel analyzer,[7] there is first an analog to digital conversion in which a number is generated in response to each pulse. This number, known as the channel address, then goes into a computer and is stored as a count in the appropriate section of the memory of the analyzer. After the radioactive sample has been "counted" in this manner for the desired (pre-set) time, with the counts per channel being stored in the memory, the resultant γ-ray spectrum can be displayed. This display can be in the form of a trace on an oscilloscope screen and/or a print-out on a chart recorder. The information can also be printed out digitally or punched onto paper tapes. Figure 2[8] shows the gamma spectrum of ^{24}Na, as it would appear on an oscilloscope screen or recorder chart.

Since the modern analyzer systems use memory-cycle storage times that are relatively long (10 to 20 μ sec.),[9] a device called a "live

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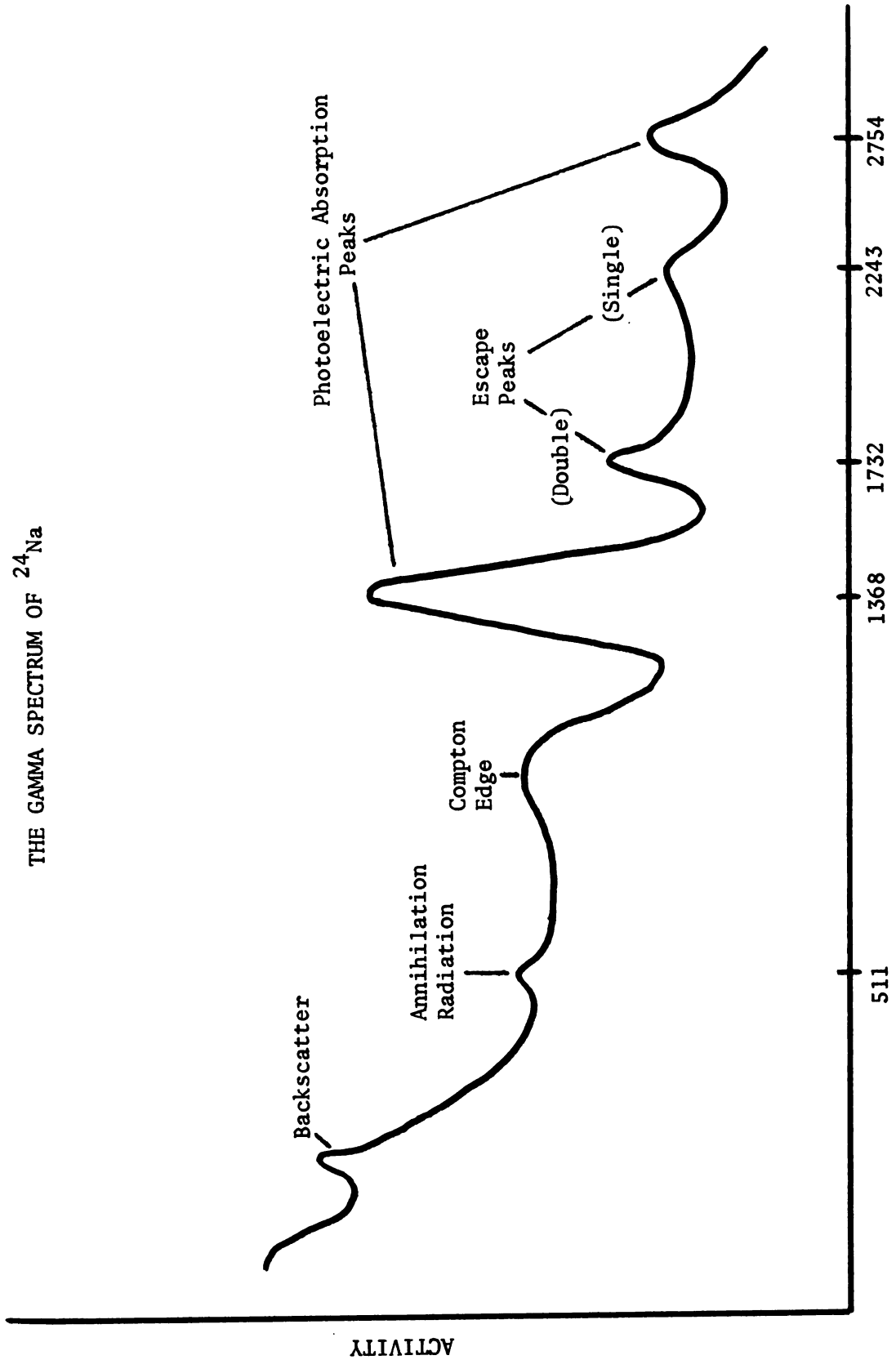
[7]Lenihan and Thomson, Chapter 5.

[8]Lenihan and Thomson, Chapter 3

[9]Lyon, Chapter 6.

FIGURE 2

THE GAMMA SPECTRUM OF ^{24}Na



timer" must be employed to correct for the time when the analyzer is busy and cannot accept additional pulses. That is, during the time when the analyzer is "analyzing" a pulse, called the "dead time," it is incapable of recording any further pulses. The dead time is dependent on the intensity of the radiation being emitted from the sample being analyzed. The greater the amount of radiation, the higher the dead time. When the dead time is relatively low, for example, less than 20%, the spectrum shape is usually undistorted and it is merely necessary to correct for the dead time in order to obtain accurate counting rates. Figure 3[10] shows a schematic diagram of the components in a detection system and multichannel analyzer.

Germanium-Lithium Detectors

When a multi-nuclide sample is being analyzed, a scintillation system with greater resolution than the NaI crystal is often required. Although the NaI(Tl) system is quite efficient in cases where the energies of the various photopeaks are reasonably separated, two peaks with very close energies will appear as one broad peak, since the peaks displayed by the NaI(Tl) detector system are relatively broad. Semiconductor detectors such as lithium-drifted germanium, Ge(Li), can be used to achieve the greater resolution desired. The Ge(Li) detector is a device in which p -type Ge (Ge doped with an electron acceptor impurity, usually gallium) is drifted with lithium. The lithium serves as an n-type (electron donor) impurity and produces a central compensated region. The ionizations produced in this compensated region lead to secondary

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[10]Lyon, Chapter 6.

