A THESIS ENTITLED

TRACE ELEMENT STUDIES USING ACTIVATION ANALYSIS

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SUMMARY

Trace Element Studies Using Activation Analysis

This thesis describes the estimation of elements at trace levels in biological samples, mainly human, by the technique of thermal neutron activation analysis.

The work falls into three main parts. In the first part the history, background, principles and details of the activation analysis technique are described; in the second part details of the methods used and their modification or development are described; and in the third part the applications made to trace element studies are described.

In neutron activation analysis the induced radioactivity of an element enables its concentration in a sample to be assessed. The advantages of this technique made it a suitable choice for the determination of the low levels of trace elements in biological tissue.

A search of the literature for neutron activation analysis techniques reveals that analysis methods for many of the elements have been described already. Methods found suitable for copper, mercury and cadmium analyses are used as described. Methods described for arsenic, gold, manganese and zinc analyses are modified to improve them and make them more suitable. For selenium and barium analyses it is necessary to develop new methods. The development of these methods is described and discussed. All methods are then applied to the trace element analyses described in the following section.

The trace element studies carried out fall into three fields of research:

(1) A study of the role in tissue of some trace elements,

(2) A study into environmental uptake by man, and clinical application of the analysis technique,

(3) A study of specimens of historical interest.

(1) The question of whether an element is essential or nonessential to a tissue may be indicated by distribution studies. Using this as a basic criterion, manganese, cadmium and selenium are investigated in the major tissues of the human body. Manganese is also investigated in Rhesus monkey tissue and levels of human and monkey tissue compared. Manganese and selenium are established as essential and cadmium is found to be nonessential. The role of barium in the human lung is investigated and it is shown to be nonessential and to accumulate in the broncopulmonary lymph nodes. The variation with time of copper levels in the nail and skin of an individual is investigated and shown to be held within a narrower range than that found in the whole population.

(2) A study is made of the occupational uptake of mercury in dental groups. Workers who were industrially exposed to mercury, arsenic and copper are also examined. A high uptake is found in all these groups. A feature, that is found common to all groups, is that a surprisingly high amount of absorption could be tolerated in many cases, without the associated occurrence of poisoning symptoms. Thus, the value

of the analysis as a means of detecting a potential health hazard is demonstrated. Arsenic analysis of hair, nail and urine is shown to be of use in monitoring a case of known arsenic poisoning. Head hair analysis enables, very elegantly, a record of previous uptake to be found. Analysis for arsenic and mercury is able to be extended into further clinical application and is proved to be useful. Zinc levels in salivary glands are investigated to see what part zinc played in their function. Lower zinc levels than in other soft tissue are found, indicating that here fewer zinc associated metabolic processes are involved. A possible association between zinc in saliva and the labial gland is found.

(3) The value of the activation technique for the examination of small historical specimens is demonstrated by the analysis undertaken of mercury in head hair from Robert Burns and of arsenic in head hair from Napoleon Bonaparte. It is thus shown that Burns may well have been suffering from mercury poisoning, but the evidence is not conclusive. It is found that there is much evidence to suggest that Napoleon was suffering from consistent arsenic poisoning during 1816.

In conclusion, the technique of neutron activation analysis is developed and applied to biological materials. It allows simple, but precise, investigation to be made into trace element levels in both living subjects and in other tissues where only small samples are available.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

This thesis describes the estimation of elements at trace levels in biological samples, mainly human, by the technique of thermal neutron activation analysis.

In the activation analysis technique comparison is made between the induced radioactivity of the element of interest and that of a similarly treated known standard, from which the concentration of the element in the sample can be determined. This technique has various advantages as a method of analysis: it can be extremely sensitive, it allows positive determination that it is the element of interest that is being assessed and not some other, and it is reasonably accurate.

It was considered that these advantages and their exploitation made activation analysis an attractive choice as an analytical technique for the determination of the low level concentrations of trace elements in biological tissue. Thus it was a suitable method for use in the studies carried out. These studies required the investigation of the elements copper, mercury, cadmium, arsenic, gold, manganese, zinc, selenium and barium. A survey of the literature showed that for some elements suitable analysis methods were already available. Some of these methods were used without change (copper, mercury and cadmium), but some required modifications (arsenic, gold, manganese and zinc). No method for either selenium or barium was considered entirely suitable, so new

methods for these elements were developed. Investigations associated with trace elements levels in tissue, using the methods selected, were carried out in the various fields outlined below; i.e. (1) surveys of trace elements in tissue, (2) environmental and clinical studies, (3) historical investigations.

1. Surveys of trace elements in tissue

As described by Liebscher and Smith (1), a "normal" or "lognormal" distribution of levels of a trace element found in different examples of the same tissue can indicate whether it is essential or nonessential to that tissue. It was considered of interest to thus investigate the role of manganese, cadmium and selenium by carrying such distribution studies in the major human tissues, and in the case of manganese also for Rhesus monkey tissues. Barium in lung was also investigated as a limited survey. It was also of interest to see whether copper levels were controlled as closely in the general population as in the individual. This was investigated by analysis for copper in nail and skin over an extended period of time.

2. Environmental and clinical studies

In the course of his daily work man can be subjected to potentially hazardous uptake of elements from his environment. It was of interest, therefore, to study such cases to enable a fuller appreciation of the subject to be made. An easily identified profession in society is dentistry, in which high amounts of mercury are handled daily; it was thus of interest

to investigate the form in which any mercury uptake occurred in dental groups. Exposure to chemicals can occur in industry, where sometimes less than adequate care may be taken. Such possible uptake in individuals was investigated, in this case for the elements mercury, arsenic and copper, to provide information on any health hazard and to allow conclusions on the problem of industrial exposure to be made.

It was seen whether the unique sensitivity and certainty of activation analysis for small samples could be usefully applied as a clinical diagnostic aid in toxicological cases. Measurement was thus made of the elements arsenic and mercury in samples of hair, nail and urine from living patients. Gold in a post-mortem liver sample was investigated to help establish the cause of death. The use of arsenic measurements in hair, nail and urine in an arsenic poisoning case was investigated.

As little is known clinically of the role of zinc in mouth tissue, an investigation of zinc in the salivary glands and saliva was made.

3. Historical investigations

The activation analysis technique is well suited to the examination of small historical specimens. A few head hairs from the Scottish "Bard" Robert Burns (1759-1796) and Napoleon Bonaparte (1769-1821) were investigated for evidence of mercury and arsenic uptake, respectively. Mercury was assessed in Burns's head hair to help establish whether his health at that time was being affected by an overindulgence of mercury

containing medication. It was of interest to see whether Napoleon may have been exposed to arsenic in 1816. To this end, hair was obtained which grew at about this time and was subjected to pooled and sectional analysis for arsenic.

CHAPTER 2

Activation Analysis

ACTIVATION ANALYSIS

Introduction

Thermal neutron activation analysis can provide a sensitive means of assaying at trace levels the concentration of an element in a given sample. Measurement of the radioactivity induced by neutron activation and emitted by the sample, when compared with that from a similarly treated known standard enables the amount of element present to be assessed.

Neutron activation is carried out by placing the samples to be analysed in a neutron flux. This is normally done by insertion inside a reactor pile. Radioactive isotopes are thus formed by neutron capture. There are other neutron sources, for example, neutron generators. Sealed neutron tubes such as the Phillips PW-5320 can provide $>10^{10}$ n/sec for a lifetime in the order of 500 hours (2). Activation is not confined to thermal neutrons; it has been done by bombardment with fast neutrons, protons, deuterons, tritons, α-particles and γ radiation. For this purpose accelerators, etc., have been used. However, for the work carried out activation was only by thermal neutrons and for this reactors were used.

After irradiation the induced radioactivity of the element under investigation is assessed. The radioactivity associated with a particular element is counted, for example, as γ rays or β emission and can be characterized by the

energy of emission and its half life. This radioactivity is counted after post-irradiation radiochemical separation is carried out to remove interference from other elements in the sample which were also activated. In certain cases a physical rather than chemical separation of the desired radioactive emissions can be made and the need for a chemical separation technique obviated. By comparison of the radioactivity of the element in the sample with that of a known standard the amount of element present can be calculated.

Nuetron activation analysis, as an analytical technique has a major advantage, that of sensitivity. The methods available allow up to many orders less than 1 μg of certain elements to be easily determined and positively identified. It is therefore a technique which is suitable to the determination of "trace" quantities of element present in some host matrix or tissue. These trace quantities are measured as parts per million (p.p.m.) by weight or less.

As an analytical technique neutron activation analysis has expanded rapidly during the last twenty-five years. The origins of the neutron activation analysis technique may be said to lie in a paper published in 1934 by Curie, Joliot and Preiswerk (3), in which the formation of new "radioelements" from Ag, Si, Zn, I and Fe by neutron bombardment was described. In 1935 Amaldi et al. bombarded gadolinium with neutrons and induced radioactivity with an 8 hour half-life (4). Sugden found that neutron irradiated europium displayed a 9.2 hour half-life, which made him suspect that the radioactivity of

the gadolinium was due to some traces of europium (5). The first analysis by such induced radioactivity occurred two years later when von Hevesy and Levi determined the content of dysprosium and europium in samples of rare earth mixtures (6). Two years later Seaborg and Livingwood used deuterons to determine gallium in iron (7), and Hevesy and Levi (8) determined europium traces in gadolinium, verifying Sugden's earlier suspicions.

In the next ten years there were relatively few publications about this new analysis technique; however, with the wide availability of reactors for neutron sources from the 1950's onwards the field expanded rapidly. The first review articles appeard in 1949 (9,10). A modern bibliography on activation analysis published by the United States Department of Commerce, listed in 1971 over 10,000 references to publications in the field (11). It lists 90 elements, the rare earth actinides and lanthanides and 107 matrixes that were investigated by an activation technique. The technique has been found useful for sensitive measurements of trace element levels in a wide range of materials, from biological tissues, industrially produced materials, to moon rock. This technique can be applied to the majority of the 90 naturally occurring elements, but it is not always necessarily the method of choice for the reasons described later.

Advantages of the technique

The major advantage of the technique was sensitivity. Depending on the element, concentrations of parts per billion by weight were detectable. Small sample sizes could therefore be used. It was a feature of the technique that one trace element could be assessed for concentration free from the influence of other elements present.

The half-life of the activated trace element and its radioactive spectrum, in particular the gamma spectrum, could be obtained during the analysis. Either or both provided an internal check that it was the element of interest being assessed and not some other.

Radiochemical separation was done by simple chemical processing in the open laboratory. Thus, large numbers of samples could be analysed at the one time. For example, up to 60 manganese analyses per day were performed. As correction could be made for any yield loss it was not necessary for time-consuming quantitative separation procedures to be used. During the post irradiation radiochemical separation any possible interference from trace quantities in the reagents used was negligible relative to the amount of carrier added (normally 10 mg).

If instrumental analysis techniques were employed, the sample need not be altered in any way. This could be important where samples were of forensic interest or historical origin.

It was not necessary that the reactor used for irradiation be close at hand. Indeed, the bulk of the activation done for this study took place 320 miles from the laboratory. Irradiated samples were returned by air and processing could therefore start within 12 hours of the samples' removal from the reactor.

Disadvantages of the technique

Not all elements will undergo thermal neutron caputre, and they could not be thus analysed. Some elements activated only to a small extent (due to a low cross section absorption coefficient or long half-life) and hence the sensitivity of the technique was not sufficient for their detection. It was preferable that the element had a half-life of reasonable length, i.e. several hours. Isotopes which activate with half-lives in the order of seconds can only be measured using specialised and limiting techniques. For my short-lived isotopes with half-lives under about one second it is possible to assess them using a pulsed neutron technique as described by Guinn (12). Guin described how the neutron flux in the reactor used for irradiation was allowed to become very large before collapsing, the temporary flux gain increasing the sensitivity obtained.

Although the technique can be extremely sensitive it is subject to an error up to $\pm$10%, as discussed later.

Access to suitable reactor facilities is required and this may not always be possible. Although only basic laboratory facilities are necessary for sample preparation and processing the counting equipment used is often complex and

expensive to buy.

Analysis is not instantaneous. The technique normally requires a certain time lag before results are obtained. Although large numbers of samples could be analysed, the unit time necessary for each analysis was often long; i.e., in the order of one to two hours per sample analysis.

A limitation of the technique is that analysis is of the total element concentration and no distinction can be made of the chemical form in which it is present.

1. The procedures carried out during the activation analysis process

These can conveniently be divided into four sections:

a) preparation

b) irradiation

c) processing

d) counting

The equipment and materials that were used are listed in each section, followed by a general description of the procedures used.

a) Preparation

Equipment

Handling and cutting of tissue material: Perspex or stainless steel forceps and surgical blades were used as normally available.

Vacuum drying of samples: This was carried out using a 14" diameter pyrex desicator and an Edwards vacuum pump with a freeze trap.

Weighings: Tissue samples were weighed on a Stanton Unimatic 4-place balance, or an Oertling 8-place quartz beam decimicrobalance. Chemical weighings were made on another Stanton Unimatic 4-place balance and a 5-place Oertling balance.

Materials

Polythene bags of various sizes including the "snap seal" variety were used. Aluminium foil for sample wrapping was of the type used for domestic purposes. Silica tubing for making packaging vials was supplied by Thermal Syndicate, Ltd. Silica gel for drying was cobalt indicated as made by BDH Chemicals Ltd. Disposable polystyrene petri dishes, 5" diameter, were used during tissue drying. Disposable vinyl examination gloves were used for handling of tissues both prior to and after irradiation.

Sample preparation

It was necessary for each sample to be prepared and suitably packaged prior to irradiation. All body tissues

were dried before irradiation with the exception of hair and nail. The wet weight of a tissue sample was found to decrease on standing at an appreciable rate which depended on various factors including the surface area/weight ratio. As a result it was considered desirable to refer concentration to dried tissue weight. There was a further advantage in that it was very much easier to irradiate tissues in a dried form.

Drying was carried out under vacuum oven silica gel. The wet samples divided into approximate one gram portions were placed on a fresh polystyrene petri-dish. The petri-dish was placed on a mesh tray inside a glass desicator. The 14" diameter glass desicator which was used held up to 20 stacked petri-dishes. The base of the desicator under the tray was filled with dry silica gel. (Very great care was exercised to exclude the possibility of contamination from silica gel. This was checked regularly.) The whole was evacuated to a pressure of 1 to 2 Torr using a vacuum pump. Where it was expected that excessive moisture would be removed an isopropyl alcohol/solid carbon dioxide freeze trap was incorporated in the vacuum line to the pump. After exhausting the air the desicator was left inside for several days. It was shown that tissues so treated had reached a constant weighing dried weight on removal. (On any further drying as described the weights remained the same.) Liquid samples analysed were blood, serum and urine. The firs- two

were dried as above, the last was not and was packaged as a liquid as described below.

On average, wet body tissues were found to lose about 70% of their weight on drying. As a typical example the figures below refer to 10 body tissues of a male human dried to constant weight for 4 days over silica gel in an evacuated desicator.

TABLE 1. Wet and Dry Weights of 9 Human Tissues

Tissue	Wet Weight gms.	Dried Weight gms.	% of Original Wet Weight
Adrenal	2.2879	1.1640	51
Aorta	1.0630	0.3703	35
Bone	0.4435	0.3441	78
Brain	2.9667	0.8655	29
Lung	3.0171	0.6234	21
Pancreas	3.3310	0.9934	30
Pectoral Muscle	2.7149	0.7983	29
Spleen	1.3350	0.4133	31
Thyroid	3.4190	1.0843	32

Other sample preparation techniques have been extensively used by other workers, for example drying in ovens and ashing of samples. Such pretreatment techniques are described widely throughout the literature and are reviewed, for example, by Girardi (13) and Bowen (14). It was considered, however, that the means selected for sample preparation, drying under

vacuum, was the optimal one for the type of biological samples analysed. Other methods were considered too prone to error inducement, by volatilisation, contamination, etc. Drying under vacuum over silica gel at room temperature was found simple and effective.

The fact that values quoted in the literature of element concentrations in tissues can refer to ashed, dry and even wet weights makes accurate comparison of results from different workers difficult. Approximate conversion factors can be used as for example given by Tipton (15). It was considered that the dry weight reference used here was the most satisfactory basis to standardise on.

Packaging

There were two different packaging techniques used depending upon whether (i) DIDO/PLUTO reactor facilities were used at the Atomic Energy Research Establishment Harwell, where the ambiant reactor temperature in the irradiation area was in excess of 70^{o} C., or (ii) the facilities of the Scottish Research Reactor Centre East Kilbride were used where the reactor was cooler.

i) For irradiations at Harwell packaging was carried out as follows. An approximately 3 cm. square of aluminium foil was cut out from a roll of domestic aluminium baking foil. The sides were pressed upwards with a plastic tool to form a slab sided boat. This was weighed to the nearest tenth of a milligram. A suitable piece of the sample of dried tissue was cut with a stainless steel blade. This

normally weighed 10 to 50 milligrams. It was placed with forceps onto the aluminium boat. Subsequent weighing gave the weight by difference of the sample. The sides of the aluminium boat were folded over the sample. The ends in turn were each folded over to form a small square encapsulating the sample. Wrapping the sample thus in aluminium foil was shown not to contaminate the sample. In this form the sample was now suitably prepared for irradiation. Each wrapped sample was numbered with a dye pencil and five to ten samples in numerical order were consolidated by wrapping as a block inside a large aluminium boat. These blocks of samples were placed inside an aluminium screw cap can. The standard was placed inside the can alongside the samples. The aluminium can was of a type approved for use at the Harwell irradiation facilities. After irradiation the samples were removed from their wrappings before counting or chemical processing. Liquid samples and liquid standards were placed inside silica vials. Sample weights were determined by weight by difference, the final weighing taking place before sealing the vials in an oxygen flame. The packaging techniques were of the type described by Bowen and Gibbons (16).

ii) For irradiations undertaken at the Scottish Research Reactor Centre where less sample heating took place, the samples were wrapped in polythene. Each sample was placed inside a marked miniature "snap seal" thin polythene bag. Sample weights were found by difference. Liquid samples were placed in polythene tubes with "snap seal" caps which were

heat-sealed to insure against any possiblity of leakage. These packaging techniques were quick and simple, in particular the unpackaging time was minimal. If irradiations were to take place in the central vertical stringer of the reactor, ambient temperatures were higher (up to 100^{o} C) and therefore Harwell packaging techniques were employed.

Any contamination taking place prior to irradiation of a sample would be reflected in the analysis result obtained. As measurements were at trace level the effect of any contamination would be great. Thus, extreme care was taken to ensure that contamination was kept at an almost irreducible minimum. Therefore the handling and wrapping tools and materials were as described. Their use produced no measurable increase in the trace levels measured. All sample handling and wrapping, with the exception of cutting, involved a plastic, aluminium, quartz or glass contact. Plastic knives, or broken quartz glass edges were found to be difficult to use as satisfactory cutting implements and their substitution by fresh stainless steel surgical blades was found to produce no measurable contamination; in particular none was found when manganese (a steel additive) was measured in tissues so cut. Fresh polythene sheets were laid out on the preparation area, which was in a laboratory not used for chemical processing and in which no chemicals were stored. As described by Bowen (14) some degree of contamination by laboratory dust is almost unavoidable. However, all sample material was normally kept wrapped inside polythene bags and was exposed

only for a short time when necessary. Dust was not found to be a measurable contaminant. (The use of a filtered air chamber would further minimise any risk of dust, however this was not found necessary.)

b) Irradiation

Equipment

Irradiation was carried out at two separate reactor installations. The facilities of the United Kingdom Atomic Energy Authority at Harwell Berkshire was used for irradiations in excess of 6 hours. The reactors known as "BEPO" (no longer available), "DIDO" and "PLUTO" were available. Packaged samples were sent by mail. Irradiations were carried out by the staff there according to instructions sent. Thermal neutron fluxes used ranged from 10^{12} - 10^{13} neutrons/cm^2/second. Active samples were returned by air. The Harwell Reactor facilities were some 300 miles from the laboratory.

The shorter irradiations of up to 6 hours duration made use of the water moderated reactor type UTR 100 at the Scottish Research Reactor Centre, National Engineering Laboratory, East Kilbride. Sample insertion was carried out by the experimenter using a vacuum "rabbit" system. Thermal neutron fluxes ranged from approximately 10^{12} n/cm^2/sec at 100 KW output to greater than 4 x 10^{12} n/cm^2/sec in the central vertical stringer (C.V.S.) at full output of 300 KW. The rabbit system was normally used except in the case of samples

irradiated for barium analysis where the C.V.S. was used. A diagram of the irradiation positions available in the reactor core is shown in Figure 1. This reactor was some 14 miles from the laboratory.

Materials

For irradiations at U.K.A.E.A. Harwell, aluminium screw top cans as supplied by Harwell were used as containers. For irradiations at the Scottish Reactor Centre "snap seal" polythene bags as described earlier were used. Chemicals irradiated as standards were "Specpure" type from Johnson Matthey Ltd. Irradiated packages were kept prior to processing in 1" thick lead pots.

Sample irradiation

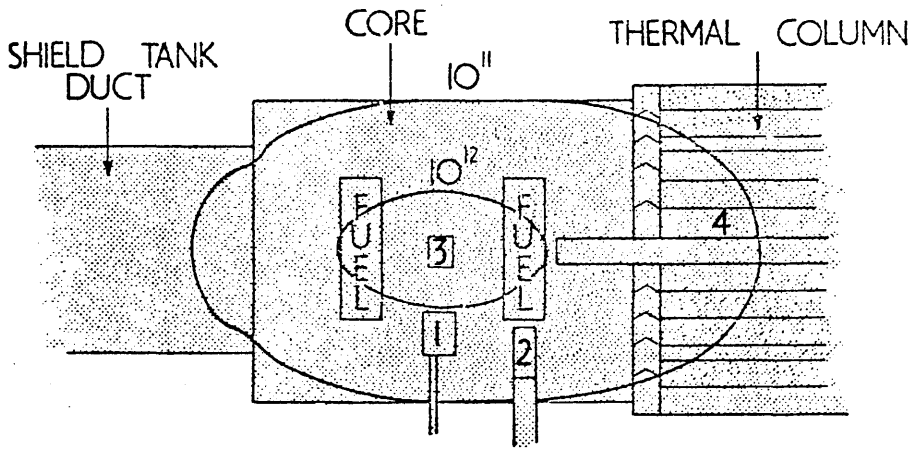
Neutrons are produced inside the reactor by the controlled nuclear fision chain reaction of, for example, Uranium-235. This fission reaction may be represented as:

$$^{235}U + n \xrightarrow{\text{via short lived } ^{236}U^*} \text{2 fission product nuclei} + 2 \text{ or } 3\ n + \gamma$$

The original reacting neutron has thermal energy. The $^{236}U^*$ is an excited short-lived intermediate isotope and the two fission products have atomic weights Z_1 and Z_2, where $72 \leq Z_1 \leq 117 \leq Z_2 \leq 161$. Using a moderator—commonly water—the fission produced high energy or epithermal neutrons are reduced to low energy thermal neutrons. This reduction is spread over a Boltzman distribution. Therefore, there will always be

FIGURE 1

IRRADIATION POSITIONS FOR ACTIVATION ANALYSIS IN THE UTR 100 REACTOR AT THE SCOTTISH RESEARCH REACTOR CENTRE.



			THERMAL FLUX AT 100 kW * NEUTRONS/CM²/SEC
1	SMALL RABBIT SYSTEM	inner end	$8{\cdot}4 \times 10^{11}$
		outer end	$7{\cdot}7 \times 10^{11}$
2	LARGE RABBIT SYSTEM	inner end	$7{\cdot}8 \times 10^{11}$
		outer end	$5{\cdot}1 \times 10^{11}$
3	CENTRAL VERTICAL STRINGER	——	$1{\cdot}4 \times 10^{12}$
4	REMOVEABLE STRINGERS IN THERMAL COLUMN	maximum	10^{12}
		minimum	10^{9}

*
MAXIMUM OUTPUT OF REACTOR = 300 KW

epithermal neutrons present in the spectrum of neutron energies. For example two of the reactors used at the Atomic Energy Research Establishment at Harwell, Didcot Berkshire the fluxes were quoted as shown below.

Table 2. Reactor Fluxes at Harwell

Reactor	"BEPO" (neutrons/cm^2/sec)	"DIDO"/"PLUTO" (neutrons/cm^2/sec)
Thermal neutrons	$10^{10} - 10^{12}$	$10^{10} - 5 \times 10^{12}$
Epithermal neutrons	$10^{9} - 10^{11}$	$10^{8} - 10^{9}$

The fission reaction is maintained with a supply of thermalized neutrons and kept at a controlled noncritical state by the use of control rods. These rods contain material such as cadmium or boron which absorb neutrons. The physical positioning of these rods with reference to the uranium fuel elements allowed the reaction to be controlled and shut down. An adequate description of the operation and type of reactor used is given by Rakovič (17).

Isotopes of the elements may be converted by neutron capture into an isotope of higher mass when placed within a thermal neutron flux inside the reactor. The nucleus of the element abosrbs a neutron, becomes excited to an unstable energy state, emits a "prompt" or immediate gamma photon and becomes an isotope of the original element with an increase of one in atomic weight. Isotopes so formed are radioactive in themselves and decay back to a stable isotope by the emission of radioactivity. A typical neutron capture

reaction is that for the element antimony, $^{123}_{51}Sb$, which by neutron capture becomes the radioactive $^{124}_{51}Sb$ isotope with the emission of a prompt gamma photon. The reaction is written as:

$$^{123}_{51}Sb + n \longrightarrow {}^{124}_{51}Sb + \gamma$$

and in shorthand notation as:

$$^{123}Sb(n,\gamma)^{124}Sb$$

Thermal neutron activation is the method most commonly applied as a means of sample irradiation. The term thermal neutron denotes that the energy of the neutron is low and corresponds to a neutron speed in the order of 2 metres/second.

Fast or epithermal neutrons react with certain elements to form radioactive products. In this case other nuclear reactions may take place, resulting in the formation of an isotope of a different element, i.e., if a neutron is absorbed and a proton given out then the atomic number of the target isotope drops by one. For example, there is the formation of $^{124}_{51}Sb$ from $^{124}_{52}Te$, as represented by the equation:

$$^{1}_{0}n + {}^{124}_{52}Te \longrightarrow {}^{124}_{51}Sb + {}^{1}_{1}P$$

The above is described as a (n,p) reaction. There are several other possibilities, one of which is important in the estimation of antimony in iodine-rich thyroid tissue. In this reaction an α particle is ejected with a resulting drop of 2 in atomic number and 3 in atomic weight. This so-called (n,α) reaction is as follows:

$$^{1}_{0}n + {}^{127}_{53}I \longrightarrow {}^{124}_{51}Sb + {}^{4}_{2}\alpha$$

Fast neutron reactions as described will always take place due to the more energetic neutrons present in the neutron flux in the reaction pile. Another example of interference from fast neutron reactions is in the manganese analysis of dried blood. This contains a significant proportion of iron in the haemoglobin; the formation of manganese, atomic number 25, from iron, atomic number 26, by (n,p) can contribute up to 20% of the recorded manganese radioactivity:

$$^{54}Fe(n,p)^{54}Mn$$

Growth and decay of radioactivity

During neutron irradiation the formation of a radioactive isotope can be shown to be given by:

$$D = \frac{\phi\, m\, N^{O}\, f\, \sigma\, (1 - 2^{-t/T})}{W} \qquad \text{Equation I}$$

where, D is the activity of the given element

ϕ is the neutron flux expressed in neutrons/cm^2/sec

m is the mass expressed in grams weight

N^{O} is Avagadro's number

f is the isotopic fraction of the given element

σ is the cross section absorption expressed in barns

t is the time of irradiation

T is the half-life of the isotope formed

W is the atomic weight of the element

As ϕ, m, N^{O}, f, σ, A are constants $D \propto (1 - 2^{-t/T})$

The saturation activity, (D satn), is achieved when the time of irradiation, $t \longrightarrow \infty$

$$\text{i.e.} \quad \text{D satn} = \frac{\phi \, m \, N^{o} \, f \, \sigma}{W}$$

It can be seen that where $t = T$,

$$D = \tfrac{1}{2} \cdot \frac{\phi \, m \, N^{o} \, f \, \sigma}{W} = \tfrac{1}{2} D \text{ satn}$$

i.e., after an irradiation time of one half-life half of the theoretical maximum activity will be obtained. It can be seen that an irradiation time of 7 half-lives provides over 99% of D satn. The period of activation was usually chosen to be one half-life. This was the most economical and often the most convenient irradiation time. Okada (18) discusses the selection of favourable times of irradiation and cooling.

After irradiation has ceased the activity of the irradiated element can be denoted by A_o and it can be shown that the activity decays exponentially as:

$$A_{t'} = A_o 2^{-t'/T} \qquad \text{Equation II}$$

where $A_{t'}$ is the activity after decay time t' and T is the half-life. By definition after decaying for time T, the activity is half the original level $\tfrac{1}{2} A_o$, as follows from Equation II.

Plotting such a decay activity against time shows an exponential fall and on a log linear scale presents a straight line.

The isotope may decay by the emission of either β rays, γ rays, X-rays, alpha particles or any combination. During the decay process there may be intermediate daughter products formed. From equations I and II it can be seen that the activity per gram of element is given by:

$$\frac{A_{t'}}{m} = \frac{\phi N^{o} f \sigma}{W} (1 - 2^{-t/T}) 2^{-t'/T}$$

The sensitivity of measurement, expressed as $\frac{A_{t'}}{m}$, is thus favoured by a large neutron flux ϕ, a high isotopic fraction f, a large cross section absorption σ, a reasonably long irradiation time t, a short as possible decay or cooling time t', and a small atomic weight W. In actual practice it is the cross section absorption coefficient of the element (a measure of the ability of the element to activate), which has the greatest order of range. This coefficient is thus of major importance in determining the suitability of an element for detection by activation. When an element is irradiated for one half-life and the activity counted within one half-life a minimum of 25% of the theoretical maximum activity is available for counting. For many of the elements assessed in the work carried out it was possible to keep within these limitations.

c) Processing

Equipment

Purely simple chemical processing was carried out. Thus the equipment required was that of a normal chemical laboratory. The centrifuges used had a head capacity of 4 x 50 ml tubes.

Materials

Chemicals used were of the "AnalaR" grade as made by B.D.H. Chemicals Ltd. 125 ml low-silicate tall form beakers were used for acid digestions. Specially made digestion tubes with 6" necks and 25 ml capacity, as described by Smith (19), were used in acid digestions for arsenic, mercury, etc. 50 ml boro-silicate centrifuge tubes were used for precipitation separations.

"Quickfit" glassware was used for the Gutzeit arsenic separation.

Other glassware was of the type normally available in a chemical laboratory.

Disposable Pasteur capillary pipettes were used for precipitate mounting on planchettes.

Procedures

These are fully described in the subsection (2.b) "Chemical Separation," page 49.

d) Counting

γ and β counting was employed.

Materials

1" flat edged aluminium or stainless steel planchettes were used to mount precipitates for both γ and β counting.

γ Counting

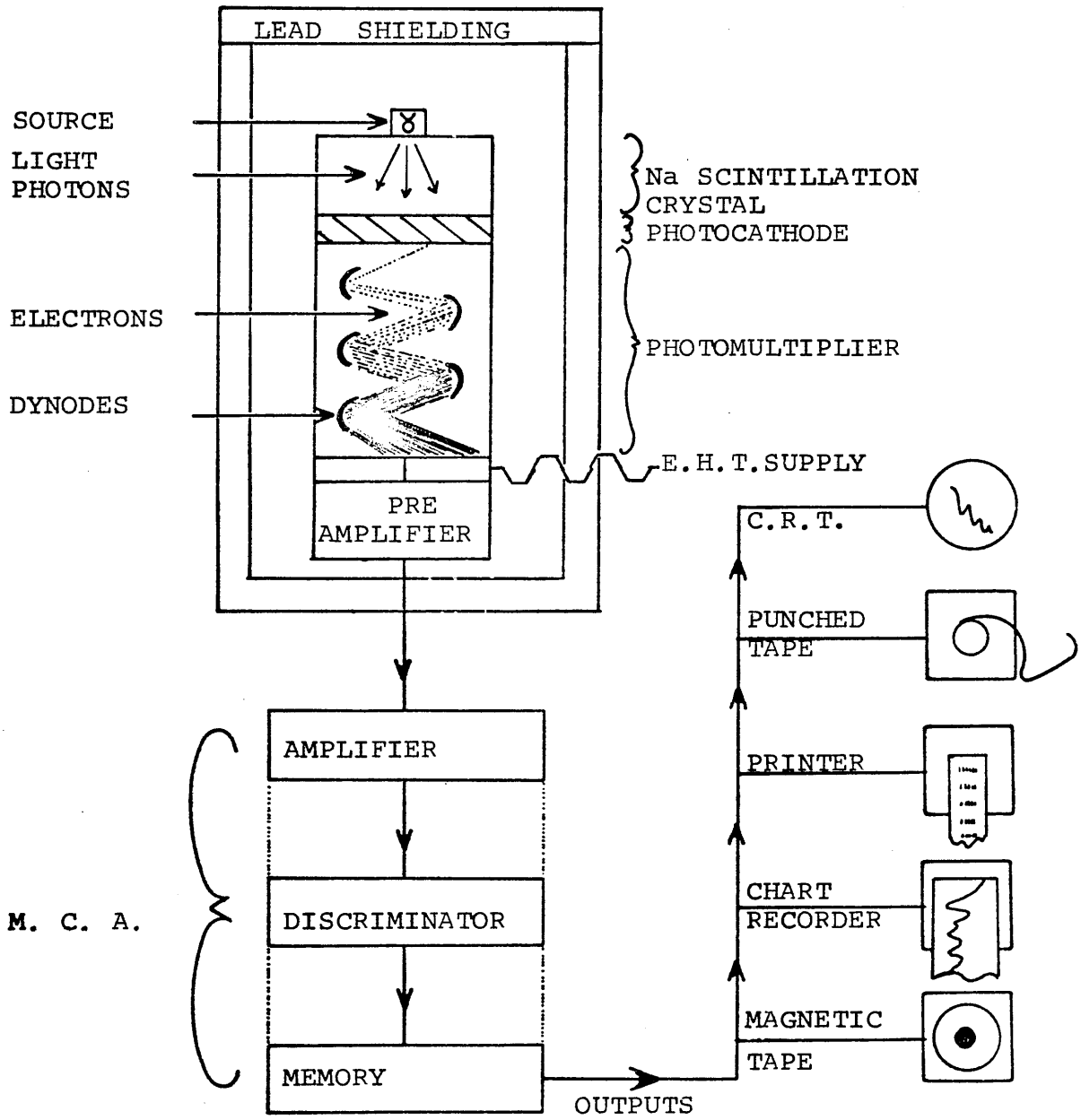
Equipment

γ counting was carried out using a 400 channel analyser (Intertechnique Ltd. type SA40B) connected to a photomultiplier and a 4" sodium iodide crystal of selected quality. Output was presented on a fast printer and spectrum storage was available on magnetic tape. The sodium iodide crystal/photomultiplier complex was mounted inside an aluminium casing. The sodium iodide crystal was encased in a film of grease as it is hydroscopic and the light transmitting efficiency would be impaired by exposure to moisture. The whole was placed behind lead shielding to help attenuate the background of external radiation from the surroundings and of cosmic origin. A diagram of the γ detection system used is shown in Figure 2.

Light photons produced within the crystal are transmitted through it. They are converted into photoelectrons by a photocathode placed at the bottom face of the crystal opposite the incident gamma radiation. An overall 10% of efficiency for photoelectron production is obtained. For approximately 10 to 20 light photons 1 photoelectron with an energy

FIGURE 2

γ COUNTING SYSTEM.


LEAD SHIELDING
SOURCE
LIGHT PHOTONS
ELECTRONS
DYNODES
Na SCINTILLATION CRYSTAL
PHOTOCATHODE
PHOTOMULTIPLIER
E.H.T.SUPPLY
PRE AMPLIFIER
C.R.T.
PUNCHED TAPE
PRINTER
CHART RECORDER
MAGNETIC TAPE
AMPLIFIER
DISCRIMINATOR
MEMORY
M. C. A.
OUTPUTS

1 to 2 eV is produced. These photoelectrons are amplified by passing through a photomultiplier.

An adequate description of γ detection using NaI crystal and a multichannel analysis is given by, for example, Neiler and Bell (20). The most important feature of the γ spectrum produced is the characteristic photoelectron peak or peaks of each γ emitting isotope.

Each planchette was γ counted by placing it manually in the centre on top of the crystal.

β Counting

Equipment

β counting was carried out using end window Geiger-Müller tubes, low background systems, and G-M tubes accepting 10 ml liquid samples in a surrounding cavity. The Geiger-Müller tubes were mounted in lead castles. Each castle incorporated a breach mechanism for planchette insertion. That of the low background counter was automatically operated and could accept 6 planchettes at one time. This facilitated decay following. The ancillary counting equipment was of modular construction.

The Geiger-Müller tube is one of the earliest radiation detection devices and its operation is well known. An adequate description is given, for example, by Price (21).

The Geiger-Müller tube was placed inside lead shielding to attenuate background radiation. The sample in the form of a chemical precipitate mounted on a circular metal

planchette was slid in and out from under the end window of the Geiger-Müller tube by use of a breach mechanism. In the case of arsenic analysis the final recovery was a liquid and a suitable Geiger-Müller tube (Mullard type M-6) was used. The background count rate for the type of end window Geiger-Müller tubes used was in the order of 0.3 counts per second. When it was desired to reduce the background further, for example when counting low activity samples, the low background Geiger-Müller tube was employed. This in principle consisted of two Geiger-Müller tubes, one surrounding the other. The electronics are arranged so that any pulse of the internal counter which is coincident with a pulse in the external counter is not registered. A β^- particle which pulses the inner counter only is registered on the attached scaler. This results in a significant reduction in the background count rate. A typical background level from such a system was found to be <0.03 counts per second, an improvement greater than one order over the simple single end window Geiger-Müller tube.

The output from the Geiger-Müller tubes of the types described above was amplified and fed into a suitable counting device. The period of counting could be varied as desired.

2. Separation techniques

The sample normally consists of a mixture of elements. A tissue sample consists of a host matrix of protein type structure with the elements carbon, hydrogen, oxygen and nitrogen and with subfractions of elements such as sodium, chlorine, phosphorous and trace quantities of others. All of these elements which can activate will do so as well as the element of interest. They too will therefore emit radiation that could interfere with the assessment of the element of interest. Therefore it is necessary to be able to separate out the radiation of the element of interest. This may be done in two ways, either by "Physical" or "Chemical" separation.

a) Physical separation

Physical separation takes place where it is possible to take the irradiated sample and count it in such a way that the activity assessed is peculiar to the element of interest. This may be done in several ways.

There is a simple method which requires neither chemical separation or mathematical separation of the gamma spectrum. It may be applied for elements whose half-lives are long (for example ^{65}Zn with a half-life of 245 days). The sample is irradiated for as long a period of time as is convenient and necessary. An irradiation time of a week is typical. The sample is then simply set aside for some time, up to six months or longer. After this "cooling" time the activity of

the short half-life isotopes formed during activation decay away. Only the activity associated with the long half-life isotopes remains. In the case of ^{65}Zn it is thus only necessary to count the activity associated with it and this may be done very satisfactorily by counting over the gamma photopeak at 1.11 MeV using a multichannel analyser.

The advantage of such a technique is the simplicity involved. The disadvantages are that the overall sensitivity is lower than the maximum that might be achieved, hence inordinately long counting times could be required. It is not always convenient to have a considerable time pass before the analysis is completed. However, where the disadvantage involved is not significant such a method is useful, particularly so if a large number of samples are being analysed.

When biological samples are irradiated the large amounts of sodium, phosphorous, etc., present activate readily and long cooling times are required to be rid of their interference. However, for other samples of non-organic origin such as rock, glass, paint, etc., where there is present only a few discrete trace elements capable of activation, long cooling times are not necessarily required. In these particular cases counting over the appropriate photopeak in the gamma spectrum produced by the activated sample may be all that is required to estimate the element of interest.

Where an element is being estimated by gamma spectrum assessment the counts under its characteristic photopeak or photopeaks are considered. Covell (22) described the simplest

and most obvious method of estimating the counts associated with each photopeak. Essentially Covell described how photopeak counts are assessed by adding up the counts in every channel associated with the photopeak. A direct arithmetic summation of the counts is made.

