INVESTIGATION OF DENTAL TISSUES USING RADIO-ISOTOPE TECHNIQUES

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by

Hugh D. Livingston, B.Sc.,

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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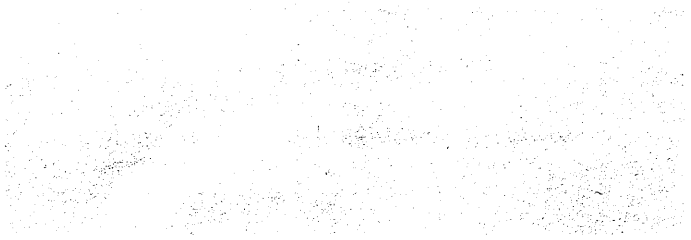

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SUMMARY

This thesis is concerned with the determination of inorganic constituents of dental tissues, mainly tooth enamel. Little is known about the occurrence and distribution of the minor constituents of teeth since analytical difficulties have prevented their estimation. As more sensitive and reliable modern analytical techniques become available more information can be obtained. This thesis deals with the development of methods of analysis for the precise and accurate determination of trace elements using the technique of neutron activation analysis.

Samples were irradiated in nuclear reactors with thermal neutron fluxes of at least 10^{12} n/cm^2/sec. In most cases a radiochemical separation was used to obtain the desired activity in a pure form for activity measurement. The efficiency of each separation was determined by decay curve and γ-spectrometric analysis. In one case no radiochemical separation was required and the element (zinc) was determined using simple γ-spectrometry by a non-destructive instrumental technique.

An analysis for vanadium was developed and shown to be valid for vanadium determination in teeth and other biological materials. A very short irradiation and a full solvent extraction radiochemical separation was used. In no tooth sample analysed (including whole teeth) could any vanadium be detected. The results indicated that vanadium, if present in teeth, occurred at concentrations less than 0.01 p.p.m.

It was found possible to measure the manganese content of over sixty samples of enamel. The method which was developed and tested used a partial post-irradiation chemical separation and final measurement by γ-spectrometry. It was found that manganese concentrated in the outer enamel of the teeth examined.

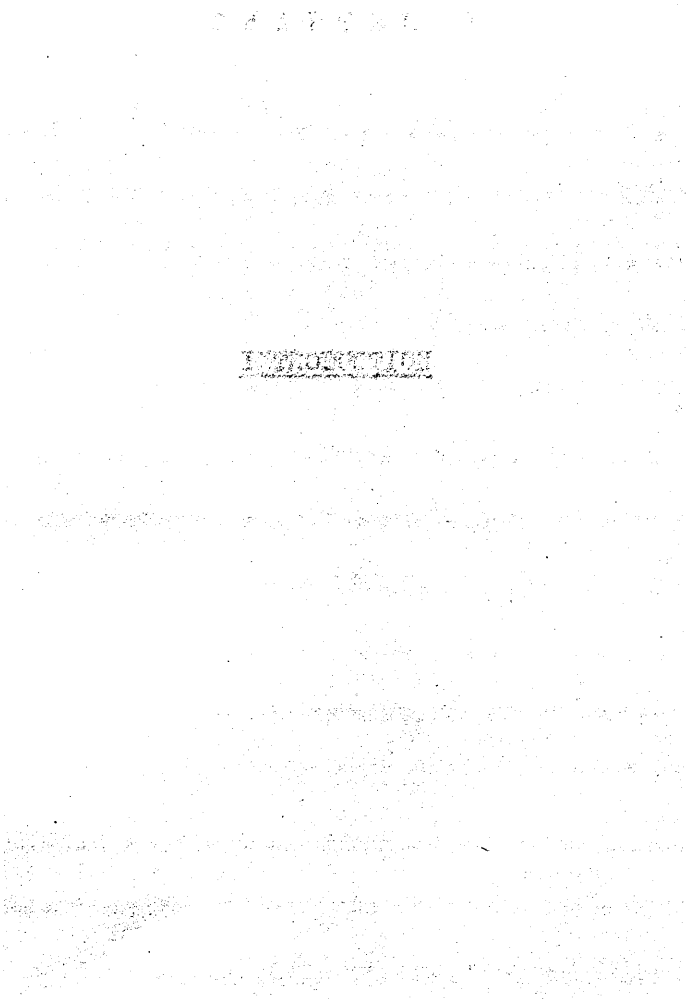
An ion-exchange separation was developed and tested which allowed the simultaneous determination of copper, zinc, cadmium and mercury in teeth or other biological materials. Final radiochemical purity was obtained by a precipitation for each element after initial separation by ion-exchange. Little cadmium was found in the enamel samples analysed - less than 0.01 p.p.m. The zinc content of thirty enamel samples was determined and, like manganese was found to be greater in outer enamel sections.

An instrumental method of zinc analysis was developed and used to measure the zinc content of a further one hundred samples. Following an irradiation for one week the samples were allowed to cool for three months when interfering activities had decayed and the zinc could be measured directly on a γ-spectrometer. It was shown that the zinc analysis results by both methods were in agreement.

The normal antimony level of enamel samples from Glasgow citizens was determined by analysis of fifty samples by a technique using full radiochemical separation and β-counting. The results were contrasted with those obtained from analysis of enamel from Egyptian citizens some of whom had been exposed to antimony in medical treatment.

A method of analysis suitable for molybdenum determination in teeth and other biological materials was devised. The radiochemical separation used both solvent extraction and precipitation. The novelty in the separation was that it was possible to extract and use the daughter isotope technetium for measurement. Two technetium separations were used. The first removed the technetium plus other extractable activities. The second, after delay to allow parent/daughter

equilibration, served to obtain the technetium in a completely pure form. It was found possible using this method to establish the normal level for molybdenum in human teeth and enamel.



CHAPTER 1

INTRODUCTION

INTRODUCTION

It has been recognised for a long time that accurate chemical analytical information is required before any useful understanding of the nature of teeth can be reached. Such information is essential to any metabolic studies of tooth processes. One of the major problems, becoming increasingly more acute, is that of tooth decay. A great deal of research effort is being, and has been, expended towards an understanding of the mechanism of caries production.

A vast literature[15] on the subject of dental caries is available. This includes investigations covering widely varying fields of scientific disciplines each contributing their specialised knowledge and skills to this basic problem. There are a large number of factors which may be related to caries production and the literature is not clear as to which is most important. The effects of age, diet (in particular carbohydrate intake), environment, use of dentrifices, fluoridation, tooth composition and structure have all been studied in considerable detail by a large number of workers.

The approach used by chemists is to consider how the composition of the tooth, both organic and inorganic, varies during formation, and how it is affected by its subsequent exposure to normal bodily processes. The permanent tooth is believed to be an unchanging entity compared with bone - a similarily calcified tissue but which is being constantly renewed. As a result, when caries starts in a tooth it is not eliminated by the formation of new healthy, non-carious tissue. The tooth is in continuing chemical interaction with the saliva in the mouth and foodstuffs entering the body. Constant mechanical processes are occurring

during mastication and related activities.

The major components of which teeth are composed have been studied in great detail. These can be divided for convenience into groups viz. (1) Inorganic (2) Organic. The ratio of inorganic to organic material varies in different parts of the tooth. The organic content becomes higher as the centre of the tooth - the pulp - is reached. In Table 1 is shown the composition of the hard tissues of the teeth[2].

Table 1 COMPOSITION OF HARD TISSUES OF TEETH

Tissue	Inorganic Content	Organic Content
Enamel	98%	2%
Dentine	75%	25%
Cementum	68%	32%

The basic elements which together make up the greatest part of the tooth matrix are calcium, phosphorus and oxygen. There has been considerable controversy over the exact nature in which these ions are present in the tooth. The theory which is now currently accepted suggests that calcium and phosphate are present in a structure similar to the mineral apatite[50]. The chief support for this is that the x-ray diffraction pattern from tooth samples is the same as that from the mineral. This means that the composition of the inorganic skeleton can be written as a double salt $Ca_3(PO_4)_2$. CaX where X = $(OH)_2$, F_2, Cl_2, O, SO_4 or CO_3 or any other equivalent ion or groups of ions. To indicate the continuous nature of the lattice some people prefer to express the hydroxyapatite structure as $Ca_{10}(OH)_2(PO_4)_6$.

This structure for teeth explains the presence and exchange of many minor elements found in teeth. Many foreign ions are capable of

isomorphous replacement of an ion or ions in the tooth lattice. As hydroxyapatite is noted for its ion exchange ability, the mechanism of replacement is established. For example, calcium may be replaced by silicon, manganese and strontium and hydroxide can be replaced by chloride, bromide and fluoride.

Animal experiments[57,65,93,94] and nutritional studies[8,60] have indicated the significance of trace elements in producing a tooth with a high natural caries resistance. The element which has been shown to have the most significant effect on caries prevention by its presence in teeth is fluorine. Water fluoridation studies[34], animal experiments[76,100] and nutritional studies[104] have all shown that there is a positive correlation between fluoride intake, tooth fluoride content and caries resistivity.

Ideally it is desirable to make an analysis of enamel, dentine etc., from caries resistant teeth to discover how their chemical composition differs from caries prone teeth. In practice this is difficult because there is no means of knowing whether a caries free tooth is resistant or whether it has dormant caries susceptibility. As a compromise solution some workers are measuring trace element levels in enamel or dentine obtained from apparently sound teeth extracted for orthodontic reasons. Differences between trace element levels in sound and carious teeth may show the value of certain elements in forming caries resistant teeth. Various chemical techniques have been used to determine the levels of different minor inorganic tooth constituents. Cremer and Voelkner[27] distilled fluorine from teeth and bones and measured the amount colorimetrically using the destruction of a ferric

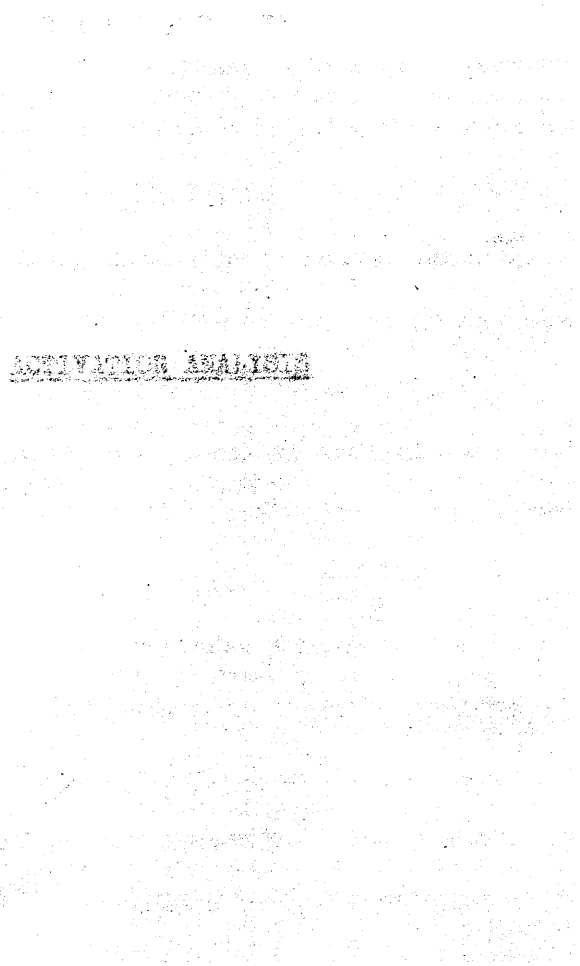
salicylate coloured complex by the collected hydrofluosilicic acid. The range of fluorine determined was 5-50µg. Cruickshank[30] separated 1-20µg. of zinc from enamel and dentine samples by a solvent extraction technique using dithizone. The separated zinc was estimated by a micro-titration. A quartz spectrograph was used by Eichoff et al[35], to measure the magnesium content of 10 mg tooth samples. The range measured was comparable to those described above for zinc and fluorine.

When the level of a trace element being determined is lower than those described above i.e. < 100 p.p.m. the amount present in enamel or dentine from a single tooth is often below the limit of the sensitivity of most techniques such as micro-analysis, colorimetry, flame photometry, emission spectrophotometry and etc. Consequently, pooled samples of enamel or dentine[16-19,41] have frequently been used so that a significant analysis would be made. The use of pooled samples results in the production of average analytical figures for a particular type of tooth section. Individual variations, or indeed, variations within a particular tooth are not detectable by these methods.

Activation analysis has the advantage that the increase in sensitivit it offers for many elements allows analysis to be made of very small samples. Given an opportune combination of activation characteristics and enamel abundance for an element it is often possible to make precise and accurate determinations on samples as small as 1 mg. Meinke[74] has published a list comparing the sensitivities obtainable using activation analysis with that using other analytical techniques for all the elements in the Periodic Table.

Only two groups of workers have made use of the technique in the

field of dental research to date. Dixon and Smith[79,79,80] have measured enamel levels of copper, arsenic and mercury while Samsahl and Soremark[96-98] have measured levels of calcium, phosphorus, chlorine, sodium, strontium, zinc, bromine, manganese, tungsten, copper and gold in enamel and dentine.



C H A P T E R II

ACTIVATION ANALYSIS

Introduction

The technique used in this thesis for the determination of trace elements in dental tissues was neutron activation analysis. The main advantage of activation analysis over conventional techniques of analysis is sensitivity. The sensitivity of activation analysis is such that for a large number of elements in the Periodic Table, a small sample of enamel from a single tooth may be used for the determination of one or more of its constituent trace elements. A sensitivity of 10^{-11} gm. is frequently obtained for many elements after irradiation at the ordinary neutron fluxes found in nuclear reactors. For this sensitivity and using samples of only 10 mg., elements present in teeth in concentrations approaching 0.01 p.p.m. can be measured. The sensitivity may be higher for some elements and can in any case be increased by using larger samples or higher neutron fluxes.

Another feature of activation analysis which facilitates trace element measurement is its simplicity. As recovery corrections are made, chemical separations do not require to be quantitative and micro-separations are not necessary, a high degree of manipulative technique is not needed. This means that with a minimum of skill it is possible to carry out a large number of analyses routinely yet with high sensitivity, precision and accuracy. In fact some current trends in the field are directed towards a wholly instrumental technique of measurement which does not require a chemical separation at all[1].

Basic Principles of Activation Analysis

The common feature in all methods of activation is the production of an unstable radioactive product which may be detected subsequently. The amount present being proportional to the amount of target material

initially present. The nucleus of an atom of the element under going analysis must be able to undergo a nuclear transformation on being exposed to particle or electromagnetic bombardment. The simplest type of transformation can be represented as the absorption of a particle or quantum of energy by a nucleus of a target atom followed by the emission of another particle or quantum to allow the intermediate, unstable nucleus formed to return to a more energetically stable state. The product nucleus will then be radioactive and may at some later period decay to a still more energetically favourable state by giving off some form of radiation eg. a beta particle or a γ-ray. It is this radiation which is detected in some instrument such as a Geiger Counter.

eg. $^{31}P(n,\gamma)^{32}P$

In this case the incident particle is a neutron which is absorbed by the ^{31}P nucleus under favourable conditions of particle energy. The energetically excited intermediate nucleus formed $^{32}P^{*}$

$$n + {}^{31}P \rightleftharpoons {}^{32}P^{*} \longrightarrow {}^{32}P + \gamma$$

can either return to the starting position by losing the neutron again or the excess energy can be dissipated by emission of a gamma ray and formation of the radioactive product nucleus ^{32}P which subsequently reaches a stable state by β-emission. The underlying principle of any method of activation analysis is based on the fact that the number of radioactive atoms of a particular element produced depends linearly in the first place on the number of atoms of the element present originally in the sample. Hence by measuring the amount of the radioactive species produced it is possible to calculate the amount of the element present.

Irradiation

In theory activation may be made with any particle which can combine with a target nucleus. Neutrons, protons, deuterons, tritons and gamma rays have all been used. The most useful and widely applied particles for activation have been thermal neutrons from reactors. Fast neutrons or accelerator produced particles have not been found to be as useful as thermal neutrons but can sometimes be used in the activation of light elements which do not undergo thermal neutron capture reactions.

The amount of activity induced in an element depends on several variables. It is related to these variables in the following way (for a monoisotopic element).

$$A = N_i (1-e^{-\lambda t}) \times \phi \sigma$$

where A - the induced activity or disintegration rate

ϕ - flux of bombarding particles (particles/1cm^2/sec)

σ - activation cross section (cm^2)

λ - decay constant (sec^{-1})

t - time from beginning of irradiation (secs)

N_i - number of inactive atoms present in target.