SCHEMATIC of DETECTION SYSTEM and MULTICHANNEL ANALYZER

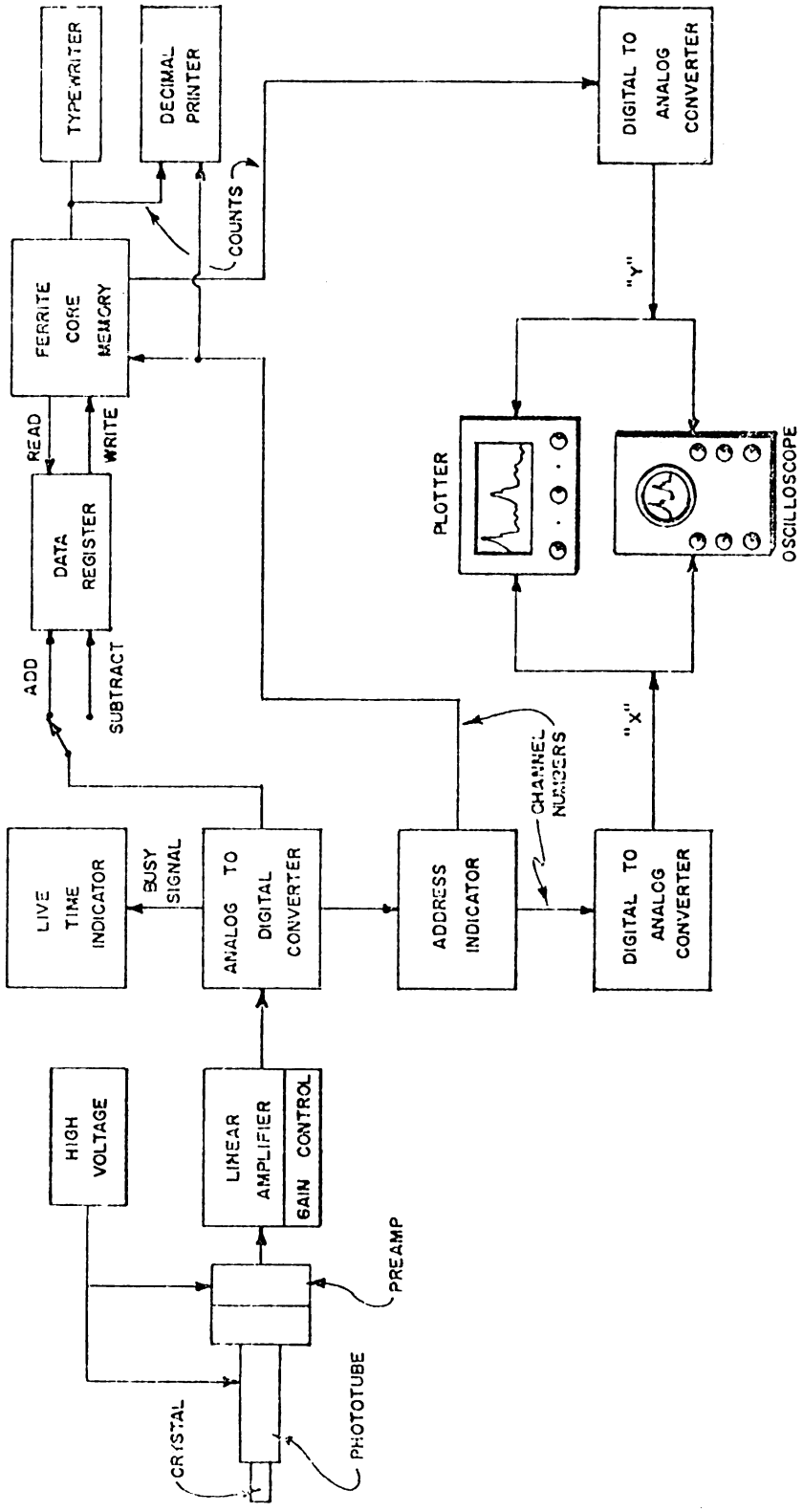

FIGURE 3

production of electron-hole pairs. An applied electrical bias across the detector causes these free charge carriers to move to the outer regions of the detectors, producing an electrical signal which is then amplified and measured.

An example of the great resolving power of the Ge(Li) detector is that, using it one can distinguish between the 1099 KeV γ-ray of ^{59}Fe and the 1115 KeV γ-ray of ^{65}Zn. This would not be possible with a NaI(Tl) detector.

Analysis of Data

The data obtained from the output of the multichannel analyzer (e.g. the plot of photopeaks on recorder paper) can be used in both qualitative and quantitative identification of the isotopes present. The qualitative analysis of the elements present in the material being analyzed is obtained by identifying the various photopeaks in the spectrum. This is accomplished by first drawing a calibration line (curve), which is obtained by counting several "standard" materials (usually long half-life isotopes such as ^{60}Co and ^{137}Cs). These "standard" isotopes give off γ-rays with precisely known energies, and the "unknown" isotopes in the matrix being analyzed are compared with these.

In many cases, this rather straightforward procedure is complicated by factors such as the presence of a large amount of an interfering element in the matrix, causing high backgrounds and poor counting efficiencies for detection of the γ-rays from the trace elements present. A radiochemical separation after irradiation and prior to counting is often

needed to remove these interferences. An example of this is the large amount of sodium present in biological materials. The large amount of ^{24}Na present, from $^{23}Na(n,\gamma)\ ^{24}Na$, $T_{1/2}$=15 hours, often precludes the identification of most, if not all, of the trace elements present.

The quantitative measurements of the trace elements present in a given matrix may be made by comparing the area under a given photopeak with that of a known standard. A known amount of standard is irradiated and counted under conditions identical to those under which the unknown was irradiated and counted. That is, both are irradiated at the same time under the same neutron flux, etc., then both are counted for set periods of time with corrections being made for radioactive decay (depending on the half-life of the materials), as well as for background radiation. The counting geometry, that is, the type of container, distance from the detector, etc., should also be kept constant for both standard and unknown.

CHAPTER VI

COMPARISON OF NEUTRON ACTIVATION ANALYSIS WITH OTHER ANALYTICAL TECHNIQUES

An evaluation of N.A.A. as a method for the qualitative and quantitative determination of trace elements is desirable to show its advantages as well as its limitations as compared with other methods of trace element identification. Although the limits of sensitivity for the various other instrumental techniques can be found throughout the literature, the values of these limits vary somewhat with different investigators, their experimental procedure, the equipment used, etc. A rough comparison is presented here to show the value of N.A.A. relative to other trace element identification techniques.

Table 4 shows the detection limits of N.A.A. and four other methods for 25 of the most common elements for which the five techniques compared can be used. The other four techniques included in the table are typical methods in general use. The values in the table actually give only an order of magnitude, since a certain normalizing factor must be used to adjust the values to a common sensitivity basis of micrograms. Some values are presented in the literature as micrograms per milliliter, some as micrograms per electrode, etc., as indicated in the table.

TABLE 4

COMPARISON OF DETECTION LIMITS OF N.A.A. AND OTHER ANALYTICAL TECHNIQUES

DETECTION LIMITS (Micrograms)

Element	Amperometric[a] Titration	Color-sensitive[a] Reaction	Direct Emission[b] Spectrograph	Atomic[c] Absorption Spectrophotometer	N.A.A.[d]
Ag	1.0	0.1	0.002	0.005	0.0001
As	0.4	0.1	1.0	0.20	0.001
Ba	25.0	---	0.4	0.05	0.05
Br	200	---	---	---	0.005
Ca	100	---	0.1	0.002	1.0
Cd	5.0	0.1	0.1	0.005	0.05
Ce	500	25.0	0.5	---	0.1
Cl	10.0	0.4	---	---	0.01
Cr	1.0	0.2	0.01	0.005	1.0
Cs	---	---	---	0.05	0.5
Cu	10.0	0.03	0.001	0.005	0.001
Fe	2.0	0.05	0.01	0.005	5[e]
Ga	---	---	0.005	0.1	0.005
Hg	---	0.8	0.3	0.5	0.01
K	100	---	---	0.005	0.05
La	---	---	0.07	2.0	0.001
Mg	---	0.6	0.002	0.0005	0.5
Mn	0.0003	0.001	0.003	0.003	0.00005

TABLE 4 (continued)

Element	Amperometric[a] Titration	Color-sensitive[a] Reaction	Direct Emission[b] Spectrograph	Atomic[c] Absorption Spectrophotometer	N.A.A.[d]
Na	---	---	0.1	0.005	0.005
Ni	0.5	0.04	0.008	0.005	0.05
Pb	3.0	0.3	0.02	0.5	10.0
Sb	10.0	0.03	0.03	0.2	0.005
Sc	---	---	0.02	0.2	0.01
Sn	2.0	---	0.01	0.06	0.5
Zn	10.0	0.016	0.005	0.002	0.1

[a]W. Meinke, "Trace Element Sensitivity: Comparison of Activation Analysis with Other Methods," Science, 121, 177, (1955). Values given in μg/ml.

[b]A. Bedrosian, et al., "Direct Emission Spectrographic Methods for Trace Elements in Biological Materials", Analytical Chemistry, 40, 856, (1968); values in μg/electrode.

[c]W. Slavin, Atomic Absorption Spectroscopy, Interscience Publishers, N. Y., 1968, p. 60. Values given in μg/ml.

[d]Gulf Energy and Environmental Systems Inc., San Diego, Calif., "Activation Analysis Sensitivities." Values are interference free sensitivities in micrograms for one hour of irradiation at a thermal neutron flux of 1.8×10^{12} n/cm^2 sec.

[e]By fast neutron reactions.

The comparison shown in Table 4 points out the many elements for which N.A.A. is particularly well suited and those for which it is not. It is noteworthy that N.A.A. is quite sensitive for many of the trace elements which are commonly found in biological materials.

Pro[1] showed a comparison of methods for the detection of lead in "moonshine." Lead is one commonly found toxic substance in illicit spirits and is introduced into the distillate when lead solder is used in still construction. The comparison data is seen in Table 5.

TABLE 5

MICROGRAMS OF LEAD /MILLILITER OF "MOONSHINE"

	Method Used		
Sample No.	Colorimetric	Atomic Absorption	Neutron Activation
1	10.7	10.3	10.1
2	28.3	28.2	27.5
3	86.4	82.0	79.0
4	5.1	4.6	4.1
5	16.4	14.9	14.7

Smales[2] summarized the advantages of N.A.A. over other analytical techniques thus:

Sensitivity: Amounts of various elements as small as 10^{-10} grams can be detected.

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[1]M. J. Pro, "Forensic Applications of Neutron Activation Analysis." Proceedings of the First International Conference on Forensic Activation Analysis, 1967, Gulf Energy and Environmental Systems, Inc., San Diego, Ca.

[2]A. A. Smales, "The Determination of Sub-Microgram Quantities of Arsenic by Radioactivation," Analyst, 77, 188, (1952).

Specificity: The identity of the nuclide used for the determination can be confirmed by decay and energy measurements.

Blanks: The technique is free from reagent blanks or contamination during chemical operations after irradiation, since only the radioactive species is measured.

Scale of Operation: The avoidance, by use of carriers, of the necessity for chemical operations with sub-microgram quantities.