Where there is not present any outside contribution to the counts under the photopeak the Covell method is accurate and works well. It is the most straightforward addition method and is used for expediency. However, where as is often the case when no chemical separation has taken place, the shape of the photopeak is distorted, Covell addition will introduce error. Photopeak distortion may be brought about when it is part of a composite gamma spectrum. The photopeak may lie on a Bremstrahlung curve, on a Compton edge, appear as a shoulder on top of another photopeak, or otherwise be distorted.

In order to circumvent this difficulty more sophisticated photopeak summation techniques have been evolved. For example, Liebcher and Smith (23) described how the photopeak in the gamma spectra output from a sodium iodide crystal is of Gaussian form and hence the area between the two points of inflexion on the photopeak is proportional to the total photopeak area. Liebcher and Smith used a computer program to carry out the otherwise lengthy calculations involved and smoothed the gamma spectra output. The points of inflexion were found and the area of that fraction of the

photopeak between them calculated. By this method of photopeak summation the effect of interference from sources other than the element of interest was removed.

Where gamma counting is employed and it is known that there is no interference over the portion of the gamma spectrum then Covell's method is suitable. Where chemical separation has taken place and the processed sample is radiochemically pure then the simple Covell summation is adequate, the need for a more complex gamma estimation is obviated. In fact the counting could be "open window" in which the entire gamma spectrum is counted.

Another approach to obtaining separation from a composite gamma spectrum is that of spectrum stripping. If the identity is known of the gamma ray sources which go to make up the spectrum, then it is possible to successively subtract or "strip out" the spectrum of each source from the whole. Consider for example the composite gamma spectrum of elements A and B as naSA + nb SB, where na + nb are the sizes of spectra SA and SB. The spectra of the single element B is obtained under similar conditions and is subtracted nb times from the composite spectra leaving the desired part of the composite spectra na SA. The factor nb is determined empirically. This stripping technique may be extrapolated to strip out more than one source.

Such stripping techniques are limited to certain particular instances where the empirical determinations involved

are straightforward. For example, if a gamma spectrum was known to consist of the photopeak of ^{65}Zn at 1.11 MeV superimposed over one of the twin photopeaks of ^{59}Fe at 1.10 MeV, then the removal of the ^{59}Fe contribution would be indicated by the disappearance of the other twin photopeak at 1.24 MeV. The ^{65}Zn photopeak at 1.11 MeV would then remain for subsequent totalling.

The actual method of stripping may be carried out in one of three ways:

(1) Mathematical subtraction is made.

(2) The composite spectrum is placed in the memory unit of the multichannel analyser. Counting of each source to be removed from the composite spectrum is carried out in a negative sense and such counting is stopped when it is seen by inspection that the unwanted portion of the spectrum has been removed.

(3) This method is an extension of the second. The spectra of the sources are stored on magnetic tape and subtracted out from the composite spectrum as desired.

In the work carried out, physical separation techniques were not employed; all separation was done chemically.

b) Chemical separation

The element of interest was isolated by chemical separation from the irradiated sample. Once isolated, its radioactivity was assessed free of interference from any of the other activated elements in the sample.

The methods employed in chemical separation generally fall into a sequence of stages, digestion, chemical processing and presentation for counting as are outlined below.

Sample digestion

In this the first step, the irradiated sample of biological origin and therefore organic, was reduced to an inorganic ionic state compatible to subsequent chemical processing. This was accomplished by dissolving the sample in a mineral acid and heating. Concentrated sulphuric acid which was used boils at 290^{o} C. Carbon thus formed (seen as charring) was oxidised away by the addition of concentrated nitric acid. Hard material such as bone and teeth may be thus successfully digested. Where sulphuric acid digestion interferes with subsequent chemical processing nitric acid alone can be used. However, nitric acid has a low boiling point of 86^{o} C and not all materials are rendered completely inorganic on boiling in it. It was often necessary to take the acid digestion mixture to dryness and add further nitric acid in the form of drops which by superheating completely reduce the sample to an inorganic form. Such digestions were carried out with the sample placed in conically shaped beakers or reflux tubes to avoid splashing. Controlled heat was applied using an electrical hot plate and acid fumes were exhausted to the atmosphere. The whole digestion process took place in a fume cupboard. All of the samples

analysed using a post-irradiation chemical separation were subjected to an acid digestion process. If the digested sample was taken to dryness, and a clear non-charred residue remained then the sample could be seen to have been rendered inorganic. All of the digestion processes in the work carried out were acid digestion.

The amount of the trace element of interest in the sample was negligible and therefore insufficient for any normal macro chemical processing. Therefore, a known non-radioactive macro amount of the element was added to the sample after irradiation. The amount introduced was in the order of 10/20 mg. It was referred to as a "carrier." This carrier equilibrated with the activated trace quantity of element present in the sample and provided sufficient bulk of the element to allow normal chemical procedures to be carried out. It was usual to introduce the carrier prior to the digestion step. Thus any possible losses of the radioactive trace element during the acid due to volatilisation, etc., would be compensated by associated losses of the carrier. For non-volatile elements, for example barium, it was sometimes found to be expedient to add the carrier after the digestion step.

Precipitation separations

If element X was to be separated out from element Y in a solution containing elements X and Y then it was better to precipitate down first element Y leaving X in the solution

to be removed later. If the reverse procedure was carried out the precipitate X could contain significant co-precipitated quantities of Y and other interfering elements. Where the unwanted Y is first precipitated the traces of X which might also co-precipitate are insignificant and the fact that other interfering elements might be co-precipitated is of advantage. When X is to be precipitated out it is often useful to introduce into the solution small amounts of "carriers" for other elements which might otherwise co-precipitate with X. These so-called "hold-back carriers" increase the amount of the respective element beyond trace levels in the solution inhibiting co-precipitation. Hence, X may be precipitated out with reduced contamination. The final form of X prepared for counting was termed the recovery. It was usual for X to be in the form of a metal organic compound. For example, copper was counted as copper quinaldate. A metal organic compound had these advantages: it was usually a bulky precipitate suitable for easy mounting on a planchette; it provided a recovery free from inorganic contamination which could occur if an inorganic precipitate was chosen. The final recovery was transferred as a slurry by Pasteur pipette onto a metal planchette using as suspension media a volatile organic liquid, typically acetone, isopropyl alcohol, etc. The planchettes were approximately 1" in diameter with lipped edges and were made of aluminium. Where the recovery was a mercury salt it was necessary to use stainless steel planchettes.

Ion exchange was a technique also employed for element separation using glass columns filled with Amberlite resin IRA-400 (Cl) analytical grade 50 mesh. The disadvantages of this method were the slowness in operation and that separation was not complete. Sometimes 25% losses were thus incurred. The advantages lay in that multi-element separation could take place in the ion exchange column allowing more than one element to be distructively estimated from a single sample. For example, such a technique was used by Livingstone et al. (24).

3. Interpretation of results

For an absolute determination of the trace element level in each sample from the measured count rate a number of experimental parameters would be required to be known. These include the integrated neutron flux and efficiency of counting. These two parameters are difficult to accurately assess. The method was greatly simplified by the use of a comparative technique wherein a known amount of element, referred to as the standard, was irradiated, processed and counted along with the sample. The corrected count rate of the sample was compared with that of the standard to find the trace element level in the sample. The concentration of the trace element in the sample was given by:

Equation A

$$\text{Concentration in p.p.m.} = \frac{S_{cr} \cdot STD_{rec} \cdot STD_{dc} \cdot STD_{dt} \cdot STD_{\mu g} \cdot Diln}{S_{rec} \cdot S_{dc} \cdot S_{dt} \cdot STD_{cr} \cdot S_{gms}}$$

where, S_{cr} is the count rate for the sample,

STD_{cr} is the count rate for the standard,

S_{rec} is the chemical recovery of the sample,

STD_{rec} is the chemical recovery of the standard,

S_{dc} is the correction factor for sample radiation decay,

STD_{dc} is the correction factor for standard radiation decay,

S_{dt} is the dead time correction factor for the sample count rate,

STD_{dt} is the dead time correction factor for the standard count rate,

$STD_{\mu g}$ is the known weight of the standard expressed in micrograms,

Diln is the dilution factor of the standard,

S_{gms} is the sample weight expressed in grams.

S_{cr} and STD_{cr} were determined as described earlier. S_{rec} and STD_{rec} were determined by weighing by the method of difference the chemical recovery mounted on the metal planchette. Where a chemical separation was not involved or where the recovery was known to be 100% complete (as was the case in the separation technique used for arsenic) S_{rec} and STD_{rec} are replaced in the equation by 1. The analysis method being comparative it was necessary that count rate determinations be made at the same time. However, only a single sample could be counted at any one time. Between the counting of the first and last sample progressive radioactive decay would occur and the count rates reduce. Therefore, it was normal to convert the recorded count rates to their equivalent at an arbitrary, but conveniently, chosen time. The times at which each count rate determination was made were noted. Knowing the decay time between the arbitrary reference time and the half-life of the element, the decay correction factor, S_{dc}, for each sample and STD_{dc} for the standard could be calculated.

With either of the two counting systems used, i.e. a Geiger-Müller tube for β detection and a sodium iodide scintillator for γ detection the following dead time effect occurs. Immediately after a count is recorded the device is unreceptive to further counting for a short space of time. This dead time was automatically made good using the Intertechnique multi-channel analysis type SA40B for γ assessment and thus the dead time correction factors S_{dt} and STD_{dt} could be removed from the equation. However, for β detection using a Geiger-Müller tube the dead time loss was adjusted electronically to a known value, normally 200 μsecs. Thus the correction factor was given as:

$$S_{dt} = (1 - S_{cr} \cdot 200 \cdot 10^{-6})$$

For count rates less than 10 counts per second the significance of the above correction factor was negligible.

To minimise contamination and avoid dilution effects the weight normally chosen of an element for irradiation as a standard was in the order of 10 mg. After irradiation the standard was dissolved and made up to 100 ml or 10,000 ml. 1 ml of this was processed and counted as were the samples. The effective standard dilution was 10^{-3} or 10^{-4}, respectively, and this was expressed as the factor Diln in the equation A.

a) Sensitivity

As can be seen from equation A, overall sensitivity is favoured by both a large sample weight, S_{gms}, and a low value for the ratio $STD_{\mu g}/STD_{cr}$. In practical terms the sample weight was limited between 10 to 100 mg. 30 mg was a typical sample weight. Large sample weights limited the number of samples that could be packaged for one irradiation. Excessive sample size was found to introduce problems in chemical processing. Very small sample weights were sometimes used. For example, less than 100 μg of hair was satisfactorily analysed for arsenic.

The ratio $STD_{\mu g}/STD_{cr}$ can be shown to be proportional to

$$W/\phi N^{o} f \sigma (1 - 2^{-t/T}) 2^{-t'/T}$$

Hence, the invariable parameters of an element that favour the sensitivity of detection are a low atomic weight W and a large value for isotopic fraction f and absorption cross section σ. Favourable variable parameters are as large a neutron flux as possible (the highest used was 9×10^{12} n/cm^2/sec); long irradiation time t (normally one half-life) and short cooling time t' (normally within one or two half-lives). The maximum irradiation time was one week at U.K.A.E.A. Harwell due to both practical and financial reasons. Thus, if the half-life T of the isotope formed was greater there was a loss in overall sensitivity. An element with a large activation cross section, for example, arsenic-76 with a cross section of 4.2 barns, can be detected with greater sensitivity

than an element with a smaller activation cross section, such as barium-139 with a cross section of 0.36 barns.

b) Errors

It can be seen from equation A that the overall error is the arithmatic sum of any error in the parameter on the right hand side of the equation. With normal laboratory care any error in the actual weighing of S_{rec} and S_{gms} was insignificant. Any error in determining S_{gms} was associated with the consistency of dried weight. During unpacking and transferring the sample after irradiation small losses could occur thus introducing error in S_{gms}. After long periods of irradiation a small weight loss was occasionally found. Error in S_{gms} was considered to be with 1%.

The main error in the weighed values of S_{rec} and STD_{rec} was associated with the accuracy of the addition of chemical carrier. With the 1 ml safety pipette normally used this error was within 2%.

The major source of error was involved in the estimation of the count rates S_{cr} and STD_{cr}. The values could be influenced in the following ways:

i) Activation errors

As described by Bowen and Gibbons (25), possible errors, quoted up to 5%, can be introduced during activation inside the reactor. These are caused, for example, by packaging attenuation of neutrons and self-shielding of samples of

high cross section absorption. Self-shielding was not significant for trace elements in a biological matrix and for the small amount of chemical standards used.

ii) Contamination

If the separation of unwanted activity was not complete the measured count rate was inaccurate to an amount dependent on the degree of contamination. The separation techniques used had been designed to produce a pure radiochemical separation. Contamination error from this source was therefore not significant. Purity of radiochemical or physical separation was demonstrated by decay following the sample.

Contamination by unwanted isotope formation by (n,p) and (n,γ) reactions was not normally significant for biological samples where only traces of possible interfering elements were present.

iii) Statistical accuracy of the measured count rate

Where c was the total number of individual counts recorded the statistical error involved was $\pm \sqrt{c}$. If c were 100 then this statistical error would be 10%; if c were 1000 the error would be 3.3%, a factor of 3 less. This counting error was intrinsic, but with a large number of recorded counts it was small.

iv) Counting geometry

If the radioactive samples were not placed in the same position relative to the counter a varying fraction of the total activity would be recorded. The apparatus used was designed to provide constant counting geometry. Error was caused by variation in the mounting of the chemical recovery on each planchette. Such error, however, was small.

v) Source self-absorption

Any error due to this effect is only significant for lower energy β emitters. By having their samples each on a similar planchette the effect of self-absorption was minimised.

c) Summary of the major problems arising in the analytical techniques employed

The greatest source of nonreducible error involved the handling of the tissue samples for analysis. Great care was necessary to ensure that negligible environmental contamination could occur during sample handling, drying, packaging and so on prior to irradiation. After irradiation no such special care was necessary. The possibility of contamination was reduced by carrying out such sample handling as was necessary using materials with low contamination influence, as described earlier (plastics, aluminium foil, etc.). The use, when necessary, of surgical blades for cutting was considered acceptable as no significant associated contamination was found to occur.

Unpackaging of samples, in particular the removal of liquid samples such as urine from silica vials was sometimes difficult. Some samples, for example hair, were difficult to remove from polythene wrappings after irradiation due to electrostatic attraction. A practical problem which was not initially apparent was that of sample deterioration during irradiation. During fission in the reactor pile heat is produced. With the reactors used temperatures in the irradiation areas of 80^{o} C were common. The resultant heating caused certain samples to change form. In particular, glandular tissues, although dried to constant weight, would exude oils and become tacky. This caused removal from their wrappings to be difficult. Other tissues, for example lung, under the combined action of heat and neutron bombardment became powdery and thus care was needed during their unpackaging. Small swabs of damp tissue paper gripped between forceps were sometimes found to be a useful aid in the unpackaging of these samples. Such problems were only significant when the irradiation periods were long, i.e. in excess of 24 hours.

Conclusion

As can be seen in the preceding pages, the underlying principles involved in the technique of neutron activation analysis are simple and straightforward. In many applications it can be both extremely sensitive and adequately accurate for assays at trace level. However, useful as it is as a technique, there are incorporated certain difficulties—such as the requirement of reactor facilities—and disadvantages: not all elements may be so assessed, no distinction can be made of the chemical form in which the element of interest is present. Hence, the technique may be restricted in its range of application. Where the technique can be usefully used it can provide a uniquely elegant means of trace element assay.

The actual methods that were used and their applications are described in the following chapters.

CHAPTER 3

Separation Methods

SEPARATION METHODS

Neutron activation analysis has expanded rapidly over the past 25 years. As a result, most of the possible elements have several methods available for their estimation by this technique (11). However, it was felt that for this study some methods required modification and some required development from the beginning if they were to be suitable. Suitability was judged on the basis of simplicity of the method (one of the great advantages of nuclear activation analysis) and applicability to organic samples. It was also necessary that as many samples as possible could be analysed in one day. The target set for this was 50 but of course this was not always possible.

Bearing these points in mind, established neutron activation analysis methods were used where possible. However, in some cases it was found that certain modifications improved their convenience of application. Finally, when it was considered that no satisfactory methods of analysis were to be found, new methods were constructed and used.

All of the analysis methods used for the trace elements investigated are described below. They are grouped into three categories. Group (i) consists of methods as described in the literature and used as so described with occasional alteration of detail. Group (ii) consists of methods described in the literature but modified where necessary to suit better their required application. Group (iii) consists of new methods.

Group (i)

This includes the elements copper, mercury and cadmium.

Copper

The separation procedure was closely based on that described by Nixon and Smith (26). Copper has an activation cross section of 3.0 barns and the ^{64}Cu isotope formed by $^{63}Cu(n,\gamma)^{64}Cu$, has a half-life of 12.84 hours. Irradiation was for one day at Harwell at a flux of 1.2×10^{12} n/cm^2/sec. ^{64}Cu is both a β and γ emitter. The final precipitate of copper quinaldate was β counted using an end window Geiger-Müller tube. The sensitivity was 0.001 μg. The method is summarised stepwise as follows:

1. The irradiated samples were unwrapped and placed in 30 ml conical beakers.
2. 10 Drops of 16 M HNO_3 were used to digest each sample. More 16 M HNO_3 was used if required to complete the digestion.
3. 10 mg of Cu carrier was added (1 ml of 1 mg/ml $CuSO_4$ solution).
4. 1 Drop of each of the following carrier solutions was added: 10% w/v $Co(NO_3)_2$; 10% w/v $Mn(NO_3)_2$; 10% w/v $NH_4H_2PO_4$.
5. The whole was transferred with washings to 50 ml centrifuge tubes.
6. 3 ml of 10% w/v Na_2SO_3 was added. (If any precipitate formed it was dissolved by adding HNO_3).
7. 1 ml of 10% w/v KSCN was added.

8. Each tube was placed in a water bath and allowed to heat.
9. The precipitate formed was spun down and washed twice with hot water.
10. The precipitate was dissolved in ½ml of 16 M HNO_3 (added quickly).
11. The following carriers were added: 2 drops of 10% w/v $NH_4H_2PO_4$; 10 drops of 10% w/v $FeCl_3$. The whole was diluted, thus also mixing the solution, and 3 drops of 10% $CaCl_2$ were added.
12. The solution was neutralised with 0.88 NH_4OH, which was initially added slowly, then in excess.
13. 3 ml of 10% w/v Na_2CO_3 was added.
14. Each tube was placed in a water bath to heat.
15. Each supernate was filtered into a fresh tube and the precipitate rejected.
16. 18 M acetic acid was added until the solution turned pale blue.
17. ½ ml of 16 M HNO_3 was added and steps 6 to 10 repeated.
18. The solution was neutralised with 5% w/v NaOH until a faint precipitate appeared.
19. 4 Drops of 16 M HNO_3 were added.
20. Each tube was placed in a water bath and allowed to heat.
21. 4 ml of 2% w/v quinaldic acid solution was added.
22. The copper quinaldate precipiate was washed with water, acetone and transferred as an acetone slurry to aluminium planchettes and dried under I.R. lamps.

23. Each recovery was counted and weighed.

Standard. A known amount of approximately 0.1 ml of a 10 mg Cu/ml $Cu(NO_3)_2$ solution was irradiated in a silica vial along with the samples. It was diluted $\times 10^{-4}$ and processed and counted as a quinaldate.

Mercury

For mercury analysis the chemical separation technique was closely based on that described by Smith (27). Naturally occurring mercury forms two isotopes on thermal neutron activation, ^{197}Hg (t ½ - 65 hours) and ^{203}Hg (t ½ - 47 days).

$$^{196}Hg\ (n,\gamma)\ ^{197}Hg \qquad ^{196}Hg(n,\gamma)\ ^{197m}Hg \xrightarrow{I.T.} {}^{197}Hg$$

$$^{202}Hg\ (n,\gamma)\ ^{203}Hg$$

The activation cross section is favourably large for ^{197}Hg, 4.5 barns, and for ^{203}Hg, 1.13 barns. Smith irradiated his samples for one week and counted over the 279 KeV γ photopeak of the ^{203}Hg isotope. The final precipitate was a copper ethylenediamine-mercury iodide complex. A higher count rate was achieved by counting instead over the low energy 77 KeV γ photopeak of the ^{197}Hg isotope. Irradiations were normally for three days (at a neutron flux of 2×10^{12} n/cm^2/sec). The sensitivity obtained was 0.003 µg.

The method is summarised stepwise as follows:

1. The irradiated samples were unpacked and placed inside 25 ml conical-bottomed flasks with 6" necks.
2. 10 mg of mercury carrier was added to each (1 ml of 10 mg Hg/ml as $HgCl_2$ solution).
3. 1 ml of 16 M HNO_3 and 1 ml of 18 M H_2SO_4 were added to digest each sample and heating was continued until all brown fumes disappeared. If any charring remained it was removed with a few drops of 16 M HNO_3.
4. The flasks were allowed to cool and the contents rinsed

into 50 ml centrifuge tubes.

5. Each solution was neutralised with 40% w/v NaOH (using universal indicator paper).
6. The tubes were heated in a water bath and 2 ml of 1% w/v ascorbic acid added to reduce the mercury to form a black metal precipitate.
7. The mercury precipitate was spun down and washed, once with water and once with acetone.
8. Each tube was placed in the water bath to dry off any acetone remaining and ½ ml of 16 N HNO_3 added to dissolve the mercury.
9. The whole was diluted and ½ ml of 10% w/v $AgNO_3$ added.
10. 2 ml of 10% w/v NaI was added to precipitate yellow AgI.
11. The precipitate was spun down and rejected, the supernate being filtered into fresh centrifuge tubes.
12. The solutions were neutralised with a few drops of 0.88 NH_4OH (using universal indicator paper).
13. 3 ml of copper ethylenediamine complex were added.
14. The precipitate formed was spun down, washed twice with cold water, once with isopropanol and transferred as an isopropanol slurry onto stainless steel planchettes.
15. Each recovery was counted and weighed.

Copper Complex

1 part 10% w/v $CuSO_4$

10 parts 10% w/v 1:2 - ethylenediamine

Standard

The silica capillary tube containing the irradiated Hg standard was placed in a 100 ml beaker and covered with a few ml of 16 N HNO_3. The tube was ground up using a glass rod and all the Hg dissolved by applying very gentle heat. The contents were rinsed thoroughly into a 1 litre flask which was made up to the mark. 1 ml of this plus 1 ml of carrier was added to a centrifuge tube, 2 ml of 10% w/v NaI added, and steps 13 onwards followed.

Cadmium

The radiochemical separation of cadmium was carried out as described by Livingston et al. (24). As the method involved multielement extraction using an ion exchange column, the copper, zinc and mercury fractions were run to waste and only the cadmium fraction was kept.

The isotopes formed by natural cadmium undergoing neutron irradiation are ^{115m}Cd, ($t_{½}$ - 43 days), ^{115}Cd ($t_{½}$ - 23 days), ^{117}Cd ($t_{½}$ - 2.9 hours) with activation cross sections of 0.04, 0.32, 0.11 barns, respectively. The capture reactions are:

$$^{114}Cd\ (n,\gamma)\ ^{115m}Cd$$

$$^{114}Cd\ (n,\gamma)\ ^{115}Cd$$

$$^{116}Cd\ (n,\gamma)\ ^{117}Cd$$

The cadmium isotopes were both β and γ emitters. Livingston et al. β counted the ^{115}Cd and ^{117}Cd isotopes.

Irradiations of one week were carried out at Harwell at the highest neutron fluxes available at the time. These ranged from 1.2 to 5 x 10^{12} $n/cm^2/sec$.

It was convenient to irradiate and process a fully packed aluminium can containing 40 samples or more. The separation by ion exchange was simple to carry out but was slow and therefore took several days or more to complete. Decay following of the samples even as long as one month after irradiation showed both that they were radiochemically pure, decaying with the half-life (43 days) of the ^{115m}Cd isotope, and that

it was quite possible to successfully count after such a long cooling period. β counting was carried out using the low background counter. Counting was normally for one hour per sample and took place two to four weeks after irradiation when all processing was complete. By this time only the activity of the ^{115m}Cd isotope was significant. As may be surmised from the above the final count rates were low, typically five times a background of 0.03 counts/second.

It was not therefore found disadvantageous to lose the greater activity of the shorter lived ^{115}Cd isotope to enable a large number of samples to be analysed in one bath, counting the ^{115m}Cd isotope ($t_{½}$ - 43 days).

It was found necessary to wash the precipitated cadmium reineckate more thoroughly than Livingston et al. indicated. Better recoveries were obtained if the ion exchange resin (Amberlite IRA-400 (Cl) Analytical Grade) was changed from time to time. The sensitivity was 0.001 μg. The method used is given stepwise below:

1. The irradiated samples were unwrapped and placed inside 25 ml conical-bottomed flasks with 6" necks.
2. 10 mg of cadmium carrier was added to each flask (1 ml of 10 mg Cd/ml $CdCl_2$ solution).
3. Approximate amounts of 10 mg of copper, zinc and mercury carriers were added as $CuSO_4$, $Zn(CH_3COOH)$ and $HgCl_2$ solutions.
4. 1 ml of 16 M HNO_3 and 1 ml of 18 M H_2SO_4 were added and the samples digested.

5. When all the nitric acid had boiled off and the flasks allowed to cool they were filled with 0.5 M HCl and the contents transferred to reservoirs above the ion exchange columns.
6. The solutions were run through each column at a flow rate around 3 ml/minute.
7. Each column was washed with 20 ml of 0.12 M HCl containing 10% w/v NaCl.
8. 40 ml of 2 N NaOH containing 2% w/v NaCl was run through each column at 3 ml/minute.
9. The cadmium was eluted using 50 ml of 1 M HNO_3 at a flow rate of 3 ml/minute; the first and last 10 ml portions collected here from the column were discarded. Only the 10-40 ml portions (containing 90% of the cadmium) were collected in 50 ml centrifuge tubes.
10. If the columns were to be re-used, the residual mercury was stripped with 75 ml of 5% w/v ethylenediamine solution.
11. Excess 5 M NaOH was added to make each solution alkaline.
12. The precipitated cadmium hydroxide was spun down, taken up in 1 ml 12 M HCl and diluted to 20 ml.
13. 5 ml of 5% thiourea solution was added followed by 8 ml of saturated ammonium reineckate containing 1% urea.
14. The precipitated cadmium reineckate, $Cd[SC(NH_2)_2][Cr(NH_2)_2(SCN)_4]_2$, was washed twice with each of the following: 1% thiourea solution, water and ethanol, and transferred as an ethanol slurry onto weighed aluminium planchettes to be dried under I.R. lamps.

15. Each recovery was counted and weighed.

Standard

Approximately 1 mg of solid "Specpure" CdO was weighed out inside a silica vial and irradiated alongside the samples. The contents were eluted out and made up to 1 litre. 10 ml of this was made up to 100 ml and 1 ml of this (a 10^{-4} dilution) transferred to a centrifuge tube. 1 ml of cadmium carrier was added, the whole diluted to 30 ml and steps 11 onwards followed.

Group (ii)

This includes the elements arsenic, gold, manganese, and zinc.

Arsenic

Arsenic activates readily to form the radioactive ^{76}As isotope from the stable ^{75}As; $^{75}As\ (n,\gamma)\ ^{76}As$, with an activation cross section of 4.2 barns. The sole method for arsenic analysis used was that of Smith (19). This elegant method enabled upwards of 100 samples to be analysed at any one time. As it was often of practical convenience to carry out at the same time analysis of other elements (e.g. copper) for other tissue samples irradiated alongside those for arsenic analysis, the average number of samples processed for arsenic analysis was around 30.

Smith describes a nitric-sulphuric acid sample digestion with a Gutzeit separation followed by β counting in a Geiger-Müller counter for liquid samples. The arsine gas was trapped in a mercuric chloride solution. Two small modifications to this separation method were made, as follows.

Smith used 8 ml of a mixture of 5 parts by volume concentrated (16 M) nitric acid and 3 parts concentrated (18 M) sulphuric acid for wet digestion of each sample. It was found that such a large acid volume was not always necessary. Instead 2 ml of concentrated sulphuric acid were added. The subsequent charred solution was cleared by the dropwise addition of concentrated nitric acid. If digestion was not complete

the process was repeated, but this was found seldom necessary. It was found that whereas it could take up to 1 hour to boil off all of the nitric acid from the 8 ml mixture, each digestion could now be completed in 20 minutes with a nitric acid free solution remaining. No nitric acid should remain, otherwise subsequent interference to the method would be caused by the formation of iodine by the oxidation of sodium iodide.

It was established that arsenic was not lost during the modified digestion procedure. A series of samples of inactive tissue to which 1 ml of a ^{76}As standard solution of known count rate was added, were so digested. Variations between final and original corrected count rates were within 1%.

Smith took his final counting solution with washings and made it up to 20 ml and counted 10 ml which was the capacity of the M-6 Geiger-Müller counter for liquid samples. With careful volume control and rinsing it was found possible to keep the total solution volume to within 10 ml and thus obviate the discarding of half the active solution.

A summary of the method, including the modifications made, is written below:

1. The irradiated and unwrapped samples were placed in the digestion tubes.
2. 2 ml of 18 M H_2SO_4 were added, heat applied, and charring cleared with the dropwise addition of 16 M HNO_3.
3. Heating was continued until HNO_3 was removed.

4. The digested samples were washed out into a 200 ml round-bottomed flask.
5. 4 ml 12 M HCl and 2 ml 18 M H_2SO_4 were added.
6. 10 µg As carrier were added.
7. 5 ml of 15% (w/v) NaI and 0.4 ml of 40% (w/v) stannous chloride in 6 M HCl were added.
8. The whole was diluted to about 150 ml, the flask placed in the boiling bath for 5 mins and the glassware for Gutzeit separation connected up.
9. 10 gms of 16 to 22 mesh zinc pellets were added and the reaction allowed to continue for 15 minutes.
10. Arsenic in the form of arsine was removed by passing through a solution of 1 ml of 1.6% (w/v) $HgCl_2$ in a trap after H_2S had been removed by allowing the gas to pass through a cotton wool filter impregnated with lead acetate.
11. 5 ml of 0.001 N iodine in 40% (w/v) NaI were added to the solution in the trap, the contents washed into a test tube and made up to 10 ml.
12. Using a M-6 Geiger-Müller tube for liquid samples, the activity of the contents of the test tube was counted.

Smith in 1959 described the wrapping of his samples and a solid arsenic standard in polythene. However under the irradiation conditions used, typically 24 hours at a neutron flux of 4×10^{12} n/cm^2/sec, polythene wrappings partially melted or became sticky. Hence aluminium foil for wrapping and a silica vial for a liquid arsenic standard were used.

Standard

Approximately 0.1 ml of a solution of 1 mg As/ml in a silica vial was used. The contents of the vial were eluted with a sodium hydroxide solution and made up to 1,000 ml. 10 ml of this was made up to 100 ml and 10 ml of this counted—an effective dilution of 10^{-4}.

To avoid prolonged refluxing of the digestion mixture with possible losses, it was ensured both that the digestion tubes were of the shape and size of those described by Smith and that the digestion stand was of a type that heated both the neck and bottom of each tube.

The sensitivity obtained was 5×10^{-5} μg.

Conclusion

The modification made improved the method. By reducing the time spent initially digesting the samples, in particular where 20 or more were being processed at one time, the final counting of the short-lived ^{76}As took place sooner and hence sensitivity was increased. Counting all of the recovery instead of half, immediately doubled the sensitivity, and where appropriate, counting times could be reduced. The overall effect of the modifications made was to reduce the average time spent on each individual sample analysis.

Gold

Gold is very suitable for activation analysis. The ^{198}Au isotope has a half-life of 2.69 days and an activation cross section of 98 barns and is an energetic γ and β emitter. The capture reaction is $^{197}(n,\gamma)\ ^{198}Au$.

The method selected was based on that described by Lenihan and Thomson (28).

Irradiation was at Harwell at a neutron flux of 1.2×10^{12} n/cm^2/sec for 3 days. Counting over the 0.411 MeV γ photopeak was carried out using the Intertechnique Multichannel Analyser.

The method required an extraction of gold into ethyl acetate and counting as a metal. Following the procedures described the final recoveries obtained were low, approximately 30%.

It was found that the acidity of the final aqueous solution from which the gold was precipitated by an SO_2 flow was critical. The carrier used, 10 mg of gold in a gold chloride solution, was made up to 25 ml. The recoveries of gold found with increasing amounts of hydrochloric acid added are shown below:

Table 3. Gold Recoveries

Acid Level	Recovery of Gold
No HCl added (pH = 2)	81%
2.5 M solution	29%
5.0 M solution	9%

Thus, for the work carried out the final aqueous solution was made just acid by the dropwise addition of concentrated HCl and final recoveries >70% were obtained. The sensitivity was 10^{-4} μg.

The method is shown stepwise below:

1. The samples were unwrapped and placed in 125 ml beakers into which 1 ml carrier of 10 mg Au/ml gold chloride solution was added.
2. The samples were digested in 15 ml of aqua regia (3 parts 12M HCl:1 part 16 M HNO_3).
3. Each solution was diluted to 30 ml and transferred to a separating funnel and shaken for 2 minutes with 30 ml of ethyl acetate.
4. The lower aqueous layer was discarded.
5. The organic layer was washed with 15 ml of 6 M HCl, shaking for 1 minute.
6. The gold was extracted from the ethyl acetate by shaking it for 2 minutes with 25 ml of NH_4OH solution (4 parts 0.88 NH_4OH, 1 part water).
7. The aqueous layer was run off into a centrifuge tube and with careful dropwise addition of 12 M HCl just made acid (using universal indicator paper).
8. Each tube was heated in a water bath and SO_2 bubbled in (at 3-5 bubbles/sec) for 15 minutes.
9. The precipitated gold was spun down, washed twice with cold 1 M HCl and transferred to weighed aluminium planchettes for counting and weighing.

Standard

20 mg of gold was dissolved in aqua regia and made up to 100 ml. 0.1 ml of this was used as a liquid standard in a silica vial. After irradiation the contents were eluted out and made up to 1 litre. 1 ml of this (0.02 μg Au) was processed as were the samples.

Manganese

The method used for manganese analysis was based on that described by Nixon et al. (29). Naturally occurring manganese consists solely of ^{55}Mn which activated readily with an activation cross section of 13.3 barns. The reaction is as follows:

$$^{55}Mn\ (n,\gamma)\ ^{56}Mn$$

Nixon et al. carried out a one-stage separation, using sodium chlorate to precipitate out manganese dioxide from a boiling nitric acid solution. It was found that good recoveries were more readily obtained when the molarity of the hot nitric acid reaction mixture was increased by the addition of fuming (24 M) HNO_3. Nixon et al. transferred the manganese dioxide to planchettes as an aqueous slurry which was dried under I.R. lamps. It was found that the black (and hence good heat absorber) manganese dioxide tended to splatter and blister on drying. The substitution of the more volatile acetone as a slurry medium was found to solve this problem. The acetone slurry was also found to be easier to transfer.

γ Counting over the 0.845 MeV photopeak was done using the Intertechnique M.C.A.

It was thus found possible to routinely carry out manganese analysis of approximately 50 samples in one day. The prepared sample package was irradiated at a flux of 10^{12} $n/cm^2/sec$ in the UTR-100 reactor at the Scottish Research Reactor Centre during the morning. The active

samples were taken back to laboratory for processing. Counting was started by the late afternoon and finished by the early evening. The principal limiting factor in the number of samples able to be analysed in one day was the time taken to count each sample. On average, including the time spent on sample changing, this was 3 minutes. With the short half-life of ^{56}Mn, 2.58 hours, the later samples became increasingly inactive and hence the required counting times progressively longer. The sensitivity was 0.002 μg.

The method used is given stepwise below:

1. The irradiated samples were unwrapped and placed inside 125 ml beakers to which manganese carrier had been added as 1 ml of 10 mg Mn/ml $Mn(NO_3)_2$ solution.
2. 3 ml of 16 M HNO_3 was added, each sample digested by being taken to dryness and any charring removed by spotting with drops of 16 M HNO_3.
3. The volume in each beaker was brought up to 10 ml of 16 M HNO_3, a further 3 ml of 24 M HNO_3 added, and the whole allowed to boil.
4. 1 ml of 50% w/v $NaClO_3$ solution was added to each beaker to precipitate out black MnO_2.
5. The contents of each beaker were transferred to centrifuge tubes, including the rinsings, the precipitates spun down, washed twice with water, once with acetone.
6. The precipitates were transferred as an acetone slurry onto weighed aluminium planchettes for counting and weighing (weighed as $MnO_2 \cdot H_2O$).

Standard

The standard was prepared by weighing out approximately 0.1 gm of a $Mn(NO)_3$ solution containing 2 μg Mn/ml onto a fresh polythene sheet. The solution was evaporated under an I.R. lamp, the polythene mount folded in four, placed inside a "snap seal" bag and irradiated alongside the samples. After irradiation the unfolded polythene mount was processed as a standard as were the samples. The polythene mount was discarded at stage 5.

Analysis of biological standard

A sample of dried powdered kale prepared by Bowen and Cawse (30) and distributed for inter-laboratory comparison was analysed for manganese. The level found was 13.0 ppm. Bowen (31) reported a mean of 14.9 ± a standard deviation of 1.8 ppm Mn for 83 measurements from various laboratories. Bowen described drying of the kale prior to analysis, giving approximately a 5% weight reduction. Here the kale was not pretreated and was analysed as obtained and therefore the Mn level found could be expected to be correspondingly reduced. Thus the accuracy obtained during analysis was shown to be adequate.

Radiochemical purity

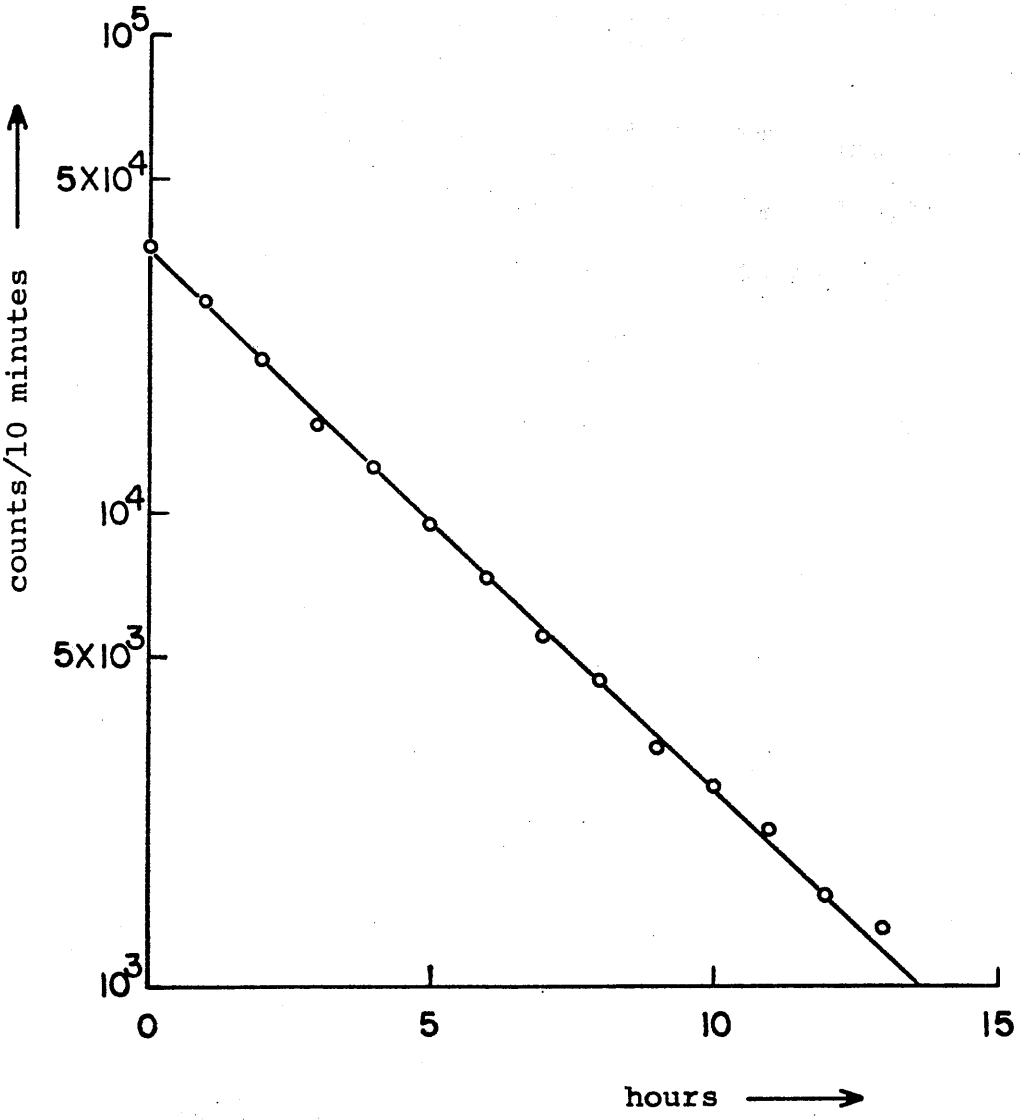
A processed monkey brain tissue was decayed followed as shown in Figure 3. Counting was carried out over the 0.845 MeV photopeak. Counting for 10 minutes every hour was started

8 hours after removal from the reactor and continued for a further 14 hours. The half-life found was 2.58 hours, which was that quoted for ^{56}Mn.