In theory it is possible to calculate the amount of an element present in a target by measuring all of these variables but in practice the errors introduced make this a difficult task. In practice it is usual to include a small amount of the element being measured as a standard of reference. Then, after simultaneous irradiation of sample and standard

$$\frac{\text{Activity in sample}}{\text{Activity in standard}} = \frac{\text{Mass of element in sample}}{\text{Mass of element in standard}}$$

It is very unusual to make a simple measurement of the activity induced in a sample to use in comparison with the activity measured in a standard of a given element. The reason is that usually measurements are made of a given element A in the presence of varying amounts of other elements B,C,D,E.... etc. Since the other elements may also undergo nuclear reactions producing other radioactive species, the activity measured will be from all of them, eg.

Determination of copper content of blood.

The copper content of blood is about 1.1μg. per gram of blood. Apart from other trace elements the following elements are present in amounts $>$ 100μg. per gram blood:

C,Cl,Fe,H,K,N,Na,O,P,S.

Although the ease with which a nuclear reaction may take place varies from one element to another the fraction of the total induced activity in a given sample due to the copper content is very small.

There are two methods of solving this problem. The method used depends upon the nature of the radiations induced in the sample and the sensitivity required for the element being measured. Sometimes a combination of the two methods can be made.

1. Instrumental. This is usually only possible for isotopes emitting γ-rays. The interaction of γ-rays with fluorescent crystals produces tiny amounts of light. By means of a photo-multiplier tube connected to the crystal the light energy may be converted into electrical energy and amplified. As γ-rays are monoenergetic and their energy values vary depending on the radioactive source, it is possible to separate the various component γ-rays in a mixture into an energy spectrum using

suitable electronic devices. It is sometimes possible to use the height of a γ-photopeak in such a spectrum as a measure of the amount of the element associated with it in the sample. The nature of γ-spectra is such that the quantitative evaluation of γ-photopeaks becomes increasingly complex as the number of components in a spectrum increases. Use has been made of computers to perform the complex calculations required in the measurement of individual γ-photopeaks in a spectrum with several components[83].

2. Chemical Separation. The instrumental technique is non-destructive and thus a given sample may be preserved for inspection or subsequent re-analysis. In the case where a chemical separation is used the sample is destroyed. Following irradiation some inactive carrier (usually about 10 mgs. of the element or elements being analysed) is added to the sample which is brought into solution by a suitable digestion technique. The inactive atoms of the carrier element should be made to equilibrate with the radioactive atoms of the sample. A chemical separation is then completed with the aim of producing the carrier element (with equilibrated active element) in a form suitable for the counting technique used and completely free from the other radioisotopes originally present in the sample. By comparison of the activity in this separated form with that of a known amount of the element being measured (irradiated with the sample and processed in the same manner) it is possible to calculate directly the amount of the element originally in the sample.

It is not always necessary to produce the required isotope completely pure. In the interests of speed it is sometimes possible to

perform a partial separation of the isotope from the total activity induced in a sample and use instrumental means eg. a γ-spectrometer, for separating the desired radiation from that incompletely removed by the chemical separation[77].

It should be emphasised that activation analysis, being concerned with nuclei of elements, takes no account of the chemical state of an atom. Whether the atom exists in covalent or ionic relationship with neighbouring atoms or is in any one of a number of valence states is of no consequence. The result of any analysis is expressed in terms of the total amount of a given element contained in a sample.

Review of Activation Analysis

The technique of activation analysis is well established. With the post-war development of nuclear science and the spread of reactors throughout the world activation analysis has so developed that it is now accepted as a proven technique of analysis. Wainerdi[108] discusses the basic principles in more detail than given above. Several text books[40,58,1,13,] have been published specifically devoted to the technique and its applications. There are many review articles[25,46,62,66,71,89,112] in the literature discussing varied general aspects of the technique. In addition the literature incorporating analytical chemistry is full of innumerable papers describing specific applications of the technique.[8,14,52]

C H A P T E R III

EXPERIMENTAL

Experimental

Gamma Spectrometric Analysis of Teeth

For very pure materials it is sometimes possible to use a method of activation analysis without a chemical separation after irradiation. The γ-radiations from the sample are measured using a γ-spectrometer and analysis made accordingly. This is usually only possible when the matrix of the sample is composed of an element which is not activated by the chosen irradiation technique. For example, Morrison and Cosgrove[75] were able to determine trace impurities in high quality silicon used for transistors by a non-destructive technique. This is made possible by the fact that silicon on being activated with thermal neutrons gives off almost entirely β-particles and hence trace impurities which emit γ-rays may be identified and measured.

A study was made of the γ-radiations emitted by teeth after various periods of thermal neutron irradiation. The purpose of this study was twofold.

(1) To discover by experiment what were the principle γ-emitting isotopes produced in teeth on thermal neutron irradiation.

(2) To investigate the possibility of elemental analysis being made for any of the minor constituents of teeth by a solely instrumental method of measurement.

The information obtained from this study was useful in relation to the development of radiochemical separations for any element. The chosen method had to be efficient in separating the required isotopes from those unwanted isotopes produced by the irradiation.

Sample Preparation. Specimens of sound human teeth which had been

extracted for orthodontic reasons were obtained. The amount of handling prior to irradiation was kept to a minimum to avoid contamination. Several specimens of different weights were obtained from each sample by fracturing the tooth. It was necessary to use smaller samples when a long period of irradiation was used otherwise an inconvenient amount of activity was induced in the specimen. The small pieces of tooth were rinsed with distilled water, dried and weighed.

Irradiation. Two reactors were used for the irradiations. For short irradiations ($<$ 6 hours) The Scottish Universities Research Reactor at East Kilbride, Glasgow, was used. For longer irradiations ($>$ 6 hours) the reactor was BEPO (Harwell). In both reactors a position was used where the thermal neutron flux was $10^{12} n/cm^2/sec$. For the BEPO irradiations the samples were packed in aluminium foil inside an aluminium irradiation can. For the East Kilbride irradiations the samples were packed in polythene envelopes inside $\frac{1}{2}$" diameter polythene tubes. A pneumatic tube system was used in the short irradiations for rapid transfer of samples to and from the reactor core. A wide range of irradiation times covered as big a range of isotopes as possible. Table 2 lists the irradiation times and cooling periods.

Table 2 IRRADIATION AND COOLING TIMES OF TEETH FOR γ-SPECTROMETRY

Reactor used	Irradiation periods	Cooling Times
S.U.R.R.	1 minute	30 secs. and 6.5 min.
S.U.R.R.	10 minutes	3 min. and 100 min.
S.U.R.R.	100 minutes	10 min. and 18 hours
BEPO	1 day (1.44×10^3 minutes)	34 hours and 7 days
BEPO	1 week (1.008×10^4 minutes)	9 days and 100 days

Cooling-periods. Two measurements of the gamma-spectrum of each sample were made. There were two reasons for this.

1. The identification of individual peaks in the spectra could be aided by an approximate indication of the half-lives of the isotopes contributing to them.

2. If the major part of a spectrum was from a short lived isotope the whole spectral pattern could have altered greatly when a second measurement was made - the short lived contribution having decayed.

For these reasons it was attempted to measure the γ-spectrum as soon as possible after the end of the irradiation and then repeat the measurement after a cooling period of about ten times the irradiation period. In the case of the one week irradiation the initial count was delayed for 7 days because experience with the 1 day irradiation had shown that the spectrum would be almost entirely that of Na^{24}.

Counting. Following irradiation the tooth samples were transferred to inactive polythene bags. These were placed on top of a 3" x 3" or $4\frac{1}{2}$" x $4\frac{1}{2}$" sodium iodide (thallium activated) crystal connected to a multi-channel pulse light analyser. The gain of the amplifier was

adjusted so that the energy range covered by the measurements was from 0.1 to 2.8 MeV. If the dead time of the instrument was too high it was reduced by either using a smaller specimen of the activated tooth, or by increasing the distance from the sample to the crystal. The spectra, including one of standard γ-sources for energy calibration of the instrument, were recorded. For very active samples a 64 second counting period was used and for samples of low activity, proportionately longer counting periods. It was found that the amount of low energy pulses produced in samples irradiated for 1 day was such that a bias voltage had to be applied to the low energy end of the spectrum to cut off these pulses. Otherwise the dead time of the instrument became unsuitably large. The principle contribution to these low energy pulses was from P^{32} bremsstrahlung radiation. P^{32} was produced in large amounts by activation of the phosphate matrix of the sample.

Gamma spectra of Irradiated Teeth.

In figs. 1 - 8 are shown the γ-spectra of teeth samples irradiated and allowed to cool as described. The spectra of the sample irradiated for one week are shown in fig. 9. The identity of the various peaks found in each spectra is listed above them. The criteria used in deducing the identity of a peak were:

(1) Photo-peak energy

(2) Decay of peak (by consideration of the changes in relative peak heights between the two measurements).

(3) The abundance of the element in teeth and its cross-section for its production in a radioactive form on irradiation.

(4) Presence of associated γ-photopeaks or associated phenomena

[illegible]

It can be seen that Na^{24} dominates all the spectra with the exception of the one in fig. 9 where it is no longer present after a cooling period of 100 days. The other main constituents of the short irradiation spectra are Mg^{27}, Ca^{49} and Cl^{38}. All of these elements occur in teeth in large enough concentrations to be readily measured by techniques other than activation analysis. The only element found suitable for direct measurement in this study was zinc. It was not found possible to apply this technique of measurement to other human tissues without modification since interference from Fe^{59}($T\frac{1}{2}$-45 days) was sometimes found. It would be possible to postpone the analysis till the iron decayed but this would prolong the analysis unduly.

A more convenient method of measuring the zinc content in the presence of iron interference was found. In figures 10 and 11 are shown the spectra of Fe^{59} and Zn^{65} respectively. Also present in the Fe^{59} spectrum is a photopeak at 0.84 MeV from Mn^{54}. This had been produced from the iron wire used as an iron irradiation standard by the fast neutron reaction

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

It can be seen that the lower Fe^{59} photopeak at 1.10 MeV interferes with the Zn^{65} photopeak at 1.11 MeV. In figure 12 can be seen the spectrum of an irradiated lung sample showing Fe^{59} contamination of the Zn^{65} photopeak. The interference was subtracted out by summing the counts in the 1.29 MeV photopeak of Fe^{59} and calculating the corresponding size of the 1.10 MeV photopeak. The ratio of the peak

sizes was obtained from the Fe^{59} standard. The true Zn^{65} peak size was obtained by subtracting the calculated Fe^{59} contribution from the apparent peak size.

Literature Review. Activation analysis has been applied to the measurement of traces of vanadium in a wide variety of materials. Kemp and Smales[56] used a sodium peroxide fusion and a cupferrate extraction in the estimation of vanadium in rocks and meteorites by neutron activation analysis. Meinke[72] used a simple cupferron extraction and γ-spectrometry for the determination of the element in biological ash. Several estimations have been made of vanadium in materials using entirely instrumental means eg. Leliart[63] et al. measured its levels in high alloy steels. Fukai and Meinke[37] used the activation technique for the measurement of vanadium in marine organism ashes using a cupferron extraction procedure. Kamemoto and Yamagishi[55] used a solvent extraction of vanadium oxinate in an activation procedure. The chemical yield was determined by re-activation. Kaiser and Meinke[53] measured the vanadium content of rat livers by activation. The method developed for vanadium estimation in this thesis has been published[64].

Vanadium Isotopes. On thermal neutron irradiation of vanadium the only isotope produced is V^{52} which has a half-life of 3.76 minutes. The cross-section for the thermal neutron reaction $V^{51}(n,\gamma)\ V^{52}$ is 4.5 barns. The activated species decays emitting a β-particle (2.5 MeV) and a γ-ray (1.43 MeV). Thus it is possible to detect this isotope by either scintillation or G.M. counting. There is a vanadium isotope V^{48} made by charged particle activation. This isotope has a half-life of 16.0 days and has been used in some procedures[59] to estimate the chemical recovery of a separation after decay, of the short lived V^{52}. This technique is only useful where fairly high

( ) (.1 microgramme) quantities of vanadium are to be determined. When the induced V^{52} activity is small its detection in the presence of V^{48} tracer activity becomes increasingly difficult because of the poor statistics involved in the activity measurement.

Irradiation. Irradiations were made at a thermal neutron flux of $10^{12} n/cm^2/sec.$ in the Scottish Universities Research Reactor at East Kilbride. The irradiation period used was usually 4 minutes. This was chosen as the optimal length for irradiation as the increase of V^{52} activity with time ceases being approximately linear after this. With an irradiation period equal to the half-life of the isotope produced, an amount of activity equal to 50% of that obtainable at saturation is obtained. Although longer irradiation times would have increased the V^{52} activity the amount of longer lived isotopes produced from the other elements of the sample matrix would be even larger and would make the chemical separation more difficult.

The samples were delivered to and from the reactor core inside a nylon 'rabbit' in a pneumatic tube rapid transfer system. The irradiation period was automatically controlled by a timer. Only one sample and a vanadium standard were packed in the rabbit per irradiation. The short half-life of the V^{52} limited the number of samples which could be processed for each irradiation. When working with such a short lived isotope as V^{52} time becomes the controlling factor determining the sensitivity of analysis obtainable. For every half-life of V^{52} (3.8 minutes) elapsed in chemical processing after irradiation the induced vanadium activity and hence the sensitivity of analysis falls by a factor of 2. This is equivalent to a ten-fold decrease in sensitivity for each 12.4 minutes of processing time. In all respects

of the development of this method of vanadium analysis time was the controlling factor.

Kemp and Smales[56] were able to begin processing the samples 3.0 to 3.5 minutes after the end of irradiation - they also used a pneumatic transfer system. It was found possible in this work to begin sample digestion only 30 seconds after the samples left the reactor.

Sample and Standard Preparation. The amount of pre-irradiation handling of the samples was kept to a minimum to avoid sample contamination. Vegetable material or animal tissues were dried in a dessicator over silica gel. Teeth samples were rinsed with distilled water, dried and fractured where required within a polythene bag using pliers. Contamination was prevented by the layer of polythene between the metal of the instrument and the surface of the tooth. Solid samples were weighed and sealed in polythene envelopes.

A large number of vanadium standards were prepared from a solution of 'Specpure' high purity vanadium pentoxide. Using a micro-pipette eg. a Hamilton syringe pipette, a small volume of this solution was placed on a 1" square sheet of thoroughly clean polythene. The polythene squares were then placed under an infra-red lamp and the water evaporated off leaving the vanadium pentoxide on the polythene. Care was taken to avoid overheating the polythene sheet. A convenient quantity of vanadium was found to be in the range 0.1 to 1.0 micrograммes. The sheet was folded and transferred to a small polythene envelope which was then sealed.

Sample and standard were enclosed in a $\frac{1}{2}$" diameter polythene tube

[illegible] for the nylon rabbit of the transfer system. The length of the polythene tube was 2¼" though the rabbit could take slightly longer samples.

Choice of Chemical Separation. The relative merits of several reagents for vanadium separation were investigated. Two general types of method seemed practicable.

(a) A solvent extraction technique

(b) Equilibration of activated vanadium in solution with an inactive precipitate of vanadium.

Three reagents were tried viz. tetraphenylarsonium chloride, tetraphenyl borate and nitron, in an attempt to find a precipitate extractable from acid solutions by an organic solvent. All were unsuitable since they are all anion precipitants and vanadium (V) is present in acid solutions as the oxy-anion VO_2^+. N-benzoyl-N-phenyl-hydroxylamine and cupferron were both found to form a precipitate with vanadium in acid solution which could be extracted by chloroform. The purple extract obtained using the N-benzoyl-N-phenylhydroxylamine reagent was found to change on standing to a brown colour. Difficulty was experienced in re-extracting the vanadium from this chloroform extract into an alkaline solution. As a back extraction of this type was favoured to give sufficient radiochemical purity in the proposed analysis this reagent was discarded in favour of cupferron. This reagent precipitates vanadium quantitatively from strongly acid solutions[38] and the precipitate is chloroform soluble. The vanadium is re-extractable from chloroform by sodium hydroxide solution. This reagent has been used widely in chemical separations of vanadium for activation analysis[8,72,37,53].

The possibility of using an inactive precipitate of vanadium pentoxide to exchange with activated vanadium was considered but discarded as it was found that vanadium pentoxide dissolved completely in solutions with the high acid concentrations resulting from tissue digestion and was still highly soluble in solutions of higher pH.

Sample Digestion. Various digestion techniques were compared to discover the best method of dissolving a tissue or tooth sample completely and rapidly. Bowen[8] used a nitric/perchloric acid digestion, Smales[56] and Meinke[59] used sodium peroxide fusion techniques. The following table (Table 3) shows the methods tried and the times taken for digestion of a 100 mg. tooth sample. Tissue samples are more easily digested than teeth and hence would be in solution in a shorter period. A sample was considered to be fully digested when the matrix was fully destroyed and no remaining traces of organic matter could be observed. Timing commenced when the sample was dropped into the hot digestion medium. This reproduced the experimental conditions which would be used with activated samples. The fastest digestions can be seen to be achieved using boiling sulphuric acid followed by addition of nitrate to oxidise charred organic material. The addition of both solid sodium nitrate by a spatula and concentrated nitric acid by a Pasteur pipette were found to be effective in oxidation of organic material. The use of the sodium nitrate was preferred because there was no spurting of hot acid from the silica digestion beaker.