CHAPTER VII

NEUTRON ACTIVATION ANALYSIS OF BLOOD

The Nature of Blood

Although the principal elemental components of all living matter are the four elements carbon, hydrogen, oxygen and nitrogen, there are many other elements present. Blood is one of the many complex compounds in the human body. Along with the four principal elements mentioned above as well as large amounts of sodium, potassium and chlorine, it contains a variety of trace elements such as iron (which serves as an oxygen-carrying component) and other elements which are bound in vitamin complexes. A tabulation of some of the various trace elements known to be present in human blood is shown in Table 6.

The human body is a large complex system of which blood is a part. The body is affected by many external variables such as diet, the environment in which the person lives and the atmosphere in which he works. The body is also affected by internal conditions which may result in disease. The trace element composition of each person's blood is very likely to be a function of these various conditions.

Since the principal matrix elements in blood (C, H, O, and N), are not appreciably affected by neutrons, N.A.A. lends itself quite readily to the trace element analysis of blood.

TABLE 6

ELEMENTAL CONTENT OF WHOLE HUMAN BLOOD[a]

Element	CONCENTRATION mg/100 ml.	μg/100 ml.
Br	2.5±.1	
Ca	9.8±.5	
Cl	285±5	
K	174.1	
Mg	3.82	
Na	196±9.4	
Si	6.4	
As		64
Co		3.7 - 16.6
Cu		94±2.2
F		120±39
Fe		43-53
Mn		12±6
Pb		27±5
Sn		14±12
Zn		880±200

[a]Cyril Long, Editor, Biochemists' Handbook, Van Nostrand Co., Inc. New York, 1961, pp. 873-888.

The Problems in Analysis

Because of the large amount of sodium in proportion to other (trace) elements in blood, a procedure for the elimination of radiosodium (^{24}Na) must be employed to remove as much of the sodium as possible, prior to counting of the samples. With its large photopeaks at 1368 and 2754 KeV as well as the associated Compton edge, single and double escape peaks, annhilation radiation and high background radiation, the presence of ^{24}Na tends to "mask" the entire spectrum, prohibiting the identification of all but the strongest trace element photopeaks. It is also desirable to remove the radiopotassium which is produced upon irradiation of biological material, by $^{41}K(n,\gamma)^{42}K$, $T_{1/2}$=12.5 hr. A third problem is presented in the form of the large amount of chlorine present in biological materials. This is chiefly associated with the sodium, but it is also combined with other elements. ^{38}Cl also produces intense peaks which tend to "mask" the spectrum.

In this work, the last two problems (^{42}K and ^{38}Cl) were overcome with a minimum of difficulty. The ^{38}Cl, with its 37.3 minute half-life, decayed quickly to a point where it was not even detected 24 hours after irradiation. Potassium, although it is presumably present to a large extent (although not as large as sodium) in blood, presented no significant interference problems, although a procedure was used to remove the ^{42}K.

The primary problem was the removal of the large amounts of radiosodium present in the blood. A chemical treatment prior to

irradiation is undesirable because of the possibility of contamination as well as loss of some of the more volatile trace elements. A technique was sought, therefore, for a post-irradiation isolation of the sodium from the other trace elements. It was desired to develop a technique which was simple, efficient, rapid, and one which would easily lend itself to routine analyses.

Review of the Literature

Although much of the work in the N.A.A. field has been done in the area of inorganic chemistry (e.g. analysis of impurities in metals), considerable work has been done in the analysis of organic matrices. Jester and Klaus,[1] for example, analyzed organic liquid lubricants (primarily esters and mineral oils) and detected trace amounts of As, Br, Cl, Co, Cu, Hg, and Mn in the oils. They had no problems with ^{24}Na interference, however, because sodium was only present in μg/ml quantities in these liquids.

Various authors have published the results of work done in the N.A.A. of whole blood, serum and plasma, as well as a variety of other biological materials. Olehy and his co-workers[2] presented a method for the determination of Ba, Ca, Cu, Mg, Mn, Sr and Zn, as well as Na and K in plasma. They used an ion exchange technique after irradiation.

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[1]W. Jester and E. Klaus, "Activation Analysis of High Purity Lubricants," Nuclear Applications, 3, 375, (1967).

[2]Olehy, Schmitt and Bethard, "N.A.A. of Human Erythrocytes and Plasma," Journal of Nuclear Medicine, 7, 917, (1966).

Kanabrocki, et al.,[3] determined quantitatively the amounts of Cu and Mn in blood serum using centrifugation, dialysis, and ion exchange techniques. Haven and Haven[4] showed a technique, using an irradiation, plus precipitation and centrifugation procedures, to be valuable in the quantitative determination of the amounts of Ca, Cu, Mn and Mg in blood serum. In another article, Haven[5] used solvent extraction and a chelating agent, as well as protein precipitation via hot picric acid prior to irradiation, to determine the amounts of Ca, Co, Mn and Mg in blood serum.

Other authors have described techniques, ranging from freeze drying[6] to merely waiting for the ^{24}Na activity to decay,[7] in an attempt to determine the presence of various elements in blood.

Girardi and Sabbioni[8] reported a unique method for the removal of sodium from neutron activated material based on the passage of the

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[3]E. L. Kanabrocki, et al., "N.A.A. Studies of Biological Fluids," International Journal of Applied Radiation and Isotopes, 15, 175, (1964).

[4]M. C. Haven and G. T. Haven, "N.A.A. of Serum," Develop. Appl. Spectrosc., 5, 459, (1965).

[5]M. C. Haven, "Simultaneous Determination of Ca, Co, Mn, and Mg in Serum by N.A.A.", Analytical Chemistry, 38, 141, (1966).

[6]C. C. Thomas, "N.A.A. of Blood Serum for Cu and Zn," Trans, Am. Nucl. Soc., 9, 69-70 (1966).

[7]H. P. Yule, "Reactor Neutron Activation Analysis", Analytical Chemistry, 38, 818, (1966).

[8]F. Girardi and E. Sabbioni, "Selective Removal of Radio-Sodium from Neutron Activated Materials," Journal of Radioanalytical Chemistry, 1, 169, (1968).

material, dissolved in a concentrated acid solution, over a column of hydrated antimony pentoxide (H.A.P.). In 1969, Haller and his co-workers[9] used this H.A.P. technique to determine Br, Cl, Cu, K, Mn, and Zn in whole human blood. They also identified Co, Cr, Fe, Hg, P, Rb, and Se in whole blood by counting samples six weeks after irradiation, when the ^{24}Na activity had decayed significantly.

In 1970, Tang and Maletskos[10] developed a simple, novel column technique for the removal of ^{24}Na and ^{42}K based on a heterogeneous isotopic exchange reaction between an organic eluting solution and crystals of NaCl and KCl. They applied this procedure, which is fast, efficient, and highly selective, to irradiated human tissues prior to gamma-ray spectrometry. The procedure consists of combining a small volume of the aqueous sample with a much larger volume of organic solvent and passing the mixture through a column of an inorganic salt containing an ion in common with the radioelement to be removed. The radioelement is exchanged for the non-radioactive species and is retained on the column while the other components are eluted.

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[9] W. A. Haller, et al., "The Determination of Elemental Concentrations in Blood by N.A.A.", Nuclear Applications, 6, 365, (1969).

[10] C. Tang and C. Maletskos, "Elimination of ^{24}Na and ^{42}K Interferences in N.A.A. of Biological Samples," Science, 167, 52-54, (1970).

CHAPTER VIII

EXPERIMENTAL PROCEDURE

Instrumentation

All irradiations were carried out using the Michigan State University "Triga" reactor which has a steady state power operation level of 250 kilowatts. It is a subsurface reactor. The core contains the following: 66 fuel elements which consist of a homogeneous mixture of zirconium hydride and 20 percent enriched uranium hydride; 19 graphite elements; and 3 control rods. The neutron flux used in all irradiations was 2×10^{12} neutrons/cm^2-sec. The samples were placed in sealed polyethylene vials which were then placed in larger plastic vials in the "lazy susan." This is a device which rotates around the core to ensure uniform radiation.

Gamma ray spectrometry was accomplished using a 3 x 3 inch NaI(Tl) crystal coupled to a Nuclear Data 4096 channel analyzer (series 2200) and a 3.5cm^3 Ge(Li) detector coupled to a Nuclear Data 512 channel analyzer (series 130).

Reagents

"Analytical Reagent" grades of NaCl, KCl, and all acids, as well as compounds, used in preparation of the standards were used. The solvents used, acetone and methyl-isobutyl ketone (hexone) were also "Analytical Reagent" grade. The water used in all cases was deionized, distilled water.

Blood Samples

The blood used in these experiments (in all cases whole human blood) was obtained from several sources. (1) Blood was obtained, in one pint plastic bags, from the Michigan Department of Public Health, Bureau of Laboratories, Lansing, Michigan. This blood was stabilized with 2250 U.S.P. units of sodium heparin per pint. These blood samples were, as were all other samples, from individual donors, not pooled blood. (2) Blood was obtained from the Olin Health Center at Michigan State University. This blood was in the form of 10 ml. samples which had been taken from the common superficial ulnar vein of the donors, using a disposable syringe with a stainless steel tip. Each of these blood samples was stabilized with 10 milligrams of Ethylene Diamine Tetracetic Acid. (3) The final source of blood was that of this writer. Blood samples were taken at Olin Health Center in a manner similar to that described above, but no stabilizing or preserving agents were added. All blood was refrigerated until it was irradiated.