FIGURE 3

DECAY PLOT FOR MONKEY BRAIN SAMPLE ANALYSED FOR MN.

Half life found = 2.58 hours
Counting started 8 hours 6 minutes after removal from the reactor.


10^5
$5X10^4$
10^4
$5X10^3$
10^3
counts/10 minutes
0
5
10
15
hours

Zinc

There are two different approaches that may be employed for zinc analysis, (1) is chemical and (2) instrumental. Such methods are described by Nixon et al. (32) and Livingston et al. (24).

Isotopes used for detection are ^{65}Zn, ^{69m}Zn and ^{69}Zn, with activation cross sections of 0.22, 0.018 and 0.186 barns and half-lives of 265 days, 13.8 hours and 55 minutes, respectively. The capture reactions are:

$$^{64}Zn\ (n,\gamma)\ ^{65}Zn$$

$$^{68}Zn\ (n,\gamma)\ ^{69m}Zn \xrightarrow{\text{I.T.}} {}^{69}Zn$$

As described by Nixon et al. (32) the greatest theoretical sensitivity is obtained with a short irradiation period and estimation of the ^{69}Zn isotope. However, in the work carried out it was experimentally convenient to count on a low background β counter the ^{69m}Zn isotope, following an irradiation period of 1 day at Harwell at a neutron flux of 9×10^{12} $n/cm^2/sec$. Sensitivity was 0.002 µg.

Separation was chemical, using an amalgamation of the similar methods employing an ion exchange column as described by Nixon et al. and Livingston et al. (Where samples can be placed aside for several months an instrumental separation as described by Nixon et al. is possible, but such separation was not found optimal to the work carried out.)

The method used is given stepwise below:

1. The unwrapped samples were placed in 125 ml beakers and zinc carrier added as 1 ml of 10 mg Zn/ml of $Zn(CH_3COOH)$ solution, followed by approximately 10 mg of copper as $CuSO_4$ solution.
2. After digestion with 16 M HNO_3 the solution was taken to dryness and taken up in 10 ml of 2 M HCl.
3. Each solution was transferred to reservoirs ontop of ion exchange columns (using Amberlite IRA-400 (Cl), Analytical Grade, 50 mesh resin) and run through. All flow rates were approximately 3 ml/minute.
4. The column was washed with 25 ml of 0.12 M HCl containing 10% w/v NaCl and the zinc eluted with 25 ml of 2 M NaOH solution containing 2% w/v NaCl and collected in centrifuge tubes.
5. The elute was made just acid with 12 M HCl and 0.5 ml of 10% w/v $FeCl_3$ solution added.
6. 3 ml of 5 M NaOH was added, each tube heated in a water bath and the scavange $Fe(OH)_3$ precipitate spun down and filtered off.
7. Each supernate was made slightly acid with 17.5 M acetic acid, heated in a water bath and 3 ml of 2% w/v quinaldic acid solution added to precipitate the zinc as a quinaldate.
8. The precipitate was spun down, washed once with water, once with acetone and transferred as an acetone slurry to weighed aluminium planchettes.

9. Each recovery was counted and weighed (as Zn $(C_{10}H_2O_2N)_2 \cdot H_2O$).

Standard

A known amount of about 2 mgs of "Specpure" zinc metal was weighed out and wrapped in aluminium foil. After irradiation the zinc was dissolved in dilute HCl and made up to 100 ml. 10 ml of this was made up to 1 litre and 1 ml of this used as the standard—an effective dilution of 10^{-4}. The standard was processed from steps 7 onwards.

Group (iii)

This includes the elements selenium and barium.

Selenium

Selenium, tellurium and polonium are the three heaviest elements of the sulphur subgroup. Selenium is known to exist in a large number of isotopic forms with mass numbers 70 to 85 inclusive and 87. Naturally occurring selenium exists as a mixture of 6 stable isotopes as listed below.

Table 4. Selenium Isotopes

Isotope	% Abundance*	Activates by neutron capture, forming:	Half-life	Activation Cross Section (barns)
^{74}Se	0.87	^{75}Se	121 days	0.23
^{76}Se	9.02	^{77m}Se	17.5 secs.	0.90
^{77}Se	7.58			
^{78}Se	23.52	^{79m}Se	3.91 mins.	0.03
^{80}Se	49.82	^{81m}Se	56.8 mins.	0.015
		$\downarrow$ I.T.		
		^{81}Se	18.2 mins.	0.25
^{82}Se	9.19			

*Data from chart of Nucleids, 2nd Edition, 1961, Kernforschungszentrum Karlsruhe.

The principal isotopes formed by neutron capture on activation of natural selenium are listed on the righthand side above. ^{77m}Se has the highest activation cross section.

However, its use for selenium detection is limited due to its short half-life of 17.5 secs, a fast transfer system from reactor to counting being required. Okada used ^{77m}Se for the analysis of Se in pure sulphur (33). Other workers, such as Fleishman and Guin (34) have used ^{77m}Se in investigation of biological material. ^{81}Se, half-life 18.2 mins., has been used for investigation of biological material as typified by Bowen and Cawse (35). In this they describe a rapid separation technique based on the specific distillation of selenium from a HCl/HBr mixture. The separation of selenium using a similar distillation technique is described throughout the literature on selenium separation techniques. The best sensitivity quoted for ^{81}Se measurement was 0.5 μg at a neutron flux of 10^{12} n/sec/cm^2. ^{75}Se although of lower activation cross section is shown by the literature to have been most often the isotope of choice for selenium detection by neutron activation. The reasons for this are as described by Mazière et al. (36); namely its long half-life enables sufficient time to be spent processing and counting for the sensitivity obtained to be greater than other described techniques.

A sound method for selenium analysis using ^{75}Se is described by Steinnes (37). Steinnes' method was based on distillation of selenium in a HCl/HBr mixture and he reported a chemical yield of 70 to 90%. Steinnes quoted a sensitivity improved from the region of 1-10 nanograms limit to a 0.5 nanograms limit.

The required application of the selenium separation and detection method used was an analysis survey of a large number of body tissues. Thus a technique was required suited to the analyses of many samples at one time with adequate sensitivity. Therefore a rapid separation technique was excluded and the ^{75}Se isotope considered.

A preliminary investigation was made during which biological samples were irradiated for one week at a neutron flux of 5×10^{12} n/cm^2/sec, and selenium was separated by a one-stage process using excess ascorbic acid to precipitate out the element. This was followed by γ counting over the 0.14 MeV photopeak of ^{75}Se. Further studies indicated adequate radiochemical purity when counting was carried out 3 to 6 weeks after irradiation. After this time significant interfering activity had decayed. The sensitivity was 0.03 μg.

It was thought that selenium might be lost during the digestion process. However, the final selenium recoveries were high, >70% was the average for over 200 analyses carried out. This figure included all transfer losses. Thus any selenium loss during digestion was not considered to be significant.

A series of separations was carried out on samples of inactive tissue to which 20 mg of selenium carrier and 1 ml of ^{75}Se tracer was added. Amounts ranging from 1 ml to 10 ml of 1% w/v ascorbic acid were used to precipitate the selenium recovery. By counting any ^{75}Se tracer remaining in the

supernate it was found that where less than 4 ml of 1% w/v ascorbic acid was added, selenium precipitation was incomplete. For any excess above that, precipitation was complete.

Thus selenium detection in the large number of samples investigated was able to be carried out in this way as detailed below:

Method for selenium analysis using ^{75}Se, half-life 121 days, activation cross section 0.23 barns

An aluminium can for Harwell irradiations was packed with 50 to 60 samples for analysis. A standard of approximately 2 mgs of solid "Specpure" SeO_2 was packaged inside a silica vial and included in the can. A week's irradiation at a neutron flux of 9×10^{12} $n/cm^2/sec$ at Harwell was given. Following such irradiation the can and contents were very radioactive (mainly ^{24}Na). To minimize shipping weight due to heavy shielding that would otherwise be required, the can was left to cool at Harwell for a further week before being returned to the laboratory. Unpackaging and processing was started two weeks after irradiation ended. The stepwise procedure as outlined below was followed.

1. The unpackaged samples were placed in digestion flasks and 2 ml 18 M H_2SO_4 and 1 ml of Se carrier added (20 mg Se/ml in 9 M H_2SO_4).
2. The above was heated until white fumes appeared and charring occurred.
3. The charring was removed by the addition of a few drops of 16 M HNO_3.

4. The contents of each digestion flask were rinsed into centrifuge tubes.
5. 10 ml of 1% w/v ascorbic acid solution was added to precipitate out the reduced black Se metal.
6. The centrifuge tubes were heated in a water bath for 5 to 10 minutes to complete Se precipitation.
7. The precipitated Se was spun down, washed twice with water and finally with ethanol.
8. The Se was transferred with ethanol into weighed aluminium planchettes and dried.
9. γ Counting over the 0.14 MeV photopeak of ^{75}Se was carried out. (This photopeak, one of the three major peaks in the ^{75}Se γ spectrum, was chosen to avoid possible interference of ^{203}Hg.)

Standard

The standard was prepared by leaching out the contents of the broken-open silica vial in water heated to below boiling point. The whole was made up to 1 litre. 1 ml of this plus 2 ml of 18 M H_2SO_4 was placed inside a centrifuge tube and made up to 25 ml. Processing as from steps 5 to 9 was carried out.

Counting was typically for 1 to 2 hours per sample. For a batch of 50 samples or so all counting was carried out over a period of approximately two weeks.

Intercomparison

Six samples of between 1 and 3 mgs of "Specpure" SeO_2 were used for cross reference of the method. Each was weighed

in a numbered silica vial which was sealed and placed in a common can. The whole was irradiated as described and the contents of each vial leached out and made up to 1 litre in an equivalently numbered volumetric flask.

One solution was treated as a standard as described.

1 ml from one of the five remaining 1 litre solutions was added to an equivalently numbered digestion flask to which was also added approximately 20 mg of inactive dried human liver. This was done for all five 1 litre solutions of irradiated SeO_2.

The five "samples" were processed as from step (1) onwards and the selenium amounts in each calculated.

The calculated values lay within 3% of the known values, illustrating that the method appeared viable within experimental error.

Conclusion

The one-stage separation method evolved was considered to have been a useful one and to possess several advantages. It was simple and straightforward to carry out. It avoided the necessity for more complex chemical separation which can make large number of analyses lengthy to perform. Although a long cooling time was thereby necessary prior to counting each sample, the actual time spent on each analysis was not excessive. The method was suited for application to biological tissue analysis.

Barium

Of the three major elements in group IIA of the Periodic Table, calcium atomic number 20, strontium atomic number 38 and barium atomic number 56, calcium is a macro element in biological tissue and is the major element in skeletal tissue. The chemical properties of strontium and barium are are very similar to those of calcium. Radioactive strontium-90 and barium-140 are products from fission of uranium-235.

There are 22 known isotopes of barium varying from ^{126}Ba to ^{144}Ba. There are seven stable isotopes of barium. Naturally found barium, atomic weight 137.56, consists of a mixture of:

0.13% ^{130}Ba, 0.19% ^{132}Ba, 2.6% ^{134}Ba, 6.7% ^{135}Ba, 8.1% ^{136}Ba, 11.9% ^{137}Ba, 70.4% ^{138}Ba

Under conditions of thermal neutron capture approximately 97% of the activity produced is due to the formation of ^{139}Ba from ^{138}Ba:

$$^{138}Ba\ (n,\gamma)\ ^{139}Ba$$

The remaining 3% is due to the formation of ^{131}Ba:

$$^{130}Ba\ (n,\gamma)\ ^{131}Ba,$$

and ^{133}Ba:

$$^{132}Ba\ (n,\gamma)\ ^{133}Ba.$$

Of the three isotopes formed by neutron capture on activation of natural barium, ^{139}Ba can be seen to be the most significant as illustrated below:

Table 5. Barium Isotopes Formed by Neutron Capture

Isotope	Half-life	Activation cross section in barns	Activity per gm natural barium at flux 10^{12} n/cm^2/sec
^{131}Ba	11.5 days	0.01	1.2 mc/gm at SATURATION 0.6 mc/gm in 11.5 days
^{133}Ba	7.2 years	0.007	60 μc/gm in 1 year
^{139}Ba	1 hr 25 min	0.36	43 mc/gm at SATURATION 21 mc/gm in 1 hour 25 min

The contribution of ^{133}Ba can in practical terms be seen to be relatively negligible. Simple calculation showed that of the other two isotopes ^{131}Ba and ^{139}Ba, the isotope that provided the greatest sensitivity was ^{139}Ba. 1 gram of natural barium irradiated for 1 hour 25 mins at 10^{12} n/cm^2/sec will produce an activity of 21 mc of ^{139}Ba. After some 7 hours from the time of cessation of irradiation approximately 0.6 mc of activity will remain. After a day or so the ^{139}Ba activity will have disappeared. It was thought that it would be possible for any necessary chemical separation to be completed within 7 hours and thus a minimum activity of the order of 0.6 mc/gm would be available. The highest neutron flux of the UTR 100 reactor available for short irradiation periods was 4.2 x 10^{12} n/cm^2/sec in the C.V.S. position (see Figure 1). This corresponded to a minimum working activity of in the order of 2 mc/gm of ^{139}Ba. The other alternate isotope ^{131}Ba would provide the equivalent sensitivity after 10 days irradiation. Thus a choice lay between selection of

the short-lived ^{139}Ba to be irradiated in the UTR 100 at the Scottish Research Reactor Centre and the long-lived ^{131}Ba to be irradiated at Harwell. ^{139}Ba was selected for three reasons: (i) Long irradiation times at Harwell required more time consuming packaging and also brought about change to the samples making unpacking difficult; (ii) A short irradiation time brought about a lower buildup of interfering activity; (iii) Finance. There was no charge made for the short irradiation times carried out at the Scottish Reactor Centre.

The separation techniques employed by the other workers were investigated. A literature search indicated that many of the techniques employed the precipitation of the insoluble nitrate based upon the method of Willard and Goodspeed (38). For example, Harrison and Raymond (39) described a radiochemical separation procedure using the precipitation of the nitrate in 19 N nitric acid. Their procedure took 2½ to 3 hours to perform for 4 samples and 2 standards and was stated to have an accuracy within 5% for the determination of 1 µg of strontium or barium. Bowen and Dymond (40) used the technique of Harrison and Raymond and quoted the limits of this method as 0.06 µg strontium 0.1 µg barium and an accuracy of $\pm$5%. In the above procedure the final separation of barium from strontium was carried out by the precipitation of barium chromate from solution. It was preferable to precipitate out any strontium before the final precipitation of barium. Therefore, a method based on that devised by Harrison and Raymond would not have been entirely suitable.

Das et al. (41) mention that the separation of barium and strontium is difficult. They avoided what they described as the cumbersome mutual separation by a final instrumental separation of the γ spectrum as recorded on a sodium iodide detector. Their chemical yield was of the order of 20 to 50% and concentrations measured were of the order of 500 ppm barium with an uncertainty of 10 to 20 ppm.

The above method had the advantage of enabling forty analyses to be performed in one day. However no separation other than instrumental was carried out between barium and strontium.

A method was described by Cherkesov and Pushinov (42) for the determination of barium with no interference from calcium, strontium and other metals. Precipitation was carried out in acetic acid solution and the precipitate contained barium and the dye brilliant yellow (C.I. Direct Yellow 4) in the ratio 1:1. The use of the precipitation agent brilliant yellow for barium therefore appeared to be of interest. However it was found that it was not possible to reproduce the results obtained by Cherkesov and Pushinov using ^{133}Ba (half-life 7.2 years) and ^{89}Sr (half-life 51 days) as tracers. The complexing agent E.D.T.A. was substituted for acetic acid in the precipitating solution. It was found that in high concentrations of E.D.T.A. solution barium would be precipitated slowly by the dye. Under the same conditions strontium did not precipitate. However the recovery of barium was low (<10%) and thus this method did not appear to be satisfactory.

Construction of a new radiochemical separation technique

In order to find a method of selectively precipitating strontium from a solution containing both strontium and barium, the solubility of salts of both were investigated. It was found that the only salt common to both strontium and calcium which had a lower solubility than that of barium was the fluoride. The solubility of the fluorides increases with atomic number of the element as can be seen below:

	Solubility*
Calcium fluoride	0.0016 g/100 ml at 18°C
Strontium fluoride	0.011 g/100 ml at 0°C
Barium fluoride	0.12 g/100 ml at 25°C

*CRC Handbook of Chemistry and Physics, 52nd Ed., 1971/1972.

Thus experiments were carried out to investigate the conditions under which strontium and calcium would be precipitated out as the fluoride leaving the barium in solution for subsequent separation.

Using the long lived (half-life 7.2 years) ^{133}Ba isotope, a tracer solution corresponding to approximately 1000 c/m was prepared. Counting was carried out over the 0.356 MeV γ photopeak. A series of fluoride precipitations shown in Table 6 were carried out using excess sodium fluoride (as 2 ml of a 10% w/v solution) and a total solution volume of 30 ml. The barium and strontium carriers were made up as $BaCl_2$ and $SrCl_2$ solutions respectively.

Table 6. Ba Co-precipitation and Sr Precipitation

Expt.	Sr mg	Ba mg	Ba/Sr ratio	% Ba co-precipitated with SrF	% SrF precipitation
1	100	50	0.20	60.1	100
2	80	50	0.63	64.5	"
3	60	50	0.83	62.2	"
4	50	50	1.00	54.1	"
5	50	60	1.20	59.9	"
6	40	60	1.50	55.7	"
7	20	50	2.50	34.6	"
8	4	20	5.00	30.2	"
9	2	20	10.0	25.2	49.9
10	1	50	50.0	21.0	82.0

The final strontium fluoride precipitate was assessed for any barium activity brought about by possible barium co-precipitation.

After the fluoride was spun down and removed the supernate rather than the fluoride was counted for any residual activity. Such liquids were first rinsed into 10 cm diameter disposable petri dishes to which matching lids were sealed using a little acetone. Each dish was placed on the sodium iodide crystal for γ counting.

Determination of strontium recovery

Experiments 1 to 10 in Table 6 were repeated using a strontium tracer (^{89}Sr, half-life 51 days) to determine the strontium recovery in the precipitation of strontium fluoride. The tracer ^{89}Sr was β counted. It was found convenient to count the liquid supernate in a "M-6" G.M. tube rather than the fluoride precipitate as the fluoride was very fine and difficult to transfer. The fluoride precipitate was removed by spinning down in a high speed centrifuge.

Results

In experiments 1 to 8 inclusive no strontium activity was found in the supernate, indicating a 100% strontium recovery by fluoride precipitation. From the above it was seen that to insure a complete strontium removal by fluoride precipitation a minimum of 4 mg of strontium in 30 ml of solution was required. Under these conditions (experiment 8) approximately 30% of the barium present was co-precipitated.

Calcium scavanging

Calcium is associated with barium and strontium in Group II of the Periodic Table. Calcium activates by thermal neutron bombardment, $^{44}Ca\ (n,\gamma)\ ^{45}Ca$. The activation cross section for ^{44}Ca is not large (0.014 barns, c.f. 0.36 barns for ^{139}Ba). However, it was considered desirable that a scavange step be constructed to be rid of any calcium.

Calcium fluoride is even less soluble than strontium fluoride and thus the fluoride precipitation separation of calcium fluoride was investigated.

A tracer solution of ^{45}Ca (half-life 165 days) was prepared to give a count rate of several hundred counts per second per ml. Counting of the soft β activity (0.254 MeV) was carried out using a beta scintillation counter.

A series of 30 ml total volume solutions was prepared each containing 20 mg Ba, 1 ml of ^{45}Ca tracer and amounts of calcium ($CaCl_2$ in solution) ranging from 1 to 10 mg.

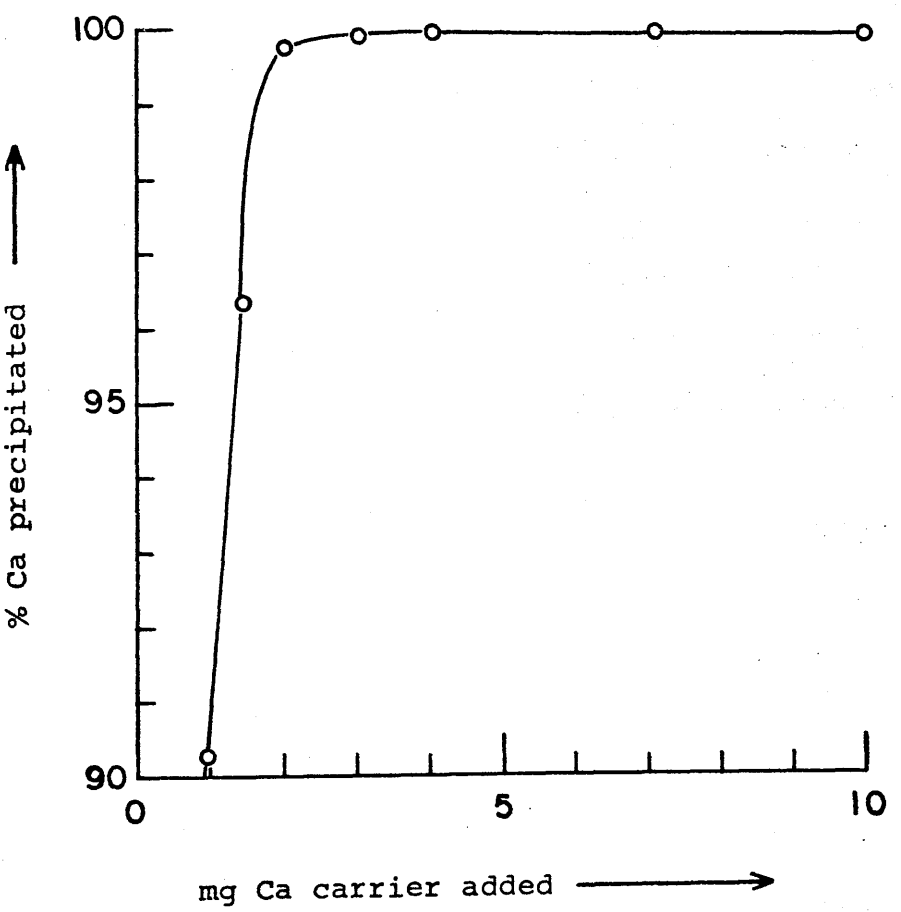
Fluoride precipitation was carried out with excess soidum fluoride (in this case 1 ml of saturated sodium fluoride). The fluoride precipitate was spun down and the supernate counted for any ^{45}Ca activity. The supernate was separated from the spun down precipitate by decanting and filtration followed by careful rinsing through Watmans No. 2 paper.

The results as displayed in Figure 4 indicated that 2 mg of calcium upwards was sufficient carrier to ensure virtually complete removal of any calcium present.

FIGURE 4

% Ca PRECIPITATED VS. MG Ca ADDED.

Each solution of 30 ml contained 20 ml of Ba, 1 ml of ^{45}Ca tracer and between 1 to 10 ml Ca.


100
95
90
0
5
10
% Ca precipitated
mg Ca carrier added

Investigation of co-precipitation of barium and strontium with calcium fluoride

A series of 12 solutions was prepared. All contained barium and calcium carriers and one-third contained strontium carrier as well. The total volume of each solution was adjusted to 30 ml. Fluoride precipitation was carried out using excess sodium fluoride (3 ml of satd. NaF solution). The effect of heating in a Bunsen flame of the total solution was also investigated. From the results shown below in Table 7 it was seen from experiments 6 and 10 that the effect of heat was adverse in that under otherwise similar conditions the amount of barium removed by co-precipitation was increased by approximately a factor of 4.

In experiment 9, Table 6, where 2 mg of strontium was present 49.9% of the strontium was precipiated by sodium fluoride. Under the same conditions, but with the addition of 2 mg Ca, an increase to 93.1% of the strontium was precipitated in experiment 7, Table 7. This would be explained by co-precipitation of strontium with calcium fluoride.

In experiment 11, Table 7, where no strontium carrier was present, substantially the same amount of strontium tracer was co-precipitated as in experiment 7, Table 7, where strontium carrier was present (88% c.f. 93.1%, respectively). Under similar condition to experiment 7, Table 7, as in experiment 9, Table 7, 81.5% of the barium remained behind in solution after the fluoride precipitation.

Table 7. Co-precipitation of Barium and Strontium with Calcium Fluoride

Soln.	Br Carrier mg	Sr Carrier mg	Ca Carrier mg	Ba Tracer	Sr Tracer	Remarks	% Copptn. Ba	% Copptn. Sr
1	40		excess	x			61	
2	40		excess		x			100
3	20		8	x			51	
4	20		24	x			83	
5	20	2	2	x			19.5	
6	20	2	2	x		Heat applied	80.4	
7	20	2	2		x			93.1
8	20	2	2		x	Heat applied		99.6
9	20		2	x			18.5	
10	20		2	x		Heat applied	77.2	
11	20		2		x			88
12	20		2		x	Heat applied		98

x denotes tracer added.

Conclusion

From the above it appeared that the use of calcium carrier alone as a precipitation agent for both calcium and strontium from a barium containing solution would be possible and thus was further investigated.

Calcium as a precipitation agent for both calcium and strontium

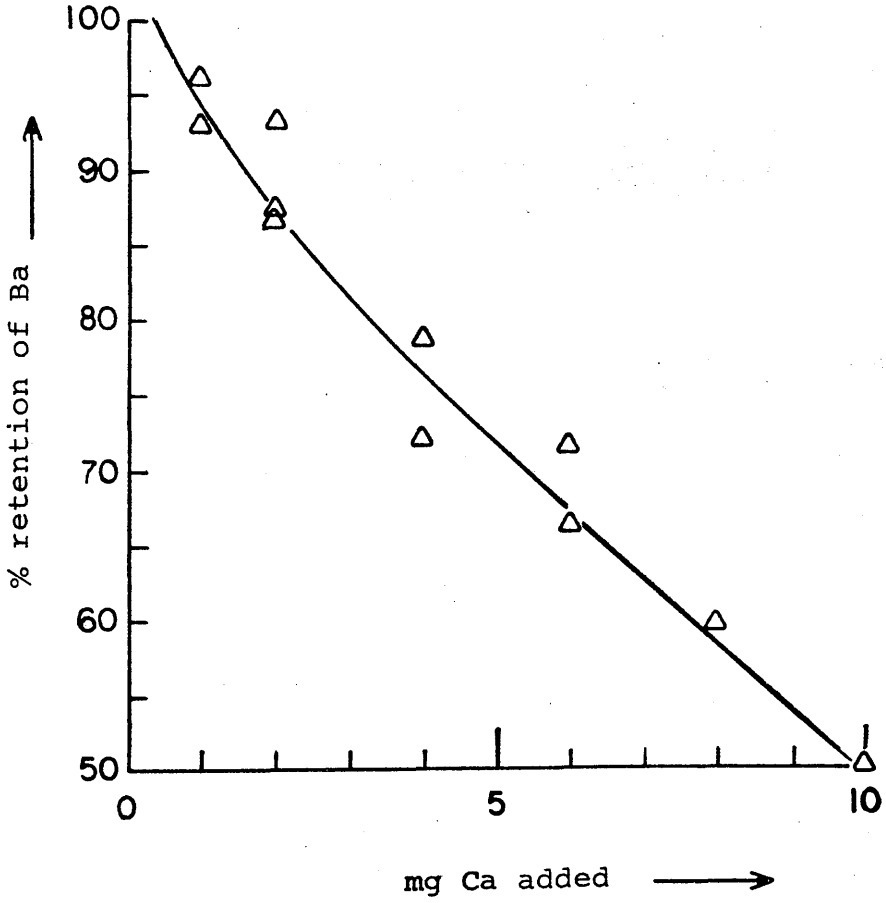
A series of solutions was prepared each containing the standardised amount of 20 mg barium (as $BaNO_3$). No strontium as carrier was added to any solution. Increasing amounts of calcium carrier (as $CaCl_2$) ranging from 1 to 10 mg were added to different solutions. Using ^{133}Ba and ^{89}Sr as tracers the retention of barium in solution and the co-precipitation of strontium traces with CaF_2 were measured on addition of excess NaF solution (3 ml saturated). The results are displayed in Figures 5 and 6.

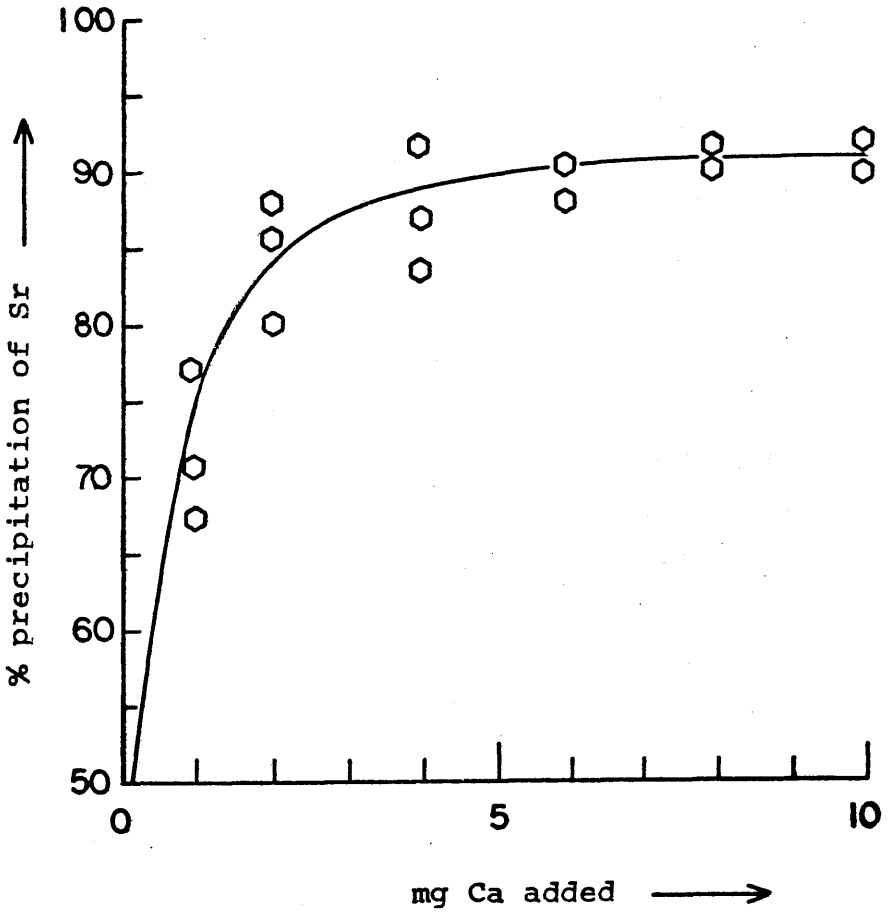
It had been shown earlier (Figure 4) that 2 mg of calcium in a carrier was required to ensure 100% precipitation of all calcium as the fluoride. All solution volumes were made up to 30 ml. The fineness of the fluoride precipitate required spinning down at a high speed centrifuge for 5 minutes followed by filtration through four thicknesses of Watmans No. 1 filter paper for its removal. It was found that 88% of the barium present was retained and 84% of the strontium co-precipitated in a solution containing 2 mg calcium to which excess NaF was added. Repeating the above after the fluoride was removed by quickly mixing in a further 2 mg calcium in a carrier

FIGURE 5

% RETENTION OF Ba VS. MG Ca ADDED.

Each solution of 30 ml contained 20 mg of Ba.


100
90
80
70
60
50
% retention of Ba
0
5
10
mg Ca added


100
90
80
70
60
50
% precipitation of Sr
0
5
10
mg Ca added

brought about 97% co-precipitation of any strontium and left 80% of the barium in solution. Thus a fluoride precipitation, as outlined above, to separate strontium and calcium leaving barium in solution was considered possible.

Digestion

As a first stage in construction of a chemical separation scheme suitable mineral acids for wet digestion were selected. H_2SO_4 could not be used as it would cause the formation of insoluble $BaSO_4$. 16 M HNO_3 could, as described earlier, be used alone for complete acid digestion. Samples of various representative biological tissues plus 20 mg barium carrier were so digested. After digestion was complete, all charring removed and the residue was taken up in water, the resulting solutions were turbid. It was found that taking up in 0.5 M HCl reduced the turbidity, but portions of the digested residue remained on the bottom of the digestion flasks. Taking up in 12 M HCl, allowing the whole to boil, followed by dilution removed all of the digested residue from the bottom of the flasks. However, the resultant solutions were still seen to be slightly turbid. On digestion of tissue alone without barium carrier the final solutions as treated alone were found to be clear. On subsequent addition of barium carrier they still remained clear.

1 ml of ^{133}Ba tracer was added to a digestion flask made up to a marked level and counted by directly placing on top of the NaI crystal. A tissue sample was added and the described

digestion sequence carried out. The solution was made up to the marked level and counted as before. The above was carried out for 6 tissues in 6 flasks. In five cases the pre- and post-count rates remained the same within the statistical variation of count rates; i.e., there was no observed barium loss on digestion in the absence of carrier. In one case there was a recorded 2% loss which was attributed to inadvertant splashing.

Thus it was found that as no barium loss on digestion took place the barium carrier could be added after digestion and the problem circumvented of post-digestion solution turbidity that would otherwise occur.

$Fe(OH)_3$ scavanging

Use of the precipitation of $Fe(OH)_3$ as a scavange to co-precipitate unwanted cations during barium separation was mentioned by Minkkinen (43).

A series of test solutions in centrifuge tubes containing barium carrier and tracer and digested tissue were prepared. The count rate of each solution was known under standardised conditions. To each, 4 drops 10% w/v Fe^{+++} was added. 0.88 NH_4OH was added dropwise with mixing until the brown gelatinous $Fe(OH)_3$ appeared. The hydroxide was spun down and removed. Each solution was counted under the same standardised conditions. It was thus found that excess NH_4OH had no effect on the retentions of barium in solution and that on average 6% of the barium was removed by the hydroxide scavange, i.e.,

the barium recovery was not significantly compromised. Further investigation using ^{89}Sr tracer in the absence of strontium carrier and presence of 20 mg barium carrier showed that as much as 40.1% of strontium was removed. Thus, if possible, the $Fe(OH)_3$ scavange prior to fluoride separation was of extra value. If this were done the fluoride separation would be carried out in an alkaline solution caused by the excess NH_4OH present. Therefore, the fluoride separation using 2 mg calcium as described earlier was carried out in such alkaline solutions. Using tracers it was found that there was no significant alteration of the previous results. Thus it was seen that fluoride separation would take place after the $Fe(OH)_3$ scavange had rendered the working solution alkaline.

Selection of a final barium precipitate for counting

No suitable organic precipitation agent was found for barium. Barium chromate, $BaCrO_4$, was frequently used in the literature as a stable barium precipitate and this was selected as a possible final barium precipitate. Using tracer and prepared representative solutions, it was established that 100% precipitation of barium in the alkaline solution occurred on addition of excess 10% w/v potassium chromate. It was shown that the precipitation of $BaCrO_4$ was unaffected by NH_4OH being present. It was found that the yellow $BaCrO_4$ transferred easily with acetone onto aluminium planchettes and dried without cracking.

The sequence: digestion, Fe scavange, fluoride separation and $BaCrO_4$ precipitation was thus shown to be compatible and capable of producing a 99% removal of any strontium, 100% of calcium and of providing a barium recovery >75%.

Thus a method based on the above sequence was used as outlined stepwise below.

1. Samples and standard were irradiated at 4.2×10^{12} $n/cm^2/sec$ for 1 hour 25 mins.
2. Each sample was placed in a 125 ml conical beaker and digested using 16 M HNO_3 to leave a dry uncharred residue.
3. The residue was taken up in 12 M HCl, allowed to boil and diluted with water.
4. 20 mg of barium were added (as 1 ml of 20 mg/ml $BaCl_2$ solution).
5. 10 drops of 10% w/v $FeCl_3$ were added.
6. The above was rinsed into 50 ml centrifugal tubes.
7. Excess NH_4OH (sp.gr. 0.88) was added, the whole was spun down and the gelatinous $Fe(OH)_3$ discarded by filtration through Watmans No. 1 paper into fresh centrifuge tubes.
8. 2 mg calcium (as 1 ml 2 mg/ml $CaCl_2$ solution) were added.
9. 3 ml of saturated NaF were added.
10. The resultant fluoride precipitate was spun down and the filtrate passed through 4 thicknesses of Watmans No. 1 paper.
11. 2 mg calcium were added to the filtrate and step 10 repeated.
12. 3 ml of 10% w/v K_2CrO_4 solution were added.

13. The resultant yellow $BaCrO_4$ was spun down, washed twice with water and once with acetone.
14. The $BaCrO_4$ was transferred with acetone onto weighed aluminium planchettes.
15. Counting over the 0.165 MeV γ photopeak of ^{139}Ba was carried out.

Standard

A standard of approximately 20 mg of "Specpure" $BaCO_3$ was weighed out in a polythene tube which was subsequently heat sealed. After irradiation the contents were leached out in dilute HCl and made up to 1000 ml. 1 ml of this was added to 20 mg barium carrier in a centrifuge tube and steps 12 to 15 carried out.

Sensitivity

The maximum limit for sensitivity was found to be approximately 0.2 µg Ba. As a result large sample weights (approximately 100 mg or more) were required for it to be possible to detect the low levels of barium in the tissues surveyed.

Tests of standard solutions

Four polythene tubes were each filled with 1 ml of an inactive standard solution containing between 2 and 20 µg of barium. A fifth tube was filled with 1 ml of distilled water as a blank. A standard was included. The whole was irradiated and each sample tube rinsed out into a numbered beaker containing approximately 50 - 100 mg of inactive dried human lung. Processing took place as from steps (2) onwards. The weight

of barium in each sample take was thus determined. Correlation between calculated and known weights was within 3%. No barium was found in the blank sample.

Radiochemical purity

To demonstrate the final radiochemical purity achieved by the separation method evolved an example of a decay curve plotted for one of the processed samples is shown in Figure 7. In this case the sample was one of wheat. γ counting was carried out over the photopeak at 0.165 MeV.

As can be seen, a straight line could be drawn from a logarithmic/linear plot and the half-life of the sample was thus found to be 1 hour 25 minutes—that of ^{139}Ba. Thus the processed samples could be considered radiochemically pure, with counting taking place over the 0.165 MeV photopeak of ^{139}Ba.

The γ spectra of the same sample and of a barium standard are shown in Figure 8.

Conclusion

The evolved method was considered to have many advantages for its particular application to biological analysis over other techniques described in the literature. It was unique in that it enabled strontium and calcium to be precipitated out, leaving the barium in solution. Other chemical separations described in the literature typically first precipitated out barium as the chromate leaving strontium and calcium in solution (e.g., Harrison and Raymond (39)). Where barium was the desired separated element it was preferable for any

FIGURE 7

DECAY PLOT OF WHEAT SAMPLE ANALYSED FOR BARIUM.

γ counted over the 0.165 MeV photopeak of ^{139}Ba. Half life found = 1 hour 25 minutes.