Table 3 DIGESTION TIMES BY DIFFERENT METHODS

Description of digestion method	Time taken for 100 mg. tooth digestion
Conc. nitric acid (5ml.)	11 minutes
Conc. hydrochloric acid (5ml.)	19 minutes
1:1 conc. nitric/hydrochloric acid (5ml.)	14 minutes
Sodium peroxide fusion (Nickel crucible)	11 minutes
1:1 conc. sulphuric acid/nitric acid (5ml.)	7 minutes
conc. sulphuric acid (10ml.). Nitrate oxidation	1.5 minutes
conc. sulphuric acid (3ml.). Nitrate oxidation	3 minutes

Although the quickest digestion used 10 ml. of sulphuric acid this was not found suitable for the subsequent cupferrate solvent extraction step. It was found that dilution to a suitable volume for solvent extraction (30-40 ml.) gave a high acid concentration which caused incomplete extraction of vanadium. The optimum volume of sulphuric acid used was 3 ml. It was necessary to perform the solvent extraction with a cool aqueous solution otherwise high pressures developed in the separating funnel due to chloroform volatility. The following digestion technique was found to:

(a) avoid hot aqueous solutions.

(b) avoid beaker damage by too rapid cooling.

(c) allow a total digestion and complete chemical separation in less than 10 minutes.

1.5 mg. of vanadium carrier (0.5ml. of a 0.3M ammonium vanadate solution), 0.5 mg. of molybdenum carrier (1 drop of a 0.1M ammonium molybdate solution) and 3 ml. of 18M sulphuric acid were heated until the acid was refluxing in a 125 ml. tall form silica beaker. The sample of tooth, which weighed up to 0.5 gm. was added and was quickly digested. 20 to 30 mg. of sodium nitrate was added to remove charred organic material. The solution, which was green to yellow and sometimes contained calcium sulphate as a white precipitate was cooled as follows: the beaker was first cooled by swirling it in air. It was further cooled by brief immersion in a boiling water bath and then brought fully to normal temperature by placing the beaker in an ice-bath. The solution was diluted with 30 ml. of an ice water mixture, the ice serving to absorb the heat released by sulphuric acid dilution.

It was not found possible to prevent precipitation of calcium sulphate during the digestion of bone or tooth samples. Ammonia, ammonium sulphate, glycerine, ammonium acetate, ammonium tartrate and ethylediamine tetra-acetic acid were all tried without success. The high acid concentration used prevented the operation of these masking or chelating agents for calcium. The precipitate was found not to interfere with the remainder of the separation by preventing vanadium extraction. A precipitate was produced in the scavenge step which followed digestion and both precipitates remained in the aqueous layer during extraction of vanadium as the cupferrate by chloroform.

Scavenge. After digestion and during cooling in the ice-bath, the solution was scavenged by adding two drops of 2M hydrochloric acid and 5 drops of silver nitrate solution (4% w/v.) The silver chloride

precipitate was formed in a solution containing excess chloride and acted as a scavenge precipitate for cations as it is negatively charged.

Molybdenum removal. Cupferron is known[38] to quantitatively extract molybdenum from strongly acid solutions. Mo^{101}($T_{\frac{1}{2}}$ - 15 mins.) is formed by neutron activation and as molybdenum occurs in biological material it is hence a potential radiocontaminant. An experiment was made using extraction conditions identical to those used for vanadium extraction to confirm this. An aliquot of Mo^{99} tracer with 1 mg. molybdenum as carrier was extracted from 10 ml. of 10% (w/v) sulphuric acid by addition of 1 ml. of 5% (w/v) ammonium cupferrate solution and 10 ml. of chloroform. The white precipitate of molybdenum cupferrate formed was extracted by the chloroform. The chloroform layer was separated from the aqueous layer and shaken up with 10 ml. of 1M sodium hydroxide solution. The chloroform layer was run off, the aqueous layer acidified with 2M hydrochloric acid and the white precipitate formed extracted into a second 10 ml. of chloroform. All layers were counted both a Geiger counter accepting liquid samples and in a scintillation well counter. It was found that most of the parent Mo^{99} was extracted to the final chloroform layer by the above procedure while the daughter Tc^{99m} remained in the initial aqueous layer. Allowance was made in counting for decay of the separated Tc^{99m}($T_{\frac{1}{2}}$ - 6.0 hours) and the equilibration of Tc^{99m} daughter from the Mo^{99} parent ($T_{\frac{1}{2}}$ - 2.8 days).

Several reagents were used in an attempt to find a suitable one which would form a compound of molybdenum extractable by an organic solvent. Zinc dithiol reacts with molybdenum to form a green product extractable by organic solvents from 6-14N sulphuric acid[5]. This

proved to be an effective method for extracting molybdenum from sulphuric acid solutions in the presence of vanadium. The vanadium could be extracted as the cupferrate after molybdenum extraction. This method was not considered suitable as it was discovered that the reaction of molybdenum with dithiol was slow in reaching equilibrium (about 1 hour) which was of no value in a short V^{52} separation[39].

It was found possible to extract molybdenum with ethyl potassium xanthate[36] but this method was unsuitable since vanadium was also extracted. Molybdenum could be reduced with sodium thiosulphate and extracted by ethyl acetate[86] but a cloudy aqueous layer was left which was thought to be undesirable for further vanadium separation. The reagent for molybdenum removal which was found most suitable was α-benzoin oxime. 0.5 mg of molybdenum was quantitatively extracted from 10 ml. of a 4N sulphuric acid by 10 ml. of a 0.1% (w/v) solution of α-benzoin oxime in chloroform. A little acetone is required to dissolve the reagent initially. Using Mo^{99} tracer all of the molybdenum activity was found in the organic layer following extraction by the chloroform solution of this reagent.

Initial Chloroform extraction. The initial separation of activated vanadium from the other isotopes in solution after digestion of an irradiated sample used the fact that cupferron reacts with vanadium in strongly acid solution to form a compound which gives a wine-red chloroform extract. When 3 ml. of a 5% (w/v) aqueous solution of cupferron was added to 30 ml. sulphuric acid solutions 10% (v/v) a red precipitate of vanadium cupferrate was formed. This was extractable by chloroform giving the wine-red extract. When the acid layer contained calcium

sulphate precipitated from the tooth digestion a yellow precipitate was formed when cupferron was added. On shaking with chloroform a wine-red extract was obtained. It was noted that more reagent was required to extract the vanadium in the presence of digested tooth. This was probably due to interference from the calcium sulphate precipitate. When the calcium sulphate was removed by centrifugation a red precipitate giving the usual wine-red extract was found.

Back-extraction. The initial chloroform extract (15ml.) was shaken with 10 ml. of 1 M sodium hydroxide containing 2 drops of ethylenediamine tetra-acetic acid solution 1% (w/v). The vanadium was extracted as vanadate from the chloroform. The EDTA was present to complex any radioactive copper which might have been extracted with the vanadium by cupferron. Cu^{64} and Cu^{66} are produced on activation of biological materials so this was a genuine source of interference. Willard[110] has used this reagent as a masking agent in determination of vanadium with cupferron in the presence of copper.

Final-extraction. The alkaline extract from the previous step was made just acid with 6-7 ml. of 2 M hydrochloric acid. The vanadium cupferrate was re-precipitated, (the reagent is also extracted from chloroform solution by sodium hydroxide). The vanadium was extracted into 10 ml. of fresh chloroform solution. This was the final form for counting.

Washing of organic layers. In any solvent extraction procedure where an aqueous/organic system is used there is always a small amount of the aqueous phase contained within the organic phase. As this reduces the effectiveness of a radiochemical separation a washing procedure was developed to remove radio-contaminants introduced in this way from the two chloroform layers used in the separation. After the separation of

each chloroform layer it was washed with 10 ml. of a dilute solution of hydrochloric and phosphoric acids. This contained phosphate and chloride ions and hence would encourage the removal of any P^{32} and Cl^{38} produced during irradiation and present in the chloroform.

Effect of standing on chloroform extract. In the early part of the work on this separation it was found that low recoveries of added vanadium were obtained after the separation. Various explanations were sought before the solution was discovered. It was noted that the wine-red extract of vanadium cupferrate initially obtained quickly became yellow. In fact the yellow colour was used for chemical recovery measurements. Crowther[28] and Kemp suggest that the change in colour corresponds to a change in the valency of the vanadium in the complex from $V^{V} \longrightarrow V^{IV}$ brought about by chloroform.

As can be seen from Table 4, the amount of vanadium recovered finally falls with the length of time in which the vanadium complex is allowed to stand in the initial chloroform extract. For these measurements the intensity of the yellow colour in the final chloroform extract (diluted to 100 ml.) was measured at 400mμ. on a spectrophotometer using 1 cm. quartz cells.

Table 4. VARIATION IN VANADIUM RECOVERY WITH TIME OF SHAKING IN CHLOROFORM

Time (minutes)	Absorbance ($\log \frac{Io}{I}$)
2	0.275
11	0.115
21	0.040
30	0.015
41	0.015

It is believed that this time dependent extraction of vanadium by sodium hydroxide is associated with the colour change of vanadium cupferrate in chloroform from red to yellow. The yellow compound did not seem to be as readily extracted by sodium hydroxide as lower recoveries were associated with stronger yellow colours in the initial chloroform extract.

The rate of formation of the yellow compound was demonstrated by measuring the change of absorbance at 400mμ of a red chloroform solution of vanadium cupferrate using a spectrophotometer with a Kodak No. 1 filter. Figure 13 shows the change in absorbance with time. It can also be seen that the yellow colour becomes fairly stable after three hours. This is the colour which is used for measuring the chemical recovery of the vanadium in the analysis.

Visible Spectra of various vanadium cupferrate/chloroform solution. Some solutions of vanadium cupferrate in chloroform became green when allowed to stand for 24 hours. In figures 14 and 15 are shown the spectral changes in the visible range of these solutions corresponding to the colour changes - red → yellow → green. The colour change red → yellow is

accompanied by a change in the peak absorption in the visible spectrum from about 500mμ.to 415mμ. The green colour produced on standing is thought to be caused by formation of a decomposition product of the reagent in chloroform solution. This is shown to be the case by comparing the spectra shown in figure 15. The upper curve was obtained from a solution of vanadium cupferrate in chloroform which was allowed to stand for 24 hours. The lower curve was obtained from a solution containing only cupferron which was prepared at the same time. The peak maxima at 750mμ.can be seen to be identical. All of these measurements were made using a UNICAM SP.800 spectrophotometer in the visible range.

Valence of extracted vanadium. Some experiments with different conditions and starting materials were made to examine the colour changes observed before and after extraction as vanadium cupferrate by chloroform. The results obtained are summarised in Table 5.

Table 5. COLOUR CHANGES OF VANADIUM AND VANADIUM CUPFERRATES

Starting material	Colours In 4N sulphuric acid (no cupferron)	In chloroform (with cupferron)
Ammonium vanadate sol. $^{n.}(V^{V})$	yellow (V^{V})	red
Ammonium vanadate sol. $^{n.}(V^{V})$ plus sodium sulphate sol. $_{n.}$	green (V^{III})	red
Ammonium vanadate sol. $^{n.}(V^{V})$ plus stannous chloride sol. $_{n.}$	blue (V^{IV})	yellow
Vanadium trichloride (V^{III})	blue (V^{IV})	red
Vanadyl sulphate (V^{IV})	blue (V^{IV})	red

The observations can be explained as follows. The trivalent vanadium chloride was oxidised to V^{IV} on solution in sulphuric acid. Vanadium was extracted as V^{V} no matter the starting valence in acid solution unless stannous chloride is present. As the stannous ion was also extracted by cupferron from strong acid it served to hold the vanadium in the IV-valent state in both acid and chloroform. In addition it was observed that the addition of a chloroform solution of stannous cupferrate to a red chloroform solution of vanadium cupferrate brought about an immediate colour change from red to yellow. This explanation is supported by Crowther and Kemp's work[28] on the reduction of vanadium (V) - cupferrates in chloroform solution.

Detection. Following washing of the final chloroform layer containing the separated vanadium cupferrate, the whole 10 ml. volume was run into a Geiger counter accepting liquid samples. The 2.6 MeV β-particles of V^{52} were counted on a scaler connected to an automatic timer (Panax Autoscaler). V^{52} also emits monoenergetic 1.44 MeV γ-rays. The sensitivity of detection by γ-counting over this photopeak was compared with that obtainable by G.M. counting. It was found that better efficiency of detection could be had for β-counting when a sample of V^{52} was counted by both methods and the count-rates compared following corrections for decay, dead-time and background radiation. The β-counting was adopted as the method of choice for detection for this reason and because of the lower background of the G.M. counter. The limit of detection obtained for a four minute irradiation at 10^{12} n/cm^2/sec. was around 10^{-9} gm. This assumed a minimum count-rate equal to background radiation and a counting time beginning 10 minutes after the end of the irradiation.

Chemical Recovery. Following counting the chloroform containing the vanadium cupferrate was transferred to a 100 ml. volumetric flask and made up with chloroform. After delay of three hours to allow colour stabilisation, the intensity of the colour was measured with a colorimeter or spectrophotometer at 400mμ. The absorbance at this wavelength was used directly as a measure of the vanadium recovered from the separation. It was shown that the absorbance was proportional to the vanadium content of the solution i.e. Beer's Law was obeyed. Recoveries were normally about 70%.

Calculation. The count-rate from a given sample was compared with that of a vanadium standard, irradiated with the sample, chemically processed at the same time and counted immediately after the sample. Each count-rate was corrected for decay, counter dead-time and background radiation. The vanadium content of the sample was then obtained from the following relationship:

$$\frac{\text{vanadium content of sample}}{\text{vanadium content of standard}} = \frac{\text{corrected sample count-rate} \times \text{absorbance of standard}}{\text{corrected standard count-rate} \times \text{absorbance of sample}}$$

Radiochemical Purity

Freiser[36] lists the elements extractable by cupferron from strongly acid solutions. The elements extracted from 10% (w/v) sulphuric acid are Tc^{II}, V^{V}, Fe^{III}, Cu^{II}, Zr^{IV}, Mo^{VI}, $Sn^{II,IV}$, Sb^{III}, Hf^{IV}, Ta^{V}, W^{VI}, Th^{IV}, Pa^{V}, and U^{IV}. For an element to interfere with the analysis for vanadium several conditions must be fulfilled.

(1) The element must be capable of becoming significantly active during the thermal neutron irradiation.

(2) It must be present in biological materials in sufficient quantities to give a detectable amount of the active form of the element.

(3) The amount of interfering activity produced under V^{52} irradiation conditions must be detectable by the G.M. counter used for V^{52} measurement (G.M. counter accepting liquid samples).

To discover which of the elements listed above were interferences in analysis of biological material, several different samples were irradiated, chemically processed and the radiochemical purity of the extract determined by γ-spectrometric and decay-curve analysis. The materials used included teeth, bone, dry liver, thyroid, parsley, ashed liver and bone. Some of these showed the following elements.

Molybdenum. In figure 16 is shown the decay curve obtained from a sample of ashed liver following a 4 minute irradiation at a flux of $10^{12} n/cm^2/sec.$ and the chemical separation for vanadium without the molybdenum extraction step. This is a composite β-decay curve showing two components. The longer lived component had a graphically measured half-life of 16.5 minutes which is consistent with Mo^{101} ($T_{\frac{1}{2}}$-14.6 minutes). The longer lived component was subtracted from the composite decay curve by extrapallation to zero time of the Mo^{101} section of the curve and subtraction from the composite curve. When the remaining curve was plotted it was found to be linear with a measured half-life of 4.2 minutes consistent with V^{52} ($T_{\frac{1}{2}}$-3.8 minutes).

In figure 17 are shown the decay curves of active parsley - irradiated as for the ashed liver. In the upper curve can be seen the decay curve obtained when no molybdenum extraction step was used. In the lower curve this step was included and the section of the curve

attributable to Ir^{194} is absent. The lower curve gives a measured half-life of 3.9 minutes which is consistent with V^{52}.