Procedure

Preliminary Investigations

Ion Exchange

An ion exchange procedure, similar to those reported in various literature articles, and that developed by Fahmy[1] was employed, in an attempt to separate the ^{24}Na from the other trace element isotopes. An

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[1]Unpublished work performed by Adel M. Fahmy, Ph.D., United Nations Postdoctoral Fellow at Michigan State University, Sept., 1969-Feb., 1970.

ion exchange column, 1.5 cm. diameter x 15 cm. high (in a standard 50 ml. burette) was packed, via a slurry with deionized water, with Dowex 50 W-X8, a highly acidic cation exchange resin. The water level was left at approximately 1 cm. above the top of the resin column.

Whole blood samples (1.0 ml. of whole human blood) were irradiated for 15 minutes. Immediately after irradiation, the blood was lysed. The lysing was performed by mixing the blood with 20 ml. (a 4:1 vol. ratio) of deionized water and allowing the solution to stand at room temperature for 5 minutes. This serves, because of the difference in osmotic pressure between the whole blood and the dilute solution, to rupture the blood cells. This aqueous solution of lysed blood was then poured slowly onto the column and the liquid allowed to flow freely through the packed resin. The column was then washed with the following solutions:

a) 20 ml (2x10 ml) of 8:2 vol. ratio of .01 N. acetic acid (HOAc) : Ethanol (EtOH).

b) 20 ml (2x10 ml) of 8:2 vol. ratio of .01 N. HCl and Ethanol.

c) 10 ml of a 1% solution of Benzidine in EtOH.

d) 20 ml (2x10 ml) of 8:2 vol. ratio of 0.1 N. HCl and EtOH.

e) 20 ml (2x10 ml) of 1.0 N. HCl.

Each 20 ml solution took approximately 20 minutes to pass through the column. Each solution was counted immediately after elution from the column.

The elution with increasingly strong acid solutions was performed to selectively remove first the blood cells and tissues (in the HOAc/EtOH

elution) and then the other cations (except Na^+) in the successively stronger HCl solutions. Apparently some ^{24}Na did come through with all the fractions, as it and ^{38}Cl, which was not expected to be removed via the cation exchange column, were all that were seen when the samples were counted.

Hydrated Antimony Pentoxide Treatment

The H.A.P. treatment of Girardi and Sabbioni[2] was also investigated. 50 gm. of hydrated Antimony Pentoxide (Sb_2O_5) was prepared by hydrolysis of Antimony Pentachloride. 55.0 ml of the liquid ($SbCl_5$) was added dropwise with stirring to 300 ml of a water-ice mixture. The resultant white precipitate was filtered off and dried @ 110° C for 20 hours.

A slurry of 5.0 gm Sb_2O_5 in 20.0 ml of 6 N. HCl was prepared, and to this the sample of irradiated, lysed blood was added. The mixture was stirred constantly for an 8.0 minute period. This is the "batch equilibration" technique. The mixture was then allowed to settle for 5 minutes and the supernatant liquid was decanted off. This liquid was then filtered through Whatman #1, fine paper, and the filtrate was counted.

The problem involved in this procedure for "selective removal" of the Na^+ ions is that, even though the technique may have been efficient enough to remove the sodium, a small amount of the finely divided Sb_2O_5 remained in all of the filtrates, even though great care was taken to filter it out. This small amount of material, however had enough ^{24}Na retained on it to interfere with the analysis.

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[2]Girardi and Sabbioni, Journal of Radioanalytical Chemistry, op. cit. p. 169.

Isotopic Exchange

The isotopic exchange procedure of Tang and Maletskos[3] was next investigated, and it, with certain modifications, proved to be the best technique for the simple, efficient, rapid removal of ^{24}Na contamination, and one which would allow the identification of the various trace elements present without further radiochemical separation.

To prepare the isotopic exchange column, granular NaCl is first sifted thru a 100 mesh sieve. These 100 mesh (and smaller) crystals were dried for 12 hours at 110°C, and stored in a vacuum dessicator containing $CaCl_2$ drying agent until they were used. A standard 50 ml burette served as the column. A small plug of glass wool was placed in the bottom of the burette, just ahead of the stopcock, to prevent any fine particles of salt from filtering through. The dried salt was slurried with acetone and an appropriate amount of the suspension was introduced into the column. The acetone was allowed to drain slowly and more slurry was introduced, until a densely packed column of the desired height was produced. The solvent was allowed to drain until there was about one inch covering the top of the salt column.

This isotopic exchange procedure is said to work for ^{42}K removal (using KCl) as well as for ^{24}Na removal, and although there was little interference in the spectra caused by ^{42}K contamination, a procedure, identical to that for NaCl was followed using KCl crystals. The eventual procedure developed actually consisted of tandem 10 cm. high columns of NaCl and KCl in the same burette. (see Figure 4)

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[3]Tang and Maletskos, Science, op. cit., pp. 52-54.

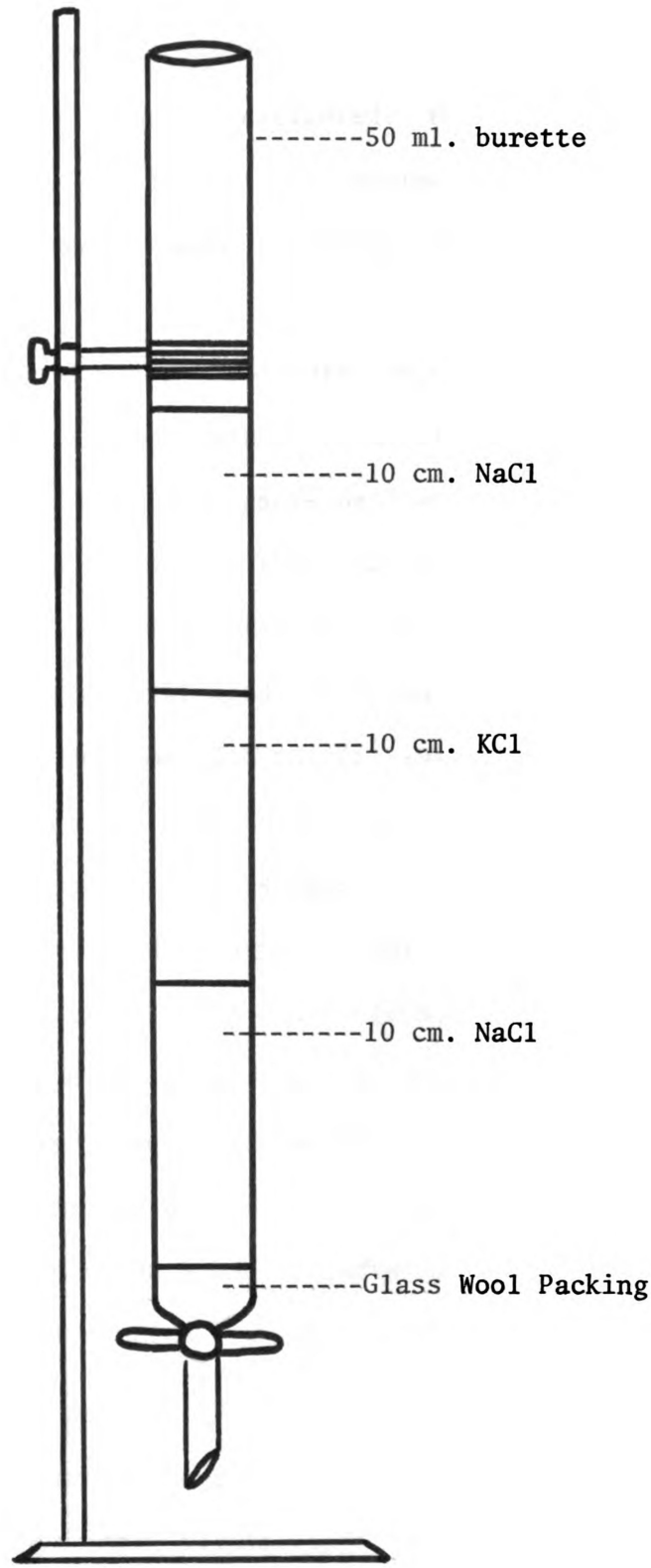

FIGURE 4

Isotopic Exchange Column

The Blood Samples

Both whole and dried blood were investigated. However whole blood was used to a greater extent. Dried blood was examined to determine what differences, if any, existed between the whole blood and dried blood from the same donor.