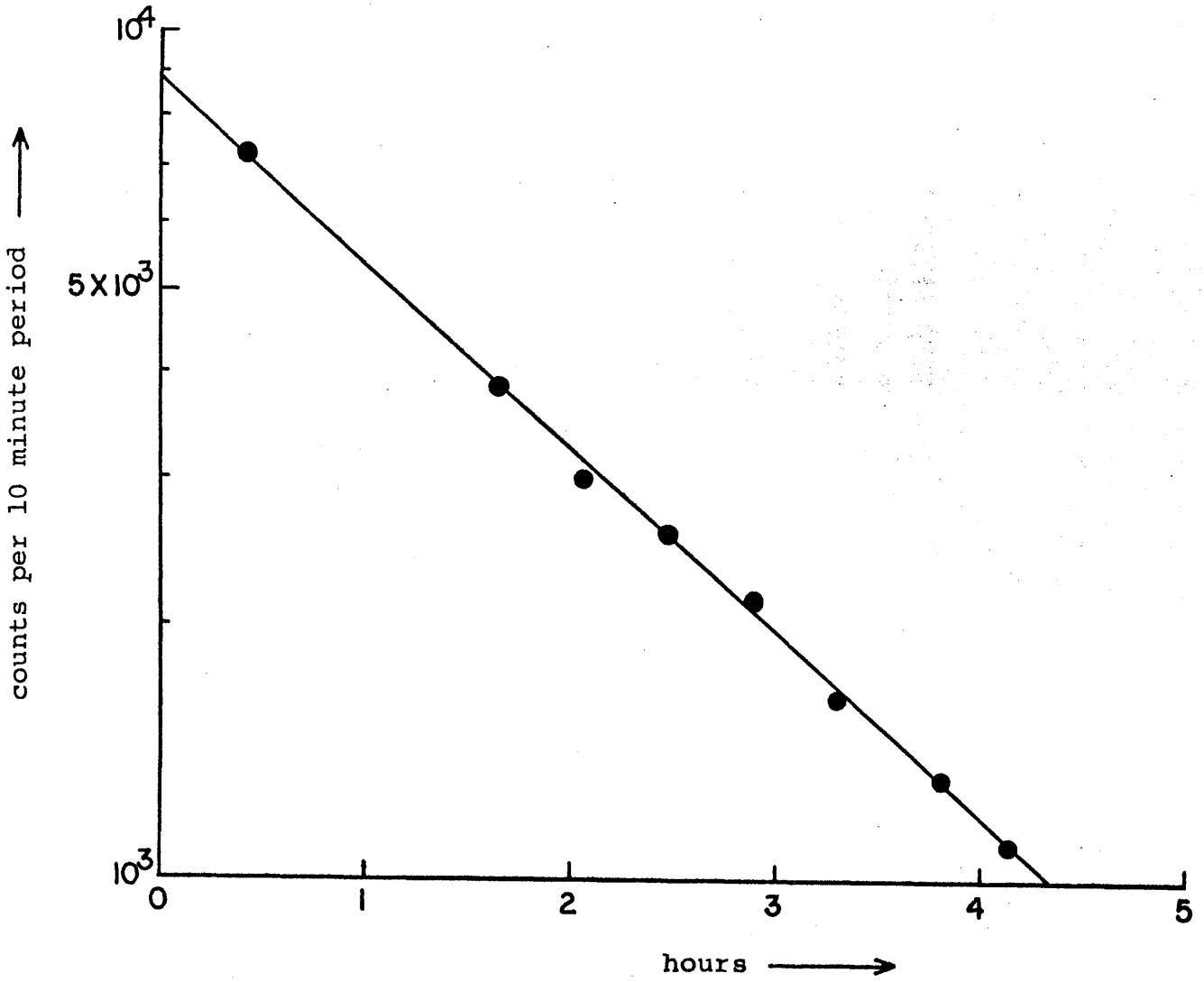
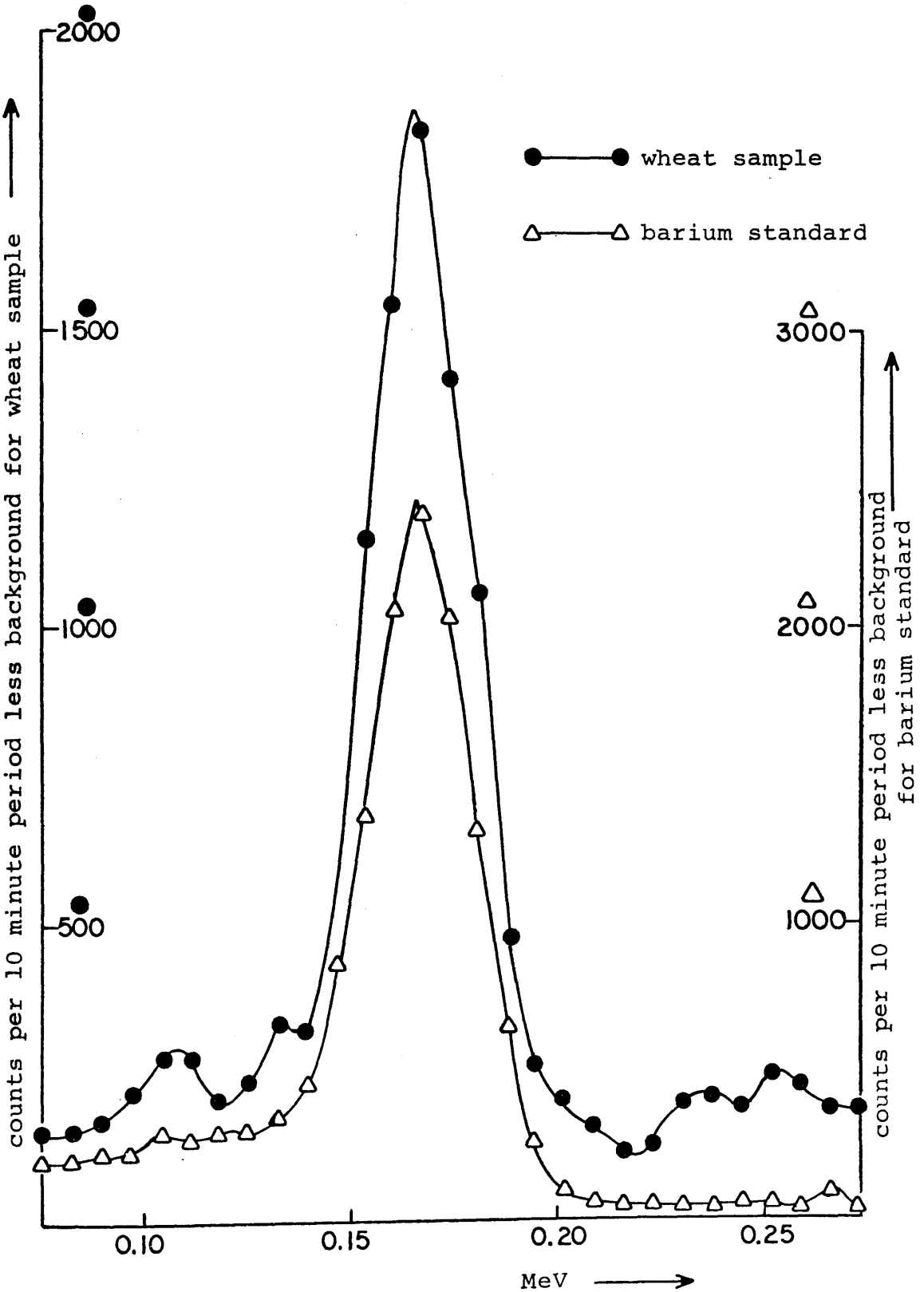

10^4
5×10^3
10^3
counts per 10 minute period
0
1
2
3
4
5
hours

FIGURE 8

SPECTRA OVER THE 0.165 MeV ^{139}Ba PHOTOPEAK FOR A PROCESSED WHEAT SAMPLE AND FOR A BARIUM STANDARD.


2000
1500
1000
500
3000
2000
1000
wheat sample
barium standard
counts per 10 minute period less background for wheat sample
counts per 10 minute period less background for barium standard
0.10
0.15
0.20
0.25
MeV

interfering strontium and calcium to be first removed. Using the fluoridation procedure derived this was able to be done.

The chemical procedures were straightforward and simple. The need was avoided, for example, for a nitrate separation from boiling nitric acid solution as described by Willard and Goodspeed (38). The method was thus able to be applied without difficulty and enabled adequate separation to take place in a short processing time. Thus it was possible to use the short-lived ^{139}Ba isotope which had the most favourable activation cross section.

The best sensitivity that is practical is required for barium analysis. Barium does not activate readily and is also present in low amounts in animal tissue. The method evolved, giving 0.2 μg sensitivity, enabled barium to be found in the tissues analysed, and as a whole was considered well suited to the analysis of biological material.

General Conclusion

Nine analysis methods were used for nine elements encompassing Groups IB, IIA, IIB, V, VIA and VIIA. The samples that were analysed were biological. Therefore sodium and chlorine were present in large amounts, both of which activate readily and were the major source of activity as ^{24}Na, half-life 12.8 hours, and ^{38}Cl, half-life 38 minutes. Thus to assay a single element it was found necessary to use a means of radiochemical separation to remove such major interference along with any other. Physical separation by instrumental means, as used by other workers and applied to geological sample analysis etc., is limited in its usefulness when applied to analysis of samples of biological origin, due principally to the Na and Cl interference. Thus the analysis techniques used in the work carried out all involved some radiochemical separation.

The nine methods selected, modified and evolved, were designed to be simple in use and appropriate to application to analysis of biological materials. They were particularly suitable to analyses of large numbers of samples, rather than one single sample, enabling the survey work which is later described to be carried out. Adequate sensitivity of detection was able to be obtained for each element.

In the selection of suitable methods for separation it was found that good use could be made of the literature which was rich in derived methods and specialised applications (11).

Thus for three of the elements, i.e. mercury, copper and cadmium it was found expedient to use methods already established. These methods required essentially little or no modification and were used as described. These methods taken from the literature proved indeed to be satisfactory, as evidenced by the large number of analyses that were able to be carried out to provide the necessary data for the environmental surveys and other measurements made. The sensitivity obtained in each case was respectively: copper 0.001 μg, mercury 0.003 μg, and cadmium 0.001 μg.

For another four elements, i.e. arsenic, manganese, gold and zinc, established methods were used but were modified since improvements were able to be discerned and were found possible to achieve. It was thus found that their applicability and usefulness were extended; for example, in the increased sensitivity obtained for arsenic enabling sectional hair analysis to be carried out, and for more analyses to be done at one time. All of these factors proved valuable. The sensitivities obtained during analysis were: arsenic 5×10^{-5} μg, gold 10^{-4} μg, manganese 0.002 μg, and zinc 0.002 μg.

For the remaining two elements, selenium and barium, it was found advantageous to construct new separation techniques. In the case of selenium it was found only necessary to have a simple one-stage separation. Here it was discovered that radiochemical purity was able to be obtained by waiting some three weeks and counting over the 0.14 MeV photopeak of ^{75}Se.

In this case it was shown that the maximum sensitivity achieved by open window counting could be sacrificed successfully for the expediency of having a straightforward separation technique, which thus enabled larger numbers of individual analyses to be carried out. The final sensitivity achieved (0.03 µg) was found adequate for the surveys made of selenium in human tissues and monkey eye tissue.

In the construction of a technique to be used for multiple analyses it was found desirable, for the sake of speed and simplicity, to confine as much as was possible of the chemical procedures involved to precipitation separations and the test tube. It was found possible to thus keep the simple method derived for selenium within these confines and avoid the more complex procedures such as HCl/HBr distillation.

It was found that in order to construct a method for barium analysis also within these "test tube" confines it was necessary to construct a very much more complex separation procedure than the one-stage method used for selenium. The difficulty of radiochemical separation of barium lies in the selective precipitation from a barium containing solution of interfering calcium and strontium. It was found that this could be done by fluoridation, calcium and strontium fluoride being relatively insoluble in water. The controlled use of calcium with no addition of strontium enabled both calcium to be precipitated and trace strontium co-precipitated as a fluoride leaving sufficient barium in solution for a final

barium recovery greater than 75% to be obtained. As can be seen, careful investigation indicated that the above method was viable. The sensitivity obtained (0.2 μg) was found adequate for the investigation carried out of barium in human lungs. Radiochemical purity was obtained by restriction to γ counting over the 0.165 MeV photopeak of ^{139}Ba. Here it was considered that any reduction in sensitivity by not counting by open window β was more than compensated by the short time able to be spent in each separation, which in turn minimised the sensitivity loss caused by radioactive decay of the short-lived (half-life, 1 hr. 25 mins.) ^{139}Ba isotope.

It can be concluded, nevertheless, that it was desirable whenever possible to be able to obtain the greater counting efficiency—and hence ultimate sensitivity—of open window β counting. Of the nine separate methods used, five involved open window β and four γ photopeak counting. It was considered, however, that the γ counting on the 4" NaI crystal, as carried out following non-rigorous chemical separation, was a successful compromise.

Thus it was considered that a viable and suitable series of analytical techniques had been established. Each technique was used for a particular application or applications involving trace elements in tissue. Arising from the actual analysis results thus obtained, further subjects were able to be examined—the role of trace elements, their toxicology, etc.—as is described in the following sections of this thesis.

Both the sensitivity of all the methods and the ability to use them after irradiation in an open laboratory immune from contamination was considered to have made the technique of thermal neutron activation analysis well suited to the trace element assays that were undertaken.

CHAPTER 4

Surveys of Trace Elements in Tissue

SURVEYS OF TRACE ELEMENTS IN TISSUE

Introduction

Carbon is distinguished amongst all the other elements by the extent of its versatility to self bond. All biological life forms contain carbon and the elements oxygen and hydrogen. These three are the basic building blocks for organic structure. The fabric materials for plant and animal tissue include nitrogen, phosphorous and sulphur. Two classifications of the elements which go together to constitute biological tissue may be made. These are the major or macro elements and the minor or micro (trace) elements.

For man, the macro element group may be said to include the fabric elements O, C, H, N, Ca, P and S and the physiological saline elements Na, K, Ca, Mg and Cl. Ca is both a fabric and saline element. There is no exact boundary between both classifications and some authors, e.g. Schütte (44), list elements such as Cl as a trace element. The first three elements, O, C, H, make up approximately 93% of the average man's weight and the group as a whole over 99.9%. Na, K, Ca and Mg together constitute 99.5% of the total metal content of the human body.

The second group, the trace elements (<0.1%) consists of all the other elements present. The concentrations of these trace elements are usually considered in terms of parts per

million by weight (p.p.m.). Some of these elements are known to be essential, e.g. Cu, Zn, Mn, Mo, I and Fe (45). Such elements are often held in co-ordinate bonds and by chelation, for example, as prosthetic groups of enzymes and in other proteins. Their free inorganic salts sometimes act as cofactors of enzymes. As the enzymes etc. are necessary to the tissue's metabolism these trace elements are in turn essential. Other trace elements are not known to be essential, for example, Hg, As, Cd and Ba. They are assumed to be present by chance due to diet, soil conditions, contamination, etc., and are not held in regulated amounts. A few accumulate passively because there is no effective way of totally excreting them, for example approximately 16 μg per day of Cd is retained by man (46). The surroundings of a living organism affects its uptake of trace elements to a certain extent; however, it is a notable feature of biological tissue, whether plant or animal, that the ratio of its constituent elements does not mirror that of its environment—the soil—diet—rocks—the sea, etc. Essential elements have been classified as macronutrients (>1 p.p.m. required), and micronutrients (<1 p.p.m.) for trace elements. Excess of any element, whether essential or not, is usually toxic, as described by Bowen (47).

Therapeutic medication of deficient trace elements in man has been carried out as long as 2000 years ago by the Greeks who used the consumption of iodine-rich ashed sponges

to cure goitre, even if they did not know the reason for its effectiveness.

The importance of many of the trace elements in biological tissue is now recognized, many of their functions known, and their need established. Those not demonstrated as essential are classified as nonessential, however it is of course possible that they may be essential at unmeasurably low levels.

It is useful to have an adequate definition of the criteria of whether a trace element is essential or not. An elegant and comprehensive definition can be quoted from Arnon and Stout (48), who in 1939 used the following to judge whether certain elements, in particular copper, were necessary to plant growth: ". . : an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium." The above criteria are as equally valid when transposed to animal tissue.

The necessity of certain trace elements to the function of a tissue was not suspected until precautions were taken to ensure absolute deficiency. For example, as recently as 1970

Thompson and Scott (49) thus demonstrated a per se requirement for selenium to chicks. Some trace elements have been identified as necessary for some time; as long ago as 1869 Raulin (50) described the necessity of zinc to plant growth. Other trace elements have only very recently been classified as essential to animal life. For example, a report in 1973 of a World Health expert committee (45) reviews that of the 14 trace elements mentioned as essential, five, i.e. Ni, Sn, Si, F and V, had only been shown to be so in the previous three or four years. The lack of a necessary trace element brings about a so-called deficiency disease. For example, copper attenuation in the diet of cattle and sheep causes damage by demyelination of the central nervous system, resulting in characteristic poor muscular coordination—the "swayback condition" (51); boron deficiency in plants as identified by Brandenburg (52) causes "heart rot"—this is the formation of necrotic regions due to meristematic damage. As Arnon and Stout's definition of essentiality suggests, demonstration that a trace element is essential can be made by introducing it to an organism that had been deprived of it, whereupon the previous manifestations of deficiency disease disappear. For example, Thomson and Scott (49), who produced severe degeneration and pancreatic fibrosis in chicks fed with a selenium deficient amino acid basal diet, were able to promote growth and prevent pancreatic fibrosis on addition of 0.02 p.p.m. selenium to the basal diet. However, such means

of confirming the essentiality of a trace element present difficulties, due to the low levels of the element that may be involved; the complete exclusion from the diet, atmosphere and environment is almost impossible (53). Thus the introduction of a trace element to an organism thought to have been deprived of it may have no significance and the possible essential nature of the element go undetected.

There is another approach in determining the role of a trace element in a tissue. That an element is essential or nonessential may be shown by the distribution pattern found of the levels measured in a sufficiently large number of different examples of the tissue. The number of results against the levels found is plotted. Such a method is described in some detail by Liebscher and Smith (1). A "normal" distribution, Gaussian in form, is associated with an essential trace element, the concentration of which is subject to control. A skewed or "log-normal" distribution indicates that the element is non-regulated and is nonessential.

Using this principle such work, described by Liebscher and Smith, was extended by the examinations that were carried out of the roles of manganese, selenium and cadmium in tissues. These were primarily the major tissues of the human body and in the case of manganese also of Rhesus monkey (macaca mulatta). The role of barium was examined to a more limited extent, mainly in human lung. The variation with time

of the essential trace element copper in the skin and nail of one individual was investigated. These studies are described element by element in the following pages.

Manganese

Manganese, classified under Group VIIA of the Periodic Table, is a member of the first transition series. It is twelfth in order of abundance and makes up approximately 0.085% of the earth's crust. It is the 14th element in order of abundance in man. The major use of manganese is as an additive in steel. It is essential as a trace element in both plant and animal tissue, as Bowen describes (54). It is the second most abundant metal found in nature (iron is the first). It is one of the few elements that is able to exist in one of eight oxidation states, of which only two, Mn(II) and Mn(III), are important in biological tissue. Its deprivation brings about deficiency diseases in animals, as described by Underwood (55), and in plants, e.g. mottled chlorosis in leaves, as described by Wallace (56). Manganese is involved in photosynthesis and enzymatic processes and its biolgoical role is adequately summarised by Cotzias (57). Only high levels of manganese absorption are toxic to man. Neurological damage thus can occur with symptoms akin to those of Parkinson disease, as described by Hunter (58). Poisoning cases, although rare, have occurred among workers in manganese mines or ore crushing plants where inhalation of dust happens, as described, for example, by Cotzias (59).

It was considered it would be of interest to carry out a survey of manganese in tissue. Samples that could be obtained enabled a comparison to be made for some of the organs

of "normal" humans and Rhesus monkeys who had lived under controlled conditions. Not all of the samples were duplicated for human and monkey. A more catholic collection of human tissue was obtained.

Analysis was carried out for most of the major organs in the human body. The tissues were taken in post-mortem examination of persons who had died in traumatic circumstances (car accidents, etc.) in the Glasgow area. Thus they were as reasonably representative of the Glasgow population as could be obtained. 28 different tissues were examined and a total of 173 samples analysed. Analysis was also carried out of tissues taken from 3-year old Rhesus (macaca mulatta) monkeys. The animals were sacrificed by guillotine. 13 different monkey tissues were examined and 187 samples analysed. The monkeys all lived under similar conditions. They were kept in an animal house at the University of Florida, USA.

Results

The results were as shown in Table 8 and Figures 9, 10, 11 and 12.

Table 8. Manganese found in human and Rhesus monkey tissue

Tissue (dried)			p.p.m. manganese					
Human	Rhesus monkey	No.	Arith. mean	Geom. mean	Median	Lowest value	Highest value	Value
Adrenal		5	1.16	0.989	1.191	0.678	1.75	
	Adrenal	11	3.54	3.42	3.24	2.39	5.25	
Aorta		5	0.481	0.386	0.383	0.196	1.16	
Bladder		1						0.639
Blood		1						0.228
Bone		5	0.442	0.277	0.233	0.108	1.44	
Brain		5	0.999	0.975	1.031	0.694	1.337	
	Brain	79	2.00	2.02	2.00	0.800	3.56	
Breast		1						0.173
Head hair		42	2.46	1.77	1.98	0.0664	9.86	
Heart		10	0.877	0.831	0.827	0.439	1.514	
	Heart	12	1.16	1.09	1.01	0.590	2.06	
Kidney		5	4.02	3.83	3.32	2.94	6.74	
	Kidney	10	3.43	3.30	3.60	2.34	4.68	
Liver		11	4.54	4.45	4.80	3.25	6.20	
	Liver	11	4.95	4.64	5.10	2.51	7.54	
Lung		16	21.82	15.81	24.1	4.70	44.4	
	Lung	13	2.67	2.29	2.48	0.843	7.73	
	Lymph nodes	7	4.61	3.74	3.27	1.64	11.61	
Nail (finger)		7	0.852	0.782	0.593	0.552	1.60	
Nail (toe)		2	0.810	0.759		0.529	1.09	
	Ovaries	4	0.630	0.614	0.611	0.447	0.850	

Table 8 (cont'd)

Tissue (dried)			p.p.m. manganese					
Human	Rhesus monkey	No.	Arith. mean	Geom. mean	Median	Lowest value	Highest value	Value
Pancreas		5	4.34	4.15	4.35	2.41	5.58	
	Pancreas	9	3.96	3.71	4.30	1.62	5.31	
Pectoral muscle		5	0.426	0.330	0.243	0.177	1.13	
	Pituitary	9	3.64	3.46	3.13	2.18	5.45	
Pleura		5	21.96	8.52	7.80	1.13	66.5	
Prostate		5	0.642	0.549	0.537	0.201	1.24	
	Prostate	7	1.18	1.11	1.29	0.540	1.66	
Pulmon. artery		3	6.28	5.72	5.20	3.58	10.05	
Pulmon. vein		2	4.78	3.99		2.15	7.40	
Spleen		5	0.568	0.546	0.528	0.413	0.904	
	Spleen	12	0.713	0.678	0.610	0.430	1.76	
	Skin	3	0.443	0.393	0.490	0.190	0.650	
Stomach		5	1.69	1.49	1.26	0.971	3.68	
Thymus		2	0.896	0.389		0.782	1.01	
Thyroid		5	0.818	0.769	0.679	0.521	1.31	
Trachea		5	5.81	5.44	5.00	4.20	10.5	
Tongue		5	8.89	7.81	9.10	3.42	16.2	
Urine		4	0.98	0.392	0.194	0.163	3.34	
Uterus		1						0.178

FIGURE 9

DISTRIBUTION OF MN FOUND IN 79 SAMPLES OF MONKEY BRAIN.

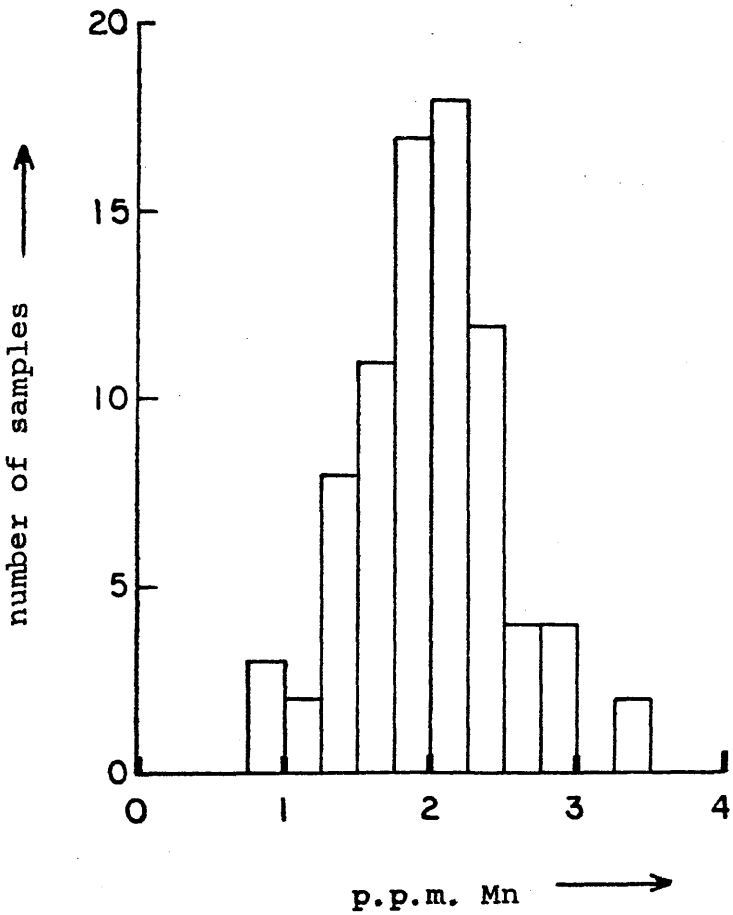

20
15
10
5
0
number of samples
0
1
2
3
4
p.p.m. Mn

FIGURE 10

DISTRIBUTION OF MN FOUND IN 42 SAMPLES OF HUMAN HAIR.

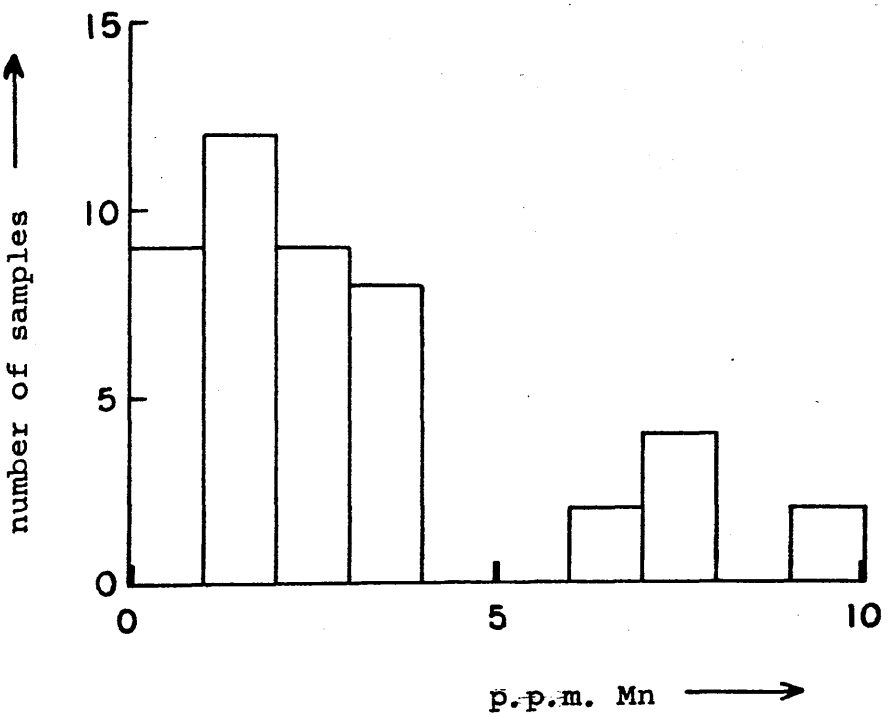

15
10
5
0
number of samples
0
5
10
p.p.m. Mn

FIGURE 11

DISTRIBUTION OF MN IN 16 SAMPLES OF HUMAN LUNG.

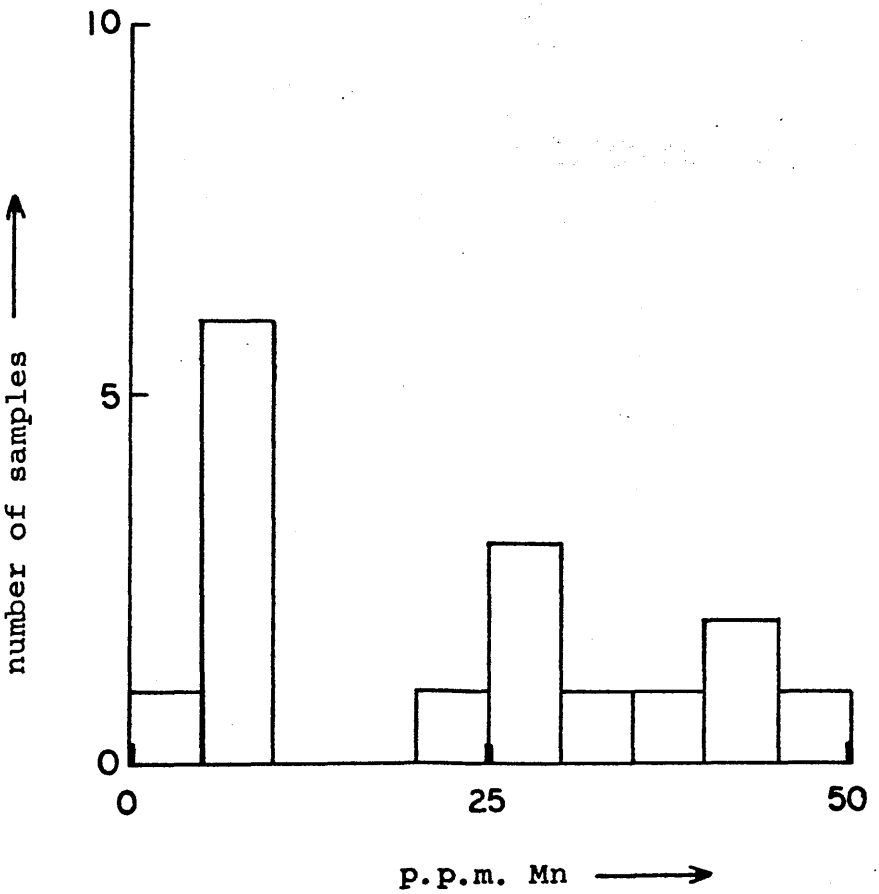
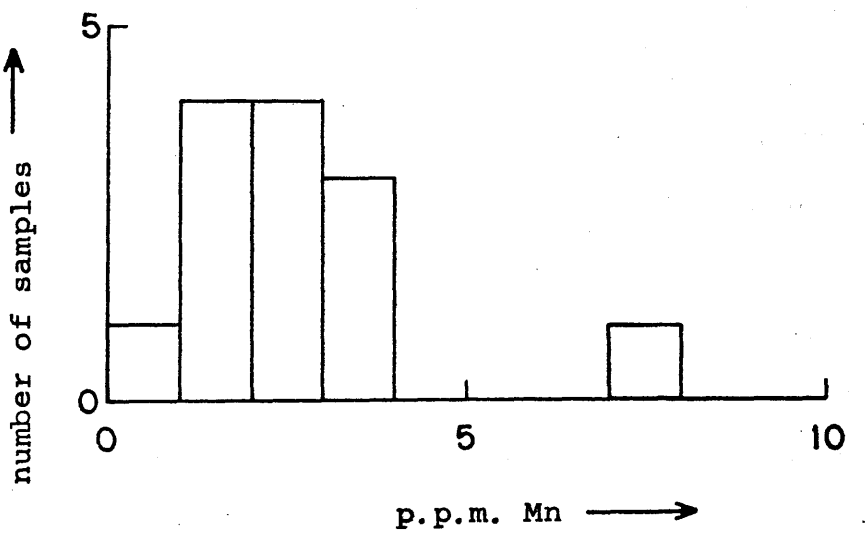

10
5
0
number of samples
0
25
50
p.p.m. Mn

FIGURE 12

DISTRIBUTION OF Mn IN 13 SAMPLES OF RHESUS MONKEY LUNG.


5
0
number of samples
0
5
10
p.p.m. Mn

Results and conclusions

It is known that manganese is considered an essential trace element in mammalian tissue (55). The median, arithmetic and geometric means of the set of levels found for the tissues analysed were found to be mostly similar as would be expected if the distributions are normal and manganese essential. The largest set of data and hence most favourable for statistical analysis was that for 79 monkey brain samples. Here the median and means were found to vary by only 1%. These values when plotted, as shown in Figure 9, were found to be normally distributed, confirming that manganese was essential to the brain. The normal distribution found was similar to those of the essential trace elements copper and zinc in heart and liver tissue, as shown by Liebscher and Smith (1).

When the values of manganese found in human hair were plotted as shown in Figure 10, a lognormal distribution was found. This indicated nonessential role for manganese in hair. This was attributed to the fact that hair is a non-living tissue in which there is no process in which manganese need play a part. The hair is merely an indicator of diet. It is also subject to unknown external contamination. If this influence was strong it could swamp any evidence of a normal distribution. In this case the lognormal distribution found was similar to the distributions of the nonessential trace elements antimony, arsenic and mercury in heart and liver shown by Liebscher and Smith (1).

The average levels found for both human and monkey in heart, kidney, liver, pancreas and spleen were similar. The average levels for monkey for adrenal, brain and prostate were approximately twice those of human origin.

The most significant and noticeable difference between the levels for humans and monkeys was found in lung tissue. Approximately 10 times as much manganese was found in human lung as in monkey lung. The distribution of manganese in human and monkey lung was plotted as shown in Figures 11 and 12. The distribution found in monkey lung appeared normal as the values shown in Table 8 suggested, whereas that in human lung was skewed and appeared lognormal as again the values in Table 8 suggested.

Molokhia and Smith (60) described the distribution of the essential trace elements copper, manganese and zinc, and of the nonessential trace elements arsenic, mercury, antimony and cadmium. For the essential trace elements (which included manganese) Molokhia and Smith reported a normal distribution and a lognormal distribution for the nonessential trace elements. Thus the skewed distribution found for manganese in human lung was not of the form described by Molokhia and Smith. This could be attributed to the fact that manganese absorption in lungs varies greatly and that the human lung samples were more grossly internally contaminated than the set analysed by Molokhia and Smith. This is also suggested by the very much higher mean levels found

(around 15 p.p.m. geometric mean for dried lung) compared to the equivalent for dried lung (about 1 p.p.m.) reported by Molokhia and Smith. The distribution found in monkey lung was similar to that described by Molokhia and Smith for human lung.

It was concluded that the higher levels and lognormal distribution in human lung was due to environmental contamination by breathing air in an industrial part of the country over a period in excess, on average, of 30 years. The monkey lung had breathed air in a rural environment for 3 years, one-tenth the average age of the human lung, and had approximately again one-tenth as much manganese. It was thought that the average level around 2 p.p.m. manganese found in monkey lung was close to a normal maintained level in such tissue. The evidence of normal distribution indicated that this was the level at which manganese was present as an essential trace element in such lung tissue. It was thought therefore less likely that the lower manganese level in monkey lung was due to its smaller age, although this may have been significant to some extent. Environmental contamination in human lung swamped the recording of any normal distribution causing large level elevation.

Selenium

Selenium is juxtaposed between sulphur and tellurium in Group VIA of the Periodic Table. The element was discovered by the Swedish chemist Berzelius in 1817 (61). Bowen (62) quotes an average level of 0.2 p.p.m. in soil. Selenium is, as discussed further on, now considered as an essential trace element to mammals. Quoted values of selenium concentrations in human (and other) tissues were found to be scattered and scarce throughout the literature. This may be attributed to difficulty in the past in determining the low levels in which selenium was present. Often when analysis methods were described their application was limited to a narrow range of materials. Dickson and Tomlinson (63) reported selenium levels in 10 adults; for liver 0.18 to 0.66 p.p.m., mean 0.44 p.p.m.; for skin 0.12 - 0.62, mean 0.27 p.p.m. and for muscle 0.26 - 0.59, mean 0.37 p.p.m. They also reported levels for the major tissues of one adult which included brain 0.27 p.p.m., muscle 0.40 p.p.m., kidney 0.63 p.p.m. and the highest level found, in thyroid 1.24 p.p.m. Dye et al. (64) using a fluorometric technique measured selenium in dried tissue from cattle, sheep and rats and found for liver 1.98 - 2.24 p.p.m., muscle 1.29 - 3.25 p.p.m., brain 1.42 - 2.64 p.p.m., heart 1.28 - 2.84 p.p.m. and kidney 0.61 - 3.19 p.p.m. Other substances analysed for selenium include hay (37), plasma (36) and blood (35).

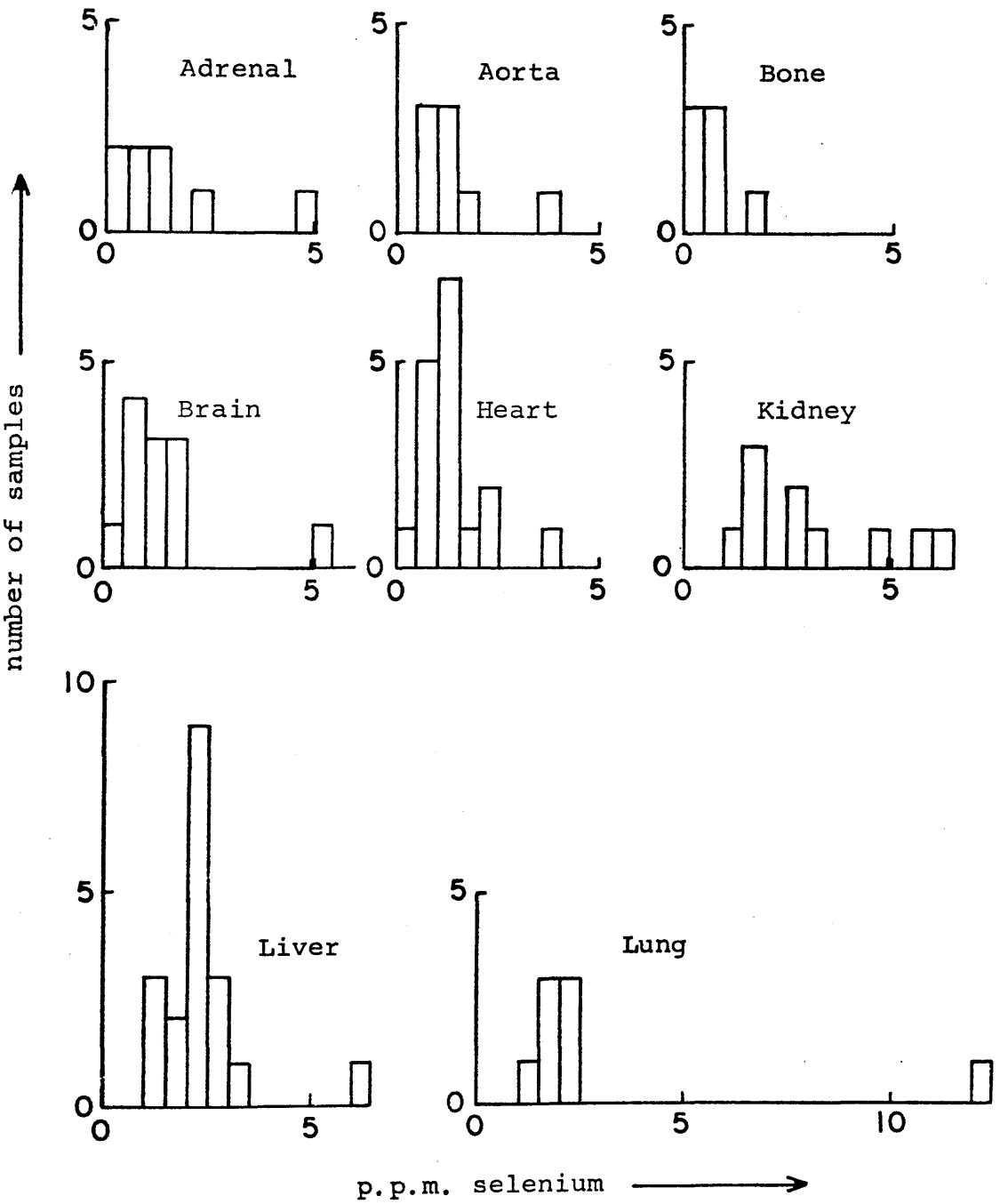
The analysis method employed provided sufficient sensitivity and a means of assaying selenium concentration in a wide range of human tissue. It was therefore considered useful for a selenium survey to be carried out and this was done. A total of 174 analyses were made and 19 human tissues analysed. The average sample weight was 50 mg. It was also thought that selenium may play some part in the function of the eye. Eyes from humans were not readily available and thus eye tissue taken from Rhesus monkeys was analysed. A total of 22 samples of 6 tissues taken from 10 such monkeys were analysed. The monkeys were 3 years old when killed and were kept as a group under normal animal house conditions.