Copper. In figures 18 and 19 are shown the decay curves and γ-spectrum obtained from a sample of ashed liver (133 mg.) irradiated and processed for vanadium. The measured half-life of the decay curve is 5.5 mins. The γ-spectrum has a large peak at 0.51 MeV and a peak at 0.30 MeV. The spectrum was obtained by counting for 256 sec. following the final decay measurement. The (n,γ) produced isotopes which have a half-life around 5.5 minutes are Ti^{51}($t_{\frac{1}{2}}$ 5.8 mins.) and Cu^{66}($t_{\frac{1}{2}}$-5.1 mins.). Ti^{51} has its principle (95%)γ-photopeak at 0.32 MeV. Cu^{66} decays principally by β-particle decay but has a small (9%) γ-photopeak at 1.04 MeV. Cu^{64}($t_{\frac{1}{2}}$-12.8 hours) decays principally by β^+ and β^- emissions with an associated 0.51 positron annihilation peak. The copper content of liver is 8.6 μg./gm. of wet tissue and the titanium content 0.14 μg./gm[87]. The probable explanation of decay curve and spectrum is that the decay curve is a composite decay curve of Ti^{51} and Cu^{66}. The photopeaks in the spectrum are associated with Ti^{51}(0.32 MeV) and Cu^{64}(0.51 MeV). The Cu^{66} photopeak has mostly decayed away by the time the spectrum was measured and only a small peak in the spectrum remains at 1.04 MeV. Calculation showed that in the ashed liver sample there should be more than 200μg. of copper. The amount of activity measured was only a small fraction of the total copper activity which would be formed from 200μg. of copper. It seems likely that the EDTA was not completely effective in this case in masking all of the copper extracted either by insufficient being present or the pH of the solution at an unfavourable value.

Iodine. Brues and Robertson[23] have reported finding a 3.8 minute half-

life activity in irradiated thyroid tissue. To discover if this observation could be supported, analysis for vanadium was made on a piece of thyroid tissue obtained from a post-mortem examination on a deceased person. The decay curve obtained from this sample after chemical separation is shown in figure 20. The measured half-life is 23.5 minutes. A γ-spectrum did not show any photopeaks of significance over background radiation. I^{128} has a half-life of 25.0 minutes and decays principally by β-emission. The iodine concentration in thyroid is 350 μg/gm. of wet tissue[87]. It seems likely that some of the radio-iodine induced in the sample by the irradiation for vanadium analysis has been separated as molecular I_2 by the chloroform solvent system.

Vanadium containing materials. The only materials analysed where V^{52} could be positively identified were bone-ash and parsley. The criteria used for identification of the isotope were half-life and γ-spectra. In figure 21 is shown the normal γ-spectrum of V^{52} obtained by irradiation of a V_2O_5 standard. The only photopeak of any significance occurs at 1.44 MeV. In figure 22 is shown the spectrum obtained following irradiation and separation of a sample of dried parsley. It can be seen to have the same appearance as the V^{52} standard. In figure 23 are shown the decay-curves obtained from a sample of parsley (17 mg.), bone-ash (255 mg.) and tooth (119 mg.) The bone-ash and the parsley show decay curves closely resembling that of V^{52}. The half-lives measured on the first part of the curves are 4.1 and 3.9 minutes respectively. The curves are linear until well into the area approaching background level when poor counting statistics make the measurements variable. The curve shown for the tooth is typical of that obtained from a large number of analyses of both samples weighing up to 0.5 gm. In no case could

activity isolated be demonstrated as V^{52}; in fact the amount of activity was seldom significantly above the background of the counter. It was concluded that the level of vanadium in teeth was less than the limit of detection of the method (10^{-9}gm.).

The following table (Table 6) shows the levels of vanadium found in some of the materials analysed. Insufficient analyses were made for this to be more than a general guide.

Table 5. VANADIUM LEVELS IN TEETH AND OTHER BIOLOGICAL MATERIALS

Material	Vanadium level (p.p.m.)
Teeth	<0.01
Ashed Bone	0.04
Parsley	2.0

Soremark[95] has found the level in parsley to be about 1 p.p.m. His estimation of vanadium levels in parsley seems comparable with the level in Table 5.

Sensitivity Improvement. The most promising method of increasing the sensitivity of the analysis is to use higher neutron fluxes for irradiations. There are available some reactors with a thermal neutron flux of 10^{14} n/cm^2/sec. Also pulsed reactors can produce an extremely high flux for milli-second periods. Because of the short half-life of V^{52} the analysis has to be carried out at the reactor. The work of vanadium estimation in teeth was concluded because the level in teeth was lower than the available sensitivity.

METHOD OF ANALYSIS

A sample and a 1 μg. vanadium standard were irradiated in a thermal neutron flux of $10^{12} n/cm^2/sec.$ for four minutes using a rapid transfer system. After irradiation both sample and standard were separately processed and counted. Digestion was made in 3 ml. 18M sulphuric acid with sodium nitrate as an oxidant. 1.5 mg. of vanadium carrier was present. A rapid cooling technique followed digestion. The solution from digestion was diluted and a silver chloride scavenge made. Solution plus precipitate was transferred to a separating funnel where molybdenum was removed by solvent extraction. Vanadium was initially separated by solvent extraction as the cupferrate. After washing, the organic layer was back extracted with 1 M sodium hydroxide. This extract was acidified with 2M hydrochloric acid and the vanadium cupferrate re-extracted into chloroform. The chloroform fraction was washed and counted in a Geiger counter. The chemical recovery was made by measuring the yellow colour of vanadium cupferrate in chloroform at 400 $m\mu$. The vanadium content of the sample was calculated by comparison of activity in sample and standard following appropriate corrections for recovery, decay, dead-time and background radiation.

MANGANESE ANALYSIS

Literature review. Bowen[9] used neutron activation analysis to determine manganese in biological material. A full chemical separation and β-counting was employed. Cotzias[81] measured manganese in biological materials using a shorter chemical separation and manganese γ-photopeak counting. Smith[92] used a solvent extraction technique for radio-manganese purification and this was specific enough to allow β-counting. A description of the method described in this thesis is available in the literature[77]. The technique involves irradiation, one-step precipitation and γ-counting using a pulse height analyser.

Manganese Isotopes. The only isotope produced by thermal neutron irradiation is Mn^{56}. Naturally occurring manganese is 100% Mn^{55} and the activation cross-section for the nuclear reaction $Mn^{55}(n,\gamma)Mn^{56}$ is 13.3 barns. Mn^{56} has a half-life of 2.58 hours and emits β-particles and γ-rays. The principal γ-ray (100%) is at 0.845 MeV and the principal β-energy is at 2.81 MeV (50%). Detection by both β and γ-counting is possible and maximum sensitivity is probably attainable by β-counting. Sufficient sensitivity for manganese determination in biological material can be made by γ-counting because of the high cross-section and element levels in biological materials.

Sample Preparation and irradiation. Samples were prepared for irradiation and were irradiated in the same manner as for vanadium analysis. The length of the irradiation varied from 1-3 hours depending on the sensitivity required.

CHEMICAL SEPARATION OF Mn^{56}

After irradiation a sample was placed in a 125 ml. conical beaker containing 10 ml. of 16M nitric acid and 10 mg. manganese carrier

(1 ml. manganous chloride solution containing 10 mg./ml. of manganese). The beaker was heated on a hot-plate until the sample was completely destroyed and no traces of organic material left.

1 ml. of sodium chlorate (50% w/v) solution was added to the hot solution to precipitate manganese dioxide. The solution and suspended precipitate were transferred to a 50 ml. centrifuge tube, centrifuged and the supernatant solution discarded. The precipitate was well washed with water and transferred with water to weighed planchets. It was then dried at 100°C. under an infra-red lamp and re-weighed as $MnO_2.H_2O$[88]. Chemical recoveries were around 85%. Water was used for transferring the precipitate as it was found that with organic liquids such as acetone, precipitate loss by crepitation could occur.

Effect of acid concentration and reagent volume on precipitation.

Mn^{54} tracer was used to examine the effects of the above factors on the precipitation of manganese dioxide from nitric acid using sodium chlorate. It has a half-life of 291 days and decays by electron capture accompanied by 0.84 γ-ray emission. The recovery of manganese dioxide obtained from precipitation under different conditions was measured by comparing the Mn^{54} activity in the precipitate with a standard amount of tracer representing 100% recovery. After precipitation and separation by centrifugation the manganese dioxide was dissolved in 3 ml. of 11 M hydrochloric acid and transferred to a counting-tube. It was counted in a $1\frac{1}{2}$" sodium iodide (Tl) well crystal and compared with the standard activity counted under the same conditions.

In both sets of experiments the total volume of solution from which precipitation was made was kept constant. In Tables 7 and 8 are shown the chemical recoveries obtained under the different conditions.

In Table 7, the total volume was 16 ml. and the nitric acid concentration 56%. In Table 7, the total volume was 30 ml. and the volume of sodium chlorate solution (50% w/v) was 2 ml. Solutions were heated in a water-basin before chlorate addition.

Table 7

EFFECT OF CHLORATE CONCENTRATION ON MANGANESE RECOVERIES

Volume of chlorate solution (ml.)	Manganese recovered (%)
0.25	33.1
0.5	62.8
1.0	88.6
2.0	87.6
3.0	88.4
4.0	82.5

Table 8

EFFECT OF NITRIC ACID CONCENTRATION ON MANGANESE RECOVERIES

Nitric acid strength (% v/v)	Manganese recovered
32.4	20.1
41.2	54.1
53.9	68.8
63.4	78.0
72.9	73.0
82.4	52.5

The conditions used in the method for activation analysis of manganese were chosen to give maximum manganese dioxide recovery i.e. 60% nitric acid concentration and 1 ml. chlorate solution.

weight is approximately linear until a sample size of about 50 mgs. is reached, and then constant recoveries, and therefore phosphate adsorption, occur.

Table 9

PHOSPHATE ADSORPTION BY MANGANESE DIOXIDE

Tooth sample weight (mg.)	Phosphate Adsorbed (mg.)
10.0	1.3
12.9	1.4
19.5	2.3
29.1	2.8
26.6	3.5
38.1	3.7
56.5	4.4
63.9	4.7
73.2	4.7

When enamel samples were being analysed the measured recoveries were reduced by a factor of 0.092 mg./mg. of tooth to give a better estimate of the manganese dioxide weight. This factor was calculated from the figures in Table 9 as an average estimate of phosphate adsorption It is only valid for samples weighing 50 mg.

Efforts were made to eliminate the adsorbed phosphate during the chemical separation. An experiment was made to examine the effect of digestion of the manganese dioxide precipitates on the amount of phosphate adsorbed. Four precipitations were made, one without digested tooth and the rest with about 50 mg. digested tooth present. Two of the samples of manganese dioxide, containing phosphate, were allowed to

digest in water on a water-bath for 30 minutes. All the precipitates were then dried and weighed. The standard precipitate weighed 15.6 mg. The undigested precipitate, containing phosphate, weighed 19.9 mg. and the digested precipitates weighed 13.8 and 19.4 mg. This result was inconclusive, but did not indicate reduction by precipitate digestion.

As an alternative to precipitation some manganese precipitates were shaken up with solutions containing Mn^{54} tracer to find out if exchange between the tracer and the manganese in the precipitate occurred. If exchange could be shown to be complete then inactive precipitate could be shaken up with the solution of an activated sample and exchange of active manganese be achieved. In the experiments summarised in Table 10 the precipitates were counted, in either a well-crystal or a flat crystal, after shaking with a solution containing Mn^{54}.

Table 10.

MANGANESE EXCHANGE EXPERIMENTS

Precipitate	Conditions for exchange	Observations
Manganese dioxide	hot 16M nitric acid	No Mn^{54} in precipitt
Tetraphenylarsonium permanganate	hot 2M sulphuric acid	"
Manganous ferrocyanide	hot 2M nitric acid	"

In no case was exchange observed. The tracer Mn^{54} and inactive manganese cannot have reached equilibrium through a common valency state.

Another method tried for avoiding phosphate adsorption was dissolving the manganese dioxide precipitate and re-precipitating it. Following the initial precipitation the manganese dioxide was dissolved

in 11M.HCl and evaporated to dryness. 16 ml. of 22.5M nitric acid were added followed, on heating, by 1 ml. of 50% (w/v) sodium chlorate solution. The total recovery after the whole separation then was under 50%. On account of this manganese loss and the increase in separation time this line of research was not pursued.

Detection. The Mn^{56} activity was measured by a 3" sodium iodide (Tl) crystal and a Laben 512 channel pulse height analyser. The spectrum of Mn^{56} is shown in figure 24. The area of the 0.845 MeV photopeak was estimated by the digital method of Covell[26]. This method is a better method of measuring γ-photopeaks, than either peak height measurement or graphical area measurement. Covell has shown that:

$$N = a_o + \sum_1^n a_i + \sum_1^n b_i - (n + \tfrac{1}{2})(a_n + b_n)$$

where N = a number representing the photopeak area.

a_o = number of counts in peak maximum channel.

a_i = number of counts in i^{th} channel below peak maximum

b_i = number of counts in i^{th} channel above peak maximum

n = number of channels below or above peak maximum.

Using a calculating machine it is a simple operation to calculate peak areas using this method.

Calculation. The peak areas were calculated for every sample and the manganese standard and these were then corrected for decay and chemical recovery. The manganese content of the sample was then obtained by comparing the corrected areas of sample and standard.

RADIOCHEMICAL PURITY

The radiochemical purity of the precipitates was determined by γ-spectrometry and decay measurements. The spectrum of an 18 mg. enamel

section after two hours irradiation and chemical separation is shown in figure 25. The degree of radiochemical purity can be observed by comparison of this spectrum with that from a piece of irradiated enamel, counted directly after 90 minutes irradiation (figure 5). In figure 26 are shown the decay curves obtained over the 0.845 MeV peaks, from two samples of irradiated enamel (88 and 61 mg.) and two manganese standards (0.072 μg. manganese) after chemical separation. The half-lives of these curves were calculated by two methods.

(1) Graphically by inspection.

(2) Statistically by a least squares method[31].

Table 11 shows the comparison of the half-lives of samples and standard obtained by the two methods. It is an indication of the radiochemical purity that the measurements of the standard half-lives are only fractionally more accurate than the sample half-lives. The least squares method of estimating half-lives can be seen to give half-lives nearer to the literature half-life of Mn^{56} (2.58 hours).

Table 11

COMPARISON OF SAMPLE AND STANDARD HALF-LIVES

Sample	Half-life (graphical)	Half-life (least squares)
Tooth A	2.67 hours (+3.5%)	2.53 hours (-1.9%)
Tooth B	2.72 hours (+5.4%)	2.69 hours (+4.3%)
Manganese A	2.53 hours (-1.9%)	2.60 hours (+0.8%)
Manganese B	2.70 hours (+4.7%)	2.61 hours (+1.2%)

N.B. figures in brackets represent % deviation from literature value of Mn^{56} half-life. (2.58 hours).

Barium contamination. In one batch of enamel samples analysed for manganese, a photopeak was observed in the spectra of some of the samples,

which had not been observed in earlier analyses. In figure 27 is shown a γ-spectrum from one of these samples. A rough decay measurement on the peak at 0.15 MeV gave a half-life of 78 minutes. These facts seemed consistent with Ba^{139} ($t_{\frac{1}{2}}$ - 85 minute and γ-photopeak at 0.163 MeV).

In an attempt to confirm the identity of this photopeak, three further specimens of the enamel samples were selected. Two of these, A and B, were specimens from a sample which had shown the 0.15 MeV peak on manganese analysis. The other, C, was a specimen from a sample in which no 0.15 MeV peak was found. All three samples were irradiated and chemically processed for barium. The separated barium activity was examined on a γ-spectrometer.

The samples were irradiated for one hour at a thermal neutron flux of $10^{12} n/cm^2/sec$. After irradiation the samples were digested in 10 ml. of 16M nitric acid, 20 mg. of barium carrier were added and the barium nitrate precipitate separated by centrifugation. After washing with 16M nitric acid the barium nitrate precipitate was dissolved in water and the solution buffered with ammonium acetate (1 ml. of 50% w/v solution). On heating, barium chromate was precipitated by the addition of 1 ml. potassium chromate solution (10% w/v). Each precipitate was washed with water and acetone and transferred to planchets for counting. Counting was carried out on a 3" sodium iodide (Tl) crystal connected to a Laben 512 channel pulse height analyser.

In figure 28 are shown the spectra obtained from the three samples. Ba^{139} photopeaks were observed in samples A and B but not in C. These observations confirm the identity of the 0.15 MeV peaks found in the manganese dioxide precipitates. It is thought that the fact that

this barium contamination was observed in a few samples from one batch of enamel sections suggests accidental contamination. On further checking it was discovered that, at the time the sections were prepared, a solution of 10% (w/v) barium nitrate had been in the sectioning area for use in some other experiment. This seemed a plausible source of the contamination and no further barium peaks were found in samples subsequently analysed. The manganese analysis in the samples showing barium peaks was not invalidated since the low energy Ba^{139} peaks did not contribute to the higher Mn^{56} peaks used for area estimations.

Manganous ferrocyanide as manganese precipitate. Precipitation of manganese from acid solution as the ferrocyanide was considered as an alternative to manganese dioxide precipitation. Several samples of powdered kale were analysed using a ferrocyanide precipitation for manganese in the radiochemical separation.