All irradiated samples of whole blood were 1.0 ml in size. The dried blood samples were prepared by placing 1.0 ml of blood (about 10 drops) on a piece of cloth (a sterile gauze pad) and allowing it to air dry, in a closed room, for 24 hours. The blood spot was then cut from the gauze pad and soaked in 2.0 ml of distilled, deionized water contained inside the polyethylene vial used for irradiation for a period of 12 hours. The cloth was then removed from the vial and the sample irradiated. This procedure for the dried blood was carried out on two different samples. Tables 7 and 8, list as samples #1 through #5 the individuals whose blood was analyzed. Sample #1 was analyzed twice using dried samples and three times using whole blood. Sample #2 was analyzed once using dried blood and three times as whole blood. Numbers 3, 4, and 5 were analyzed three times, each as whole blood.

All irradiations were carried out in sealed polyethylene vials for 3.0 hours (except for one case of an 8 hour irradiation which will be discussed later), at a thermal neutron flux of 2×10^{12} n/cm^2-sec.

The Removal of ^{24}Na

The samples were put through the radiosodium decontamination procedure between 3 and 5 days after irradiation. When the samples were removed from the reactor, immediately after irradiation, they had a

radiation reading (at the surface of the vial) of 200-400 mR/hr. They were stored in a lead vault until much of this initial high radiation (mostly due to ^{38}Cl, but partially from ^{24}Na) had decayed. It required about 3-5 days for the samples to reach a value of less than 5 mR/hr., at which point they were analyzed.

The blood, which had clotted during irradiation, was removed from the vial with a stainless steel spatula and placed in a 50 ml beaker. 4.0 ml of concentrated nitric acid were added to the vial, to wash out the traces of the coagulated blood, and this, along with 1.0 ml of concentrated hydrochloric acid, was added to the beaker. This mixture was heated on a low Bunsen burner flame until a clear (yellow) solution resulted. This usually took 5 to 10 minutes. The solution was allowed to cool to room temperature, and two ml of distilled water were added to dilute the acid. This material was then added to a solution consisting of 35 ml of acetone and 5 ml of hexone. The result was a clear (pale yellow) solution.

A variety of conditions for the isotopic exchange column were evaluated before the optimum conditions were found. Variables such as height and density of the salt column as well as retention time on the column were investigated before conditions which would deliver efficient, reproducible ^{24}Na removal were determined. The optimum column was determined to be one consisting of 10 cm. of NaCl, 10 cm. of KCl, and another 10 cm. of NaCl on top of that.

The pale yellow acetone/hexone solution was poured onto the column and allowed to elute through the column at a rate of 10 drops per minute.

It took about 90 minutes for the entire solution to pass through the column. The column was then washed with a mixture of 20 ml of acetone and 3 ml of hexone, at the same flow rate. For those solutions which contained a measurable amount of radioactivity (greater than 1 mR/hr.), the column was placed behind lead bricks for shielding during the decontamination process.

Gamma Ray Spectra

It was determined, by examining the γ-ray spectra, that the first 15 ml of solution to elute from the column emitted no radioactivity (this was merely the acetone originally contained in the column); the next 30-35 ml contained the bulk of the trace element isotopes, and the last 30-35 ml (which included the wash material) contained the same isotopes, but at a much lower concentration. The forerun was therefore discarded in all cases, and the last two fractions were counted.

All samples were counted for periods of 720-800 min. using the NaI(Tl) or Ge(Li) detector, or both. The samples were counted in stoppered glass flasks which had a geometry such that the bulk of the solution was kept as close to the detector surface as possible. All of the spectra were recorded on chart paper; a "standard" spectrum was counted under identical conditions and printed on the same chart as the "unknown."

Most samples, particularly in the early stages of the work, were counted as many as 12 times while gamma ray energy values were being verified by repetitious counting and half lives were being checked by decreases in peak heights.

One sample of blood was irradiated for 8 hours, in an attempt to bring some of the longer-lived isotopes closer to saturation, so that they could be determined for a longer period of time. This was particularly valuable in the case of such isotopes as ^{134}Cs and ^{113}Sn which were present in only very small amounts and which are difficult to see in all but the very best cases of ^{24}Na decontamination.

Figure 5 shows the gamma ray spectrum of whole human blood before the radiochemical separation. The sample had been counted, using the Ge(Li) detector, two days after a 3-hour irradiation. Essentially all that is seen is the spectrum of ^{24}Na as is evidenced by the large photopeaks at 1368 and 2754 KeV, as well as the Compton edge and escape peaks. The lower spectrum in the figure is that of ^{137}Cs, used as a standard.

Figure 6 shows the various photopeaks which can be seen after the ^{24}Na has been removed. The lower spectrum is that of the standard peaks from ^{133}Ba, ^{137}Cs and ^{60}Co, which are used to draw the calibration line, which is used to determine the energies of the "unknown" peaks.