Results

The results were as shown in Tables 9 and 10, and Figures 13 and 14.

FIGURE 13

DISTRIBUTION OF SELENIUM FOUND IN 17 DIFFERENT HUMAN TISSUES.


Adrenal
Aorta
Bone
Brain
Heart
Kidney
Liver
Lung
number of samples
p.p.m. selenium
0
5
10

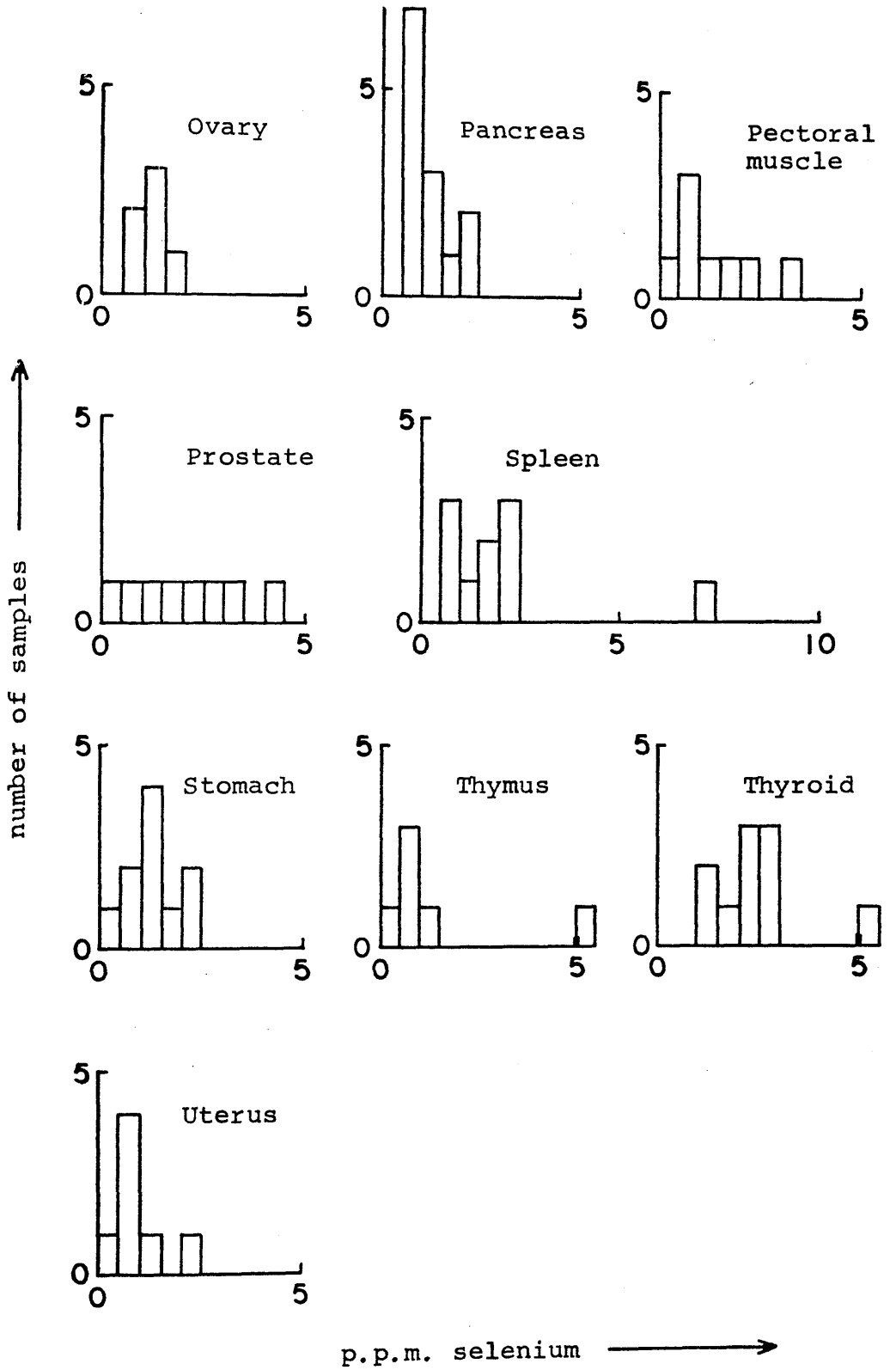

Ovary
Pancreas
Pectoral muscle
Prostate
Spleen
Stomach
Thymus
Thyroid
Uterus
number of samples
p.p.m. selenium
0
5
10

FIGURE 14

DISTRIBUTION OF SELENIUM FOUND IN LENS AND RETINA TISSUE OF RHESUS MONKEYS.

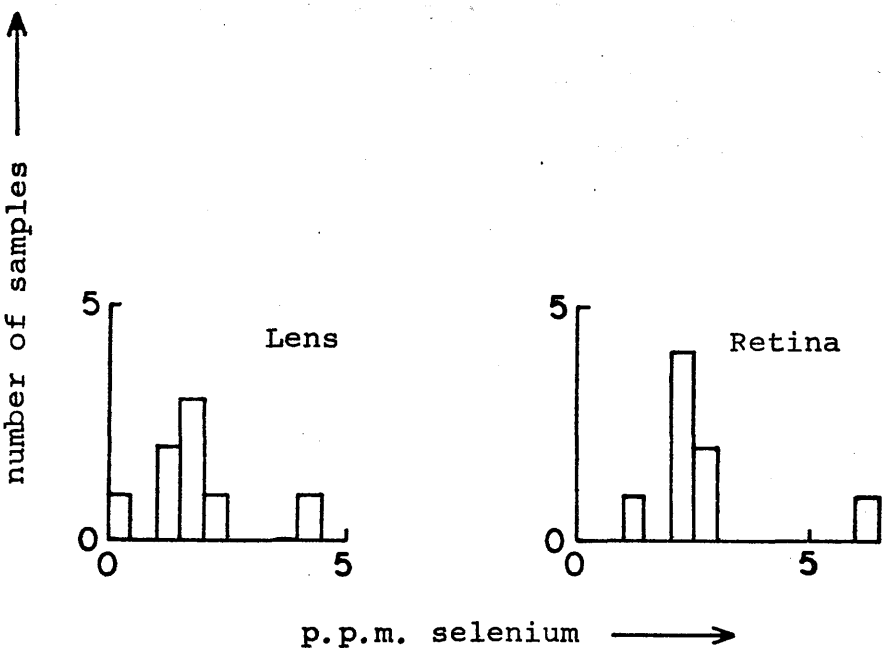

number of samples
5
0
0
5
Lens
5
0
0
5
Retina
p.p.m. selenium

Table 9. Selenium levels found in human tissue

Tissue	No.	p.p.m. selenium					
		Arith. mean	Geom. mean	Median	Lowest value	Highest value	Value
Adrenal	8	1.50	1.16	1.07	0.38	4.20	
Aorta	8	1.54	1.33	1.29	0.62	3.96	
Bone	7	0.612	0.483	0.595	0.20	1.67	
Brain	12	1.51	1.21	1.45	0.36	5.16	
Breast	1						0.56
Hair	1						3.71
Heart	17	1.37	1.17	1.15	0.41	3.96	
Kidney	10	3.47	3.09	3.26	1.17	6.15	
Liver	19	2.38	2.20	2.31	1.09	6.30	
Lung	13	2.39	1.64	1.62	0.684	12.4	
Ovary	6	1.20	1.15	1.20	0.770	1.76	
Pancreas	13	1.13	1.01	0.92	0.530	2.31	
Pectoral muscle	8	1.38	1.14	1.14	0.404	3.10	
Prostate	8	2.09	1.64	2.02	0.382	4.31	
Spleen	10	2.03	1.58	1.62	0.710	7.27	
Stomach	10	1.33	1.20	1.32	0.445	2.28	
Thymus	6	1.48	0.941	0.647	0.487	5.40	
Thyroid	10	2.44	2.24	2.41	1.25	5.35	
Uterus	7	0.978	0.865	0.875	0.382	2.00	

Table 10. Selenium levels found in monkey eye tissue

Tissue	No.	p.p.m. selenium					
		Arith. mean	Geom. mean	Median	Lowest value	Highest value	Value
Cornea	2	5.14	4.11		2.06	8.22	
Iris	1						9.35
Lens	8	1.88	1.56	1.76	0.36	4.43	
Retina	8	2.73	2.49	2.39	1.21	6.10	
Sclera	2	1.55	1.53		1.35	1.74	
Vitreous body	1						0.200

Discussion

Selenium has been shown to be an essential trace nutrient in animal diet, as described by Schwarz (65). A selenium requirement was first shown by Schwarz and Foltz in 1957 (66), who found that 0.1 p.p.m. selenium in the diet of vitamin E deficient rats prevented liver necrosis. The so-called Factor 3 selenium, thoght to originate in the diet, has a biological activity similar to vitamin E (the tocopherols, the main natural antioxidants) and is thought to function as an alternate to it (67). Selenium occurs in a variety of proteins and some amino acids, as described by Rosenfeld and Beath (67). Selenium as a requirement, per se, to chicks from selenium depleted hens is described by Thompson and Scott (49). Cattle and sheep fed on feedstock containing little trace selenium, which happens if it is grown in an area with low levels of selenium in soil—as in parts of eastern USA—develop muscular

dystrophy which may be prevented by a selenium dietary supplement (67). Growth of children suffering from kwaskioskor has been increased by administration of sodium selenite, as Majaj (68) described, and of 4,4'-di-seleno-di-valeric acid, as Schwarz (69) described.

Selenium in high levels is toxic (67). The so-called alkali disease in livestock from South Dakota was first identified in 1934 as due to the toxicity of naturally occurring selenium, found there in high concentrations in plant foodstuffs, by Franke (70). Its toxicity to animals is described, for example, by Rosenfeld and Beath (67). In alkali disease, shedding of hooves and nails, loss of teeth and hair, and paralysis can occur.

To man, selenium salts if directly ingested are highly toxic; they are poorly absorbed through the skin. Injury to heart, kidney and liver can be caused (67). Included among the poisoning symptoms of coughing, gastroenteric disorder, etc., is the occurrence of conjunctivitis, as Deichmann and Gerarde describe (71).

As analysis was carried out for a "normal" population not knowingly exposed to selenium, it would be expected that evidence of a normal distribution of selenium in tissue would be found. The results as shown in Table 9 and Figure 13 appeared to indicate this. The average levels which could be cross referenced to levels found in similar tissue by other workers correlated in the main (for example, to levels in cattle, sheep and rat tissue found by Dye et al. (64)). The

average levels found in the 17 samples of heart tissue were lower. The highest average levels were found in the kidney, liver and thyroid. Liebscher and Smith (1) reported selenium levels in dried human heart and liver. They found the following arithmetic means, geometric means and medians for 10 samples of heart: 1.14, 1.05, 1.17 p.p.m. Se, and for 12 samples of liver 2.40, 2.34, 2.34 p.p.m. Se, respectively. These levels were in good agreement with those found here.

The number of monkey eye tissues that were analysed was small. Nevertheless the results in Table 10 and Figure 14 showed evidence of a normal distribution of selenium in the lens and retina.

Conclusion

The evidence found indicated that selenium was an essential trace element in human tissue. This correlates with the evidence described elsewhere of the necessity of selenium at trace level in diet. It was observed that the mean values for the human tissues were all within a fairly close range of 1.5 ± 1.00 p.p.m. This suggests that selenium is used to perform a similar function in all soft tissue. The lowest overall selenium levels were found in bone (in this case rib bone), they were less than half those in any other tissue investigated. This is in agreement with Bowen (54). This may be caused by the greater association of soft tissue with proteins and the physiological functions of the tocopherols and Factor 3 selenium. Again the fact that selenium appeared to be essential within the tissues indicates that it plays a part

in metabolic and physiological processes (for example, as found by Rotruck et al. (72), participating in the action of the glutathione peroxidase of rat erythrocytes).

Selenium levels found in eye tissue were not significantly higher or lower than those found in other human tissue. Thus there was no direct evidence found that selenium played any part in the operation of the eye complex other than in the physiological functions already mentioned.

Cadmium

Cadmium is the closest member of the Group IIB series to zinc and is found alongside zinc in nature. Bowen (54) quotes a Cd/Zn ratio in minerals in the order of $1:10^3$. Zinc is an essential trace element in biological tissue, as Bowen (54) and Vallee (73) describe. It is well known that zinc has an association with enzymes, proteins, amino acids, etc. Where there is zinc present it might therefore be expected that cadmium would be found. Cadmium and zinc may compete for certain intracellular ligands and it is probable that metabolically significant interactions take place (74). There is ample evidence that cadmium levels in various tissues accumulate with age and at birth is virtually absent (46, 75). The biological half-life of cadmium is estimated as being between 16 to 33 years and it is mainly bound to the protein, metallothionein, which is involved in its transportation and storage (74). It has not been shown to be essential. For example, cadmium in kidney tissue was assumed lognormal by Perry et al. (76). It is calculated that man has an average accumulative daily retention of 16 µg cadmium, representing a 3% to 8% absorption of a daily diet of between 20 to 50 µg cadmium. The average adult man contains approximately 25 mg cadmium in his body (46). The most significant source of cadmium is food. However, workers such as Nandi et al. (77) have shown that there is significant amounts of cadmium to be found in cigarettes (1.2 µg per cigarette) of which 70% passes

into smoke. It can be shown that an average smoker can absorb 0.75 µg of cadmium per day. Cadmium is toxic and injection of sufficient amounts causes such effects as gastroentric distress, liver injury; breathing of cadmium fumes causes nausea, dyspnoea, etc. (71). As described by Friberg et al. (46) it is considered that the interrelationship of zinc-cadmium plays some part in the toxic effects of cadmium.

A survey of cadmium was carried out in 17 of the major human tissues. Nine different examples on average of each tissue were analysed. The tissues came from a "normal" Scottish population as described earlier.

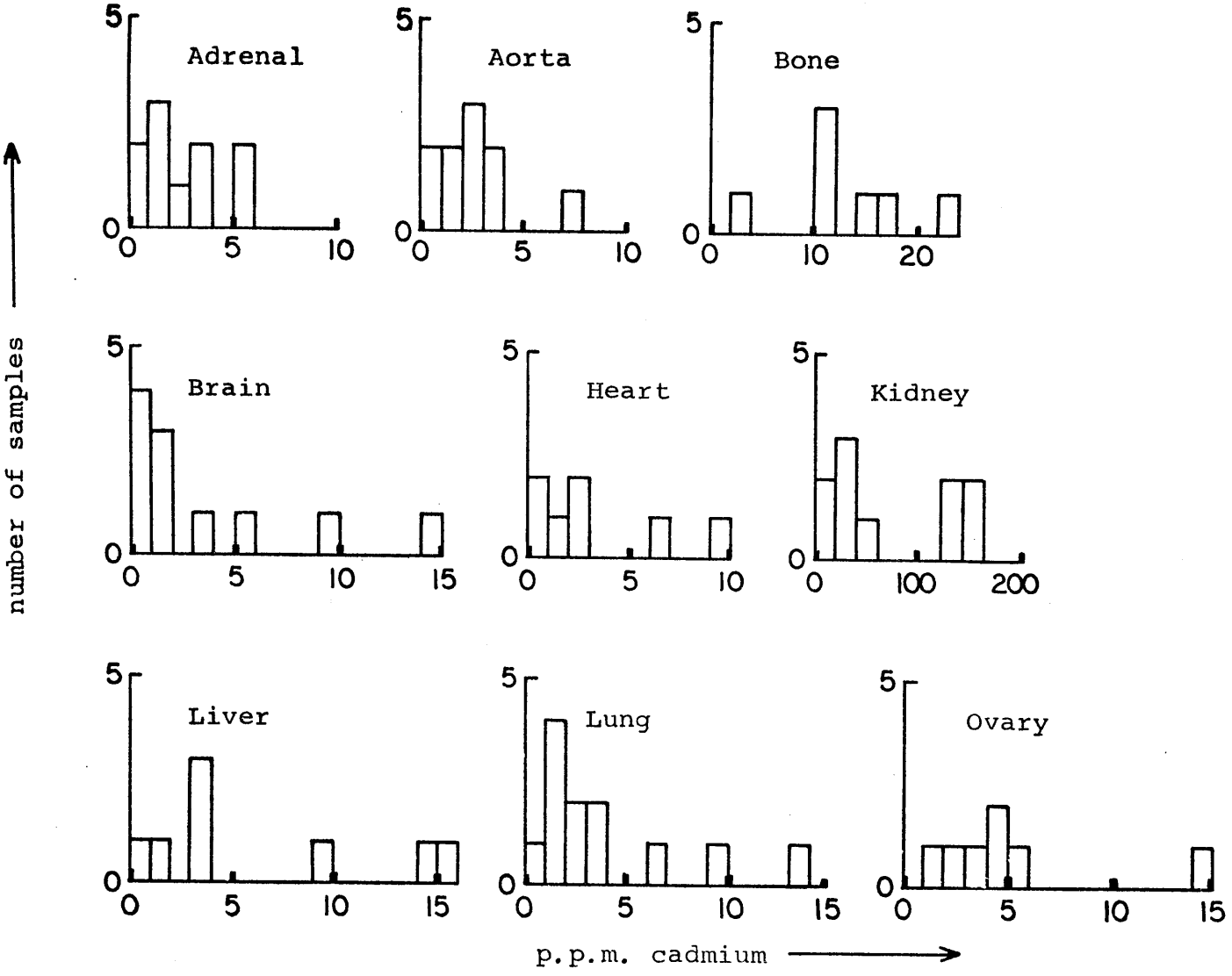
It was of interest, therefore, to see whether the results found would reflect a nonessential, lognormal distribution, as would be expected. Also it was of interest to compare the results found with those reported in the literature.

Results

These were as shown in Table 11 and Figure 15.

FIGURE 15

DISTRIBUTION OF CADMIUM FOUND IN 17 DIFFERENT HUMAN TISSUES.


Adrenal
Aorta
Bone
Brain
Heart
Kidney
Liver
Lung
Ovary
number of samples
p.p.m. cadmium

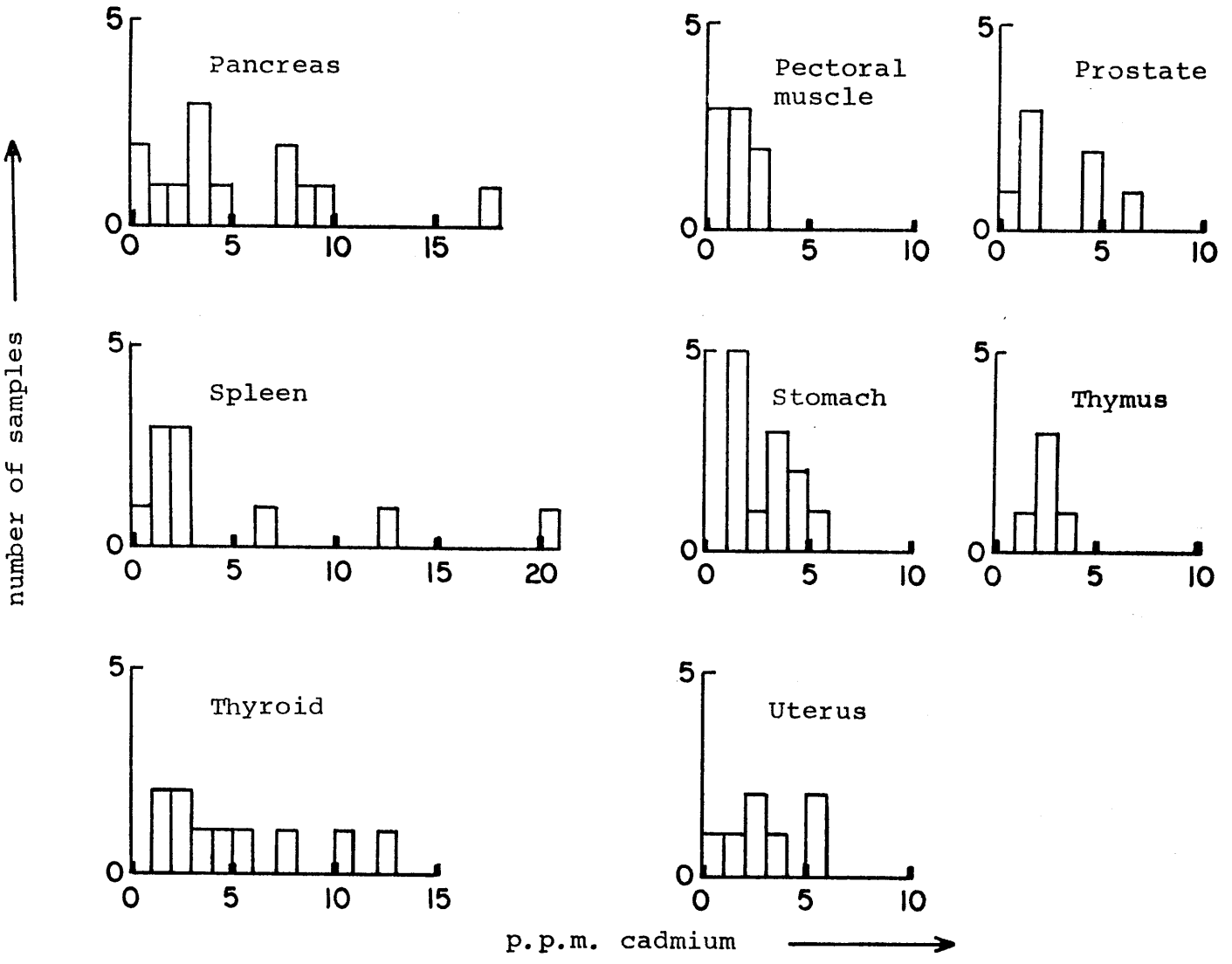

Pancreas
Pectoral muscle
Prostate
Spleen
Stomach
Thymus
Thyroid
Uterus
number of samples
p.p.m. cadmium

Table 11. Cadmium levels found in human tissue

Tissue	No.	p.p.m. Cadmium Arith. mean	Geom. mean	Median	Lowest Value	Highest Value
Adrenal	10	2.47	1.75	2.07	0.224	5.70
Aorta	10	2.60	2.05	2.12	0.550	7.40
Bone	7	12.7	11.0	11.5	2.71	22.1
Brain	11	3.62	1.75	1.39	0.340	14.3
Heart	7	3.34	2.08	2.11	0.400	9.80
Kidney	10	66.4	36.9	34.7	1.63	145
Liver	8	6.39	4.09	3.32	0.800	15.1
Lung	12	3.90	2.55	2.52	0.453	13.9
Ovary	7	5.31	4.16	4.40	1.20	14.9
Pancreas	13	5.43	3.65	3.92	0.540	17.5
Pectoral muscle	8	2.85	1.13	1.44	0.369	2.80
Prostate	7	2.96	2.15	1.55	0.490	6.16
Spleen	10	5.22	2.97	2.51	0.680	20.4
Stomach	12	2.74	2.39	2.57	1.20	5.16
Thymus	5	2.28	2.12	2.19	1.07	3.53
Thyroid	10	5.28	4.20	4.24	1.64	12.5
Uterus	7	3.09	2.64	2.19	0.990	5.81

Discussion

There was very little evidence found shown in Table 11 or Figure 15 of any normal distribution of cadmium in tissues. Thus, by the method of Liebscher and Smith (1), a nonessential role for cadmium was confirmed.

There is a certain paucity and variation of quoted cadmium levels to be found in the literature. A reasonably comprehensive list of cadmium levels in human tissue, by spectrographic analysis, is given by Tipton and Cook (15) and by Tipton et al. (78).

Tipton and her collaborators quote a dry weight equivalent of around 160 p.p.m. mean for kidney and less than 2 p.p.m. for most other tissues (dried weight equivalent). For many tissues Tipton did not measure absolute values. Lieberman and Kramer (79) quoted higher kidney levels than Tipton.

The results obtained were found to be in approximate agreement when they could be compared on a dry weight basis to literature levels. There was one significant difference, the highest cadmium concentration was found as expected in the kidney, but the mean levels were approximately one-quarter of those quoted by Tipton. The lowest value (1.63 p.p.m.) found came from the kidney of a young adult.

The next highest concentration was found in bone. This did not support Friberg et al. (46) who stated that cadmium does not accumulate in osseus tissue. However, cadmium's associate, zinc, is known to be important in the formation of bone. (Cadmium exposure is associated with the bone disease Itai-itai found in Japan (46)). The next highest concentrations were found in the liver and thyroid. This is in accordance with the abstracted data of Friberg et al.

The levels for muscle tissue were higher than those quoted by Lieberman and Kramer. Lung levels found lay between the higher levels quoted by Lieberman and Kramer and the lower levels quoted by Molokhia and Smith (60) and by Liebscher and Smith (1).

Conclusion

The results obtained provided a comprehensive list of cadmium levels in human tissue for a sample of "normal" Scottish population. The tissues were taken from 17 different humans, with an average age of around 40 years. The levels found were in broad agreement with those quoted by other workers. The variations found can be associated with the known dependence of cadmium concentration on age, geographical location, diet, etc. The distribution of cadmium was found in all the tissues examined to be lognormal, indicating its nonessential role. Such a consolidated and comprehensive listing for cadmium levels was not found quoted in one place in the literature.

It was found that cadmium accumulates to a much greater extent in the kidney than any other organ and also in bone liver and thyroid to a diminishing extent. The reason for this preferential accumulation could be associated with a zinc/cadmium interaction in the biochemical functioning of the tissue.

Barium

Barium lies below strontium and calcium in Group IIA of the Periodic Table. It is present at an average level of around 500 p.p.m. in soils and rocks. Das et al. quote values between 700 and 1200 p.p.m. in the standard rocks they analysed (41). The work of Turekian and Johnson (80) indicates that barium is present in the sea at a fairly uniform level of around 20 μg/litre. Bowen and Dymond (40) reported levels of 0.5 to 40 p.p.m. (ashed weight) for plants grown on normal soils. A survey of the literature shows that no real evidence has been found to prove that barium is essential to human tissue. A marked concentration of barium in the iris and choroid of the eyes of some animals was reported by Sowden and Pirie (81). They reported a lower value in human eye tissue, more comparable to that in soft tissue. Some evidence may be construed from the work of Rygh (82) that barium was necessary to the growth of guinea pigs and rats. Chemically, barium, strontium and calcium behave very similarly and all three are found together in tissue with calcium present in the greatest amounts, as for example, Bowen and Dymond found in plants, and the values found in human tissues by Tipton (15,78) indicate. Sowden (83) reported the highest barium and strontium levels were found in bone and aorta and Sowden and Stitch (84) showed that a greater amount of strontium than barium was found in bone.

Calcium is also found concentrated in bone and aorta tissue (15,78). The barium levels quoted by most workers are, in the main, low for human soft tissue, typically <0.1 p.p.m. dry weight (15, 78, 83).

Much higher levels (around 1 p.p.m.) are quoted for lung than for any other tissue. (Tipton and her colleagues (15, 78) show that along with barium, chromium, titanium and vanadium are concentrated in human lung.) As this appeared of interest, a study was made of barium in adult lungs obtained from a "normal" Glasgow area population. With the above facts in mind the following investigations were carried out:

1) A study was made to see if there would be found a lognormal distribution of barium in lung which would thus indicate a nonessential and non-regulated role (1). Seventeen different lung pairs were examined. The lungs on post-mortem removal had the blackish mottling usual in town dwellers.

Results

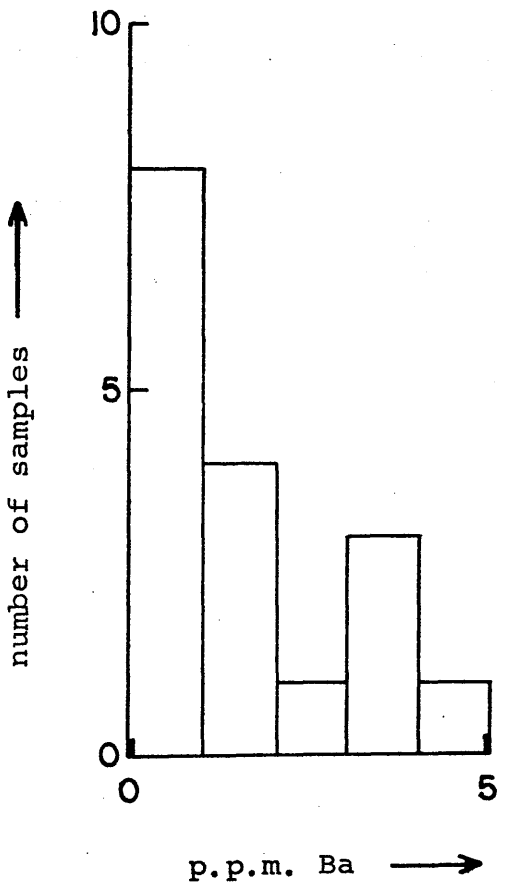
The results are shown in Table 12 and Figure 16.

Table 12. Barium found in human lung

		p.p.m. Barium				
Tissue	No.	Arith. mean	Geom. mean	Median	Lowest value	Highest value
Lung	17	1.63	1.14	1.38	0.250	4.18

FIGURE 16

DISTRIBUTION OF BARIUM FOUND IN 17 HUMAN LUNGS.


10
5
0
0
5
number of samples
p.p.m. Ba

Discussion

The values in Table 12 and the distribution shown in Figure 16 strongly indicated a lognormal distribution in lung. The Davies test showed a logarithmic distribution. This in turn suggested that the barium was nonessential to the lung and was present by chance. It was suspected therefore that its presence was due to environmental absorption by the lungs.

2) Barium levels within different parts of the lung were measured to find if there was any significant variation throughout the lung itself.

Results

The results were as shown below:

Table 13. Distribution of barium found within 3 lung pairs

	p.p.m. Barium		
Lung part	Pair 1	Pair 2	Pair 3
Right superior lobe	0.576	1.50	1.07
Right middle lobe	0.840	1.96	*
Right inferior lobe	0.750	1.12	0.950
Left superior lobe	0.964	1.83	*
Left inferior lobe	0.794	1.39	*
Right bronchus	0.794	*	*
Left bronchus	0.671	*	*
Trachea	1.20	0.082	*
Right bronchopulmonary lymph nodes	2.36	*	*
Left bronchopulmonary lymph nodes	2.23	2.40	2.20
Pleura	*	*	0.397
Pulmonary artery	*	*	0.280

*Sample not available for analysis.

Discussion

The results in Table 13 very much indicated that environmental contamination does occur. The higher barium levels found in lobe tissue were fairly constant, indicating an even distribution between left and right lobes. In Pair 1, where bronchus tissue was analysed the barium level was similar to that in the lobes. A complete range of tissue samples was not obtained from each lung pair, the greatest number being taken from Pair 1. In Pair 3 where pleura and pulmonary artery tissue were analysed about one-third less barium than in lobe tissue was present.

By far the highest levels were found in the broncho-pulmonary lymph nodes, >2 p.p.m. This was considered to have been caused as follows. Dust and other inhaled particles, not discharged in mucus from the lungs, are taken up by phagocytes cells and transported via the lymphatic vessels to the lymph nodes. In time the nodes become enlarged, inky black and packed with minute foreign bodies. Thus, in a way, the nodes may be considered as providing an accumulation of some of the contamination breathed over the life of the lungs. In this case the lymph nodes were found to contain more than twice as much barium as elsewhere in the lung. This correlates with the details concerning the relatively rare occupational disease of baritosis. As described by Hunter (58), baritosis, a form of benign pneumoconiosis, is caused by long-term inhalation of finely ground $BaSO_4$ by grinders and packers. X-rays

show small sharply circumscribed nodules evenly distributed throughout the lung. It would appear possible that this might be a more advanced form of the above finding.

An increase in trace element level in lymph tissue in the lung has also been reported by Molokhia and Smith (60). Molokhia and Smith reported a pronounced increase of the non-essential trace elements antimony and arsenic and of essential manganese in lymph tissue. They found no such effect for nonessential cadmium and mercury and essential copper and zinc. That this was so they attributed to the varying ease of absorption and removal of particulate contaminants by the normal body processes. That some trace elements including barium accumulate in lymph tissue may well be because there is no other mechanism in which they may be removed in high amounts from the lung.

The overall levels found in lung were similar to those reported by Tipton (15, 78) and elsewhere in the literature.

3) The analyses of some other tissues, namely aorta, bone and stomach, were carried out to compare the levels found with those reported by workers as described earlier (83, 15, 78).

Results

The results were as shown below:

Table 14. Barium levels found in 3 human tissues other than lung

Tissue	p.p.m. Barium
Aorta	0.330
Bone	2.58
Stomach	0.441

Discussion

The barium levels found in the aorta, bone and stomach samples showed that the analysis method gave results in agreement with literature values. More barium was found present in bone than other tissue.

4) Wheat, barley and garden peas were also analysed to see what level of barium was present in dietary items. All the samples were analysed in a dried form.

Results

The results were as shown below:

Table 15. Barium levels found in dietary items

Sample	p.p.m. Barium
Wheat grain	3.82
Barley grain	1.42
Garden peas	0.54

Discussion

The levels of barium found were in the lower range of those quoted for plant material (35). The levels are of the same order as the tissue samples which tends to suggest little if any discrimination against barium by the human system. In other words, it may be said that barium is a random contaminant which reflects the environment.

Conclusion

The survey indicated that the distribution of barium in lung tissue is lognormal and therefore that barium is nonessential at the levels recorded. (It must, however, be left open that it could be essential at a low level, and that the observation of this would have been masked by absorbed contamination.) Within the lung itself barium was found to be concentrated in the bronchopulmonary lymph nodes. Thus it was thought that barium is found at higher levels in lung than in other soft tissues, due to its absorption and accumulation there. The lungs studied came from a section of the population living in an industrially polluted area and therefore were probably subject to greater lifetime contamination than those from a rural population.

All of the barium levels found in the tissues analysed, mainly lung and the three examples each of other human tissue and plant material, were similar to values reported elsewhere.

Copper

Copper, in Group IIB of the Periodic Table, is the lowest valued of the three coinage metals. However, it is second only to iron as the most useful metal throughout man's history. It constitutes approximately 70 p.p.m. of the Earth's crust. The major use of copper is in the electrical industry. It is used extensively in alloyed forms, brass, bronze, etc.; over 1000 alloys are known.

Copper is an essential trace element in animals and plants, as described, for example, by Stiles (85), Liebscher and Smith (1) and by Underwood (86). It is found as a component in enzymes and proteins (86). It is the 13th most abundant element in man, around 80 mg is estimated by Cartwright and Wintrobe (87) to be contained in a 70 Kgm man. Grinstead (88) states that 2 mg of copper per day is required by the adult human. The 14th report of the Joint F.A.O./W.H.O. Expert Committee on Food Additives mentions that a copper intake of up to 0.5 mg/kgm body weight is considered acceptable (89). As is reviewed in 1973 in a report of a W.H.O. Expert Committee (90), copper deficiency in adult humans is unreported, but is known in infants. Cordano (91) describes the effects of such deficiencies. As described by Schütte (51), lack of copper causes deficiency diseases in animals and retardation in growth. Deficiency disease can be related to decreased concentrations of some copper enzymes (86).

The toxicity of copper is reviewed in Chapter 5, page 228.

The work by Liebscher and Smith (1) shows a normal distribution of copper in human skin and nail taken from a random selection of healthy subjects (Table 17). It was felt that the variation of copper level in one subject over an extended period would be of interest. The question was whether the distribution was as wide as for the normal population or whether it would be held in a much narrower range by the personal metabolic process.

To investigate the variation in copper levels samples of fingernail, toenail and skin were collected from one individual over a 15-month period. Fingernail samples, which were easily collected, numbered 31, i.e. approximately one sample every two weeks; 22 toenail samples were taken; skin was collected more infrequently, 11 samples were taken. The individual concerned led a normal professional life and was not industrially exposed to copper, although he was occasionally in contact with copper and its salts. All copper levels were measured at the same time.

Results

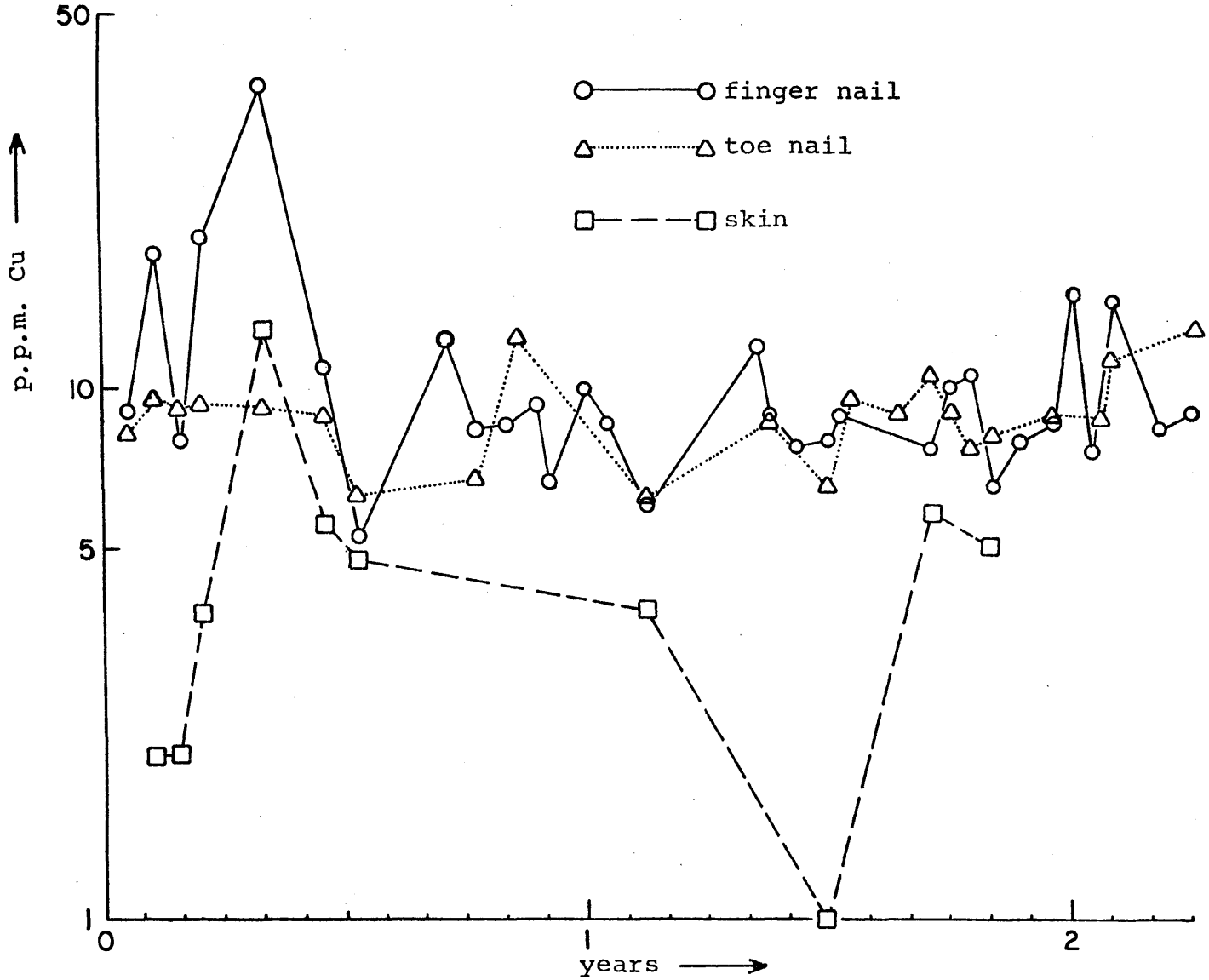
The levels found were plotted as shown in Figure 17.

Discussion

No observable pattern was found in the copper levels with time. Indeed it could be seen that considerable variation could take place from one measurement to the next. However, it could also be seen that greater variation took

FIGURE 17

VARIATION OF COPPER IN SKIN, FINGER AND TOENAILS TAKEN FROM THE SAME SUBJECT FOR MORE THAN 2 YEARS.


50
10
5
1
0
1
2
p.p.m. Cu
years
finger nail
toe nail
skin

place in fingernail levels as might be expected where the influence of external contamination would be strongest.