The samples and a manganese standard were irradiated at a flux of 10^{12} neutrons/cm^2/sec. for one hour. Each sample was digested by heating in 3 ml. of 18M sulphuric acid containing 10 mg. manganese carrier. Solid sodium nitrate (about 30 mgs.) was used to oxidise charred organic material. After digestion the solution was diluted to 36 ml. and 6 ml. of potassium ferrocyanide solution (10% w/v) added to precipitate manganous ferrocyanide. The precipitate was washed with water and acetone and transferred to aluminium planchets. Counting was carried out as for manganese dioxide precipitates.

In figure 29 is shown a γ-spectrum of one of the samples. The spectrum can be seen to be mainly that of Mn^{56} (γ-photopeaks at 0.845, 1.81, 2.12 MeV). There can also be seen other peaks at 0.50 and 1.58 MeV. A recount of the sample after 1.2 hours was made and is also shown

PRECISION AND ACCURACY OF ANALYSIS

Two types of finely divided biological material were analysed for manganese to measure the reproducibility and accuracy of the method. The first type was dry, powdered kale prepared as a homogeneous biological standard by, and obtained from, H.J.M. Bowen[3]. The second type was powdered human teeth, obtained by crushing 20 sound human teeth in a steel mortar until a fine powder was obtained. It was expected that manganese could be introduced into the powdered teeth from the crushing process since manganese is a steel additive. However, as long as the distribution was uniform the material still served as a check on analytical reproducibility. The results obtained from analysis of these materials are shown in Table 13.

Table 13

MANGANESE CONTENT OF POWDERED KALE AND TOOTH

Sample	Manganese content (p.p.m.)	Mean value (p.p.m
Powdered Teeth	4.06,4.09,4.10,4.11,4.26, 4.44.	4.19
Powdered Kale	13.7,14.3,14.4,14.5,14.7, 14.8,15.1,15.2,15.5,15.7.	14.9

The results from the powdered tooth are reproducible to within 5% and do in fact show manganese contamination when compared with manganese levels in whole specimens of enamel from the same batch of teeth (Table 42). The kale results are also reproducible within 5% and show good agreement with the other measured values of manganese in kale 14.9 ± 1.8 p.p.m.[10].

MANGANESE STANDARDS

Comparison of results obtained using two types of manganese standard were made using kale as a reference material.

(a) Micro-standard.

A solution of 'SPECPURE' manganese metal was made by dissolving a weighed amount of the metal in 16M nitric acid and diluting with distilled water so that the final manganese concentration was about 1 µg./ml. Using a Hamilton micro-syringe 0.1 ml. aliquots of this solution were placed on thoroughly clean (nitric acid/distilled water washed) 1" squares of polythene. The liquid was evaporated under an infra red lamp and the polythene squares were folded and placed into small polythene envelopes and then sealed. Each envelope thus contained a 0.1 µg. manganese standard and was enclosed with other samples in a polythene capsule for irradiation.

(b) Macro-standard.

About 1 mg. of manganese sulphate monohydrate (ANALAR) was weighed and sealed in small polythene tubes. After irradiation the manganese sulphate was dissolved in 1 M nitric acid and diluted to 10,000 ml. A 1 ml. aliquot (equivalent to approximately 0.1 µg.of manganese) was chemically processed and counted in an identical manner to the samples.

The specific activity was calculated for each standard in terms of peak area. The manganese content of each kale sample was calculated with reference to each standard. Two samples of each type of standard were used. For the micro-standards two volume sizes were included; (1) 0.01 ml. of a 10µg./ml. manganese solution.

(2) 0.1 ml. of a 1 µg./ml. manganese solution.

The results obtained are given in Table 14.

Table 14

INTERCOMPARISON OF MANGANESE STANDARDS

Standard type	Specific Activity (peak area/0.1 μg. manganese)	Kale A	Kale B
Micro-standard			
0.01 ml.	5420	15.2	16.1
0.01 ml.	6000	13.7	14.5
0.1 ml.	5560	14.8	15.7
0.1 ml.	5760	14.3	15.1
Macro-standard			
$Mn.SO_4H_2O$	4980	16.5	17.5
$Mn.SO_4H_2O$	4995	16.4	17.4

It can be seen that lower specific activities and hence higher kale figures are found for the solid macro-standards. The micro-standards were thought to be more accurate since their use gave kale analyses nearer to the known figure of 14.9 p.p.m.[10]. Two possible explanations of the low specific activities of the macro-standards were considered.

1. The larger mass of the macro sample caused some self-shielding from the neutron flux and caused low specific activity production.

2. The macro-standard ($MnSO_4H_2O$) may have gained some of its water content during storage. This would produce apparent low specific activities.

To consider which of these explanations was more likely, a kale analysis was made using a micro-standard made from a solution of $MnSO_4H_2O$. This gave a figure for the kale of 12.2 p.p.m. As this figure is lower than the previous figures it seems more probable that

the self-shielding explanation is correct.

For the rest of the work on manganese analysis the micro-standard from metallic manganese was used, as it appeared to give more accurate results and was simple and convenient. It was also shown that a 'blank' standard prepared in the same manner as the manganese standards, only using distilled water in place of the manganese solution, showed on analysis 0.0012 μg. of manganese. This was in fact the limit of detection of the method and was less than 1% of the manganese content of the standards normally used.

CALCULATION OF MANGANESE CONTENT

The peak areas obtained using the digital method of calculation described earlier were corrected for decay and chemical recovery. Where necessary a correction for phosphate absorption was made on the chemical recovery. The manganese content of each sample was then calculated by comparison of the corrected sample peak areas with the corrected standard peak area.

SUMMARY OF ANALYSIS

Samples and a 0.1 μg. manganese standard were irradiated in a thermal neutron flux of 10^{12} n/cm^2/sec. for 1-3 hours. Following irradiation samples and standard were separately processed and counted. Digestion was made in 10 ml. of 16M nitric acid containing 10 mg. of manganese carrier. When solution was complete the manganese was precipitated as manganese dioxide, by addition of a solution of sodium chlorate. This precipitate was separated by centrifugation, washed with water and transferred to a weighed planchet with water. It was dried at 110°C and weighed as MnO_2H_2O.

The activity of samples and standards was measured with a sodium iodide (Tl) crystal and a multi-channel analyser. The area of the 0.845 MeV Mn^{56} photopeak was estimated using a digital method of peak area evaluation. The manganese content of each sample was calculated by comparison of peak areas of sample and standard after corrections for decay and chemical recovery had been made.

[illegible] material activation analysis technique.

COMBINED COPPER, ZINC, CADMIUM & MERCURY ANALYSIS.

Literature Review

Copper has been determined in biological materials by a full radiochemical procedure by Bowen.[9,11] It has also been determined in serum along with chromium, zinc and cobalt by Hoste[43]. Copper levels in human enamel have been measured by Nixon[78] and Smith. Copper and zinc are frequently determined together in analysis of biological material since Cu^{64} and Zn^{69m} have similar half-lives.[62,113] In the same paper[62], Leddicotte describes the determination of cadmium in animal tissue. Cadmium has been determined by chemical separation and γ-spectrometry on biopsy kidney samples, although the sensitivity obtained is not very high (0.1 μg.).[109] Mercury has been determined in biological material by various workers using neutron activation analysis by a variety of analytical techniques involving at least some degree of radiochemical separation.[42,90,105] This element has been studied in human enamel by Nixon[79].

Isotopes.

In Table 15 are listed the isotopes of copper, zinc, cadmium and mercury produced by thermal neutron irradiation which are suitable for biological material activation analysis techniques.

Table 15

USEFUL ISOTOPES

Isotope	Half-Life	Actσ(Barns)	Usable Decay Products
Cu^{64}	12.8 hours	3.0	β^+, β^-
Zn^{65}	245 days	0.22	γ
Zn^{69}	57 mins.	0.186	β^-
Zn^{69m}	13.8 hours	0.018	β^-, γ
Cd^{115}	53 hours	0.32	β^-, γ
Cd^{115m}	43 days	0.04	β^-, γ
Cd^{117}	2.9 hours	0.11	β^-, γ
Hg^{197}	65 hours)	4.5	γ
Hg^{197m}	24 hours)		γ
Hg^{203}	47 days	1.13	γ

Sample Irradiation. Irradiations were carried out in reactors with a thermal neutron flux of 10^{12} n/cm^2/sec. BEPO (Harwell) was used for irradiations longer than 6 hours, and the Scottish Research Reactor (East Kilbride) for irradiations shorter than 6 hours.

In the short irradiations only zinc and cadmium were determined as there are no isotopes of copper and mercury which give sufficient analytical sensitivity under experimental conditions, with these irradiations. One day irradiations using Cu^{64}, Zn^{69m} and Cd^{115}, and seven day irradiations using Cu^{64}, Zn^{69m}, Zn^{65}, Cd^{115}, Hg^{197} and Hg^{203}, completed the range which this work covered.

Samples for the short irradiations were prepared as described for vanadium and manganese irradiations. For the Harwell irradiations samples were weighed and wrapped in 1" squares of aluminium foil and

packed with standards in a Harwell aluminium irradiation can.

Standard preparation. For short irradiations 1 µg. standards of cadmium and zinc were prepared from dilute solutions of 'Specpure' cadmium oxide and zinc, in a similar manner to that used for manganese and vanadium standard preparation. When the length of irradiation was 1 day, the form of the standard was a sealed silica ampoule containing 1 mg. of the appropriate element in 0.1 ml. of solution. 'Specpure' reagent grade materials were used in making solutions (usually effected with 16M nitric acid). Following irradiation the ampoule was opened and its contents diluted. An aliquot of the diluted standard was used for comparison with the samples after chemical separation. It would be possible to use a standard solution containing all four elements being analysed, but it was found convenient to irradiate each standard separately, since the diluted active material could be used in tracer experiments.

For one week irradiations solid samples of high purity copper, zinc, cadmium oxide and mercury were irradiated in silica ampoules. Following irradiation the ampoules were opened, the active metal dissolved and diluted in a similar manner as for the liquid standards. It may be possible to irradiate liquid standards for one week, but the dangers of ampoules 'blowing', under the increased pressure induced by the reactor temperature and radiolysis products, are eliminated when solid standards are used. Harwell safety regulations required the mercury metal standard being doubly sealed in silica ampoules.

Chemical Separation

Digestion. Two digestion techniques were used in this work.

(1) Nitric/Sulphuric. This technique was used when mercury analysi was required, since it was known to give minimal losses of both active

and carrier mercury during digestion[90]. The sample was heated in a 25 ml. conical bottomed flask with 6 inch neck, which contained 10 mg. mercury and 2 ml. of a 1:1 mixture of 18 M sulphuric and 16 M nitric acids, until the tissue was destroyed and all charring removed. On cooling 10 mg. each of copper, zinc and cadmium carriers were added and the solutions diluted to 36 ml. with 0.5 M hydrochloric acid.

(2) Nitric acid. When no analysis for mercury was required the sample was digested by heating it in a beaker containing 10 mg. each of copper, zinc, and cadmium carriers and 3 ml. of 16 M nitric acid. When digestion was complete the solution was evaporated to dryness, 1 ml. of 11 M hydrochloric acid added and again taken to dryness. The residues were then dissolved in 0.5 M hydrochloric acid. When this digestion was used with mercury carrier, total loss of mercury occurred during evaporation to dryness, by the formation of highly volatile mercury salts.

Preliminary phosphate scavenge.

It was found by tracer experiments using P^{32} that no phosphate was held on the anion exchange column used in the initial separation of copper, zinc, cadmium and mercury. This was in accordance with published data on the adsorption of elements from dilute hydrochloric acid on a strongly basic anion exchange[60]. Nevertheless using a 1 day irradiation P^{32} was detected in the decay curves of the separated zinc and cadmium activities. Figure 31 shows decay curves of separated zinc activity from a representative enamel sample and a zinc standard counted at the same time. Figure 32 shows the corresponding decay curves of the separated cadmium activity from the same samples. In both sets of curves the decay can be seen to be levelling out, indicating the presence of a long lived contaminant. These curves were obtained

from data obtained using an end window Geiger counter. The level of the contaminant can be seen to be greater in the zinc samples. Three days after irradiation the samples were examined on a γ-spectrometer to try to identify the long lived contaminant. Little γ-activity of any significance could be detected in any of the samples. In Figure 33 is shown the γ-spectrum from one of the cadmium samples. Although this sample was counted for over 30 minutes only small Cd^{115} and daughter In^{11} photopeaks could be detected. The lack of γ-activity from the long lived impurity seemed consistent with P^{32}. Also, the higher level in the zinc samples was not surprising, since zinc is eluted before cadmium in the ion-exchange separation. It is more likely to contain residual phosphate incompletely washed from the column during adsorption of zinc and cadmium.

A phosphate separation step was introduced after sample digestion, to remove this interference. Following digestion 5 drops of 10% (w/v) ammonium dihydrogen phosphate were added to the solution and 8 drops of 10% (w/v) zirconyl chloride solution. After heating on a water-bath the solution was centrifuged and the zirconyl phosphate precipitate discarded. The completeness of precipitation was checked by further addition of zirconyl chloride solution. No precipitate formed if the phosphate was fully removed.

It was known that the removal of phosphate was complete if the hydrochloric acid concentration was less than 1 M[107]. Since sulphuric acid was present, after digestion, for mercury analysis the effect of the presence of sulphuric acid was examined using P^{32} tracer. Solution of 0.5 M hydrochloric containing various amounts of sulphuric acid were used. Each solution (20 ml.) contained 10 mg. of copper, zinc, cadmium and mercury carrier and a known amount of P^{32} tracer. Zirconyl phos-

phate was precipitated from each solution as described above, and the P^{32} left in solution determined by counting 10 ml. aliquots of each supernate in a Geiger counter accepting liquid samples. In Table 16 are shown the figures obtained for the unprecipitated phosphate. It was concluded that the sulphuric acid remaining after sample digestion would not constitute a serious interference in phosphate removal at the acid concentration at which precipitation was made i.e. less than 1 M.

Table 16

EFFECT OF SULPHURIC ACID ON ZIRCONYL PHOSPHATE PRECIPITATION FROM 0.5M HYDROCHLORIC ACID

Sulphuric acid concentration (M)	Phosphate Unprecipitated %
0.09	1.20
0.18	0.64
0.36	0.00
0.72	1.24
0.90	2.62
1.80	5.88

Ion-exchange separation

The ion-exchange separation was based on that reported by Kallmann, Steele and Chu[54] for the separation of zinc and cadmium. 1 cm. diameter columns containing 7 cms. of the strongly basic Amberlite IRA - 400 (Cl) anion exchange resin were used. The solution of a sample in 0.5 M hydrochloric acid from the digestion or phosphate scavenge step was transferred to a gravity operated reservoir fitted on top of the columns. When phosphate precipitation had been made the solution was filtered into the reservoir to avoid accidental transfer of zirconyl phosphate precipitate. The solution was passed throug

the column which was then washed with 25 ml. of 0.12 M hydrochloric acid containing sodium chloride (10% w/v). Zinc, cadmium and mercury are retained on the column as their chloro-complexes eg. $ZnCl_4^{=}$ while cations and non-adsorbable anions are washed through. Kraus and Nelson have published a list of the elements held on a strongly basic anion resin under these conditions[60].

Elution. The elution pattern of the four elements was studied using tracers. The tracers used were Cu^{64}, Zn^{69m}, Cd^{115} and Hg^{203}. Each tracer was used in a separate experiment from the others and its distribution during elution studied. 10 mg. of each carrier were present in every experiment. 10 ml. fractions from the column were collected and counted either by (a) a Geiger counter accepting liquid samples (for β-counting) or (b) a $1\frac{1}{2}$" sodium iodide crystal (for γ-photopeak counting The flow-rate used was 3 ml. per minute.

In Table 17 are shown the results of these experiments. The reagents and their order of use was as listed in the Table. They show that (a) copper was not retained on the resin.

(b) over 75% of the zinc was eluted in 30 ml. of sodium hydroxide (solution C)

(c) over 95% of the cadmium was eluted in 50 ml. of nitric acid (solution D)

(d) almost 70% of the mercury was eluted in 50 ml. of ethylene-diamine (solution E).