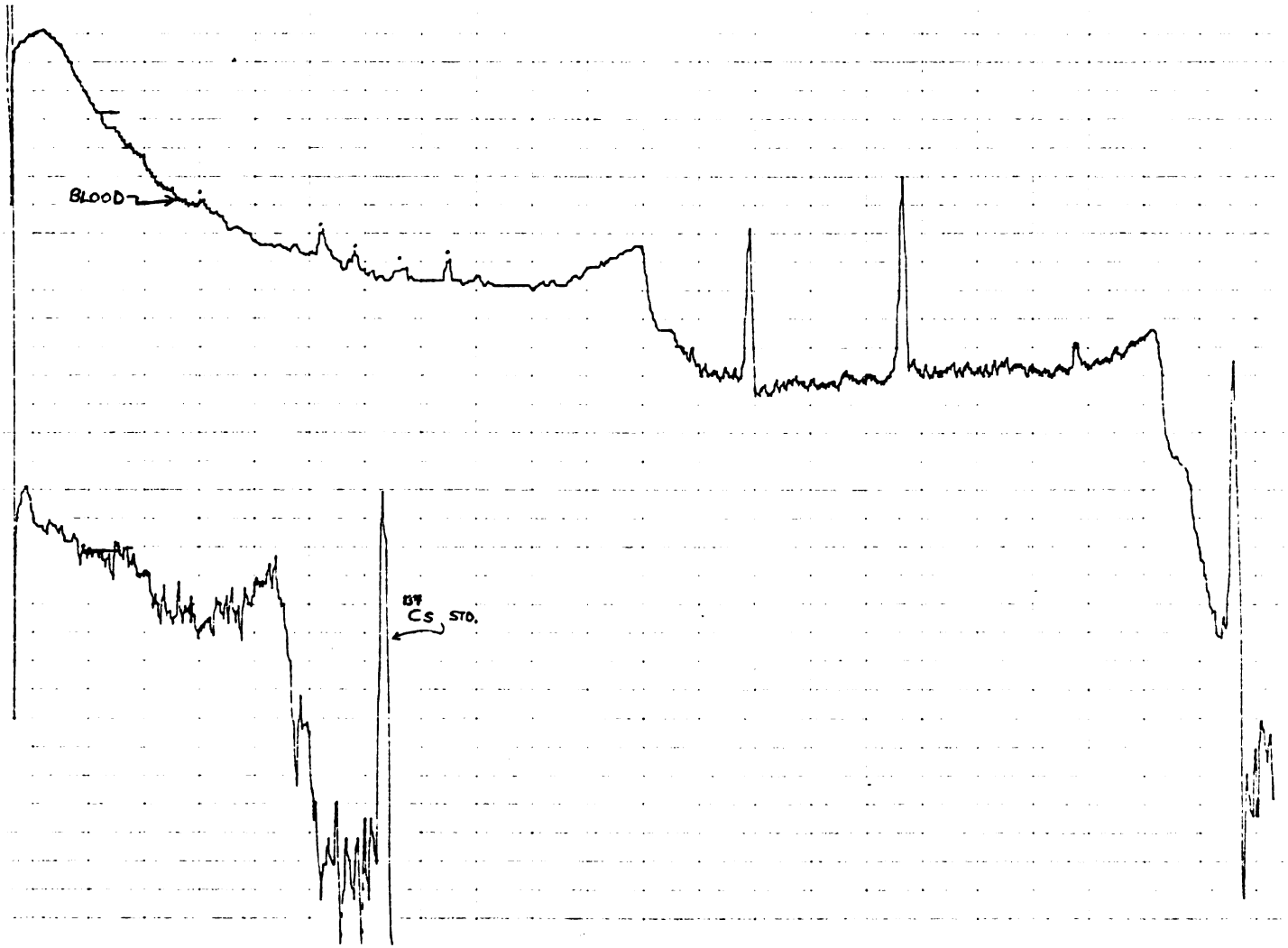

FIGURE 5

GAMMA RAY SPECTRUM OF WHOLE HUMAN BLOOD BEFORE RADIOCHEMICAL SEPARATION

Sample #2, Analysis #1

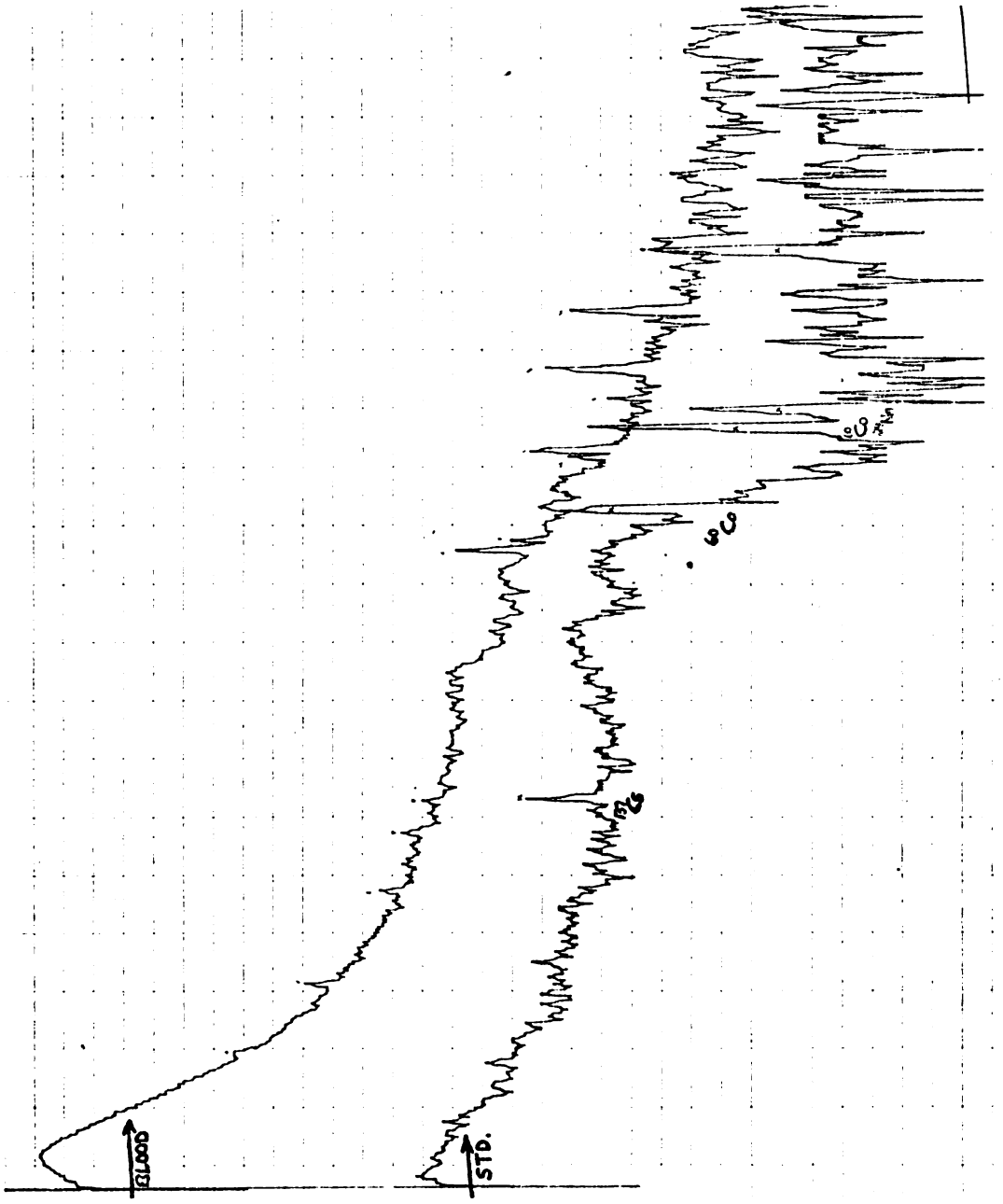

FIGURE 6

PHOTOPEAKS VISIBLE AFTER ^{24}Na REMOVAL

Sample #2, Analysis #1

CHAPTER IX

RESULTS AND DATA

Photopeaks corresponding to the gamma-ray energies of fourteen different elements were identified in the five individual blood samples analyzed. Twelve of these elements were definitely identified by virtue of the existence of multiple peaks, e.g., bromine (^{82}Br) with peaks at 554, 619, and 778 KeV, or by half life measurements, or both. The two other elements, barium and silver, are strongly suspected as being present, but have not been definitely identified because of the low intensity of the peak, lack of existence of complimentary photopeaks, etc.

Of the twelve elements definitely identified, seven elements are common to all of the samples. Of the remaining five elements, one or more is present in each of the five samples. No sample contained less than eight elements which were identified, nor more than ten elements.

Table 7 lists the elements which were identified and the blood samples in which they were found. Table 8 shows the differences in the number of elements identified between pairs of blood samples. In almost all of the cases, there existed a difference of two, three, or four elements among the various samples, but in one case, between samples four and five, there was only one element beyond the seven common elements which allowed a differentiation to be made.

TABLE 7

ELEMENTS DETECTED IN HUMAN BLOOD

	Definitely Detected												Suspected not verified	
Sample	As	Br	Cd	Ce	Cs	Fe	K	La	Sb	Sc	Sn	Zn	Ag	Ba
1	X	X	X		X	X	X		X	X	X	X		X
2	X	X		X	X	X	X	X	X		X	X		
3	X	X		X	X	X	X		X			X	X	
4	X	X		X	X	X	X	X	X	X		X	X	
5	X	X			X	X	X	X	X	X		X		

Note: Iron was only seen once in sample #4, due to experimental or instrumental error. It is presumed to be there, since it is present in all blood.

TABLE 8

DIFFERENCES IN NUMBER OF ELEMENTS FOUND
BETWEEN PAIRS OF SAMPLES

Samples	Number of elements Different Between Samples
1 and 2	4
1 and 3	4
1 and 4	4
1 and 5	3
2 and 3	2
2 and 4	2
2 and 5	3
3 and 4	2
3 and 5	3
4 and 5	1

Of the five individual blood samples analyzed, number one was actually put through the entire experimental procedure seventeen times, number two was analyzed four times, and numbers three, four and five were analyzed three times each. Table 9 lists the elements seen (as the radioactive isotope) in each of the five individual blood samples and the number of times that the element was seen in separate analyses, along with the gamma-ray energy and half-life of each isotope. Only the last five analyses run on sample one are included in the table because the first dozen runs were made while the optimum conditions of such variables as irradiation time, height of the isotopic exchange (salt) column and retention time on the column were being determined. Because of these varying conditions and the different degrees of radioactive sodium removal, all of the elements were not seen identically until the first twelve "trial" runs were completed. For example, bromine and antimony which have photopeaks sufficiently removed from the peaks caused by sodium contamination were seen in all seventeen of the samples, even though in some cases sodium removal was very poor. Arsenic and tin, however, were only identified five and six times respectively, after the sodium decontamination procedure was optimized. The last five analyses of sample number one and all of the analyses of the other four samples were run under the optimized and standardized conditions and show very good reproducibility. (see Table 9).

TABLE 9

THE ELEMENTS FOUND IN INDIVIDUAL BLOOD SAMPLES

Isotope	γ-Energy in Kev		Half Life	Number of Times Found in Each Sample				
	Literature Value[1]	Found		#1	#2	#3	#4	#5
^{76}As	559	560-565	26.3 hours	5	4	3	3	3
^{82}Br	554	550-555	35.9 hours	5	4	3	3	3
^{82}Br	619	615-620	"	4	3	2	1	2
^{82}Br	778	780-785	"	5	4	3	3	3
^{115}Cd	527	525-530	2.3 days	4	0	0	0	0
^{115m}Cd	935[a]	930-935	43 days	5	0	0	0	0
^{143}Ce	294	290-295	33 hours	0	3	3	3	0
^{134}Cs	796[b]	795-800	2.1 years	5	3	3	3	3
^{59}Fe	1099[c]	1100-1105	45 days	5	4	3	1[d]	3
^{59}Fe	1291	1290-1295	"	5	4	3	0	3
^{40}K	1461	1455-1465	1.2×10^9 yr.	5	4	3	3	3
^{140}La	1597	1595-1600	40.3 hours	0	3	0	2	3

TABLE 9 (Continued)

^{124}Sb	603	600-610	60 days	5	4	3	3	3
^{124}Sb	1691	1690-1710	"	5	4	3	3	3
^{46}Sc	888[e]	885-895	85 days	5	0	0	3	3
^{113}Sn	253	250-255	118 days	5	4	0	0	0
^{65}Zn	1115	1115-1120	245 days	5	4	3	3	3
		Elements suspected, but not verified						
^{131}Ba	216	215-220	11 days	4	0	0	0	0
^{111}Ag	342	340-345	7.5 days	0	0	2	2	0

[1]Dams and Adams, "Gamma Ray Energies of Radionuclides Formed by Neutron Capture," Radiochimica Acta, 10, 8-10, (1968).