Further analysis of the data obtained

When the distribution of the copper levels measured in each tissue was plotted as shown in Figures 18, 19 and 20 some evidence of normal distribution was found. Ideally, more single observations would have been required to make analysis of the available data more statistically viable. However, in the case of the fingernail analyses where the largest number of observations was made, the Davies test gave a coefficient of 0.36, indicating a normal distribution. For the toenail and skin values, where there were insufficient observations for the Davies test to be really valid, it failed to show a normal distribution (coefficients were <0.2). The means and median values shown in Table 16 below were, however, in accordance with an assumed normal copper distribution in each tissue.

Table 16. Copper levels found in the same individual over a 27-month period

		p.p.m. Copper					
		Lowest		Highest	Arithmetic		Geom.
Tissue	No.	value	Median	value	Mean	Std.Dev.	mean
Fingernail	31	5.24	8.98	37.1	10.75	5.89	9.10
Toenail	22	5.73	8.88	12.9	8.81	1.88	8.62
Skin	11	1.03	3.84	13.0	4.59	3.19	3.79

FIGURE 18

DISTRIBUTION OF COPPER IN 31 SAMPLES OF FINGERNAILS TAKEN FROM THE SAME SUBJECT OVER A 2-YEAR PERIOD.

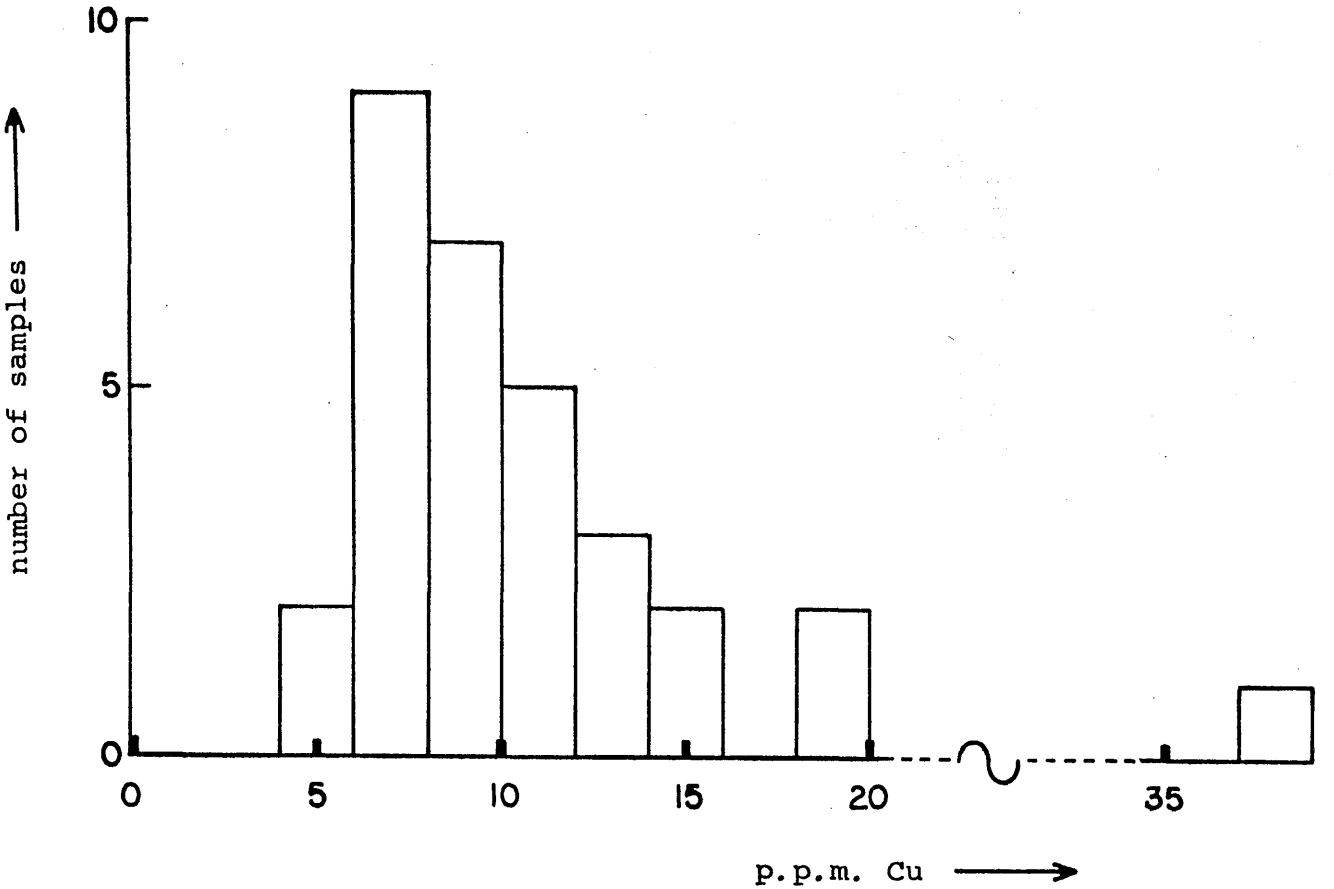

10
5
0
number of samples
0
5
10
15
20
35
p.p.m. Cu

FIGURE 19

DISTRIBUTION OF COPPER IN 22 SAMPLES OF TOENAILS TAKEN FROM THE SAME SUBJECT OVER A 2-YEAR PERIOD.

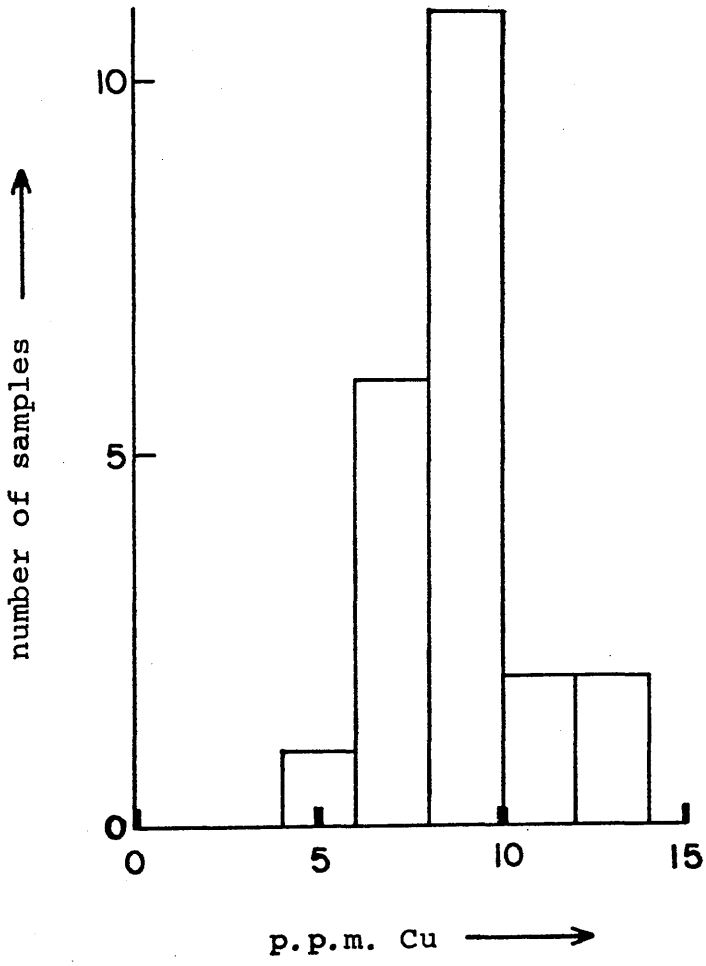
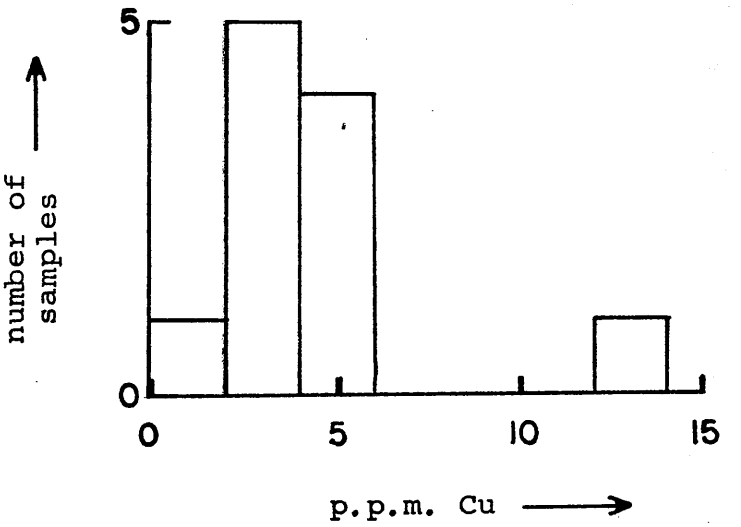

10
5
0
number of samples
0
5
10
15
p.p.m. Cu

FIGURE 20

DISTRIBUTION OF COPPER IN 11 SAMPLES OF SKIN TAKEN FROM THE SAME SUBJECT OVER A 2-YEAR PERIOD.


5
0
number of samples
0
5
10
15
p.p.m. Cu

The "normal" values of Liebscher and Smith are shown below:

Table 17. Copper levels found in a normal population*

		p.p.m. Copper					
		Lowest		Highest	Arithmetic		Geom.
Tissue	No.	value	Median	value	Mean	Std.Dev.	mean
"Nail" (i.e. fingernail)	33	3.18	14.9	58.2	18.1	12.1	14.7
Skin	10	0.29	1.76	5.39	1.98	1.69	1.30

*From Liebscher and Smith (1). The nail samples analysed were fingernails.

The F test was used to estimate the evidence from the sample group of observations of individual copper levels, and the sample group of normal population, of any difference of variance (s^2) between individual levels and population levels. It would follow that it could thereby be shown if indeed the personal metabolic process of the individual might restrict copper variation within narrower limits than those of the population as a whole.

The calculated F values are shown below:

Table 18. Variances and F values for fingernail and skin from sample groups of a normal population and an individual

Tissue	Sample groups	No.	Variance	F
Fingernail	norm. pop.	33	146.40	4.21
Fingernail	individual	31	34.74	
Skin	norm. pop.	10	2.86	3.56
Skin	individual	11	10.17	

For the fingernail groups (F = 4.21) there was very strong evidence at the 0.5% level that the variance of total population and the variance of the individual were different; the variance in the individual being the smaller.

For the skin groups the evidence at the 5% level showed that variances were different; the variance in the individual being the greater.

Thus it could be seen that copper levels in fingernail were maintained by the individual within a narrower spread of those found in the population. This could be due to the influence of a personal metabolic process. It should also be noted that external contamination in the population may be more widespread and hence a narrower individual range found. The evidence from the skin measurements showed with a lower degree of certainty greater individual copper variation than that in the population; however, the number of observations here was small.

Conclusion

It was considered that the evidence thus derived, showed on the whole, with certain qualifications, that copper variation was probably wider in the population than in the individual.

General Conclusion

Surveys of the widest range of tissues were carried out for the trace elements manganese, selenium and cadmium. A more limited survey of barium was made, principally in lung. Copper levels in one individual were monitored over an extended period to find out whether any variation was kept within a narrower range than the distribution found in the general population.

In the case of manganese, selenium and cadmium the major human organs and some Rhesus monkey tissues were examined. The results found illustrated the essential nature of manganese in human and monkey tissue and selenium in human tissue and the nonessential nature of cadmium in man. Where a comparison was made of manganese levels of human and monkey origin they were found to be similar apart from the adrenal, brain and prostate levels, which were higher in monkeys, and for lung, which were lower in monkeys. As the function and structure of these tissues were similar there was no immediate explanation for this except in the case of lung tissue. The lower level of manganese found in monkey lung was attributed to the greater external contamination from the air breathed in an industrial region by man for more than ten times as long as was the rural air surrounding the monkeys. Such influence was considered to have swamped any evidence of a normal distribution of manganese in human lung, as was found in monkey lung. It was thought of interest that the survey

results were in agreement with an essential role for manganese with the exception of human lung and of hair. In the case of hair it was concluded that manganese must be present in a non-regulated role merely as an indicator of diet, by absorption in the lungs or by possible external contamination.

Selenium was shown to be essential in the 17 major tissues of the human body examined. Not a great deal of variation was found between the mean levels of selenium in each tissue, the average being around 1.5 ± 1.00 p.p.m., suggesting that selenium performs a similar function in all soft tissue. The lowest concentration of selenium found in the body was not in soft tissue but in bone, indicating that there selenium played a lesser role. Selenium levels found in monkey eye tissue were similar to those of other soft tissue.

The cadmium levels surveyed in human tissue had a lognormal distribution which was in agreement with the non-regulated and passively accumulated role of cadmium in man. The results enabled a comprehensive listing of levels to be made. They were in broad agreement with those of other workers, except for bone in which higher levels were found.

Where barium in human lung was investigated, it was found to be concentrated in the lung lymphatic nodes. Barium was proved to be present in human lung as a nonessential and as an accumulated trace element. The concentration of barium in the lymph nodes was presumed due to transportation of inhaled contamination by the phagocytes cells via the lymphatic vessels.

The survey of copper was of interest, in that unlike the other surveys carried out, it concerned copper levels in skin and nail in one individual only, monitored for more than a two-year period. There was no obvious time pattern observed for copper levels in any one tissue and considerable variation from one measurement to the next was found. What was noticeable was that the highest copper levels occurred in fingernail, due it was assumed to the influence of contamination from the environment, and the lowest in skin. 95% of all the levels found were within the range 1 to 15 p.p.m. copper. Evidence of Gaussian distribution was found, indicating that there was a control of copper levels in each tissue which was long term. Evidence was shown that there was a smaller variance of copper levels in fingernail of one member of the population than the variance of copper levels in fingernail of the whole population. It was thought that this might reflect the influence of the personal metabolic process in tissues.

CHAPTER 5

Environmental and Clinical Studies

ENVIRONMENTAL AND CLINICAL STUDIES

Introduction

During the course of his daily work man is exposed to the influences of the surrounding environment. If materials, such as chemical agents, are prevalent some absorption of them by man may occur. Such uptake may be potentially hazardous and the extent to which this is so is becoming more and more appreciated. In 1969, Mayers (92) examined in some detail the subject of occupational health hazards. Some concerted attempt nowadays at industrial or occupational hygiene is normally made in order to reduce such uptake to acceptable limits. Maximum allowable concentrations ("MAC") or threshold limit values ("TLV"), below which it is considered there is no health hazard by exposure, are discussed in a recent World Health Organization publication (93). Health hazards now known to be present during all kinds of daily work are listed and described, for example, by Stellman and Stellman (94). Many of the diseases of occupations and their associated symptoms typified by forms of pneumocosis and dermatitis are well known and studied as described by Hunter (58).

Over the course of time the need for industrial hygiene to be exercised has been recognized and legislation has been made to enforce it. A new era in the protection of workers in dangerous trades started when the first Act of Parliament concerning a specific occupational disease was passed in

Britain over ninety years ago. This was the Factories (Prevention of Lead Poisoning) Act, 1883, which proscribed standards to be conformed with by white-lead factories. The first known legislative measure concerning industrial hygiene occurred some two hundred years prior to that, when in 1665 hand tremors of the mercury miners in Idria, Italy, led to a reduction of their working day to six hours (58).

The degree of any uptake depends on the magnitude and duration of exposure. Hazards may arise from the handling of solutions and exposure to fumes, dusts and vapors of chemicals (58). Sufficient care is not always taken or the need for it recognized where long-term exposure is concerned. Industrial and occupational hygiene has improved from earlier unenlightened days, however there is sometimes need for further upgrading of the precautions already taken.

Activation analysis is a technique ideally suited to investigation of this type of uptake. Its sensitivity allows precise analyses to be made of small, easily available and socially acceptable samples taken from workers, such as hair, nail and urine. The detection therein of elevated levels of elements can provide some measure of any uptake which has occurred.

A number of incidents of industrial exposure involving the elements mercury, arsenic and copper were thus investigated. One group was chosen whose common profession involved known lifelong exposure to mercury and a study was made into their absorption of that element. This investigation concerned the

uptake of mercury among dental groups.

Evolving from the work already described, the sensitivity and convenience of the analytical techniques used enabled extension into more direct clinical applications to be made. Samples were provided from hospitalized patients. These studies involved the measurement of the trace elements mercury, arsenic and gold. They were associated with the monitoring of poisoning cases and as an aid to diagnosis of disease. Clinical study was also carried out in an investigation of the role of zinc in the human mouth.

Mercury

Mercury is classified under Group IIB of the Periodic Table. It has the unique property of being a liquid metal at normal temperatures. It is among the earliest metals known by man. A small vessel containing mercury was found in a tomb at Kurna, Egypt, and was dated around 1600 B.C. The ancient Greeks used mercury compounds in ointments. Reference to mercury is found in Aristotle's "Meteorology" 320 B.C. The chemical symbol Hg comes from "hydrargyrum", the name given by Pliny. Mercury greatly intrigued the alchemists who considered it as a possible key to the transmutation of gold. The symbol they gave to "hydrargyrum" was that of the messenger of the Gods, Mercury; hence the metal's final name. The metallic nature of mercury was first proved in 1759 by J.A. Braun who froze it to a solid.

Extraction of mercury from its principal ore cinnabar, HgS, has therefore been undertaken for over 3000 years. Its average proportion in the earth's crust of 0.5 p.p.m. places it 62nd in order of abundance. All industrial mining occurs in the mercuri-ferrous belts of the globe. The world's principal mine is at Almaden, Spain. Other major mines are in Italy, Yugoslavia, USSR, USA, Canada and Mexico.

The process of natural land erosion cycles mercury to the sea where it is present at an average concentration of around 0.03 µg/ℓ (95). The wide range of uses of mercury brings about its release by man into his environment both by

deliberate (e.g. waste water discharges) and accidental means. Vostal (96) describes in detail the release of mercury into the environment by nature and man. Its uses include the electrical industry—switches, lamps, batteries, etc., catalytic production of chemicals—caustic soda, chlorides, etc., paint manufacture, general laboratory use, mercury chemicals such as agricultural fungicides, dental preparations, pharmaceutical and cosmetic applications, amalgamation extraction of metals such as gold and many other applications ranging from felt manufacture to spacecraft instrumentation. Present world consumption is in excess of 10,000 tons per year. About one-third of this is used as the metal, the rest in chemical form.

Elemental mercury is virtually non-toxic in the native form: it passes through the gastro-intestinal tract without absorption (97, 98). Only ionic mercury is considered capable of absorption by the formation of complexes in the tissues of the body (97). It is a protoplasmic poison. By linkage with sulphydryl groups it is able to protein bond and can produce potassium ion loss from cells. The lethal oral dose of mercuric chloride is given as 1 to 4 gms (71). Mercury vapour has been shown to be readily absorbed in the lungs, where it is ionised (with an 80% efficiency) (97). At normal room temperatures air in equilibrium with mercury contains approximately 150 times the maximum safe concentration, given as 0.012 p.p.m. The threshold limit value (TLV) is now given in the U.S.A. as half this level (99). Mercury is absorbed

through the skin to some extent (97). Small amounts are absorbed in the gastro-intestinal tract (97). The main excretion route for Hg^{++} is via the gut and kidneys (97). The average daily dietary intake in England is given by Abbott and Tatton as 20 µg (100). The normal route for mercury intake is via the diet. Concentration of airborne mercury is normally insufficient for this to be a significant source for man (101). Organo-mercury compounds (e.g. alkyl mercury compounds) are readily absorbed and highly hazardous and can cause among other effects irreversible brain damage (102). Having a non-polar nature they easily pass biological membranes. Their maximum safe concentration is given as 0.001 p.p.m. in air.

In general, poisoning by mercury is of the chronic type requiring exposure over weeks and months before the symptoms become apparent. Symptoms, however, may appear after a few hours exposure to very high concentrations, as described for example by Milne et al. (103).

Characteristic poisoning symptoms include softening of the gums, loose teeth, gastric pain, vomiting, renal failure, insomnia, tremor, irritability, changes in personality and hallucinations (71, 104). Symptoms vary and depend on the extent of poisoning.

Mercury is known to occur as a nonessential trace element in human tissue. "Normal" levels in tissue are described for example by Howie and Smith (105) and some of the same data by Liebscher and Smith (1). They found the highest concentration of mercury to be in the kidney.

Work undertaken

Mercury being such a widely and regularly used metal and toxic as a result of accumulation, a study of human uptake was considered of interest. As dentistry is one of the professions in which mercury exposure is a hazard, an investigation of dentists and dental students was undertaken as follows.

Mercury levels were surveyed in dentists and dental assistants in the Glasgow area.

Dental students were monitored for several years to investigate mercury accumulation.

Other cases of toxicological interest which arose during the course of the study are also reported.

Mercury in dentists

An amalgam is very extensively used in restorative dentistry to fill teeth. The amalgam consists of equal parts of mercury and an alloy of approximately 70% silver, 25% tin and 5% copper and zinc. This material has almost ideal physical properties for its purpose.

The drilling out of old fillings, mixing of amalgam, filling and polishing is carried out in the confines of the dental surgery. Mixing of amalgam is normally carried out by the dental assistant. Mixing and squeezing out of excess mercury is sometimes carried out by hand. A surgery witnesses on the average around 4,000 fillings every year. Environmental contamination occurs through spilled mercury, mercury dust and vapors in the air. It is the vapor from accidentally dispersed mercury that is the principal source of absorption which thus takes place in the lungs. As mentioned earlier elemental mercury is non-toxic and thus any swallowed by the patient during treatment has no ill effect. The patient's exposure to other contamination is brief and no evidence is found that the amount of mercury thus absorbed is harmful. For example, Frykholm (106) showed that there was a minimal hazard associated with amalgam treatment.

The care taken in the dental surgery to avoid environmental contamination is variable. Pools of spilled mercury between floorboards and behind radiators are not unknown (105).

Howie and Smith (105), mentioned the measurement of mercury in hair and nail from 20 dental assistants. They

found elevated values.

The following samples were taken from dentists and their assistants in surgeries in the Glasgow area: head hair, pubic hair, fingernail and toenail. These samples were selected for two reasons, (i) they were easily obtained and (ii) the relationships between head hair/pubic hair and fingernail/toenail would provide a measure of external and internal mercury uptake. A total of 320 samples were analysed.

Results

The results obtained are shown below both in graphical form, in Figures 21, 22, 23 and 24, and in summarised form in Table 19.

Table 19. Mercury found in dental personnel

		p.p.m. Mercury			
Sample	No.	Geometric mean	Median	Highest value	Lowest value
Head hair	81	10.02	9.96	288	1.20
Pubic hair	81	2.97	3.13	27.4	0.585
Fingernail	80	27.5	21.3	2580	1.33
Toenail	78	4.28	3.31	147	0.614

Mercury levels quoted by Howie and Smith (105) and Liebscher and Smith (1) for a normal population are shown in Table 20.

FIGURE 21

MERCURY DISTRIBUTION FOUND IN HEAD HAIR FROM DENTAL PERSONNEL.

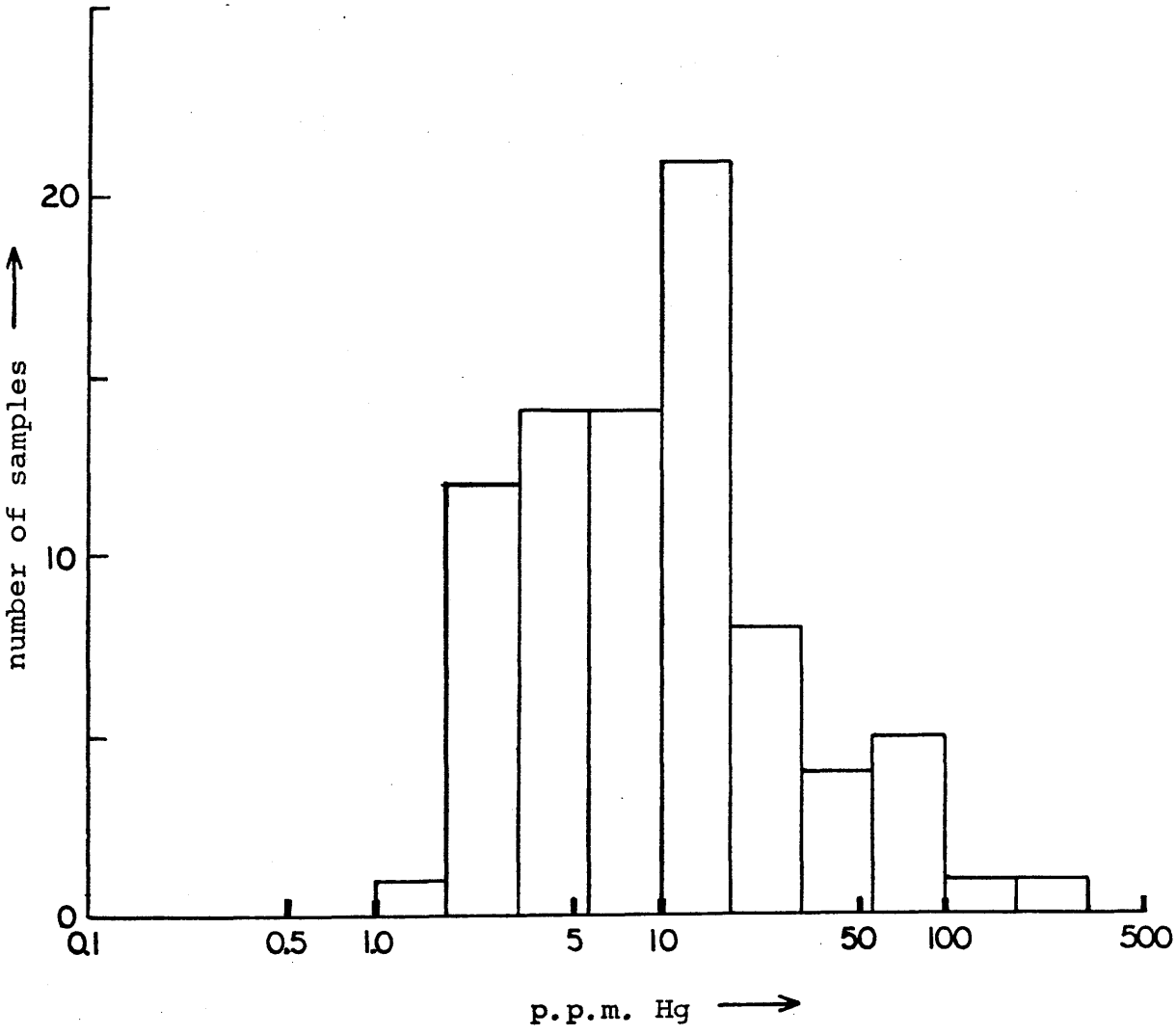

number of samples
20
10
0
0.1
0.5
1.0
5
10
50
100
500
p.p.m. Hg

FIGURE 22

MERCURY DISTRIBUTION FOUND IN PUBIC HAIR FROM DENTAL PERSONNEL.

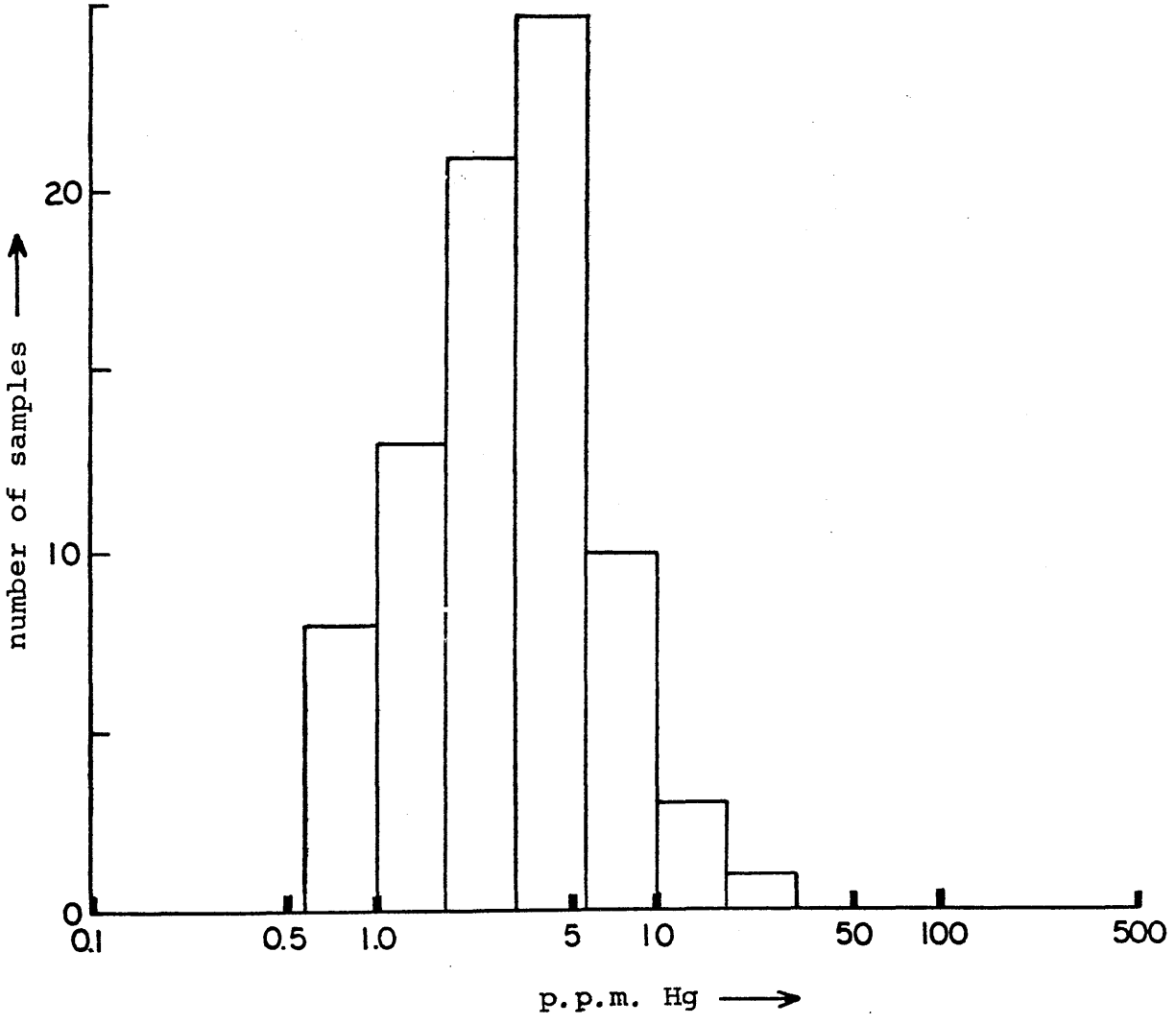

number of samples
20
10
0
0.1
0.5
1.0
5
10
50
100
500
p.p.m. Hg

FIGURE 23

MERCURY DISTRIBUTION FOUND IN FINGERNAIL FROM DENTAL PERSONNEL.

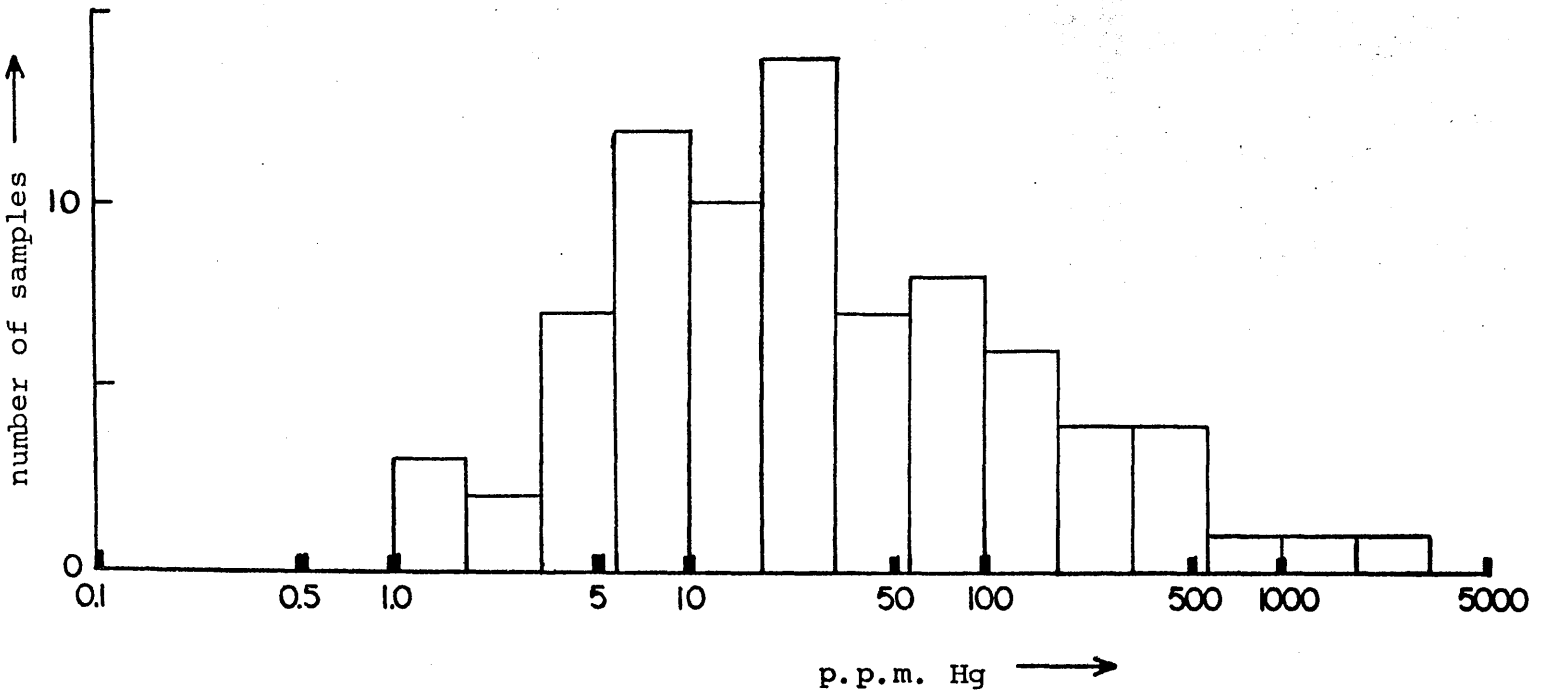

number of samples
10
0
0.1
0.5
1.0
5
10
50
100
500
1000
5000
p.p.m. Hg

FIGURE 24

MERCURY DISTRIBUTION FOUND IN TOENAIL FROM DENTAL PERSONNEL.

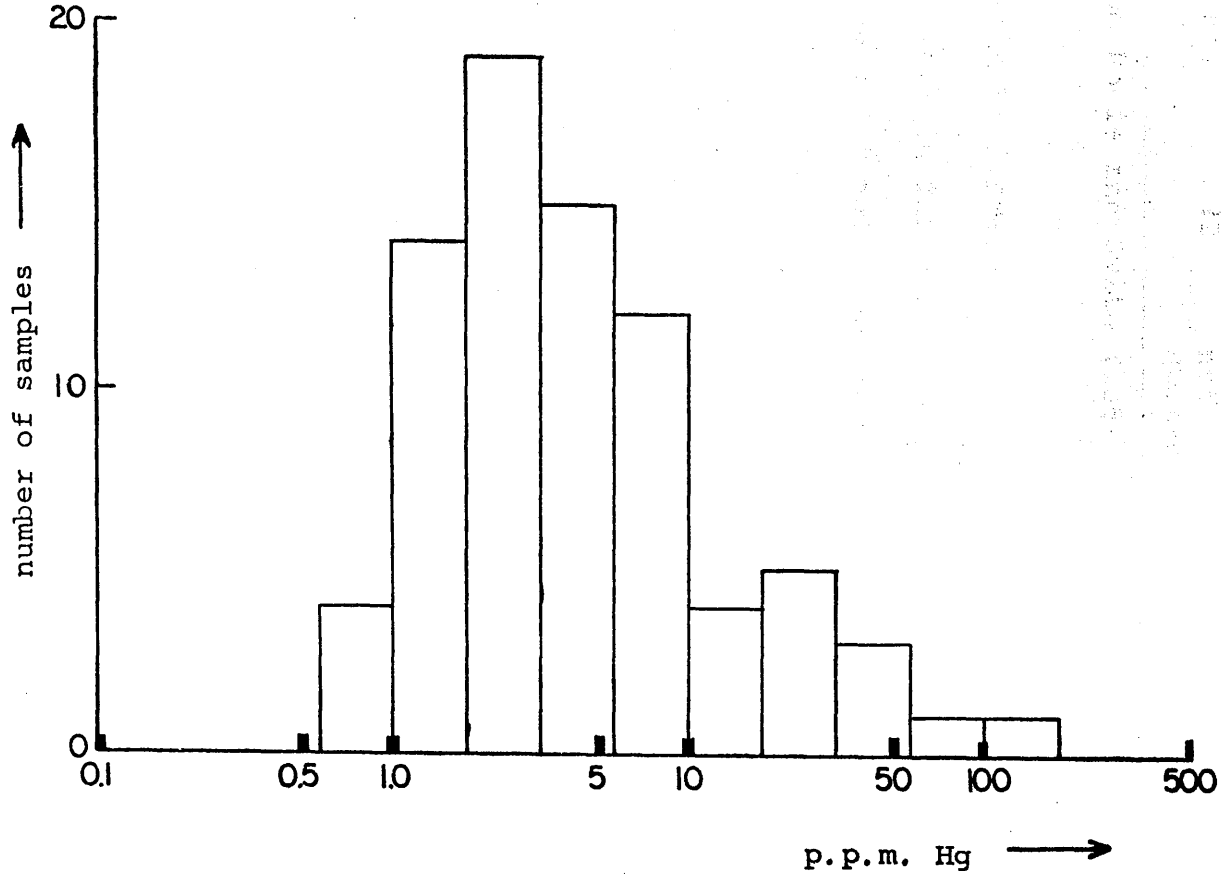

20
10
0
number of samples
0.1
0.5
1.0
5
10
50
100
500
p.p.m. Hg

Table 20. "Normal" mercury levels*

Sample	No.	p.p.m. Mercury: Geometric mean	Median	Highest value	Lowest value
Head hair	70	3.51	4.20	24.4	0.03
Pubic hair	22	not quoted	1.21	5.43	0.28
Fingernail	25	4.50	4.76	33.8	0.80
Toenail	22	not quoted	2.75	5.00	0.05

*From Howie and Smith (105) and Liebscher and Smith (1).

Discussion of results

As seen in Table 19, a general elevation of mercury levels in the hair and nail samples as a group was found. The highest value recorded was, as might be expected, for fingernail. The level found was 2580 p.p.m. Hg. The same individual had 50.4 p.p.m. Hg in toenail, the second highest level recorded; 67.8 p.p.m. Hg in head hair, the third highest level recorded; and 27.4 p.p.m. Hg in pubic hair, the highest level recorded. With these levels the individual concerned should display evidence of mercury poisoning. The graphical presentation provided a useful illustration of the trends observed. The distribution of mercury in the hair and nail samples appeared basically Gaussian and lognormal. As might be expected, the head hair and fingernail sample groups showed strong evidence of high (external) contamination. Their distribution pattern was broader than that of the matching pubic hair and the nail

samples. In particular, levels in fingernail had the widest range. The pubic hair and nail groups showed evidence of higher than normal levels, indicating internal mercury uptake. The head/pubic hair and finger/toenail graph pairs were basically similar showing the same evidence of external/internal mercury uptake. However in the nail sample pair there was found two observable trends. The mercury in fingernail distribution pattern deviated a little from lognormal Gaussian, showing a "tail" of high values. This indicated that there was a greater probability of external contamination than might be expected if the group was not a selected one.

There was also found a high value "tail" in Figure 24 for toenail, especially when compared to Figure 22 for pubic hair. This indicated that external contamination may have occurred in one sample group, i.e. some toenail samples were exposed to some outside contamination as were fingernails. A reason for this was not hard to find. Mercury contamination of the surgery was at its highest at floor level due to spillage, dust, etc. accumulating there. Some dental assistants were in the habit of wearing sandals or other open-toed style footwear and hence were exposed to contamination. The high levels in the nails were found to come from such an "open-toed" group.