Table 17

ELUTION OF COPPER, ZINC, CADMIUM & MERCURY

Eluant	Eluant volume (ml.)	Total tracer in fraction (%) Copper	Zinc	Cadmium	Mercury
A.					
0.5M hydrochloric acid	0 - 10	43.4	-	-	-
	10 - 20	53.9	-	-	-
B.					
0.12M hydrochloric acid/	0 - 10	2.2	-	-	-
Sodium Chloride (10% w/v)	10 - 20	-	-	-	-
C.					
2M sodium hydroxide/	0 - 10	-	29.7	1.8	7.6
Sodium chloride (2% w/v)	10 - 20	-	33.8	1.5	5.4
	20 - 30	-	12.5	1.0	5.4
	30 - 40	-	7.0	0.5	4.0
D.	0 - 10	-	4.8	2.3	0.5
1 M nitric acid	10 - 20	-	5.7	45.5	-
	20 - 30	-	4.8	36.0	-
	30 - 40	-	2.8	9.0	1.1
	40 - 50	-	-	3.8	3.6
E.	0 - 10	-	-	-	22.2
Ethylenediamine (5% w/v)	10 - 20	-	-	-	18.3
	20 - 30	-	-	-	13.9
	30 - 40	-	-	-	8.8
	40 - 50	-	-	-	6.6
	50 - 60	-	-	-	1.5
Total tracer recovered		99.5	101.1	101.4	98.9

(e) some contamination of the zinc fraction with cadmium and mercury occurred.

(f) some zinc and mercury were found in the cadmium fraction.

Zinc was eluted from the column by displacement of the chloro-complex of zinc and formation of the basic zincate ion. Only amphoteric elements capable of forming alkali soluble salts would be eluted with this fraction. Many other elements on being displaced from the resin by the hydroxide, would form insoluble hydroxides or oxides.

In the case of mercury and cadmium this could be seen by the formation of a yellow band of mercuric oxide or a grey band of cadmium hydroxide on the resin. Kallmann, Steele and Chu[54], did not find cadmium in the zinc fractions from the separation of zinc and cadmium using this ion exchange technique. They tested neutralised portions of the zinc fraction with dithizone and found no positive test for cadmium. The amounts of zinc and cadmium separated were usually of the order of 1 gm. each. They noted that the mechanism of the cadmium retention by the resin has not been established. They suggested that the presence of sodium chloride has some effect. The slight leakage of cadmium and mercury from the resin with sodium hydroxide, (Table 17) leads to a suggestion of the manner in which these elements are retained. Presumably this would apply to other elements held on the resin and capable of forming insoluble oxides and hydroxides. When the alkaline fraction used to elute zinc in the presence of cadmium tracer and carrier were examined, no activity was detected in the Geiger counter accepting liquid samples used. When the fractions were made acid with hydrochloric acid, 4 - 5% of the cadmium tracer used was detected with the zinc fraction. (Table 17).

It is probable that, in the alkaline medium, the cadmium was adsorbed on the walls of the collecting tube as cadmium hydroxide and was not transferred to the counting tube. When the fraction was made acid, the cadmium was held in solution and was transferred and counted. This problem did not arise in the case of zinc elution in the presence of mercury tracer and carrier as the eluted fractions were counted directly in a scintillation well crystal without being transferred from the collecting tube.

It is suggested that the retention of the cadmium and mercury bands on the resin is a mechanical retention of the insoluble hydroxide and oxide respectively. The slight loss detected in this work may be attributed to small particles of precipitate being separated from the main bulk of precipitate and washed down through the resin. Kraus and Nelson[61] report that mercury is difficult to elute from an anion exchange resin where it is adsorbed as its chloro-complex. Various solutions (Table 18) were used in an attempt to remove the mercury from the resin following cadmium elution. The figures show the amount of mercury tracer eluted from the column by 50 ml. of eluant.

The mercury carrier precipitated on the column as mercuric oxide during zinc elution and was not eluted in 1 M nitric acid, though mercuric oxide dissolves readily in this concentration. In addition, tracer experiments showed that mercury was not retained on the resin when added to it in the nitrate form.

The reason for the ease of mercury elution using ethylenediamine is thought to be the formation of the cationic species $Hg(en)_3^{2+}$ which is not held on an anion resin. As most complexing agents produce a negatively charged complex they would not be of value in mercury elution

from this type of resin.

Table 18

MERCURY ELUTION

Eluant	Mercury tracer in 50 ml. eluant (%)
Nitric acid 2M	19
Thiourea 0.1M/hydrochloric acid 0.01M	17
Hydrochloric acid 9M	7
Sulphuric acid 4.5M	8
EDTA (5% w/v) pH 4	11
" " pH 7	14
" " pH 9	19
" " pH 11	18
Ethylenediamine (5% w/v)	71

The effect of the pH of the ethylenediamine solution used to elute mercury was studied. Using $Hg^{197/197m}$ tracer, an experiment was carried out in the same manner as the tracer experiments used to collect the elution data of Table 17. In three columns copper, zinc and cadmium carriers were eluted by the reagents described in Table 17 and each fraction was collected for counting. The mercury fraction left on each column was eluted with ethylenediamine solution (5%) at different pH values. The pH was adjusted using a pH meter and 16M nitric acid for increasing acidity. In Table 19 is shown the amount of mercury in each fraction. It can be seen that the mercury was best eluted by an alkaline solution of ethylenediamine i.e. no nitric acid addition. 50 ml. of acid, neutral and alkaline solutions eluted 27, 49 and 65% respectively of the mercury added to the resin.

Table 19

EFFECT OF pH OF ETHYLEDIAMINE SOLUTION ON MERCURY ELUTION

Eluant	Volume (ml.)	Mercury eluted (%)		
		pH 2.1	pH 7.0	pH 11.7
0.12 M hydrochloric acid/sodium chloride (10% w/v)	30	0.4	0.4	0.6
2 M sodium hydroxide/ sodium chloride (2% w/v)	30	27.4	25.0	22.1
1 M nitric acid	50	6.0	2.9	2.9
Ethylenediamine (5% w/v)	10	6.1	12.3	21.2
	20	5.0	18.9	17.3
	30	6.4	8.4	12.9
	40	5.1	4.7	7.8
	50	4.6	5.1	5.6

A check was made to ensure that no mercury was left on the resin after an elution cycle. After a set of experiments using $Hg^{115/11}$ tracer the columns were washed with a further 25 ml. of elution reagent (ethylenediamine). The columns were washed further with water, and converted to the chloride form with 25 ml. of 2 M hydrochloric acid. 10 mg. of mercury carrier were added to each column and elution of mercury with ethylenediamine made as before. The eluted mercury was precipitated as the mercury iodide/copper ethylenediamine precipitate

used in the final chemical processing step for mercury and its activity measured. No activity above background was detected, indicating that no tracer mercury had been left on the column in the first set of experiments.

Final Separation.

Each fraction containing an element eluted from the resin was further processed to produce the element in a radiochemically pure form suitable for counting.

Copper. The copper was further processed using the hydrochloric acid column effluent, using a precipitation technique described by Nixon & Smith[78]. This entailed four steps as follows;-

1. Precipitation of copper as the cuprous thiocyanate in the presence of 'hold back' carriers.
2. Ferric hydroxide scavenge.
3. Reprecipitation of cuprous thiocyanate.
4. Final precipitation of the copper as the quinaldate.

The quinaldate is a weighing form and was used for calculating recoveries and for activity determinations.

Zinc. Of the elements adsorbable on the resin from dilute hydrochloric acid solutions, Cd, Zn, Ag, Pb. Hg^{II}, Pd^{II}, Pt^{II}, and Fe^{III} are capable of forming quinaldates. Of these, only zinc and lead form an alkali soluble salt and hence are separated from the above elements on elution of the zinc fraction. Under the activation conditions lead did not interfere but, as noted before, (Table 17) there was a leakage of some of the other elements into the alkaline fraction. To avoid this contamination, it was necessary to introduce a scavenging step before the

final precipitation. The following scheme resulted in complete radiochemical purity as will be shown by γ-spectrometry and decay studies.

The alkaline zinc fraction was made just acid with 11 M hydrochloric acid, and 0.5 ml. of iron carrier (ferric chloride 10% w/v) added. 3 ml. of sodium hydroxide solution (20% w/v) were added, the solution heated on a water bath, and the resulting ferric hydroxide precipitate centrifuged off. The supernatant was filtered to ensure complete removal of any precipitate and neutralised with 17.5 M acetic acid to the phenolphthalein end point. About 1 ml. of acetic acid was added after neutralisation. The solution was placed in a boiling water bath and 3 ml. of quinaldic acid solution (2% w/v) added to precipitate the zinc. The precipitate was washed once with water and once with acetone, and slurried on to a weighed aluminium planchet with acetone. Following drying under an infra-red lamp at 100°C, the precipitate was weighed as zinc quinaldate $Zn(C_{10}H_6O_2N)_2 \cdot H_2O$.

Cadmium. Of the elements held on the resin with cadmium by formation of insoluble oxides or hydroxides, only Ag, Hg^{II}, Tl and Bi are precipitated with cadmium by the Reinecke's salt[32]. As shown in Table very little mercury was eluted with the cadmium and it was not found as a contaminant in any of the samples analysed. It may well prove to be a contaminant in analysis of material relatively rich in mercury. If silver, mercury or thallium prove to be a problem, they can be removed by a suitable silver chloride scavenge, prior to the reineckate precipitation. Due to its poor activation characteristics, bismuth does not interfere in usual biological materials. The zinc eluted with the cadmium is not precipitated with cadmium and does not contaminate

the final precipitate.

Satisfactory radiochemical purity of the cadmium reineckate precipitate as shown by γ-spectrometry and decay studies, was given by the following method. The cadmium effluant was made alkaline with sodium hydroxide solution (20 % w/v) to precipitate the cadmium as the hydroxide. Following centrifugation, the supernatant solution was discarded. The precipitate was dissolved in 1 ml. of 11 M hydrochloric acid diluted to 20 ml. and 5 ml. of thiourea solution (5% w/v) added. 8 ml. of a saturated solution (at 18°C) of ammonium reineckate containing thiourea (1% w/v) were then added to precipitate the cadmium as the reineckate. The precipitate was washed with thiourea (1% w/v) and absolute alcohol. It was slurried on to a weighed aluminium planchet with alcohol, dried at 110°C and weighed as cadmium reineckate $\left\{Cd(SC(NH_2)_2\right\} \left\{Cr(NH_3)_2 \ (SCN)_4\right\}_2$.

The ammonium reineckate solution used was prepared before each analysis, since it decomposes readily (in distilled water it forms a greenish blue chromium ammonium complex). The volume of ammonium reineckate solution required was found by varying the volume of reagent used to precipitate 11 mg. of cadmium carrier from 25 ml. of 0.5M. hydrochloric containing thiourea (1% w/v). In Table 20 are shown the weight of cadmium reineckate obtained on washing and drying each precipitate. A volume of 8 ml. was found to be optimum.

Table 20.

VOLUME OF AMMONIUM REINECKATE REAGENT REQUIRED TO PRECIPITATE CADMIUM

Volume used (ml.)	Weight of precipitate (mg.)
2	20.9
4	30.7
6	77.7
8	86.4
10	85.4

Mercury. As shown in Table 17, the mercury carrier left on the resin can be completely removed in 50 ml. of ethylenediamine solution (5% w/v). It was then precipitated as the copper ethylenediamine - mercuric iodide complex,[90] by addition of 1 ml. of sodium iodide solution (10% w/v), and 1 ml. of copper sulphate solution (10% w/v). The precipitate was washed with water and isopropyl alcohol, transferred to stainless steel planchets, dried at 90°C and weighed. The chemical recovery may be only 60% because of carrier losses during separation. In particular significant amounts of mercury are eluted from the resin with the zinc fraction (Table 17).

The conditions for mercury precipitation in this method are slightly different from those used in the precipitation method used by Smith[90]. The reagent volumes required were determined by precipitating 10 mg. aliquots of mercury carrier from 30 ml. of ethylenediamine solution (5% w/v) using various volumes of copper sulphate (10% w/v) and sodium iodide solution (10% w/v). 1 ml. of either reagent was added to each 50 ml. centrifuge tube while the volume of the other reagent was varied. The results (shown in Table 21) show that 1 ml. of each reagent gives

adequate precipitation of the mercury.

Table 21.

REAGENT VOLUMES FOR MERCURY IODIDE/COPPER ETHYLENEDIAMINE PRECIPITATION

Volume of copper sulphate sulphate solution (ml.)	Volume of mercuric iodide solution (ml.)	Mercury precipitated (mg.
1	0.25	23.2
1	0.5	36.1
1	0.75	42.0
1	1.0	42.9
1	2.0	44.3
1	3.0	44.5
0.1	1	25.4
0.25	1	38.2
0.5	1	38.7
0.75	1	40.1
1.0	1	41.8
2.0	1	43.4

Detection. The activity in the final precipitates was measured by either an end-window Geiger counter or a sodium iodide (Tl) crystal connected to a pulse-height analyser. The Geiger counter was used for detection of Zn^{69}, Zn^{69m}, Cu^{64}, Cd^{115} and Cd^{117}. The sodium iodide (Tl) crystal was used for detection of Hg^{197}, Hg^{197m}, Hg^{203} and Zn^{65}. It was also used where appropriate in checking radiochemical purity of the Geiger-Muller determined isotopes.

RADIOCHEMICAL PURITY

Copper. The radiochemical purity was known to be good since a complete separation of a proven method[78] followed the ion-exchange separation.

Zinc. It was found using the short lived Zn^{69} that the amount of P^{32} produced in an hour irradiation did not interfere significantly with the Zn^{69} measurement. In figure 34 is shown the decay curve obtained after zinc separation was made on an enamel sample irradiated for 1 hour at a thermal neutron flux of $10^{12} n/cm^2/sec$. Several such curves were obtained using a PANAX six sample comparator (sample changer) in conjunction with PANAX module counting equipment. A standard zinc sample is shown to have an identical decay curve. The decay is initially that of Zn^{69}($t_{\frac{1}{2}}$ - 55 minutes) but levels off onto a section of the curve corresponding to Zn^{69m}($t_{\frac{1}{2}}$ - 13.8 hours). The first recorded count on the curve was made 3.8 hours after irradiation, so the Zn^{69} has decayed for approximately four half-lives before the start of the measurement.

When an irradiation of 24 hours was made for a zinc determination using Zn^{69m} the amount of P^{32} produced was much greater than in the short irradiation. When a decay curve was plotted from samples irradiated for 24 hours, and processed and counted as in the short irradiation separation procedure, a long lived contaminant was observed. This was identified as P^{32} and removed by a zirconyl phosphate scavenge as described earlier (figs. 31-33).

Cadmium contamination. As was noted in Table 17 there was slight leakage of cadmium when zinc was being eluted from the resin during ion-exchange separation. It was found that this leakage led in some

cases to radiochemical contamination of the zinc quinaldate precipitates with cadmium activity.

Human kidney tissue is known to contain considerably higher amounts of cadmium than other body tissues. A value of 32 μg/gm. of wet tissue is reported[87] which is over ten times higher than the next highest tissue level measured. When dry kidney samples were analysed for zinc by the ion-exchange separation technique following a seven day irradiation, deviation from the expected Zn^{69m} decay curve was noted. Figure 35 shows the decay curves from two zinc quinaldate precipitates from kidney samples and one from a zinc standard. The standard can be seen to be decaying faster than the samples (on the Zn^{69m}/Zn^{65} curve). A γ-spectrum of the activity from one of the kidney samples is shown in Figure 36. As well as the characteristic Zn^{65} photopeak at 1.11 MeV there can be seen peaks at 0.34 and 0.54 MeV. These correspond to the γ-rays from Cd^{115} and daughter In^{115n} at 0.52 MeV and 0.34 MeV respectively.

Removal of cadmium contamination.

When a sample containing large amounts of cadmium, such as kidney, was being analysed, the cadmium contamination of the zinc column fraction was removed as described previously, before quinaldate precipitation. The basis used for cadmium removal was the difference in the solubilities in alkaline solution of zinc and cadmium. Cadmium was precipitated as the hydroxide whereas in excess of sodium hydroxide zinc hydroxide was soluble by formation of the zincate.

$$Cd^{2+} + 2OH^- \longrightarrow Cd(OH)_2$$

$$Zn^{2+} + 2OH^- \longrightarrow Zn(OH)_2 \xrightarrow{2OH^-} ZnO_2^{2-} + 2H_2O$$

When iron was precipitated as ferric hydroxide in the presence of excess sodium hydroxide the cadmium was precipitated or co-precipitated along

with it. The zinc remained in solution as the zincate and, after removal of ferric hydroxide precipitate, was precipitated as the quinaldate.

In Figure 37 is shown the γ-spectrum of a zinc quinaldate precipitate obtained after zinc separation of a sample of irradiated enamel. The three peaks appearing in the spectrum correspond with the 1.11 MeV and 0.44 MeV photopeaks of Zn^{65} and Zn^{69m}, and the 0.51 MeV positron annihilation peak of Zn^{65}.

In Figure 38 are shown the decay curves of zinc activity separated from three tissue samples - spleen, stomach and lung, and a zinc standard. Over a six day period of measurement good comparison was observed between sample and standard decay curves of the Zn^{69m}/Zn^{65} couple.