[a]^{115m}Cd also has a peak at 1295 but this is superimposed on ^{59}Fe at 1291

[b]^{134}Cs also has a peak at 605 but this is superimposed on ^{124}Sb at 603

[c]^{59}Fe at 1099 was seen only on the Ge/Li detector; when using the NaI crystal, this peak was Superimposed on ^{65}Zn and ^{46}Sc at 1115.

[d]^{59}Fe was seen only once in this sample, due to experimental or instrumental error. Iron is presumed to be present in sample #4.

[e]^{46}Sc also has a peak at 1119 but this is superimposed on ^{65}Zn at 1115.

The results of the three analyses of the dried blood samples are included in the table, since their elemental content was identical with the whole blood samples from their respective donors. Two out of the five "#1" samples were dried blood, as was one of the "#2" samples. All of the rest were whole blood samples.

CHAPTER X

DISCUSSION OF RESULTS

The results obtained from these experiments indicate that at least twelve, and possibly more, trace elements can be identified in both whole and dried human blood. Since seven elements were common to all samples, the five (or more) additional elements can provide the basis for individualization of samples. None of the samples contained more than three of these additional elements and one sample contained only one element beyond the seven common ones. It would obviously be advantageous from a statistical point of view to have a larger number of trace elements identified in order to make a more thorough differentiation among samples. These figures may be enhanced when the presence of the "suspected, but not confirmed" elements, Ba and Ag, can be added to the table, or when additional trace elements are confirmed in future studies.

The most definite differentiation between blood samples occurs in the case of sample #1, versus samples 2, 3 and 4, as seen in Table 8. There is a difference of four trace elements being present or absent in individual samples. That is, of the five trace elements used to differentiate between samples, they are present in various combinations in samples #1, 2, 3, and 4, such that a difference of at least four elements always exists.

The reproducibility of the technique is very encouraging. As seen in Table 9, all of the trace elements which were detected in a particular sample were seen in all replicate analyses (at least three per sample), with very few exceptions. Occasionally (less than 10% of the time), the isotopic exchange procedure would provide a degree of ^{24}Na removal which was slightly less than normal and this additional ^{24}Na in the material being counted would provide sufficient interference so that the peaks from some of the trace elements which were present in only very small amounts could not be seen. Examples of this are that in sample #1, all of the elements were seen five times, except for ^{115}Cd, which was seen only four times. ^{115}Cd, with its photopeak at 527 KeV, and 2.3 day half-life was masked by the radiosodium background in one of the five analyses of sample #1.

The 619 KeV photopeak from ^{82}Br was also not seen every time in every analysis (e.g. only two times out of three in samples #3 and 5), but this was due to its low intensity. The other two complementary ^{82}Br peaks, at 554 and 778 KeV, respectively, were seen every time.

The validity of the data obtained in this study is seen in the fact that it reinforces the findings of Wester[1] who analyzed human heart and liver tissue via N.A.A., and made a qualitative determination of 23 trace elements present in these tissues. The 23 elements which he detected included the 12 "confirmed" elements determined in this work, as well as the "suspected" Ag and Ba.

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[1]P. O. Wester, "Radiochemical Recovery Studies of a Separation Scheme for 23 Elements in Biological Material," Int. Jour. Appl. Rad. and Isotopes, 15, 59-67, (1964).

Sources of Error

There are several sources of error in this and any N.A.A. procedure. The largest source of error is pre-irradiation contamination. As in all trace element work, samples must be handled in such a way that contamination is avoided. Errors can occur during the irradiation procedure, such as not irradiating all of the samples to be compared, and the standards, at the same time and for the same length of time, under the same neutron flux.

In the radiochemical separation procedure, material can be mechanically lost in transfer and by spillage. Other losses could include, for example, in the procedure used herein, if the irradiated blood is heated in the acid for an unduly long period, such that some of the more volatile trace elements are lost.

Errors can occur in the counting procedure, such as those caused by variations in the geometry of the vessel used to contain the sample while it is counted, not counting the sample and standard for the same period of time or not correcting for the time difference, and excessive dead time on multichannel analyzer systems.

Another source of possible error arises from fast-neutron-induced nuclear reactions[2] in other elements which also yield the isotope of interest. For example, if a greater amount of manganese than is expected is detected in a sample which also contains iron, some of the ^{56}Mn may be from the reaction $^{56}Fe(n,p)^{56}Mn$.

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[2]W. Lyon, pp. 14-31.

CHAPTER XI

CONCLUSIONS AND RECOMMENDATIONS

From the foregoing it is evident that a good comparison or differentiation among two or more blood samples can be made by trace element analysis. This can be of great potential significance to the forensic scientist seeking to relate a sample found at the scene of a crime with similar material from a suspected individual. Although the Neutron Activation Analysis of blood may prove to be a major breakthrough in an attempt to individualize blood samples, care should be exercised with respect to its forensic applications. The hypothesis which was advanced on page 3 of this work has been partially proven. The fact that Neutron Activation Analysis can be used to identify the trace elements in human blood and that differences among the trace element contents of individual blood samples can be detected has been demonstrated. The results presented here, however, represent a very small population sampling.

Before any conclusions can be reached concerning the applicability of the technique to the populace, a much larger sampling must be made, perhaps ten to twenty times larger than that which has been made here. Because of the variety of influences within his environment which can affect the trace element content of an individual's blood, samplings

should be taken from different geographical areas, with their possible accompanying dietary variations, as well as blood samples from persons with a diversity of occupations.

What the present work does show is that, even though N.A.A. cannot at this time be used to completely individualize a blood sample, it could be of value in making a more quantitative identification of blood than is now possible using the various groups, types, etc. That is, by using N.A.A. in conjunction with the various chemical typing and identification tests, the blood from victims and/or suspects in a criminal investigation can be put in much narrower categories than is presently possible. Some suspects could be positively eliminated.

Another technique of N.A.A. which can be of tremendous help in the individualization of blood samples is that of quantitative as well as qualitative trace element analysis. The statistical value of being able to detect the amount of a trace element, not just its presence, is obvious. The great precision which N.A.A. possesses as a quantitative tool would enable the analyst to differentiate even among samples with similar trace element content (as in samples #4 and 5 in this work), by determining how much of each element is present. A complete elemental analysis would not be necessary in all cases; a comparison of the ratios of various elements in different samples might suffice.

Jervis and Perkons[1] wrote, concerning the quantitative elemental analysis of hair via N.A.A.: "The probability of establishing, with a

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[1] R. E. Jervis and A. K. Perkons, "Applications of Radio-Activation Analysis in Forensic Investigations," Journal of Forensic Sciences, 7, 4, 449, (1962).

high degree of certainty, identity or nonidentity in the comparison of two specimens would depend on the following factors:

1) the precision of the analysis for the various trace elements of interest,

2) the extent of variation in concentration of these elements among many different individuals, and,

3) the reproducibility in concentration of these elements in different specimens of a similar nature from the same individual."[2]

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[2]Ibid.

BIBLIOGRAPHY

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Periodicals

Bedrosian, A., et al., "Direct Emission Spectrographic Methods for Trace Elements in Biological Materials," Analytical Chemistry, 40, (1968).

Burbidge, E. M., et al., "Synthesis of the Elements in Stars," Rev. Mod. Phys., 29, (1957).

Dams and Adams, "Gamma Ray Energies of Radionuclides Formed by Neutron Capture," Radiochimica Acta, 10, (1968).

Dragel, D. T., "Science v. the Law," Analytical Chemistry, 37, (1965).

Girardi, F., and Sabbioni, E., "Selective Removal of Radio-Sodium from Neutron Activated Materials," Jour. of Radioanalytical Chemistry, 1, (1968).

Guinn, V. P., and Pro, M., Transactions of the American Nuclear Society, 12, (1970).

Guinn, V. P., et al., Nucl. Sci. and Eng., 20, (1964).

Haller, W. A., et al., "The Determination of Elemental Concentrations in Blood by N.A.A.," Nuclear Applications, 6, (1969).

Haven, M. C., "Simultaneous Determination of Ca, Co, Mn, and Mg in Serum by N.A.A.," Analytical Chemistry, 38, (1966).

Haven, M. C., and Haven, G. T., "N.A.A. of Serum," Develop. Appl. Spectrosc., 5, (1965).

Jervis, R. E., and Perkons, A. K., "Applications of Radioactivation Analysis in Forensic Investigations," Journal of Forensic Sciences, 7, (1962).

Jervis, R. E., and Perkons, A. K., "Trace Elements in Human Head Hair," Journal of Forensic Sciences, 11, (1966).

Jester, W., and Klaus, E., "Activation Analysis of High Purity Lubricants," Nuclear Applications, 3, (1967).

Kanabrocki, E. L., et al., "N.A.A. Studies of Biological Fluids," International Journal of Applied Radiation and Isotopes, 15, (1964).