Conclusion

The evidence found showed that the dental surgery group had higher mercury levels in the tissues examined than had a normal group. External contamination was shown by the high

values for fingernail and head hair. Although no symptoms of mercury poisoning were reported from the group examined, a significant mercury uptake was found. For a few individuals the measured levels were dangerously high and although no symptoms were reported it was reasonable to conclude that syndromes were there. It was considered that the survey was useful as it provided strong evidence of potential health damage from environmental exposure.

Dentists and other people who are thus continuously environmentally exposed to mercury are normally aware of the hazard and take some precautions, though not always. However, an annual monitoring of easily collected samples of pubic hair (as an internal indicator) and fingernail (external), would be a valuable and preventive aid for the maintenance of good health.

Mercury in dental students

A smaller, but similar, survey was made of mercury levels in one class of dental students. Samples were taken from the group as first year students prior to any surgical training and resultant mercury exposure. Three years later when the same group had graduated and had been so exposed, further samples were taken. The object of the survey was to investigate any mercury increase.

The first samples taken were of head hair from each of the 30 students. In retrospect collection of pubic hair, finger and toenail would have been useful, but this was not

carried out. Head and pubic hair, finger and toenail samples were taken 3 years later from the 27 students remaining in the class. A direct comparison could therefore only be made in the case of the head hair samples; however the monitoring of the other tissues collected from the graduated students provided a more complete measure of any mercury uptake.

Results

The results obtained are shown below.

Table 21. Mercury levels found in 1st year dental student group prior to exposure

		p.p.m. Mercury			
Sample	No.	Geometric mean	Median	Highest value	Lowest value
Head hair	30	3.51	3.54	94.7	0.187

Table 22. Mercury levels found in 3rd year dental student group

		p.p.m. Mercury			
Sample	No.	Geometric mean	Median	Highest value	Lowest value
Head hair	24	5.22	3.97	47.3	1.0
Pubic hair	23	4.81	4.61	122	0.9
Fingernail	27	21.24	24.5	546	1.30
Toenail	25	2.37	1.91	32.0	0.25

Discussion of results

The levels found for the 1st year student group (Table 21) were similar (c.f. Table 20) to those of a normal group (the geometric means of the head hair values were in fact identical). The mercury levels in the group after training (Table 22) were found to be similar to those of practicing dental personnel (Table 19). The geometric mean in head hair rose from 3.51 p.p.m. to 5.22 p.p.m. The elevated levels in the other tissues measured (Table 22) were found to be typical of an exposed group. However the geometric means were found to be less overall for the graduated dental students than practicing dentists.

Conclusion

The result showed that the dental student group, over their three years of surgical training, accumulated an elevated background of mercury. Three of the students were found to have rather high mercury levels. Thus the occupational handling of mercury, even during training, was shown to be a potential health hazard. The accumulation of mercury during training could be predicted. What was not expected, however, was the extent of mercury uptake found after the limited exposure during training.

Industrial exposure to mercury

Industrial exposure to mercury is a continuing hazard even though examination and strict control is now the rule. The following study illustrates the problem and how analysis can be of diagnostic use. It must be appreciated, however, that there is little correlation between mercury levels and symptoms. However, any undue exposure can be uncovered.

History

Six workers were exposed to mercury vapour. They were employed in an electric light factory. They had to clean and maintain the pumps which automatically injected a very small quantity of metallic mercury into the lamps before these were sealed and filled with inert gas after evacuation of air. Working conditions were clean and the process had been carried out for over 20 years without reported mishap.

Periodic medical examinations took place and were fairly detailed and included retinoscopy, examination of the central nervous system and urine analysis. An increase in mercury excretion was found from urine analysis during such an examination. Five workers, B, C, D, E, and F, out of the group of six reported no symptoms of ill health. The sixth, A, did. It later transpired that of the first five, one, B, had had albuminuria and had been treated for 3 years for hypertension. Details of each workers are listed below.

A. Age 46

Employed for 18 years in the process.

Had had recent morning headaches for about 3 weeks.

Blood pressure was 140/80 mm Hg. Examination was negative except for rather brisk reflexes.

B. Age 50

Employed for 21 years, 6 months.

Looked fit and denied symptoms but had been treated for hypertension for 3 years.

Blood pressure was 180/100 mm Hg, 6 months earlier it was 210/100 mm Hg. In the previous year was off work for 4 months for haemolytic anaemia, apparently due to one of the hypertensive drugs.

The diagnosis was polycystic kidneys.

C. Age 41

Employed for 7 years.

Looked fit, had no symptoms other than a very slight tremor.

Had brisk reflexes.

D. Age 51

Employed for 9 years.

Had no symptoms.

Blood pressure was 125/80 mm Hg.

E. Age 31

Employed with mercury for 2 years, 6 months.

Had no symptoms.

Blood pressure was 120/76 mm Hg.

F. Age 56

Employed with mercury for 1 year.

Had no symptoms.

Blood pressure was 160/90 mm Hg.

Results

Samples of head hair, pubic hair, fingernail and toenail were taken from each worker and analysed for mercury levels.

Table 23. Mercury found in 6 lamp factory workers

	p.p.m. Mercury						
Tissue	A	B	C	D	E	F	Geometric mean
Head hair	36.1	6.6	4.5	10.2	30.6	13.4	12.84
Pubic hair	21.6	3.2	11.0	23.4	19.6	2.89	10.01
Fingernail	132	17.8	19.5	13.9	80.1	316	50.26
Toenail	5.13	6.85	6.7	16.2	8.0	36.3	10.17

Discussion

Worker A, who had complained of headaches was found to have high mercury levels. However it was found that the group as a whole also had elevated levels, as seen by the geometric mean values. All had high fingernail levels indicating external contamination. F, who had the least time of exposure to mercury, was found to have the highest fingernail level. It was noted that A and B, who had some record of symptoms,

were the two workers who had worked with the mercury pump for the longest time.

Conclusion

All of the group were shown to have had signficant mercury uptake. Prior to tissue analysis this was only detected by an increase in mercury excretion in urine. The potentially hazardous levels of mercury uptake found were not necessarily associated with prior warning by the occurrence of poisoning symptoms. The group had high but not chronically dangerous levels when compared to levels found in poisoning cases (e.g. Howie and Smith (105)). The elevated levels found illustrated that the routine medical examinations indicated only the overall state of health of each subject and were a poor indicator of the latent hazard of mercury uptake.

The hair and nail samples were obtained for analysis through H.M. Factory Inspectorate, Glasgow, and the results obtained were forwarded in case any action was considered necessary to improve the working conditions of the group investigated.

A similar case involving one worker was investigated.

History

A male, G, worked with a Regional Gas Board. His work for several years concerned gas pressure governors in which mercury was used.

G, when medically examined was observed to have a distinct personality change—a classic mercury poisoning symptom, which can occur after long-term exposure. Samples of head hair, fingernail and toenail were obtained for mercury analysis.

Results

The results obtained are shown below.

Table 24. Mercury in worker "G"

Tissue	p.p.m. Mercury
Head hair	7.1
Fingernail	31.9
Toenail	4.56

Conclusion

The above confirmed that some mercury uptake had occurred. The levels, however, were not dangerously elevated. Nevertheless the uptake observed could be associated with the reported personality change of G.

What was of interest was that the general levels for G were found to be no higher than those of workers C, D, E, and F, who on similar industrial exposure displayed no poisoning symptoms.

The evidence found therefore indicated that the level of mercury uptake at which poisoning symptoms would start to appear varied greatly from person to person. This is in keeping with the work reported by Howie and Smith (105) and by West and Lim (107).

Clinical investigations

(1) Mercury as the metal and simple salts has been used as a medicine from the distant past. A common remedy of only a few years ago for teething troubles was a mercury containing teething powder. As reviewed by Skerfving and Vostal (104) the occurrence of pink disease (acrodynia) has been correlated with the use of such powders. These are no longer commercially available, but the results of their use still occur occasionally. The following is such an example.

Urine samples were taken on two consecutive days from a baby male child aged 1 year and 2 months and thought to be suffering from pink disease (acrodynia). Symptoms included photophobia, weight loss and constipation.

Results

The results of the urine analysis are shown below.

Table 25. Mercury found in urine of baby

Sample	µg Hg/ml
Urine, first day	0.137
Urine, following day	0.442

Discussion

These levels were similar to those obtained by Howie and Smith (105) for suspected acrodynia in children. Howie and Smith quote an arithmetic mean of 0.023 µg/ml, a maximum of 0.133 µg/ml and minimum of 0.00097 µg/ml in 46 normal urine samples. Monier-Williams suggests a lower limit of 0.1 to

0.2 µg Hg/ml urine to be indicative of mercury poisoning (108). The elevated urine levels found helped to confirm pink disease.

(2) A male patient, age 33, was under investigation for progressive renal failure. He had had a recent pulmonary embolism from deep venous thrombosis. There was no obvious explanation for his deteriorating renal function and he was not hypertensive. Renal failure has an association with chronic mercury poisoning (109).

It was known that he had possible mercury exposure at his work. Therefore a sample of a 24-hour urine volume (590 ml) was taken for mercury analysis, the result of which is shown below.

Result

Table 26. Mercury in patient's urine

Sample	µg Hg/ml
Urine	0.0085

Discussion

The level found was well within normal (105). Thus the analysis indicated that mercury poisoning was an unlikely cause of the patient's renal failure and that the cause lay elsewhere.

Conclusion

The great sensitivity of the activation analysis technique was able to be usefully applied to the analysis of urine samples, the collection of which from the patient was very simple and straightforward. Its value thereby as a clinical aid to confirm or indicate mercury exposure was demonstrated.

General conclusion

The ease with which activation analysis of hair, nail and urine was able to ascertain mercury exposure in the subjects concerned, was well demonstrated in the investigations described. Evidence of the degree to which the subject may have absorbed mercury was given by such measurement and was usefully available before poisoning symptoms might appear.

A significantly high mercury uptake was found amongst the dental surgery population group. Evidence of potential health hazard was thus shown. Mercury uptake was found to have occurred to a surprising extent in the dental student group exposed to mercury for 3 years during training.

The mercury levels found in the 6 lamp factory workers showed high, but not dangerously high, uptake had occurred. It was established therefore that a mercury hazard was present. Possible poisoning symptoms existed in two of the workers. The mercury analyses carried out made it unnecessary that chronic symptoms had to develop in the future before this hazard was appreciated. Thus the value of the analysis was illustrated.

The mercury levels found in the gas worker, G, although elevated, were less than those for four of the lamp factory workers, who displayed no poisoning symptoms. Yet G was probably suffering from mercury poisoning, illustrating that the level at which symptoms start to appear varies from person to person.

Mercury analysis of the urine samples of clinical origin was shown to be a valuable supportive aid.

The suitability of the activation analysis technique for mercury to environmental studies was seen.

Arsenic

Arsenic is in Group V of the Periodic Table. Reference is found in early Chinese civilisations to arsenic compounds used for pigments and poisons. Greek alchemists knew of the red and yellow pigments realgar As_4S_4 and orpiment As_2S_3. In 1733 Brandt isolated arsenic by reduction with oil from "white arsenic" which he recognized as the calx or oxide of the element. Today arsenic is principally obtained by roasting arsenic containing minerals in air or as a by-product from lead or copper smelting. Minerals containing arsenic are found in many parts of the world. Traces exist in many soils. The principal use of arsenic is as a constituent in industrial fluids, insectiside, weedkiller, sprays, alloys, pigments, etc. Significant traces have been found in materials such as detergents, as described by Lenihan et al. (110). Arsenic was, and still is, contained in some medications (e.g. Diarsenol, for the treatment of syphilis), and large quantities have been thus consumed. Arsenic in organic forms is used as a growth stimulant to fatten chickens and pigs, as described by Frost and his colleagues (111, 112). Arsenic is the traditional poison, fatal when ingested in large quantities. Associated exposure symptoms include skin irritation, dermatitis, conjunctivitis and dryness of the throat (71). Prolonged exposure to arsenical dusts over many years can produce "raindrop" pigmentation in industrial workers (58).

From such sources arsenic uptake in humans can occur. The literature indicates that arsenic is a nonessential trace element in human tissue. Distribution is described by Leibscher and Smith (1). The levels in normal tissue described therein and elsewhere (15, 17, 78) provided a comparison to which those found in the work undertaken could be related.

Arsenic is present in tissue by chance at an average concentration around 0.05 p.p.m. as seen from the levels given by Liebscher and Smith (1) and also quoted by Smith elsewhere (113). Significantly higher levels (around 0.4 p.p.m.) are reported in hair and nail tissue. Arsenic has an affinity for the sulphydryl groups in proteins in tissue. The acceptable maximum of daily arsenic intake from diet is given as 0.05 mg As/Kgm body weight (114).

The association of occupational disease with long-term arsenic poisoning by exposure is known. For example, arsenic has been used as a herbicide and insecticide for many years and was used in vineyards extensively prior to its prohibition in 1942. Roth (115) described the autopsies performed on 24 vineyard workers who had had long-term exposure. Chronic poisoning progressed even if no more arsenic was found in the body. Neoplasms were present with a predominance of bronchogenic carcinoma. Late poisoning stages are characterised by severe hyperkeratosis and a high incidence of malignant tumors of the skin and internal organs.

The association of arsenic as a carcinogen to man, producing lung and skin cancer, has been known for some time.

The observed association of the symptom of dermatitis with the arsenic exposed groups studied has been mentioned. Snegireff and Lombard (116) in 1951 statistically showed that the incidence of lung cancer was greater in workers who had been exposed over a long term to arsenic. Tseng et al. (117) described the increasing prevalence with age to skin cancer, hyperpigmentation, etc., in an area with a high arsenic concentration in the water supply. Lee and Fraumeni's (118) findings about male smelter workers supported the hypothesis that inhaled arsenic is a respiratory carcinogen. Thus it may be concluded that there is evidence that a group shown to have a high arsenic uptake may well have an increased probability of eventually suffering from lung, skin cancer, etc.

Arsenic uptake and the interpretation of the levels found in humans was investigated from the toxicological and clinical viewpoint. The work fell into three categories:

1) Arsenic poisoning by internal consumption.
2) Industrial exposure to arsenic.
3) Clinical applications of the analytical technique.

1. Poisoning

Arsenic is a famous poison for both homicidal and suicidal purposes, but in actual practice it is seldom used. A unique opportunity to monitor a severe case of arsenic poisoning was presented and investigated as follows. A young man, aged 18, swallowed a quantity of arsenious oxide on a known date

("Day 1"). The material was obtained from the laboratory of a school where he was employed as a technician. He said that the arsenious oxide was taken from a glass bottle about 3 cm in diameter and that it occupied a depth of 1-2 cms. The bottle and any remaining contents were not recovered. Thus the best estimation of the ingested dose that could be made was that a fatal amount could certainly have been taken. Deichmann and Gerarde quote a minimum dose of 180 mg arsenious oxide (71). A fatal range of 60-180 mg with wide individual variation is given in The Encyclopedia of Biochemistry (119).

The man was later hospitalised and subsequently treated with penicillamine 50 days after swallowing arsenic ("Day 50" onwards). Penicillamine, 2,2-dimethylcysteine, is a chelating and excretion agent. He recovered from his experiences and samples were freely available.

The following samples were obtained:

a) A few head hairs pulled from the scalp on "Day 49"

b) Beard shavings (from an electric razor) were collected between "Day 47" and "Day 69"

c) Urine samples between "Day 46" and "Day 69"

d) Nail clippings taken on "Day 69"

(b) and (c) provided specimens before and after administration of penicillamine.

(a) Head hair

One hair strand, 6.6 cms long was laid between clear thin plastic sheets and cut into 3 mm lengths. Each length represented 1 week to 10 days average hair growth (Saitoh et al. (120)). The arithmetic mean weight of each hair segment (excluding the root) was 27.33 µg. The arsenic found in each segment was plotted in Figure 25.

94.4 p.p.m. As was found in the fifth segment from the root. Apart from each segment adjacent to the fifth, all other arsenic levels found along the hair were normal. Smith (121) states an arsenic concentration >3 p.p.m. should be suspect. Liebscher and Smith, as shown in Table 27, report a geometric mean of 0.460 p.p.m. for 1250 samples of "normal" hair.

The location of the high arsenic level, 12-15 mm from the hair root, was consistent with an arsenic intake 7 weeks earlier. The higher than normal levels in the adjacent 4th and 6th segments were considered to be caused by arsenic excretion during the time represented by each hair segment or by sweat washing or both.

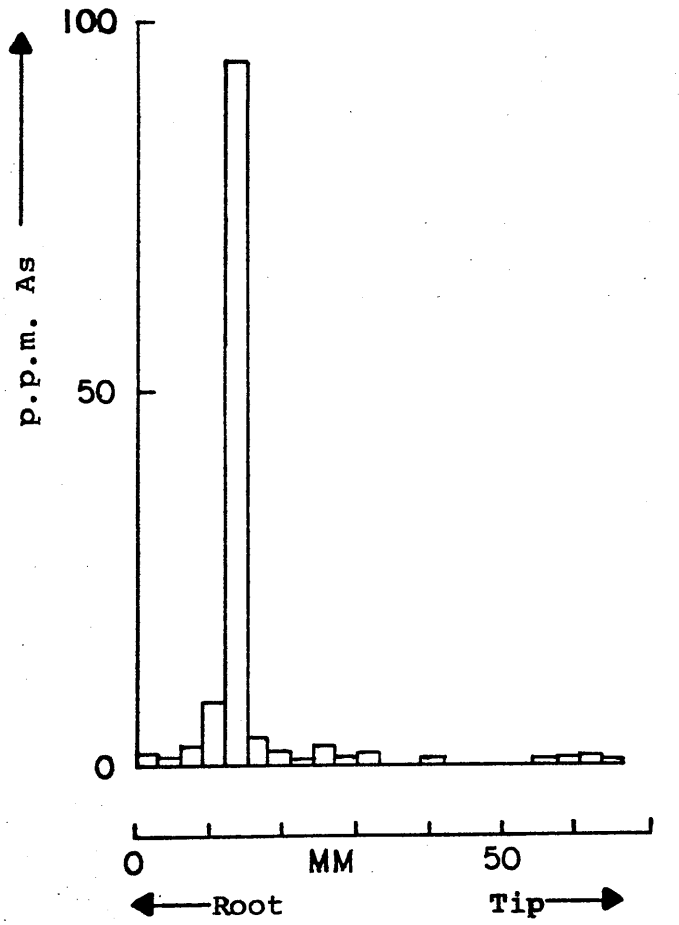
Table 27. Arsenic levels of a normal population*

		p.p.m. Arsenic			
Tissue	No.	Geometric mean	Median	Highest value	Lowest value
Head hair	1250	0.460	0.460	8.17	0.020
Fingernail	124	0.283	0.300	2.90	0.020
Urine	25	0.053	0.042	0.770	0.006
Skin	76	0.080	0.090	0.590	0.009

*From Liebscher and Smith (1).

FIGURE 25

ARSENIC FOUND IN CONSECUTIVE 3 MM SEGMENTS OF HAIR STRAND PULLED FROM SUBJECT 7 WEEKS AFTER INGESTION.


100
50
0
p.p.m. As
0
MM
50
Root
Tip

(b) Beard shavings

Figure 26 shows the arsenic levels found in beard shavings by electric razor between "Day 47" and "Day 69". Some evidence of an increase of arsenic excretion after penicillamine treatment was found. However, the qualification need be made that samples for only 4 days prior to treatment could be provided. Smith (121) showed that arsenic levels in beard shavings returned to normal after 3 weeks from ingestion.

(c) Urine

Arsenic concentrations found in the urine are shown in Figure 27 and total arsenic excretions in urine are shown in Figure 28.

No firm evidence of an As excretion pattern could be seen. The levels appeared to be normal, c.f. Table 27.

(d) Nails

The analysis results in Table 28 below show that the nail samples taken 2 months after ingestion gave evidence of arsenic uptake.

Table 28. Arsenic found in nail samples

Tissue	p.p.m. As
Right index finger	2.83
Left index finger	1.38
Right toenail	2.53
Left toenail	0.42

FIGURE 26

ARSENIC FOUND IN BEARD SHAVINGS.

Beard shavings were not taken on every day.

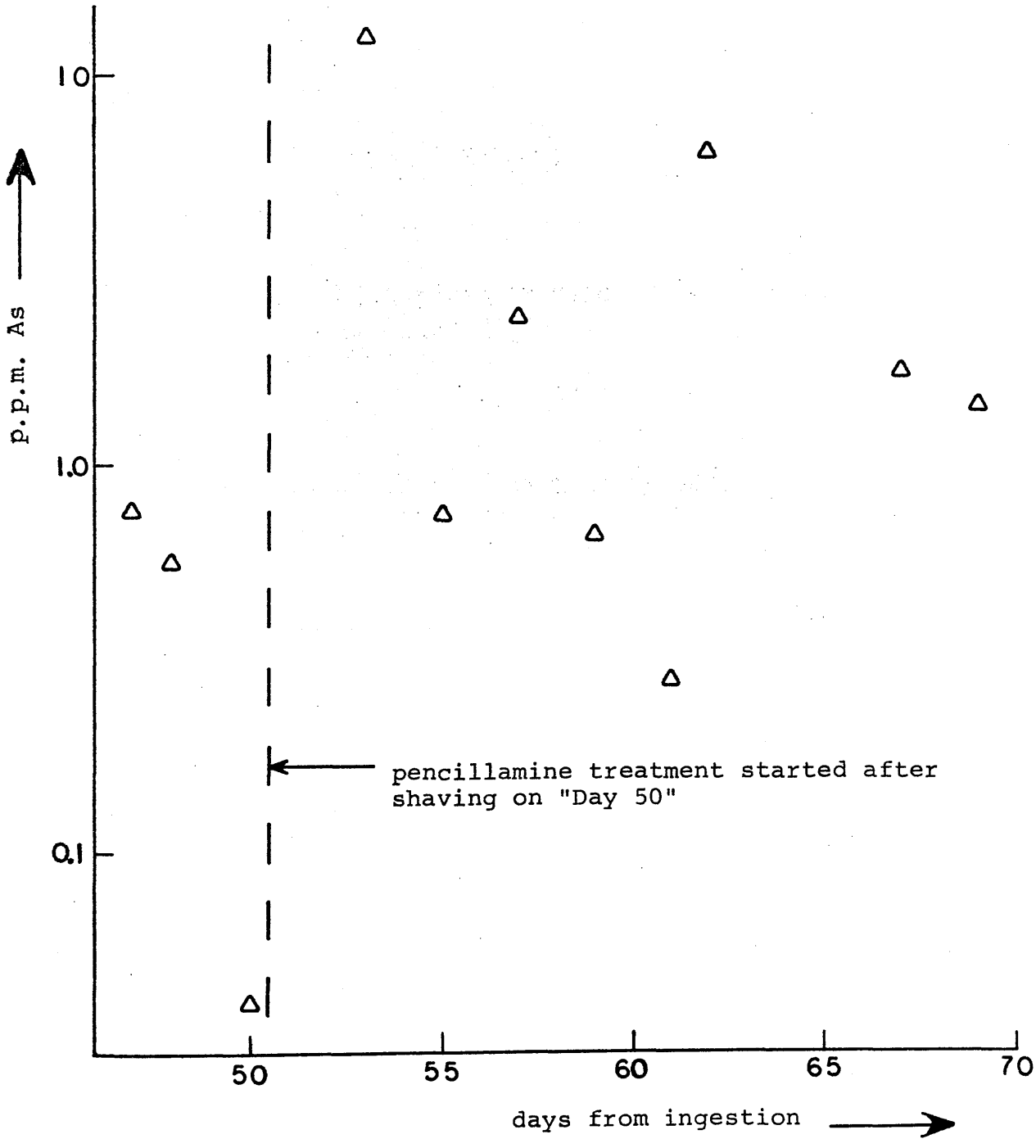

10
1.0
0.1
p.p.m. As
pencillamine treatment started after shaving on "Day 50"
50
55
60
65
70
days from ingestion

FIGURE 27

ARSENIC LEVELS FOUND IN URINE TAKEN BETWEEN "DAY 46" AND "DAY 69".

Urine was not collected on "Day 50", "51" and "52".

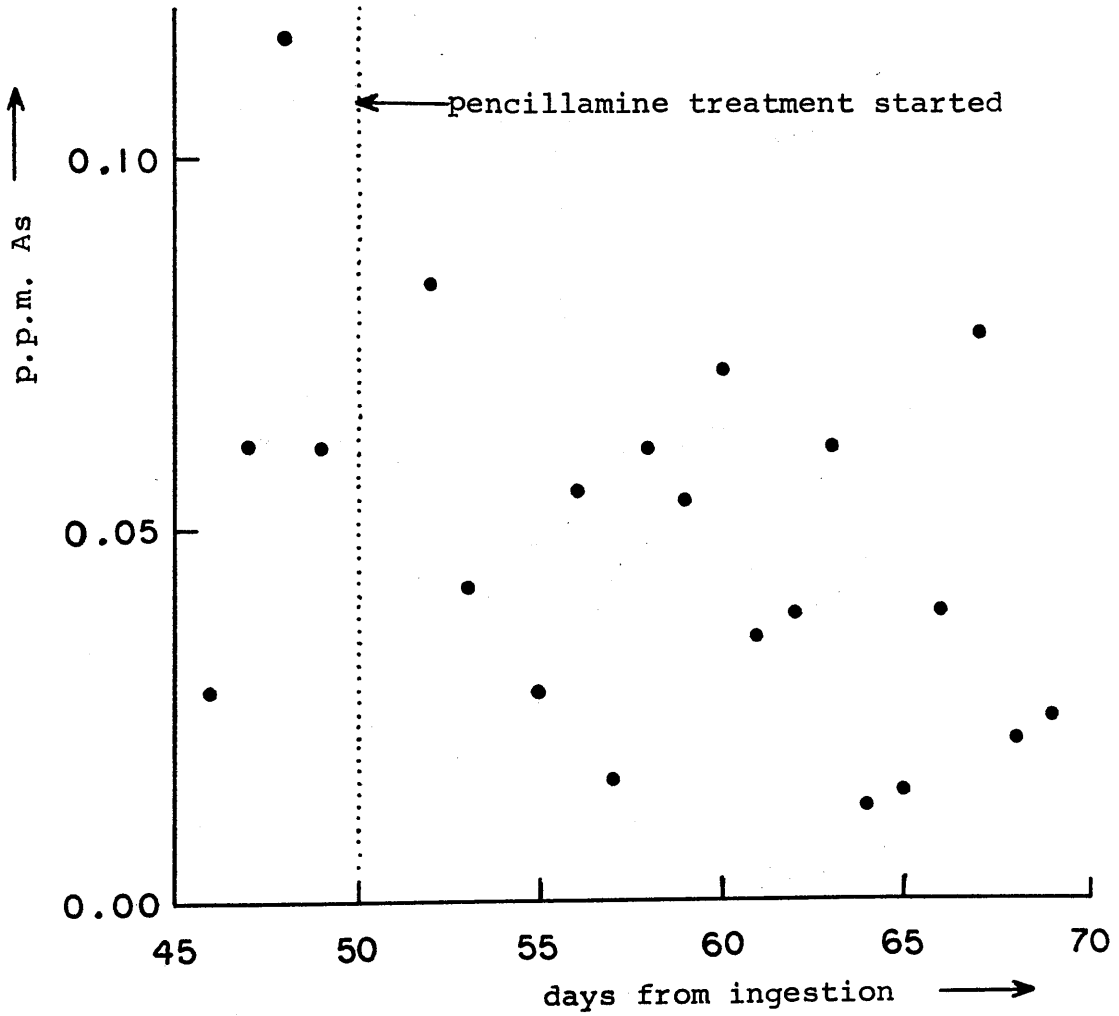
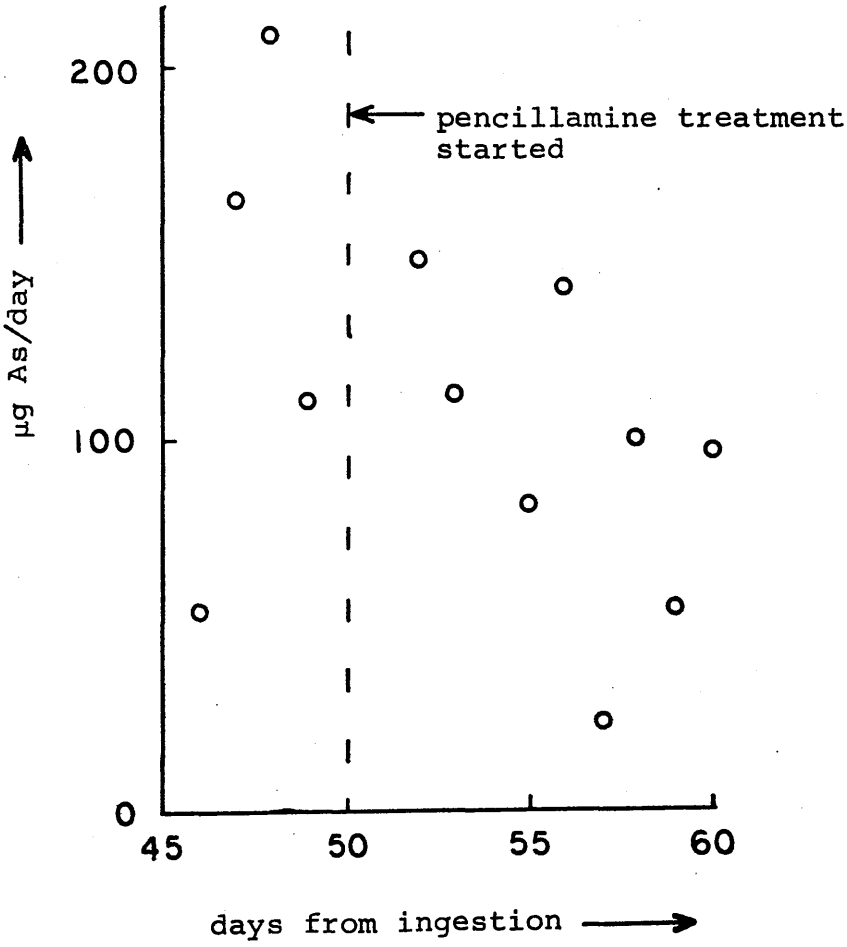

pencillamine treatment started
p.p.m. As
0.10
0.05
0.00
45
50
55
60
65
70
days from ingestion

FIGURE 28

TOTAL ARSENIC EXCRETED PER DAY IN THE URINE TAKEN BETWEEN "DAY 46" AND "DAY 60".


200
100
0
µg As/day
pencillamine treatment started
45
50
55
60
days from ingestion

The levels found in finger and toenail were similar, indicating internal absorption.

Conclusion

Sectional analysis of head hair was seen to provide an elegant time record of arsenial poisoning in the subject. Smith (121) carried out such analysis of a single hair from a subject suffering from arsenic poisoning. However, Smith's results did not present such a clearcut graphical form as in this case. Monitoring of beard shavings and urine levels was of interest to follow the penicillamine treatment. The results found here were inconclusive and it is felt that there is nothing to be gained by treatment at this distance in time from the poisoning. Analysis of the nail samples taken 2 months after dosage provided further evidence of arsenic poisoning.

2. Industrial exposure

As mentioned earlier, the hazards of prolonged arsenic exposure are known. Nevertheless, they are not always fully appreciated by those accustomed to work with arsenic over long periods of time. Arsenic poisoning and the occurrence of associated symptoms can still happen where inadequate care and poor industrial hygiene take place. Ideally protective clothing—gloves, headgear—should be worn to avoid absorption through the skin and respiratory masks where dust is present.

Under the Factories Act, 1961, poisoning by arsenic is one of the 16 notifiable poisonings to the Chief Inspector of Factories. The hazards of industrial exposure to arsenic are described, for example, by Hunter (58). Poisoning can occur due to contact with arsenic in inorganic forms, dust, liquids, etc., as arsine gas or organic arsenic compounds. The cases investigated here involved exposure to inorganic and some organic arsenic.

Timber workers

Wood preservative fluids can contain high amounts of arsenic. Mixtures containing sodium arsenate and arsenic pentoxide are common. Therefore workers who daily handle such preservatives and timber might unknowingly accumulate arsenic if sufficient precautions are not taken.

High arsenic levels were found in samples obtained from workers so exposed. Not all workers with high arsenic levels displayed symptoms of arsenic poisoning. Cases of interest illustrating such industrial poisoning are outlined below.

Case 1. Two males, A and B, each worked at a timber yard. A dipped wood into an arsenic preservative. B, a carpenter, sawed up the wood, often wet. Both males displayed arsenic poisoning symptoms. A had ulcers and dermatitis. B had dermatitis. Samples of head hair and fingernail from A, and head hair, fingernail and skin from B were analysed. It was expected that A, who had the greater likelihood of arsenic

exposure than B and who had the more pronounced poisoning symptoms, would have higher arsenic levels. The results found shown in Table 29 confirm this.

Table 29. Arsenic found in hair and nail of timber workers

Person	Tissue	p.p.m. As
A	Nail	161
A	Hair	70.8
B	Nail	2.93
B	Hair	8.25
B	Skin	2.96

The higher levels of A than B reflected the degree of exposure and of probable external sample contamination.

Samples of head hair, pubic hair, fingernail, toenail and urine from 5 male workers in another sawmill were analysed as shown in Table 30. The years each worker had been employed were known. Each did similar work. Arsenic wood preservatives were used daily. The study was to determine whether any correlation between exposure time and observed arsenic levels existed. The results are shown below:

Table 30. Arsenic levels found in more timber workers

		p.p.m. Arsenic				
Worker	Duration of employment	Head hair	Pubic hair	Finger-nail	Toe-nail	Urine
C, age 27	7 months	12.1	10.6	44.7	26.7	0.88
D	2 years	37.4	16.7	Lost	32.2	0.39
E, age 48	3 years	4.03	Lost	*	1.50	0.42
F, age 54	3 years	25.2	17.9	22.5	0.8	Lost
G, age 47	7 years	21.6	38.8	13.7	24.2	0.35

*Not provided.

Three samples were lost during analysis due to a spillage. None of the above workers had symptoms of arsenic poisoning such as stigmata of arsenic irritation.

All the levels were similar and high, in particular urine, indicating considerable arsenic absorption. It appeared that the workers under daily exposure became to a certain extent arsenophagists. The arsenic levels did not appear cumulative, they reached a high plateau state independent of the years of exposure.

Another independent case of observed high arsenic levels in a subject consistently exposed without associated poisoning symptoms was investigated:

A male, H, aged 43, was engaged in timber preservation for 3 years using an arsenical preparation. He spent about 60% of his time of this, otherwise he was a sawmill and estate worker. He appeared in good health, had not consulted his doctor for years, did not complain of any gastro-intestinal symptoms, had no dermatitis or stigmata. The results of arsenic analysis in hair, nail and urine are shown in Table 31 below:

Table 31. Arsenic levels found in Worker "H"

Tissue	p.p.m. As
Head hair	15.40
Pubic hair	13.71
Fingernail	1.30
Toenail	8.05
Urine	0.214

Again the elevated levels found showed the subject's ability to tolerate arsenic absorption without any adverse reactions.

In one case it was possible to measure the concentration in urine for one timber worker, I, both during and after arsenic exposure. For more than one year I had used fir wood preservation "Celcure A", a fluid not only containing 26% of arsenic, but high levels of copper and chromium. I was being treated as an outpatient for dermatitis while still being exposed.

Arsenic concentration was measured in urine taken then and found to be elevated as seen in Table 32. Exposure ceased, the dermatitis disappeared and 7 months later a urine sample was analysed. The normal level found confirmed the simple effectiveness of removing the subject from exposure.

Table 32. Arsenic levels in urine of Worker "I"

Description	As in urine p.p.m.
Prior to removal from As exposure	0.456
7 months after exposure ceased	0.018

Other cases of industrial exposure

An example, similar to the above, of the use of arsenic estimation to confirm both the suspected source of dermatitis and the return to normal was carried out as shown in Table 33.

The male labourer, J, aged 22, erupted in dermatitis. He had carried bags containing arsenic for the preceding 3 months.

Table 33. Arsenic found in hair and nail of Worker "J"

Description	As in head hair, p.p.m.	As in finger-nail, p.p.m.
Immediately following period of exposure	39.2	63.1
8 months later	3.32	0.13

The highest arsenic level found in the cases of industrial exposure investigated occurred as follows: A small firm had a chemical plant for the manufacture of arsenic containing anti-fouling paint. The initial form of the arsenic was arsenic pentoxide and the final form was diphenylamine chloroarsine. The sole operator of this plant for five years was a male, K, aged 52. The work was intermittent. In the intervals he did work in which carbon black was handled. A previous operative on this plant died of bronchial cancer although it was never shown whether the condition had any connection with the man's occupation.

Samples of axillary hair, pubic hair and toenail were analysed as shown in Table 34.

The remarkable fact was that K was in good health and had no relevant medical history.

Table 34. Arsenic found in Worker "K"

Tissue	p.p.m. As
Toenail	865
Axillary hair	1612
Pubic hair	1.51

The toenails provided were discoloured black from the carbon black. The operator, K, worked under dirty conditions. The exceptionally high arsenic levels found in the nail and axillary hair indicated external contamination. The smaller, although still elevated, level in pubic hair was a more reliable indicator of internal arsenic absorption.

K was therefore seen to have had a very high level of external arsenic contamination. He nevertheless appeared to absorb insufficient to affect his health and produce any poisoning symptoms. Smith (121) mentioned that arsenic dust as an external hair contaminant was found in cases of high recorded levels.

General conclusion

The significant feature in 7 of the subjects out of the 11 described was their tolerance to high levels of arsenic. It was supposed that routine exposure builds up some immunity. The subjects become partial arsenophagists. However it was of interest to note that their levels were similar to, sometimes higher than, subjects who had poisoning symptoms. Evidence was found that a high plateau of uptake could be reached independent of the years of exposure. Removal of the poisoned

subjects, I and J, from the source of arsenic exposure rid them of further poisoning.

Some simple precautions would have greatly reduced the level of arsenic exposure for the subjects examined, for example, the wearing of plastic outerwear and the taking of a shower after work. It was of interest that not one of the subjects examined had normal arsenic levels.

(3) Clinical investigations

Arsenic analysis was used as an aid to medical diagnosis.

Samples of head hair and scales from the abdomen of a patient were provided. The patient was female, aged 67. She had recently developed exfoliative dermatitis and arsenial type keratosis of the hands and feet.

The results of arsenic analysis are shown in Table 35:

Table 35. Arsenic found in hair and skin of patient

Tissue	p.p.m. As
Head hair	10.6
Scales from skin of abdomen	4.70

The elevated values found showed that the patient was indeed suffering from arsenic poisoning. This was subsequently traced to the use of an arsenical tonic.

Another case of medical origin concerned a patient, female, aged 48, who had had a mild polyneuritis. It was thought that this was due to arsenic medication over a period of time. Samples of fingernail, toenail, head hair and pubic hair were analysed as shown in Table 36.

Table 36. Arsenic levels found in another female patient

Tissue	p.p.m. As
Head hair	27.1
Pubic hair	16.0
Fingernail	20.2
Toenail	4.68

As seen, the levels were significantly high. To observe any pattern of arsenic medication a sample of head hair, 6.9 cms long, pulled from the scalp was cut into 23 3 mm sections (each approximately 1 week's growth) and each analysed. The results were plotted as shown in Figure 29.

The results indicated a fairly constant level of arsenic medication over six months or so. Evidence of a gradual increase prior to hospitalization was seen. Such an increase could have been instrumental in bringing about the polyneuritis.