Cadmium. In Figure 39 are shown the decay curves of the cadmium activity separated from five irradiated human tissues, and a cadmium standard. They represent the decay over ten days after a one week irradiation at a neutron flux of $10^{12} n/cm^2/sec$. The radiochemical purity of the cadmium reineckate precipitate is demonstrated by the close resemblance in the shapes of the sample and standard curves. The initial rise in the measured activity in both samples and standards is caused by the growth of In^{115m}($T_{\frac{1}{2}}$ - 4.5 hours). This isotope is the daughter of Cd^{115} and does not follow cadmium through the separation. It is neither retained by the ion-exchange resin nor precipitated as the reineckate and hence parent/daughter equilibration begins after final precipitation. Equilibration will be practically complete 9 - 12 hours after precipitation.

In Figure 40 is shown the γ-spectrum of the cadmium activity from a sample of human kidney after one week's irradiation. The two photo-

peaks present correspond to the 0.34 MeV and 0.52 MeV photopeaks of In^{115m} and Cd^{115} respectively. The γ-spectra and decay curves indicate that the chemical separation is efficient enough to give adequate radiochemical purity from biological samples after a one week irradiation.

Identification of silicon contamination of enamel samples.

When cadmium analysis of enamel samples was made using Cd^{117} ($T_{\frac{1}{2}}$ - 2.9 hours) it was observed that the separated activity was not Cd^{117} activity nor any related cadmium isotope. This was immediately evident since it was observed that the level of the contaminant activity was very much greater if the cadmium hydroxide precipitate was counted, instead of the final cadmium reineckate subsequently separated. In addition the decay and γ-spectra of the contaminant bore no resemblance to that from a cadmium standard irradiated and processed with the samples.

The γ-spectrum of the contaminating activity was studied carefully to help identify the nature of the contaminant. Several measurements were made over a wide range of energies eg. 512 second counts were made over three ranges covering the energy range 0.05 - 2.50 MeV. No γ-activity in the form of photopeaks could be detected in any case. Since the same sample (a precipitate of cadmium hydroxide separated from 28.8 mg. of enamel irradiated for 1 hour at $10^{12} n/cm^2/sec$. gave around 100 counts/second on an end-window Geiger counter at that time it was concluded that the contaminating isotope must be principally a β- emitter.

Several attempts at β-decay curve analysis were made. In the early part of the work only a few measurements could be made on the

activity, because of the absence of an automatic instrument for following decay. An early estimate of the half-life by a least squares analysis of a decay curve gave a figure of $T_{\frac{1}{2}}$ - 2.43 hours. A more sophisticated measurement was made when the PANAX sample comparator became available. Using this instrument a decay curve of the contaminant was obtained covering the period 3 - 85 hours following irradiation. The decay curve obtained is shown in figure 41. This can be seen to be a two component decay curve with short and long lived components. A graphical estimate of the half-life of the longer lived component gave a value of $T_{\frac{1}{2}}$ = 12.6 days. It was concluded that this would be P^{32}($T_{\frac{1}{2}}$ - 14.8 days) since it is a common contaminant in bone or teeth analysis and is a pure β -emitter.

The contribution of the P^{32} to the early part of the curve was calculated by extrapallation of the later linear section of the curve back to zero time. The calculated P^{32} contributions to the early activity were subtracted from the original measurements and the early section of the curve re-drawn. In figure 42 is shown the result which can be seen to show a linear decay of 2.55 hours (graphical estimate).

Several possible candidates for the contaminant identity were considered. These were the only isotopes produced by a (n, γ) reaction which appeared to have suitable nuclear characteristics. The isotopes appropriate nuclear data are listed in Table 22.

Table 22.

NUCLEAR DATA OF POSSIBLE ISOTOPES FOR CADMIUM CONTAMINATION

Isotope	Half-Life	Beta (MeV)	Gamma (MeV)
Si^{31}	2.62 hr.	1.47 (100%)	1.26 (0.07%)
Sc^{49}	55 min.	2.0 (100%)	-
Dy^{165}	2.3 hr.	1.31 (80%)	0.095 (3.2%) and 0.36 MeV (1.7%)

Sc^{49} is the daughter of Ca^{49}($T_{\frac{1}{2}}$ - 8.8 min.) which is produced in fairly large amounts from irradiation of teeth because of the high levels of calcium in teeth. Neither silicon nor dysprosium are known to be present in teeth in large amounts, although dysprosium is readily activated by thermal neutrons and hence small amounts could be readily detected (activation cross-section for Dy^{165} production - 790 barns).

Scandium - 49. During analysis for cadmium in teeth using Cd^{117} an experiment was made to determine whether the β-emitting nuclide found in cadmium hydroxide precipitates could be Sc^{49}. 10.3 mg. of ANALAR calcium nitrate was irradiated and processed along with the tooth samples. The amount of calcium in this sample was greater than that present in the enamel samples being analysed. If Sc^{49} was the contaminant found in the teeth samples it was expected that, being produced in the activated calcium nitrate, it would also be found in the cadmium hydroxide precipitate. In the short irradiation (1 hour) only Ca^{49} and Sc^{49} would be produced in significant amounts. No significant activity was detected in the cadmium precipitate separated from the activated calcium nitrate, and it was concluded that the con-

taminant in the teeth samples was not Sc^{47} nor any other calcium derived isotope formed on activation. In addition the half-life of Sc^{49} (55 minutes) is considerably shorter than that found for the contaminant.

Dysprosium - 165. The characteristics of this nuclide appeared very similar to those found in the contaminant. Dy^{165} was made by irradiation of a few μg. of Dy_2O_3 and used as a tracer. A known amount of Dy^{165} activity was digested in the presence of inactive enamel and copper, zinc and cadmium carrier. The ion-exchange separation was made and 10 ml. aliquots from the column collected and counted in a Geiger-counter accepting liquid samples. By comparison of the activity found in each aliquot with the amount of activity in a standard aliquot, it was possible to determine the path of the Dy^{16} during the column separation. In Table 23 is shown the amount of Dy^{165} found in each column fraction.

Table 23.

ABSORPTION AND ELUTION PATTERN OF Dy^{165} ON AMBERLITE IRA-400 RESIN

Aliquot No.	Eluant in use	Dy^{165} found
1	0.12 M hydrochloric acid	55%
2	"	43%
3	"	1.2%
4	"	-
5	2 M sodium hydroxide	-
6	"	-
7	"	-
8	1 M nitric acid	-
9	"	-
10	"	-

It can be seen that the dysprosium tracer is not held on the resin. This agrees with the published data on rare earth absorption on anion exchange resins from hydrochloric acid[60]. It was concluded that Dy^{165} was not the nuclide found in the cadmium hydroxide precipitates since it would not be retained on the resin with cadmium during separation. In addition, the amount of contaminant activity generally found was high enough to enable the low abundant Dy^{165} γ-photopeaks to be detected, if they had been present.

Silicon - 31. The nuclear characteristics shown in Table 22 of Si^{31} are similar to those found in the cadmium contaminant. Since the cross-section for the reaction $Si^{30}(n,\gamma)\ Si^{31}$ is small, the silicon content of the enamel samples must have been high if Si^{31} was the contaminating nuclide. A radiochemical separation for silicon was made on an activated enamel sample to determine if there was enough Si^{31} activity produced to account for the contaminant.

The chemical separation was the distillation of silicon tetrafluoride from an acid solution of an irradiated enamel sample. The separation used is described by Mullins and Leddicotte[84]. An enamel sample weighing 36.1 mg. was irradiated with a standard consisting of ammonium silicate solution (10 μg. of silicon) for two hours at $10^{12} n/cm^2/sec$. After irradiation sample and standard were separately processed and counted. The enamel sample was dissolved by heating in 5 ml. of 16 M nitric acid. The acid solution was transferred to a high density polythene distillation flask similar to that described by Mullins and Leddicotte[84]. The flask contained 5 ml. of 18 M sulphuric acid and 10 mg. of silicon carrier (as sodium silicate solution). 2 ml. of 17.5 M hydrofluoric acid were added via a

delivery funnel and the evolution of silicon tetrafluoride started by immersion of the flask in a boiling water bath. A stream of air from a pump was used to blow the silicon tetrafluoride evolved from the distillation flask to a centrifuge tube containing 10 ml. water used to collect the gas. The reactions involved are:

$$SiO_3^{2-} + 6\,HF \longrightarrow SiF_4 + 3H_2O + 2\bar{F}$$

$$SiF_4 + 3H_2O \longrightarrow H_2SiO_3 + 4HF$$

5 ml. of aluminium nitrate solution (50% w/v) were added to the centrifuge tube to complex the fluoride ion. The solution containing the silicic acid was transferred to a beaker, 10 ml. of 18 M sulphuric acid added and heated until gelatinous silicon dioxide was precipitated. The hydrated silicon dioxide precipitate was centrifuged and separated and washed with water. It was collected by filtering through a Whatman No. 1 filter paper, transferred to a nickel crucible and ignited at 1000°C to SiO_2. This was mounted on a weighed planchet, weighed and coated with a film of polyvinyl acetate for counting. The silicon standard was similarly processed with the omission of the distillation step. Both sample and standard were counted using an end-window Geiger-counter. The decay of the sample was followed using the PANAX decay follower.

In figure 43 is shown the decay curve obtained from the SiO_2 precipitate from the enamel sample. The decay can be seen to be consistent with Si^{31} ($T_{\frac{1}{2}}$ - 2.62 hours). This showed that a large amount of Si^{31} activity was being produced by the irradiation process. By comparison with the standard it was calculated that the enamel sample contained about 700 µg. of silicon.

Source of Silicon in enamel samples.

The silicon content in the enamel section analysed corresponded to a silicon level in enamel of about 2%. This value seems exceptionally high compared with enamel silicon levels determined by Brudevold[16]. He found that enamel levels of silicon were usually less than 10 micromoles per gram i.e. less than 30 p.p.m. A concentration of 2% is much higher than even the silicon content of outer enamel from teeth which had lain in the soil for hundreds of years. Steadman[99] found that the highest concentration in outer enamel samples from ancient Indian teeth was 2500 p.p.m. On consideration of the method used in sample preparation it was found that the cutting disc used to section enamel samples was made of carborundum. As this is essentially silicon carbide the samples must have been heavily contaminated with carborundum dust which accounted for the exceptionally high silicon level found in the enamel on analysis.

Fast neutron production of Si^{31}.

This was an alternative explanation investigated as a possible source of Si^{31} from irradiated enamel. Phosphorus is a major constituent element of the tooth or enamel matrix as phosphate. Si^{31} can be produced from phosphorus by the fast neutron induced nuclear reaction $P^{31}(n,p)\ Si^{31}$. Naturally occurring phosphorus consists of 100% P^{31}. The cross-section for the reaction using fast neutrons in the range 5 - 10 MeV is 142 millibarns[58].

An amount of solid ammonium phosphate (10.3 mg) containing a comparable amount of phosphorus to that present in the enamel samples was irradiated in an identical manner as the enamel samples. Following irradiation this sample was processed and the cadmium hydroxide

counted in the same fashion as the enamel samples. Only 1.3 counts per second were detected in the cadmium hydroxide precipitate. This is fully twenty to forty times less than the amount of Si^{31} activity found in enamel samples containing similar amounts of phosphorus.

It was concluded that while some Si^{31} is undoubtedly produced by the fast neutron reaction with P^{31} the bulk of the activity found in the cadmium precipitates from irradiated enamel originated from a thermal neutron reaction Si^{30} (n,γ) Si^{31} on silicon introduced to the sample during sectioning in the form of carborundum dust.

Adsorption of Silicon on Anion Resin.

No information could be found in the literature describing the adsorption of silicates from hydrochloric acid solution on anion resins. A tracer experiment, using Si^{31}, was made to determine the adsorption behaviour of silicates by experiment. The tracer was made by a solution containing about 1 mg. of ammonium silicate for 1 hour at a flux of $10^{12} n/cm^2/sec$. Aliquots of the diluted solution were used as the tracer. The aliquot of Si^{31} tracer was added to the resin with copper, zinc and cadmium carriers and the elution carried out as in the normal analysis. 10 ml. column fractions were collected and their activity measured in a Geiger counter accepting liquid samples. The percentage of the total tracer found in each column fraction is shown in Table 24.

Table 24

ADSORPTION OF Si^{31} FROM HYDROCHLORIC SOLUTION

Fraction	Eluant	Percentage tracer in aliquot
1	0.12 M HCl	51
2	"	39
3	"	5
4	"	0.9
5	2 M NaOH	-
6	"	0.5
7	"	-
8	I M HNO_3	1.1
9	"	0.6
10	"	-
11	"	-
12	"	0.8

The same experiment was repeated (a) in the presence of silicon carrier (sodium silicate) and (b) with silicon carrier and following nitric acid digestion. In neither case were significant differences from the results shown in Table 24 found. It was concluded that silicon in the form of silicate was not adsorbed on the resin. This conclusion raised the problem of how Si^{31} activity from the enamel samples was being found in the precipitates of cadmium hydroxide, obtained following ion exchange separation of copper, zinc and cadmium. To examine this problem further, analysis of known amounts of silicon were made. Samples containing 0.8, 8.0 and 80 µg. of silicon were

prepared by evaporating sodium silicate solutions on polythene squares. Following 2 hours irradiation at 10^{12} n/cm^2/sec. these samples were processed using the normal digestion and separation conditions. Each silicate sample was duplicated - one analysed with inactive tooth carrier and one without it. The activity found in each cadmium hydroxide precipitate after separation was measured on an end-window G.M. counter and is shown (corrected for background) in Table 25. The decay of the activity detected was checked and found to be consistent with Si^{31}.

Table 25

Si^{31} CONTENT OF CADMIUM HYDROXIDE PRECIPITATES

Silicon taken µg.	Activity in precipitate c/s with inactive tooth	Activity in precipitate c/s without inactive tooth
0.8	0.05	0.02
8.0	0.2	0.02
80.0	1.98	2.1

The presence of inactive tooth as carrier did not appear to affect the amount of tracer found with the cadmium hydroxide. The amount of Si^{31} which appeared with the cadmium can be seen to be small compared with the silicon irradiated. This is in accordance with the non-adsorption of silicates by the resin as shown in Table 25. A similar situation was found for the Si^{31} activity in the cadmium hydroxide precipitates from irradiated enamel samples. The activity found was only a small fraction of that being produced by the irradiation.

It is suggested that the origins of both the Si^{31} contamination from the enamel samples and the traces of Si^{31} found with cadmium in the

tracer experiment are the same. The major part of the activity is not held on the resin. A small fraction is not completely removed by the washing of the column with dilute hydrochloric acid and is subsequently washed off during nitric acid elution of cadmium.

Mercury.

The radiochemical purity of the mercury precipitates was determined by γ-spectrometric analysis of the sample activity using a Laben multi-channel analyser and a 3" sodium iodide (Tl) crystal. In figure 44 is shown the γ-spectrum of one of mercury precipitates. It can be seen that only mercury photopeaks show in the spectrum indicating radiochemica purity.

EQUILIBRATION OF ACTIVATED ELEMENTS WITH CARRIERS

During digestion of an activated sample equilibration takes place between activated atoms of an element in the sample and inactive atoms of the element added as a carrier. Such exchange is very rapid when the active and inactive forms of the element are in the same chemica state. It is essential that this exchange takes place when a chemical form of activation analysis is used. This is because the recovery correction applied to the measured sample count-rates assumes that active and inactive atoms are completely homogeneous so that the specifi activity of the carrier always remains constant despite carrier loss during separation.

An experiment was made using Zn^{69m}, Cd^{115} and Hg^{197} tracers to establish that exchange took place between tracer and carrier. The experiment also served as an indication of the efficiency of separation of the tracers obtained by the full chemical process. Various aliquots

of each tracer were added to digestion flasks containing carriers and inactive powdered kale to simulate the conditions normally used in analysis. Each sample was digested and processed in the manner described previously and the activity in each separated precipitate determined by the appropriate counting technique. In Table 26 is shown the data obtained from this experiment. The constancy of the measured specific activities of each tracer is an indication that exchange was complete between tracer and carrier.

	"	2.0	6.9
	"	6.0	20.60
	"	10.0	33.78
	[illegible]	1.0	22.2
	"	1.0	31.8
	"	1.0	28.4
	"	1.0	33.0
	"	1.0	31.8
	"	1.0	29.4

Table 26.