Meinke, W., "Trace Element Sensitivity: Comparison of Activation Analysis with Other Methods," Science, 121, (1955).

Olehy, Schmitt and Bethard, "N.A.A. of Human Erythrocytes and Plasma," Journal of Nuclear Medicine, 7, (1966).

Seaborg, G., and Livingood, J., "Artificial Radioactivity as a Test for Minute Traces of Elements," Journal of the American Chemical Society, 60, (1938).

Smales, A. A., "The Determination of Sub-Microgram Quantities of Arsenic by Radioactivation," Analyst, 77, (1952).

Smith, Forshufvud and Wassen, "Distribution of Arsenic in Napoleon's Hair," Nature, 194, (1962).

Spencer, R., "Medical Applications of Neutron Activation Analysis," International Journal of Applied Radiation and Isotopes, 3, (1958).

Tang, C., and Maletskos, C., "Elimination of ^{24}Na and ^{42}K Interferences in N.A.A. of Biological Samples," Science, 167, (1970).

Thomas, C. C., "Neutron Activation Analysis of Blood Serum for Cu and Zn," Transactions of the American Nuclear Society, 9, (1966).

Time, August 7, 1964, "Atomic Fingerprints".

Wainerdi, R., and DuBeau, N., "Nuclear Activation Analysis," Science, 139, (1963).

Watkins and Watkins, "Identification of Substances by Neutron Activation Analysis," American Jurisprudence, 115, (1964).

Wester, P. O., "Radiochemical Recovery Studies of a Separation Scheme for 23 Elements in Biological Material," International Journal of Applied Radiation and Isotopes, 15, (1964).

Yule, H. P., "Reactor Neutron Activation Analysis," Analytical Chemistry, 38, (1966).

Books

Bowen, J., and Gibbons, D., Radioactive Analysis, Oxford University Press, New York, 1963.

Kirk, Paul L., Crime Investigation, Intersicence Publishers, New York, 1960.

Lenihan, J., and Thomson, S., Activation Analysis, Academic Press, London, 1965.

Lyon, W. S., Guide to Activation Analysis, Van Nostrand Co., Inc., New York, 1964.

O'Hara, C. E., Fundamentals of Criminal Investigation, 2nd ed., Charles Thomas Co., Springfield, Ill., 1970.

Slavin, W., Atomic Absorption Spectroscopy, Interscience Publishers. New York, 1968.

Miscellaneous References

Biochemists' Handbook, Cyril Long, Editor, Van Nostrand Co., Inc., New York, 1961.

Work performed by Adel M. Fahmy, Ph.D., United Nations Post-doctoral Fellow at Michigan State University, Sept. 1969, Feb., 1970.

Guinn, V. P., and Pinker, R. H., The World-Wide-Status of Forensic Activation Analysis, paper presented at the 19th Annual Meeting of the American Academy of Forensic Sciences, Honolulu, Hawaii, February, 1967.

Gulf Energy and Environmental Systems, Inc., San Diego, Calif., "Activation Analysis Sensitivities."

Hahn, O. J., Applications of Neutron Activation Analysis in Criminal Investigations, Bulletin 89, College of Engineering, University of Kentucky, Lexington, Ky., 1969.

Pro, M. J., "Forensic Applications of Neutron Activation Analysis," Proceedings of the First International Conference of Forensic Activation Analysis, 1967, Gulf Energy and Environmental Systems, Inc., San Diego, California

U. S. v. Stifel, No. 19958, U. S. Court of Appeals (6th Circuit), Oct. 29, 1970.

Additional Bibliography

Curry, A. S., ed., Methods of Forensic Science, Volume Three, Interscience Publishers, Inc., New York, 1964.

Semat, Henry, Introduction to Atomic and Nuclear Physics, Fourth Edition, Holt, Rinehart and Winston, New York, 1962.

Taylor, Denis, Neutron Irradiation and Activation Analysis, D. Van Nostrand Company, Inc., London, 1964.

Walls, H. J., Forensic Science, Frederick A. Praeger, New York, 1968.

APPENDICES

APPENDIX A

CURRENT FORENSIC APPLICATIONS OF N.A.A.

The Internal Revenue Service Laboratory in Washington, D. C., has, in recent years, employed N.A.A. to compare many types of physical evidence specimens[1] such as drugs, soils, paint, gunshot residues, hairs and fibers, metals, rubber, glass and plastics. The I.R.S. lab has done a particularly large amount of work in the area of analyzing non-taxpaid spirits, soils and drugs. Trace elements in soil have been used to connect a suspect to a crime 1000 miles distant.

In criminal cases involving the firing of a pistol or rifle, N.A.A. has been employed, instead of the nonspecific dermal nitrate test, to detect gunshot residues deposited on the gun hand.[2] This is based on the determination of antimony and barium from the primer of the cartridge.

Jervis and Perkons have done considerable work on the identification of trace elements in human head hair, including one study in which they analyzed and compared 600 individual hair samples.[3]

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[1] M. J. Pro, "Forensic Applications of Neutron Activation Analysis," op. cit.

[2] V. P. Guinn, et al., Nucl. Sci. and Eng., 20, 381, (1964).

[3] R. E. Jervis and A. K. Perkons, "Trace Elements in Human Head Hair," Jour. of Forensic Science, 11, 1, 50-63, (1966).

APPENDIX B

QUANTITATIVE ANALYSIS

The quantitative analysis of a trace element present in blood (or any element in any matrix) is accomplished by comparing the unknown with a standard irradiated at the same time. If both standard and unknown are subjected to the same flux for an identical period of time, then:

$$\frac{\text{Activity in Standard}}{\text{Weight of Standard}} = \frac{\text{Activity in unknown}}{\text{Weight of unknown}}$$

Since the standard and unknown are usually analyzed at different times, a correction for radioactive decay [4] must be made:

$$\frac{A_s\ (e^{-\lambda t_o})}{W_s} = \frac{A_u}{W_u},$$

where t_o is the time interval between analyses, A is activity, W is weight, s is standard, u is unknown, and λ is the decay constant of the induced nuclide.

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[4] R. Spencer, "Medical Applications of N.A.A.," Int. Jour. of Appl. Rad. and Isotopes, 3, 104, (1958).

APPENDIX C

THE USE OF NEUTRON ACTIVATION ANALYTICAL EVIDENCE IN COURT

The presentation of activation analysis results in court is merely a part of the larger problem of the introduction of new scientific methods in court. Some of the difficulties of introducing scientific evidence into legal testimony have been discussed by Dragel[5] where he points out that as late as 1946, a murder conviction was appealed on the grounds that the science of emission spectroscopy was so little known that it lacked the degree of certainty necessary to justify its use in a criminal case. In the past, other scientific techniques such as microscopy, fingerprints and ballistics tests, which have been relatively incomprehensible to the lay judge and jury, have met with similar opposition until their true value was proven and accepted.

In the United States, activation analysis results have been presented in over sixty cases[6] to date, and in some of these, the introduction of the N.A.A. results has met with great opposition because of its "newness" as an analytical tool. There have been a few cases to date[7] where poor quality N.A.A. data have been presented or where the invalidating poor conditions of the evidence specimens

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[5] D. T. Dragel, "Science v. the Law," Analytical Chemistry, 37, 27A-32A, (1965).

[6] Sixty cases as of January, 1970. - V. P. Guinn and M. J. Pro, Trans. Am. Nucl. Soc., 12, 506, (1970).

[7] Ibid., p. 507.

was not recognized by the analyst. The forensic scientist who employs N.A.A. must, therefore, be extremely careful, not only with respect to the accuracy with which he analyzes a particular piece of evidence, but also in the manner in which he interprets the results. As with all scientific techniques which have only recently been accepted by the courts, the material being presented is under very close scrutiny.

A decision handed down by the U. S. Court of Appeals for the 6th Circuit in Ohio on October 29, 1970, is considered to be the "landmark" decision regarding the final recognition of N.A.A. as a forensic technique. In this case, United States v. Orville Stifel II(#19958), the defendant appealed a previous conviction (he was convicted of murdering a man by sending a bomb through the U. S. mails) on the grounds that the judge committed reversible error by admitting, over vigorous objection by the defense, the expert testimony concerning N.A.A. results concerning pieces of the bomb package fragments which were analyzed. The Court affirmed the conviction and cited American Jurisprudence's "Proof of Facts"[8] concerning N.A.A. which cites the "extreme accuracy. . . and versatility" of the process and describes it as "one of the most promising techniques in forensic science."

The Ohio court also noted that N.A.A. evidence had not, to that time, been rejected by any court because the technique was "too new," "too unreliable," or "lacking in general acceptance" in its own scientific field.

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[8]Watkins and Watkins, "Identification of Substances by Neutron Activation Analysis", 15 Am. Jour. "Proof of Facts", 115, 116-119, (1964).