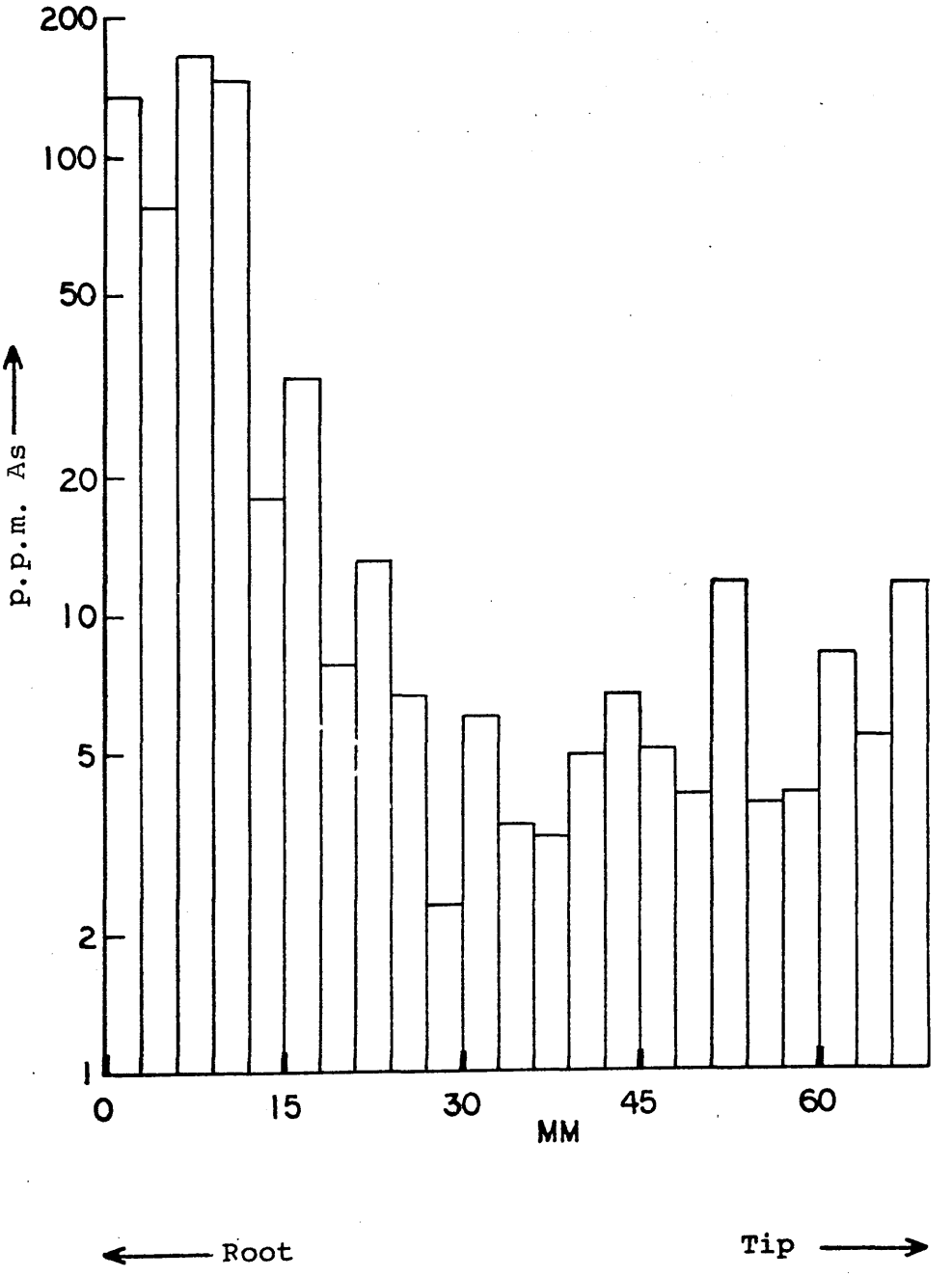
It was confirmed, therefore, that the patient had been absorbing appreciable amounts of arsenic.

Conclusion

Self-medication with arsenical tonics is an old remedy, but one that is still sometimes used. The result is a general development of ill health with symptoms of chronic arsenical poisoning. An earlier case of this type was described by Dewer and Lenihan (122) and the values reported are comparable.

FIGURE 29

ARSENIC LEVELS ALONG ONE HAIR STRAND OF FEMALE PATIENT.


200
100
50
20
10
5
2
1
p.p.m. As
0
15
30
45
60
MM
Root
Tip

Thus activation analysis for arsenic was found to be a valuable diagnostic aid. The small samples of hair, nail and skin scales could be easily obtained without distress to the patient and their analysis prove the poisoning source. The sensitivity of the technique enabled small successive sections of hair to be estimated for arsenic allowing in one case the history of uptake to be revealed.

General conclusion

The application made of the activation analysis technique to the assessment of arsenic uptake yielded valuable and interesting results. The measurement in hair, nail and urine of arsenic was able to provide a good assessment of any uptake in the subject concerned. Such samples could be easily and readily obtained.

The analysis of head hair in successive 3 mm segments was found to be able to provide a unique time record of arsenic poisoning. In the case of the laboratory technician, where one single dose was taken, a very clearcut elevation of arsenic level along the hair was found. When such a method of hair analysis was carried out for the female patient examined, a pattern of high self-administration at gradually increasing levels over 6 months was able to be seen.

All of the subjects examined for industrial exposure to arsenic were found to have elevated levels. It was thought significant that 7 out of the group of 11 displayed no associated poisoning symptoms. It was considered that the exposed

workers could become arsinophagists. Very few, if any, cases of notifiable industrial arsenic poisonings are reported on average each year to the Chief Inspector of Factories. For example, H.M. Chief Inspector of Factories Annual Report 1973, states that no such cases were recorded for that year (123). The evidence found from the small number of workers examined suggests that in fact many more cases go unreported. A long term study into the development of any carcinogenic disease in the workers studied would be of interest.

The application of the technique to the analysis of arsenic in tissue samples from patients was found to be a valuable clinical aid. It enabled the cause of the patients' condition to be confirmed as being due to arsenic self-administration.

Copper

Copper is not particularly toxic to man; a minimum lethal dose of copper sulphate is said to be 10 gms (71). Ingestion of a toxic dose causes symptoms such as vomiting, coma, respiratory failure, prostation, cramps and convulsions to occur. Excess copper is toxic to plants and bacteria (47). The present wide domestic use of copper for cooking vessels and plumbing shows that such use is considered acceptable; 0.2 mg Cu/litre in water supplies is considered safe (71). Cartwright and Wintrobe (87,124) report that copper is more readily absorbed orally. Relatively little is known about the actual mechanism of copper absorption in man (86,90). However, more is known about the factors concerned in the genetic diseases of Menke's syndrome, where absorption of copper is adversely affected and of Wilson's disease in which copper is internally retained. Wilson's disease is characterised by increased urinary excretion of copper. Cartwright and Wintrobe (124) indicated some correlation between copper in serum and ceruloplasmin concentrations. As described by Underwood (86) alteration of copper levels is manifested in various diseases and chronic infections in man and he lists measurements of hypercupremia that have been made. Fell et al. (125) described their application of activation analysis to the study of Wilson's disease and reported a mean level of copper in urine of 521 μg/24 hours before treatment.

Ravesteyn (126) in 1944 found no demonstrable increase of copper in urine after oral administration of copper sulphate. Included amongst their findings Bush et al. (127) in

1955 showed that a mean of 72.5% of an administered copper dose was excreted in stools by their normal subjects, whereas only 0.1% was found in their urine. Cartwright and Wintrobe (87) in 1964 estimated that of the 2-5 mg daily intake man absorbs 0.6 to 1.6 mg, and of this excretes 0.5 to 1.3 mg in the bile, 0.1 to 0.3 mg passes directly into the bowel and 0.01 to 0.06 mg is excreted in the urine. Thus there is certainly evidence to suggest that as an indicator of copper absorption urine should not be first choice. The main route for copper excretion appears to be the intestinal tract and not via the kidneys.

Industrial exposure

Problems caused by copper exposure in industry seldom arise, so it was with some interest that the following two cases were received for investigation. In the first case samples of hair and nail were provided and in the second case where gross external contamination existed, urine was given.

Four factory workers were exposed to finely divided metallic copper and in some cases to ground copper sulphate and copper carbonate dust. Specimens of head hair, pubic hair and the nails were taken. Fingernail specimens were not obtained as in most cases they had been cut very short and were grossly contaminated. Some of the toenail parings had a distinct greenish discolouration suggestive of copper staining.

Individual histories

A - Aged 48. Employed in the factory for 18 years. Exposed to metallic copper. Had no symptoms or abnormal signs apart from a cough (was a heavy smoker).

B - Aged 42. Employed in the factory for 11 years. Exposed to copper sulphate and copper carbonate dust. Had no symptoms or abnormal signs apart again from a cough (also a heavy smoker).

C - Aged 49. Employed for 4 years. Smoked 30 cigarettes per day. Had a cough and had some dyspnoea.

D - Aged 60. Employed for 2 years. Exposed to mixed dusts. Had no symptoms or abnormal signs.

Results

The results of the analyses are given in Table 37.

Table 37. Copper levels found in 4 workers

Worker	Years employed	Tissue	p.p.m. Cu
A	18	Head hair	240
		Pubic hair	1840
		Toenail*	610
B	11	Pubic hair	1220
		(no other samples obtained)	
C	4	Head hair	3940
		Pubic hair	4030
		Toenail	6770
D	2	Head hair	228
		Pubic hair	932
		Toenail*	873

*Discoloured green.

For convenient comparison normal copper levels reported by Liebscher and Smith (1) are shown below in Table 38.

Table 38. Copper levels in hair and nail from a normal population*

		p.p.m. Cu				
Tissue	No.	Arithmetic mean	Geometric mean	Median	Highest value	Lowest value
Head hair	29	23.1	20.6	19.1	54.5	7.64
Fingernail	33	18.1	14.7	14.9	58.2	3.18

*From Liebscher and Smith (1).

Discussion

The levels found were remarkably high. In fact, the processed samples had to be cooled for 4 days (8 half-lives) before the dead time was sufficiently reduced for counting to take place. It was apparent that a great deal of external contamination had taken place as the working conditions suggested (the nails discoloured green, etc.). However, associated with this external contamination at least some copper absorption must also have taken place in all of the workers. The levels found for C were noticeably greater than those of A, B, or D, and C had dyspnoea—respiratory difficulties, a symptom of copper toxicity. The coughs of A, B and to a lesser extent C, were likely to have been caused by their heavy smoking, although their exposure to copper dusts may have been contributory. D, who worked in the factory for only 2 years, had high copper levels but had no toxic symptoms.

Conclusion

All of the four workers who had been exposed to copper dusts for periods varying from 2 to 18 years were shown to have been grossly contaminated. However none, with the exception of C, appeared to have absorbed sufficient copper to have developed obvious toxic symptoms. The high levels found in C made it reasonable to assume that his dyspnoea was associated with copper poisoning. It appeared that in the cases of A, B and D, copper was absorbed through the skin and lungs and yet did not give rise to toxicity.

Another similar case, in which this time urine samples were analysed, further illustrated the above finding. For 20 years a factory had been making very fine copper dust for pigmentation in paints, etc. Five workers concerned in the process had been working there from 6 to the full 20 years. Their bodies were covered red all over with copper during each working day. Hence no hair or nail samples were taken as they would have been grossly contaminated. None of the workers showed any symptoms of copper poisoning.

Results

The results of the urine analyses are given in Table 39.

Table 39. Copper in urine of more workers

Worker	Years employed	µg Cu/ml Urine
E	20	8.82
F	15	5.84
G	6	1.12
H	9	0.236
I	6	0.107

Discussion

There is variation in the copper levels reported for normal urine. Some of the quoted mean values are listed below.

	µg Cu/24 hrs
Bearn and Kunkel (128)	48*
Butler and Newman (129)	18
Ch'en (130)	18
Fell et al. (125)	120
Giorgio et al. (131)	21
Ravesteyn (126)	30
Schroeder (132)	60

*For children

Fell et al. reported levels up to 3,800 µg Cu/24 hours being excreted during penicillamine treatment in Wilson's disease.

Normal 24-hour urine volume ranges between 600 to 1600 ml (133). Thus from Table 39 it can be seen that all 5 workers, in particular E, F and G, had vastly increased copper urine levels, at about 3 to 50 times normal. As described earlier, urine reflects only a small proportion of copper intake, and therefore it could be seen that each worker must have been absorbing a great deal of copper. The concentrations of copper found in the urine were comparable or greater than subjects being treated with penicillamine for Wilson's disease (125).

Conclusion

All of the workers were shown to have been absorbing high amounts of copper. It was not difficult to see why, as they each had a daily coating of red copper dust. Howe·er,

there appeared to be no adverse effects on the health of any worker. Thus there was proven a tolerance to excessive exposure in this case. It was of interest to note, however, that the workers with the longest years of service excreted the greatest amounts of copper, indicating a possible accumulation in the lungs and other body sites.

General conclusion

Of the 9 workers examined only one had probable poisoning symptoms. It was demonstrated that there was a great deal of tolerance to high copper exposure. Nevertheless, it was also demonstrated that a great deal of copper uptake was occurring in each subject. Copper is not an industrially hazardous metal, but there were indications at least of more care being required. Such excessive absorption could be reduced by simple application of industrial hygiene. The use of the activation technique enabled analyses to be made of samples which could be easily provided and the results thereof made a useful insight into the working conditions of their donors.

Zinc

Zinc is the first member of the Group IIB series in the Periodic Table which contains also cadmium and mercury. Zinc comes immediately after the first transition series of metals. It has been long recognized as an essential trace element in plant and animal tissue in which it widely occurs. Raulin (50) first identified the necessity of zinc for the growth of "Aspergillus niger" in 1869. Zinc occurs in high trace levels in the organs of the human body. It is found at its highest levels in teeth, the soft tissue organs of kidney, liver, muscle, etc., from 300 p.p.m. dry weight downwards (1, 15, 78). Widdowson and her colleagues report that the average adult human has approximately 2 gms of zinc in his body (134, 135).

Zinc has been shown to occur in proteins, nucleic acids, amino acids, etc., and is widely associated with enzymes as metalloenzymes and more loosely as metalenzyme complexes. It is considered that through its enzyme association zinc plays a part in tissue metabolism. The complete metabolism of zinc is not as yet fully understood and it is known that it is influenced by cadmium and calcium. The essential nature of zinc is described, for example, by Vallee (73). Liebscher and Smith (1) demonstrated the normal distribution of zinc in human tissues that would be associated with an essential trace element.

Average daily zinc intake by humans was reported as 10-15 mg by McCance and Widdowson (136). Spencer et al.found that half the zinc intake was excreted directly from the intestine and little in the urine (137). Zinc is most rapidly passed through the liver, spleen and kidney, as described by Heath and Liquier-Milward (138).

Arwill et al. (139) found that levels of Na and Mn were significantly higher in the saliva of subjects, stimulated by chewing wax than in unstimulated saliva, approximately 6 times and twice, respectively. They observed no similar differences for Cl, Br, Cu and Sr. It was thought of interest to see whether the salivary glands played any part in maintaining the distribution of zinc in the human body. Beck and Gillings (140) demonstrated that zinc was present in human saliva. They qualitatively found ^{69}Zn in the γ spectrum of irradiated ashed saliva.

Saliva glands and mucosa tissue inside the mouth were obtained from 6 adults during post-mortem examination. In order to compare the levels found to ordinary skin tissue a skin sample was taken from the abdomen in each case. The gland tissue on drying remained slightly oily and was irradiated as such.

Saliva itself was also assessed for zinc. It was obtained from 7 adult subjects, mixed from the central cavity of the mouth where the labial and submandibular glands excrete ("mixed"), and from the upper back region of the parotoid glands ("paratoid").

It was taken as resting saliva and stimulated. Stimulation was by enacted chewing. After irradiation some sedimentation in the saliva samples was found to have taken place inside the silica vials used. However, all of the contents were able to be eluted for subsequent processing.

Results

The results are shown in Tables 40 and 41.

Table 40. Zinc levels found in mouth tissue and abdomen skin from 6 humans

		p.p.m. Zn, dry weight				
Tissue	No.	Arith. mean	Geom. mean	Median	Lowest value	Highest value
Right Paratoid	6	37.7	24.5	39.6	2.31	62.7
Left Paratoid	6	41.1	29.4	43.0	3.31	68.8
Right Submandibular	6	36.0	33.0	34.7	15.8	59.0
Left Submandibular	6	37.6	32.2	35.3	13.3	71.5
Labial Gland	5	87.5	78.1	99.5	37.1	126.3
Buccal Mucosa	6	49.1	40.8	38.0	13.5	110.1
Tongue Mucosa	6	132.7	115.4	101.2	65.5	316
Skin (abdomen)	6	19.5	18.0	20.2	9.70	27.7

Table 41. Zinc levels found in saliva taken from 7 subjects

Saliva obtained as	p.p.m. (wet weight) Zn			
	Stimulated paratoid	Resting paratoid	Stimulated mixed	Resting mixed
Subjects				
A, ♂, 18 yrs.	0.02	0.03	0.04	0.05
B, ♂, 28 yrs.	0.005	0.005	0.05	0.03
C, ♂, 33 yrs.	0.04	0.05	0.06	0.05
D, ♀, 17 yrs.	0.02	0.03	0.02	0.05
E, ♀, 18 yrs.	0.01	0.03	0.02	0.05
F, ♀, 22 yrs.*	0.005	0.11	0.31	0.17
G, ♀, 44 yrs.	0.04	0.05	0.07	0.06
Mean values excluding F*				
Arithmetic mean	0.02	0.03	0.05	0.04
Geometric mean	0.02	0.03	0.05	0.04
Median	0.02	0.03	0.06	0.05

*Saliva from F was coloured brown and was considered contaminated with foodstuffs or other material. Hence these results were disregarded.

It was found that the mean zinc levels in the paratoid and submandibular glands were in the lower range of those found in other tissues, c.f. Liebscher and Smith (1) and Tipton et al. (15, 78). The labial gland appeared to contain significantly great (>x2) zinc than the other salivary glands. The highest zinc levels were found in the mucosa tissue of the tongue. Buccal mucosa (i.e. tissue from the cheek) contained similar levels of zinc as the paratoid and submandibular glands.

All of the mucosa and gland tissue was found to contain a greater amount (>x2) of zinc than normal skin, in this case skin from the abdomen.

The zinc levels found in saliva indicated marginally more zinc was present in mixed than paratoid saliva and there was a marginal increase in stimulated mixed over resting mixed. It was thought possible that the labial gland, containing more zinc than the other glands, was instrumental in these effects.

Conclusion

Such a pronounced element level increase in stimulated saliva over resting saliva as found by Arwill et al. for sodium and manganese (139) did not occur for zinc. Compared to the high levels of zinc in such tissues as prostate kidney, liver, etc. (circa 200 p.p.m.) it was of interest that less zinc appeared in the salivary glands, indicating that here zinc associated metabolic processes were not so important. However, it was also of interest that amongst the salivary glands

markedly higher zinc levels were found in the labial gland, indicating a more active zinc role therein. A possible zinc association between the labial gland and saliva was indicated.

Zinc levels in similar tissue from the mouth were not found reported in the literature. Therefore the values determined were of unique and particular interest. Mean values found for skin were in agreement with those of others (1, 15, 78). Zinc is normally present at fairly high trace levels in tissue, thus making ultimate sensitivity measurement unnecessary. However, the zinc concentration in the saliva samples analysed was approximately one-thousandth that in the gland tissue. Nevertheless, the sensitivity of the method was such that these levels could just be measured. Thus the major advantage of the activation analysis technique—sensitivity—was of use in enabling this clinical survey to be carried out.

Gold

Gold is classified under group IB of the Periodic Table. It is a so-called "coinage" metal, the others being silver and copper. It was the earliest pure metal known by man. Gold is untarnished by air and is the least reactive metal. Because of its permanent attractiveness and rarity it has been a metal much coveted and hoarded throughout the ages. Even today it is of unique economic importance as it is the basis of a world economic standard. Gold that is used is mainly made up in jewelery, etc., less than 20% is industrially consumed in electrical and manufacturing processes, etc.

Gold therapy is used as a treatment for rheumatoid arthritis. It is administered as an injection in the form of solutions such as gold sodium thiosulphate ("Sanocrysin"), gold sodium thiomalate ("Myochrysine"), aurothioglucose ("Solganol") and gold chloride. Its associated toxicity has been widely reported throughout the medical literature. Reported effects include nephritis, hepatitis, blood dyscrasias and aplastic anemia. Typical examples of such effects were given by McCarty et al. (141), in which they reported a fatal case and reviewed the literature, and by Wohlenberg (142) who included a review of 32 case reports in world literature in his paper.

In this study one case of gold toxicity was investigated. A liver sample was taken from a hospital patient who had had one known dose of gold chloride. She had then died of acute hepatitis. As the circumstances of her dosage were far from clear it was suspected that she might have died from me al

toxicity and not due to any allergic phenomenon.

The specimen was dried, analysed and the result was as show below.

Result

Table 42. Gold Found in Patient's Liver

Tissue	Gold p.p.m.
Liver	0.975

Conclusion

Parr and Taylor (143) analysed 32 samples of wet liver and found a range of values of 13 to 790 µµg/gm and a median of 57 µµg (equivalent to around 0.003 p.p.m. for dry tissue). In comparison therefore to this and other literature values a very high level of gold was found in the liver. Thus it was thought that there was some justification for the belief that death was associated with gold toxicity, but on the whole, with such slender evidence, the final decision must be left open.

General Conclusion

The sensitive analysis at trace level of small samples by the activation technique enabled much pertinent information to be gained and insight to be made into metal uptake by man from his industrial and occupational environment.

A more clinical application of the technique was able to be made, to detect poisoning, as a diagnostic aid and as a study to investigate the role of zinc in the mouth.

In the cases examined for uptake it was found that the choice of head/pubic hair and finger/toe nail and urine as samples for analysis had many advantages. The principal advantage was that their collection was straightforward by being simple, painless and fast, causing little social inconvenience. The head/pubic and finger/toe ratio both gave an indicator of the ratio of external contamination to internal uptake.

One part of the work done, concerning mercury exposure of dentists and dental surgery personnel, produced a finding which was not anticipated. It was expected that dentists under daily mercury exposure would, as a group, show elevated levels in the samples of head and pubic hair, finger and toe-nail examined, as earlier work, for example by Howie and Smith (105) indicated. This indeed was proved to be the case. However, what was not expected was the extent to which many of the levels were found to be elevated, some being dangerously high, associated with the fact that there were no reports of related poisoning symptoms. This pattern of high

heavy metal uptake to people routinely exposed, without obvious occurrence of the poisoning symptoms that would be expected at the levels measured, was found to reoccur throughout other cases examined for different elements. The actual uptake of mercury to even potentially hazardous levels was shown to be able to be quickly achieved by the dental student group after three years surgical training. In all, it was therefore concluded that there was a demonstrated health hazard that was not otherwise apparent by any obvious manifestations of poisoning. It was found that mercury uptake by dental groups was more extensive than might be thought and therefore although there may be no poisoning syndromes the possible long-term detriment to health associated with the demonstrated mercury accumulation should not be ignored.

Uptake of mercury, arsenic and copper was shown to occur during occupational exposure to various chemical forms of these elements by industrial and other workers. In the 11 cases described, involving 27 people, certain common patterns were found to emerge. Again, as for the dental group, in certain cases a remarkable degree of tolerance was found to high absorption of each of the three elements. Lamp factory workers exposed to mercury were found to have high mercury levels in hair and nail and yet most had good health. It was concluded that many of the arsenic exposed groups examined had become partial arsenophagists, i.e. capable of high uptake

independent of ill effect. Also it was found that for timber workers exposed to arsenic that their degree of uptake appeared to rapidly (within a year) reach a plateau. This paralleled the rapid and extensive mercury uptake found in dental students. Copper exposed workers who were daily covered in red copper dust also appeared remarkably resistant to the high absorption found and showed no obvious signs of health damage.

Less than one-third (8 out of 27) of all the known to be exposed workers examined for either mercury, arsenic or copper uptake, had any possible associated poisoning symptoms. It appeared that routine exposure enabled the large levels accumulated of mercury, arsenic and copper, to be often tolerated and to produce no obvious ill effects on health.

In the cases examined where occupational poisoning was confirmed the removal of the subject from the source brought about the disappearance of symptoms and a cure. Subsequent analysis proved the uptake reduction. It was noticed that dermatitis was a symptom strongly associated with arsenic exposure in the cases examined. Persistent dermatitis is often a precursor of skin cancer.

In the case concerning the young male science technician, who was known to have swallowed a large arsenic dose, of all the analyses of body samples obtained, the sectional analyses of head hair proved to be the most informative. It was shown to be able to provide an elegant time record of the degree of arsenic absorption that had occurred. Similar analysis of any

pulled hair could thus be seen to be able to perform two functions: (1) provide a means of measuring any uptake, (2) provide an approximate dating of any uptake. Fingernail analysis also confirmed arsenic poisoning. The monitoring undertaken of arsenic levels in beard shavings and urine taken during hospitalisation of the above subject, prior to and during penicillamine treatment, provided no evidence that penicillamine treatment at so late a stage of 6 weeks after ingestion, caused an increase in arsenic excretion. After such a period most arsenic would have been removed from the body (121).

In the one and the only analysis carried out for gold, a considerably elevated level was found in the liver of a patient who had been treated with gold chloride. It was able to be concluded, therefore, that the death of the above from acute hepatitis could have been associated with gold toxicity.

In two separate cases, analysis of tissue samples for arsenic was able to show that it was the cause of the exfoliative dermatitis and polyneuritis in each patient respectively. For the second patient sectional hair analysis was found valuable in that it showed the way in which arsenic had been absorbed over a period of six months prior to hospitalisation.

Mercury analysis in urine was shown to be a valuable aid in the confirmation of pink disease in a 1-year old baby. Similar urine analysis of a patient showed a normal mercury level, indicating that mercury absorption, previously suspected, was not the cause of the patient's deteriorating renal function.

In the case of zinc it was of interest that the levels in the salivary glands of the mouth were in general lower than in many other tissues and that amongst the glands a significantly higher zinc concentration was found in the labial gland. An association was considered possible with the labial gland to the zinc concentrations that were measured in saliva. It was thus thought that the higher zinc levels observed in stimulated and resting mixed saliva may have been due to the zinc contained in labial gland excretion.

In the toxicological studies made it could be seen that there was no precise correlation of recorded uptake to disease symptoms, there was variation from person to person. The evidence from the occupational studies carried out indicated that often poor industrial hygiene existed and that future improvement was necessary. Activation analysis was found to be a useful tool in the studies made.

CHAPTER 6

Historical Applications

HISTORICAL APPLICATIONS

Introduction

The circumstances of the illnesses and deaths of many persons of historical importance are sometimes rather suspicious when regarded from the modern viewpoint. In particular, evidence of metal poisoning in some cases has been found. For example, Wolbarsht and Sax (144) analysed hair from Charles II of England and discovered very high levels of mercury to be present. Charles II had a private laboratory and distilled copious amounts of mercury during his researches. His death in 1685 is now believed to have been due to chronic mercury poisoning precipitating renal failure. Forshufvud et al. (145, 146) and Smith et al. (147) analysed for arsenic, hair cut from Napoleon Bonaparte during his exile on St. Helena. From hair samples cut in 1816 and on his death in 1821, they found evidence of arsenic administration. Westermark and Sjöstrand (148) found evidence of arsenic and mercury uptake when they examined some remains of Eric XIV of Sweden.

Tissue material from the subject is required for analysis and this is generally available only in very small amounts. The sensitivity of the technique of activation analysis makes it highly suited, therefore, to this application. The analysis material, for example, hair, can very often be well authenticated. It was a custom for small locks

of hair to be distributed as keepsakes. Death was such an occasion where this was done. Such samples are sometimes preserved to this day where they have been kept as family heirlooms.

Problems are associated with the analyses of historical tissue samples. It is necessary that the sample be known to be authentic. Both the possibility of contamination and of loss of the element of interest from the sample over its years of storage need be considered.

That activation analysis is ideal for this type of work is shown by the following two investigations that were carried out, each on samples of hair.

Mercury in hair of Burns

An authenticated sample was obtained of head hair taken during the late adult life of the Scottish "Bard", Robert Burns (born 1759, died, aged 37, in 1796). The exact date of the cutting of the hair sample was not known. There were 7 hair strands in all, each approximately 4 cms long, representing about 3 months growth (120). The hair was examined microscopically and had the cracked and fissured characteristics of age. It had a red colour. Three of the strands were pooled to provide sufficient sample for analysis and the total weight was 0.60 mg.

Burns lived in Dumfries from 1790 onwards. In 1795 there was a marked decline in his health and in the winter of 1795-6 he was ill with rheumatic fever. It was of

interest to see whether a high mercury level would be found in hair. Contemporary reports of the poet's health and reckless behavior could be associated with symptoms of mercury poisoning (mercury was a common constituent of medications of the time).

Result

Analysis of the sample gave the following result:

Table 43. Mercury found in hair of Burns

Tissue	p.p.m. Hg
"Rabbie" Burns head hair	8.02

Conclusion

The above was higher than normal, but not outstandingly elevated. The level was within one standard deviation of the geometric mean of 3.51 p.p.m. ($X \div 3.03$) quoted by Liebscher and Smith (1) for 70 normal hair samples. It showed, however, that Burns could have been suffering from some mercury poisoning. For example, Worker G, described under "Industrial exposure to mercury," on page 201 , had a head hair level of 7.1 p.p.m. Hg and was suffering from personality change. It is also possible that the level measured in Burns's hair reflected a minimum value as mercury loss could have taken place over nearly two centuries of storage.

The evidence of possible mercury poisoning perhaps provides a useful clue to the poet's state of health in late life. His early death was diagnosed as due to heart disease.

Arsenic in hair of Napoleon

After his defeat at Waterloo, 1815, to his death in May 5, 1821, Napoleon was kept in exile on St. Helena by the British. A few strands of hair taken from Napoleon's head during this period were available. Their history was as follows:

The hair was cut by Napoleon's manservant, Santini, in October 1816. It was not known how far from the scalp Santini cut these hairs. However, it was most probable that they were taken from the nape of the neck and cut off close to the skin, as Santini was concerned mainly in turning out a well-groomed ex-emperor and not in changing the hair style by shortening the hairs on the crown. Napoleon liked his hair cut close and was called "Le Petit Tondu"—"The Little Crop Head." The hair strands ranged from 1.4 to 2.1 cms in length and would thus provide a record of through the months of August and September 1816. Alternatively, if, as was more unlikely, they were cut off as much as 10 cm from the scalp they would cover the period from January to the beginning of March 1816.

The hairs were first examined microscopically and matched the characteristics of other known samples—a reddish tinge, cracking of age, etc. One of the longest hairs (2.1 cms)

was cut as described earlier into seven 3 mm segments. The hair samples were analysed for arsenic and the results are shown in Table 44 and Figure 30. There were 4 hair strands.

Results

Table 44. Arsenic found in hairs "A", "B" and "C" of Napoleon

Description	Weight µg	p.p.m. As
Hair "A" - 1.9 cms long	110.0	9.22
Hair "A" - 1.4 " "	91.1	9.81
Hair "B" - 1.7 " "	91.0	30.4
Hair "B" - 1.3 " "	74.5	13.84
Hair "C"*- 2.1 " "	37.4	25.4

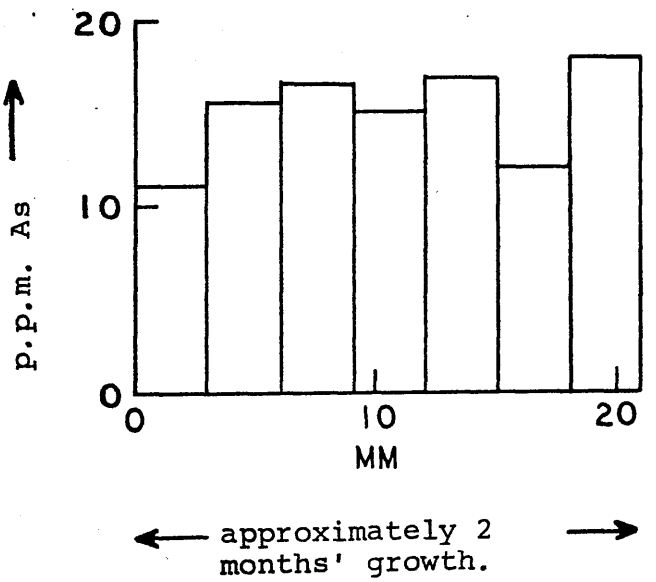
*Very fine hair, which looked like the end of a hair strand. Hairs "A" and "B" were obtained broken in two. The direction of growth in the hairs was not known.

The high arsenic levels found showed evidence of arsenic poisoning (c.f. Smith (121) and Liebscher and Smith (1)). The concentration found in each segment of hair "D" indicated consistent arsenic intake over some 2 months.

If the hair samples were cut off close to the skin, the results prove that Napoleon ingested quantities of arsenic regularly from roughly 31st July to 1st October 1816. This fits in very well with contemporary reports of his health by Balmain (149), Stürmer (150) and Montholon (151). From mid-April 1816, Napoleon no longer went riding long distances, his swollen legs no longer supported him. It was noticed how he became quite changed. For a few days in August and

FIGURE 30

ARSENIC CONCENTRATION FOUND IN 7 CONSECUTIVE 3 MM SEGMENTS OF HAIR "D" FROM NAPOLEON.


20
10
0
p.p.m. As
0
10
20
MM
approximately 2
months' growth.

from 9th to 13th September 1816 he was more seriously ill. On October 1, 1816 he fell ill for six successive weeks. From 1816 onwards Napoleon began to look progressively more like an overweight alcoholic, although he drank only small amounts of wine. Napoleon could have become an arsenophagist, i.e. capable of ingesting arsenic without absorbing it all and becoming sick.

If, as was more unlikely, the hair was cut off up to 10 cm from the scalp, reports of Napoleon's indifferent health during the earlier part of 1816 do not contradict the results found. However, as already described, the available evidence is in favour of the hair having grown later in the year, and certainly there is a strong match between his deteriorating health at that time and the evidence found of continuous arsenic ingestion.

Conclusion

The results found indicated that Napoleon was subjected to consistent and high amounts of arsenic poisoning during 1816. Forshufvud et al. (145) found from the analysis of other hairs cut from Napoleon in 1816 high arsenic levels which they concluded had been deposited there before his exile to St. Helena. Thus the findings now made showed that further and intensive administration of arsenic to the ex-emperor continued after his arrival on St. Helena.

Forshufvud et al. (146) found 10.38 p.p.m. arsenic in hair cut from Napoleon probably on his death in 1821. Hair from another source and authenticated as being cut from Napoleon on the day after his death was analysed by Smith et

al. (147). They found a range of 1.06 to 11.0 p.p.m. and an average of 4.91 p.p.m. arsenic for 1 cm lengths of a 9 cm hair. The sensitivity that they could obtain made these results for small samples subject to error. Analysis by Smith et al. of two pooled samples gave values of 3.75 and 3.27 p.p.m. arsenic. Thus, as a higher arsenic level range was found in the hairs analysed here (9.22 to 30.6 p.p.m. arsenic), it was concluded that Napoleon had ingested greater amounts of arsenic during 1816 than towards the end of his life.

Whatever the exact cause of Napoleon's death in 1821, aged 51, the combined evidence shows that prolonged and at times intensive arsenic consumption must have played a significant if not major part. Cartwright (152) describes Napoleon's death as due to cancer of the stomach, which had left an aperture in the wall of the stomach sufficiently large to admit a finger. Cartwright also mentions that arsenic might have been the causitive agent of the cancer if it had been taken for any length of time. As evidence of such protracted arsenic ingestion was found, arsenic may in this way have been an agent of Napoleon's death.

General conclusion

Activation analysis is an invaluable research technique for assessing uptake of mercury or arsenic that took place by historically important figures. That this is so is primarily because it can be successfully applied to the minute samples which are the only ones now available. For

example, less than 100 μg portions of hair were assessed for arsenic. Positive findings in the form of elevated values can support other evidence of poisoning or excessive medication—the evidence found of a high level of arsenic ingestion by Napoleon during 1816 correlated with reports of his poor health during that era.

Information on uptake at a particular period in the life of the subject may be obtained where there is available a hair sample known to have been cut at that time.

Authentication of origin may be excellent, but qualification need be made about possible prior contamination of the sample or loss of elements during its years of storage. However, the findings made may be helpful, in particular when placed alongside other known information. The fact that such fresh evidence from the past can be established allows new fields of speculation and research be made into the circumstances concerning epochs in the life of the subject. Absolute and firm statements regarding causes of death which occurred long ago are, however, difficult to completely justify at this distance from the event.

CHAPTER 7

Conclusion

CONCLUSION

Neutron activation analysis techniques are selected and developed for the determination of the trace elements copper, mercury, cadmium, arsenic, gold, manganese, zinc, selenium and barium in biological tissue. These methods all have the advantages of neutron activation analysis, namely: they are sufficiently sensitive to enable trace element studies to be made in small samples of biological tissue, they provide absolute certainty that it is the element of interest that is being assessed and not any other, they are reasonably accurate, only simple chemical processing is necessary, they enable analyses of large numbers of samples to be carried out at one time.

The sensitivities obtained in the methods as finally used are as follows:

Copper	0.001 µg
Mercury	0.003 µg
Cadmium	0.001 µg
Arsenic	5×10^{-5} µg
Gold	10^{-4} µg
Manganese	0.002 µg
Zinc	0.002 µg
Selenium	0.03 µg
Barium	0.2 µg

These methods are applied to various biological samples and enabled the following studies to be made.

Surveys of trace elements in tissue

Distribution studies can enable the role of an element to be established. A normal distribution is found for such essential trace elements as manganese. Measurements of selenium concentrations throughout a wide range of human tissues indicates that it plays an essential role, which recent evidence from other sources confirms. Selenium is also seen to be held at a level of 1.5 ± 1.0 p.p.m. in soft body tissue, which suggests that it performs a similar function in each tissue. In eye tissue, from Rhesus monkeys (macaca mulatta) selenium is found at a comparable level to other soft tissue and it is thought that its role in eye tissue is not special. A comprehensive lisitng of cadmium levels for 17 body tissues is given, thus filling a gap observed in the literature. A nonessential role for cadmium, known to accumulate in the body, is confirmed. The essential role of manganese in both human and monkey tissue is shown. Comparison between mean levels in tissues from both species shows that manganese concentrations are in the main the same and thus that manganese may be identically involved in the metabolic processes of man and monkey. An accumulation of manganese in human lungs at higher levels than that in monkey lung is shown. This is attributed to external absorption from the general atmosphere during the lifetime of the human lung. Barium is shown to be accumulated preferentially in the lymph nodes as a nonessential trace element. This shows that the

phagocytes cells are probably responsible for its transportation to the lymph sites from the lung body. Studies of copper levels in nail and skin of one individual over an extended period of time indicates that the variation is less than that found in the population as a whole.

Environmental and clinical studies

The use of hair, nail and urine analyses is shown to be valuable in the carrying out of studies of occupational and industrial uptake. Similar analysis is able to be usefully employed as a clinical diagnostic aid and is demonstrated by monitoring a case of arsenic poisoning. A surprising amount of occupational uptake of mercury is found in the dental groups investigated. It is seen that there is an obvious need for greater care to be taken about working conditions. It is a feature of this and further studies of uptake of other elements, that long term exposure appears to create tolerance to high absorption. Poisoning symptoms that might be expected are often not visible. This tendency is found in the group of exposed industrial workers, of whom only 30% display any symptoms. The levels of either mercury, arsenic or copper that are absorbed are shown to be high. The need for greater industrial hygiene is indicated because latent damage may be taking place. The possibility of delayed and cumulative effects is not limited to any particular element or agent and also it must be considered that the appearance of adverse symptoms may require a combination of factors.

Examples of general clinical diagnostic applications are as follows. Sectional hair analysis is proved to be valuable as a monitor in the examination of arsenic poisoning. Such a technique is also shown to be clinically informative in assessing arsenic previously administered and in confirming the cause of a female patient's condition. A case of pink disease is confirmed by mercury analysis of urine. Measurement of a high gold level in a post-mortem liver sample gives valuable corroborative evidence that gold toxicity was possibly the cause of a fatal acute hepatitis. A surprising amount of information can be extrapolated from such analysis of easily obtained hair and urine samples. The study made of zinc levels in mouth tissue shows that levels are lower than in many other tissues in the body, indicating a lesser involvement of zinc. Zinc is shown to be present at a much greater level in the labial gland than other salivary glands and a possible association of it with zinc in saliva is indicated.

Historical investigations

Application of the activation analysis technique to historical specimens provides results of interest. When head hair from Robert Burns is analysed for mercury it is seen that he could have been suffering from mercury poisoning at the stage of his life represented by the hair growth. However, the elevated level is within one standard deviation of normal and therefore the evidence for this is by no means conclusive,

although previous analysis of an industrial worker exposed to mercury, who developed poisoning symptoms, shows a similar level of mercury in hair.

Arsenic analysis of a few head hairs from Napoleon provides much fascinating information on his life during 1816. It is demonstrable that during that period the unfortunate ex-emperor was being subjected to consistent arsenic exposure whilst exiled by the British on St. Helena. Here sectional hair analysis proves invaluable in that it shows that exposure was consistent.

The biological function of trace elements in human tissue and their importance in clinical assessment is one of the major problems now facing medical science. Neutron activation analysis is a versatile, sensitive technique ideally suited to this investigation.

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