EQUILIBRATION OF TRACER AND CARRIER ZINC, CADMIUM AND MERCURY

Sample	Tracer	Volume used (ml.)	Measured activity (c/s.)	Specific Activity (c/s/ml. tracer)
1	Zinc	0.2	0.30	1.49
2	"	0.6	0.58	0.97
3	"	1.0	0.98	0.98
4	"	2.0	1.86	0.93
5	"	6.0	5.42	0.90
6	"	10.0	8.72	0.87
1	Cadmium	0.2	0.72	3.58
2	"	0.6	2.23	3.72
3	"	1.0	3.61	3.61
4	"	2.0	6.95	3.48
5	"	6.0	20.60	3.43
6	"	10.0	33.78	3.38
1	Mercury	1.0	28.2	28.2
2	"	1.0	31.8	27.8
3	"	1.0	28.3	28.8
4	"	1.0	33.8	26.1
5	"	1.0	31.8	26.5
6	"	1.0	29.4	27.3

THE DETERMINATION OF KNOWN AMOUNTS OF ZINC

Some measurements were made using the short lived Zn^{69} to determine by analysis a known amount of zinc. Samples containing a known amount of zinc were prepared by evaporating small volumes of a zinc solution onto

polythene in a similar manner to that used in zinc standard preparation. These samples were analysed with a zinc standard in an identical manner to that used in enamel analysis for zinc using Zn^{69}. A blank sample prepared using distilled water was also analysed to check if any zinc was being introduced into the samples by the method of preparation. The blank sample was not processed with the samples on the ion-exchange column but was only chemically treated by digestion and final precipitation as zinc quinaldate. A small amount of activity was found in the blank sample when it was counted (< 3 counts per second above the counter background). A few decay measurements on this activity indicated that it had a longer half-life than Zn^{69}($t_{\frac{1}{2}}$ - 55 mins.). The half-life observed of the activity was in the range 2-3 hours and it was thought likely to be Mn^{56}($t_{\frac{1}{2}}$ - 2.58 hours). This seemed likely since a little Mn^{56} was found in identical blanks used to test the method of manganese standard preparation. In addition, manganese in solution is capable of forming a quinaldate[107] and could, therefore, have been precipitated or co-precipitated with the zinc.

The comparison between the taken amounts of zinc and the amounts found on analysis are shown in Table 27.

Table 27.

ANALYSIS OF KNOWN AMOUNTS OF ZINC

Sample	Amount of zinc in sample (μg.)	Total zinc found on analysis (μg.)
1	4.77	4.41
2	0.954	0.920
3	0.477	0.458
4	0.239	0.177
5	0.0477	0.0424

Inspection of the results shows a slight systematic error in the analytical results. The zinc content found was consistently low in each sample analysed. The reason for this is not known but since all samples are in error by a similar fraction (in the region of 5% excepting sample 4), it is thought that the error lies in the standard used. The amount of zinc in the standard may have been slightly greater than intended due to errors in preparation of the micro-standard and so low results would occur.

MATERIALS ANALYSED FOR COPPER, ZINC, CADMIUM AND MERCURY

The material which was used as a standard biological material was the powdered kale, supplied by H.J.M. Bowen, described earlier[3]. This was the best available material for testing the analysis as it is believed to be homogeneous and so allows analytical reproducability to be tested. It has been analysed widely by various laboratories so that the accuracy of determination may be assessed. Five samples weighing in the range 80-120 mg. were irradiated for 1 week at 10^{12} n/cm^2/sec. Following irradiation simultaneous analysis was made for each element using the techniques described earlier in this work. It was found convenient to

analyse the two elements giving short-lived nuclides after irradiation on the day the samples were received i.e. copper and zinc. The cadmium and mercury, having longer half-lives could then be analysed on the following day without significant loss in analytical sensitivity due to nuclide decay

In Table 28 are shown the results obtained following analysis of powdered kale. In sample 4 the zinc and cadmium analysis was invalidated by extreme P^{32} contamination of the final precipitates caused by accidental transfer of the phosphate scavenge precipitate to the ion-exchange column. At the bottom of the table are shown the mean concentrations and ranges for each element as determined by other workers[10]. The figures in brackets after each elemental range indicate the number of determinations on which the results are based.

Table 28.

ANALYSIS OF POWDERED KALE FOR COPPER, ZINC, CADMIUM AND MERCURY

Sample	Copper content (p.p.m.)	Zinc content (p.p.m.)	Cadmium content (p.p.m.)	Mercury content (p.p.m.)
1	4.52	27.4	0.671	0.212
2	4.54	34.1	0.895	0.256
3	4.94	36.8	0.604	0.235
4	5.12	-	-	0.208
5	7.07	32.8	0.625	0.243
Comparative results from H.J.M. Bowen	4.81 ± 0.74 (88)	31.9 ± 4.8 (77)	0.69 ± 0.3 (8)	0.15 ± 0.01 (9) (0.012 by colorimetry)

The radiochemical purity of the copper, zinc and cadmium fractions was checked by following the decay of each sample using an end-window Geiger counter. A little P^{32} was found to be present in the cadmium precipitate from sample 2. As this sample showed an apparently higher

cadmium content than the others the P^{32} content of the measured activity has raised the measured figure slightly. On the other hand, the copper precipitate from sample 5 showed correct Cu^{64} decay so this result was regarded as valid and representing a higher copper content in the sample itself. The decay of the other samples was in accordance with the nuclide being measured.

As an additional check on the mercury analyses, two kale samples were analysed by the established analytical technique for mercury analysis of Smith[90]. These gave analyses of 0.218 and 0.246 p.p.m. which show good agreement with the results obtained using the ion-exchange separation of mercury during analysis.

In addition to kale, analyses for zinc and/or cadmium were made on other materials as shown in Table 29. Where possible a figure obtained from the literature is included for comparison. No indication of range of results is available except for teeth, since only a few measurements were made.

MATERIALS ANALYSED FOR ZINC AND CADMIUM

Material	Zinc content*	Literature value	Cadmium content	Literature value
Dried human liver	211	46 (wet tissue)	5.7	2.4 (wet tissue)
" " kidney	174	48 (")	150	32 (")
" " spleen	108	19 (")	0.80	0.7 (")
" " stomach	106	18 (")	0.88	0.4 (")
" " lung	46	14 (")	0.74	0.7 (")
Human enamel	364	276	0.03	not found
Serum from a patient with zinc phosphide poisoning.	5.98	1.4	-	-

* All figures are parts per million except serum which is $\mu g/ml$.

The tissue levels of zinc and cadmium are from work by Tipton[87], the zinc level in enamel from work by Soremark[96] and the serum zinc figure from a review by Bowen[11].

INSTRUMENTAL ZINC ANALYSIS

It was found possible to measure the zinc content of a large number of enamel samples by an activation analysis method which did not require a chemical separation prior to counting. By using a long cooling period after irradiation it was possible to measure the Zn^{65} photopeak at 1.11 MeV directly on a γ-spectrometer.

The samples were irradiated for 1 week at a neutron flux of $10^{12} n/cm^2/sec.$ along with a zinc standard. Afterwards they were allowed to decay for a period of 100 days so that the short-lived activities decay. In Figure 9 is shown the γ-spectra of a 40 mg. sample of human enamel made 9 and 100 days respectively after the end of a 1

week irradiation. In Figure 9 (a) the principal features of the spectrum are:-

1. A Zn^{65} photopeak at 1.11 MeV.
2. A Na^{24} photopeak at 1.37 MeV.
3. Gross P^{32} bremsstrahlung in the lower (< 1 MeV) end of the spectrum.

In Figure 9 (b) the Na^{24} photopeak ($t_{\frac{1}{2}}$ - 15.0 hours) has disappeared and the brehmsstrahlung effect from the P^{32}($t_{\frac{1}{2}}$ - 14.2 days) is greatly reduced. The spectrum above 0.5 MeV is essentially that of Zn^{65} ($t_{\frac{1}{2}}$ - 245 days) and the 1.11 MeV photopeak was used for the zinc estimation.

Method.

The active sample of tooth was placed on top of a 3" sodium iodide (Tl) crystal connected to a single channel pulse height analyser with the window set over the 1.11 MeV photopeak of Zn^{65}, i.e. from 1.00 to 1.25 MeV. The sample was counted for a suitable period and the zinc content calculated by comparison with the count-rate from an aliquot of the zinc standard irradiated with the sample.

Accuracy.

As a check on the accuracy of the instrumental method a series of samples analysed by two methods was compared. The samples analysed were sections of the inner enamel cut from sound extracted human teeth . One batch of these samples was analysed by the method mentioned above for zinc using a 1 hour irradiation. The other batch of results was obtained by the instrumental method. A comparison of the results obtained is given in Table 29.The weight of sample used is in the range 10 - 30 mgs. The results show a good comparison of the two methods with

reasonable agreement between the mean and median values. The spread of results is greater for the batch analysed by the instrumental method but this is not unexpected since more samples were analysed by this method.

TABLE 29

COMPARISON OF ZINC CONTENT OF INNER ENAMEL SECTIONS USING TWO METHODS OF ANALYSIS (RESULTS p.p.m. ZINC)

	Chemical Method	Instrumental Method
No. of samples analysed	16	60
Range (p.p.m.)	110 - 435	58 - 584
Mean value (p.p.m.)	255	236
Median value (p.p.m.)	224	209

SENSITIVITY

The sensitivities varied according to the experimental conditions chosen. The major factors are neutron flux and irradiation time which in turn depend on the isotope used. The limit of sensitivity was taken to be a sample with a count-rate equal to the background count rate of the detection instrument. The results are shown in Table 30.

TABLE 30

TRACE ELEMENT SENSITIVITIES FOR COPPER, ZINC, CADMIUM & MERCURY

Condition	Sensitivity (μg) at a neutron flux of 10^{12} n/cm^2/sec. Copper	Zinc	Cadmium	Mercury
1 hour irradiation	not used	0.01	0.1	not used
1 day irradiation	0.0005	0.1	0.01	not used
1 week irradiation	0.0005	0.1	0.001	0.0005
Instrumental Analysis	-	1.0	-	-

As can be seen from the table, the choice of irradiation period affected the sensitivity attainable for both zinc and cadmium. For maximum sensitivity of analysis for zinc a short irradiation of $\frac{1}{2}$ - 2 hours was required. Maximum sensitivity for cadmium analysis was obtained by irradiating for a week using Cd^{115}. The instrumental method of zinc analysis will often prove adequate for zinc determination in biological material. It has the advantage that a large number of samples may be analysed at a time since there is no problem of completing the analysis in a short-time interval, as the long-lived Zn^{65} is used. From experience in zinc analysis of dry human tissue from various organs the amount of zinc in a 50 mg. sample will be in the range 1 - 20 μg. which is readily detectable by this method. When tissue samples are analysed by this method some samples showed interference caused by Fe^{59} - a nuclide not in evidence in irradiated enamel. It was possible to calculate the amount of interference in zinc measurement by measuring the activity in a higher (1.29 MeV) Fe^{59} and from it calculating the amount of Fe^{59}

activity in the Zn^{65} photopeak range. When this was subtracted from the total activity in the Zn^{65} peak range the zinc content of the tissue could be calculated.

SUMMARY OF ANALYSIS

Samples and standards were irradiated for various time intervals from 1 hour to 1 week in a thermal neutron flux of $10^{12} n/cm^2/sec$. Digestion was made by heating in conical digestion flasks containing 2 ml. of a 1:1 mixture of 16 M nitric and 18 M sulphuric acids containing 10 mg. each of the various carrier elements. On dilution to 0.5 M with respect to hydrochloric acid a phosphate scavenge was made by the addition of 5 drops of ammonium phosphate solution (10% w/v) followed by 8 drops of zirconyl chloride solution (10% w/v). The supernate was filtered onto a strongly basic anion-exchange column in the chloride form and washed onto the column with 25 ml. of 0.12 M hydrochloric acid containing sodium chloride (10% w/v). Zinc, cadmium and mercury were retained on the column while copper was washed through without adsorption. The copper in this eluant was precipitated as the thiocyanate twice (with an intermediate ferric hydroxide scavenge) and precipitated and counted as copper quinaldate by the method described by Smith and Nixon[78].

Zinc was removed from the column by elution with 25 ml. of 2 M sodium hydroxide containing sodium chloride (2% w/v). Cadmium was removed with 50 ml. of 1 M nitric acid and mercury was eluted in 50 ml. of ethylenediamine solution (5% w/v).

After an iron hydroxide scavenge zinc was precipitated from a slightly acidic solution as the quinaldate by addition of 3 ml. of quinaldic acid solution (2% w/v). It was mounted and counted in the same manner as the copper quinaldate precipitates. 12 - 15 ml. of sodium hydroxide solution (20% w/v) was added to the cadmium fraction to precipitate cadmium hydroxi It was separated, dissolved in 0.5 M hydrochloric acid and precipitated

as the thiourea reineckate complex by addition of 8 ml. of saturated ammonium reineckate solution to the solution (which itself contained thiourea (1% w/v). The cadmium thiourea reineckate was transferred to aluminium planchets with ethyl alcohol (95% w/v) and after drying was counted using an end-window G.M. counter.

To the fraction from the column containing the eluted mercury was added 1 ml. sodium iodide solution (10% w/v) and copper sulphate solution (10% w/v). The copper ethylenediamine/mercuric iodide which was precipitated was washed and transferred to stainless steel planchets with isopropyl alcohol. The activity of the precipitate over the 77 KeV Hg^{197} photopeak was measured using a $1\frac{1}{2}$" sodium iodide (Tl) and a pulse height analyser. The elemental content of each sample was calculated by comparing the sample count-rate with the appropriate standard count-rate. The standard was irradiated and processed in an identical fashion to the sample. Corrections for background radiation, chemical recovery, counter dead-time and nuclide decay were made where appropriate

After a three month cooling period the zinc content of some teeth samples was determined by direct measurement of the Zn^{65} activity of an active sample using a $1\frac{1}{2}$" sodium iodide crystal and pulse height analyser. A zinc standard was used as a comparison standard for the calculation of the zinc concentrations.

MOLYBDENUM ANALYSIS

Literature Review

Bowen[12] has measured molybdenum in biological material using a precipitation technique and the isotope Mo^{99}. Meinke[73] used Mo^{101} in a solvent extraction method of molybdenum analysis. The former method is more sensitive because of the higher activation cross-section for Mo^{99} production. Van Zanten[114] used both isotopes in an analytical technique for molybdenum determination in plant material such as clover. The chemical separation used a solvent extraction of molybdenum by Tri-n-octylamine in kerosene. The sensitivity using the Mo^{101} isotope was found to be 0.1 μg. after a 15 minute irradiation at a thermal neutron flux of $1.4 \times 10^{12} n/cm^2/sec$. The sensitivity of activation analysis methods does not appear to be significantly greater than that obtainable by the most sensitive colorimetric analysis methods eg. in a technique developed for analysis of minimal amounts of molybdenum, Johnson[51] claims a sensitivity of 0.1 μg.

Molybdenum Isotopes.

On thermal neutron irradiation of natural molybdenum Mo^{93}, Mo^{99} and Mo^{101} are produced by (n, γ) reactions. In Table 31 are shown the activation data for each reaction and the relevant decay properties of the active product nuclides.

TABLE 31

MOLYBDENUM ISOTOPES PRODUCED BY THERMAL NEUTRON IRRADIATION.

Target Isotope	Natural Abundance %	Thermal Neutron Capture Cross-section (barns)	Product Half-Life	Decay
Mo^{92}	15.9	0.006	6.95 hrs.	γ 0.26 MeV 0.69 MeV 1.48 MeV
Mo^{98}	23.8	0.45	67 hours	β 1.23 MeV γ 0.74 MeV 0.14 MeV (Tc^{99m})
Mo^{100}	9.6	0.20	14.6 mins.	β 1.6 MeV γ(Multiple 0.31 MeV (Tc^{101})

As the reaction producing Mo^{99} gives the most sensitive method for molybdenum detection and analysis it was chosen for this work since molybdenum was not expected to be present in enamel in very large amounts.

Irradiation

Teeth samples were irradiated in BEPO (Harwell) at a thermal neutron flux of $10^{12} n/cm^2/sec.$ for periods ranging from 3 days to 7 days. The samples were weighed and wrapped in aluminium foil and irradiated along with a molybdenum standard in an aluminium irradiation can.

MOLYBDENUM STANDARDS

Two types of molybdenum standard were used - one a solid and one a molybdenum solution.

(a) Solid standard. About 1 mg. of SPECPURE molybdenum trioxide was weighed in a silica ampoule, heat sealed and irradiated with the samples

in a Harwell aluminium irradiation can. After irradiation the ampoule was opened and the molybdenum trioxide dissolved in 16 M nitric acid. The solution was diluted to 1000 ml. and 1 ml. processed and counted along with the samples.

(b) Solution. About 0.1 ml. volumes of solutions of ammonium molybdate (ANALAR) or molybdenum trioxide (SPECPURE) containing about 1.0 mg. of molybdenum were weighed and sealed in silica ampoules. After irradiation the ampoule was opened and the contents diluted to 1000 ml. 1 ml. of the dilute solution was used as a standard as for the solid standard.

CHEMICAL SEPARATION

Digestion. After irradiation samples were placed in 125 ml. conical beakers containing 3 ml. of 18 M sulphuric acid and 5 mg. of molybdenum carrier (as ammonium molybdate solution). They were heated on a hot-plate until fumes appeared and the matrix of the sample was destroyed. About 10-20 mg. of solid sodium nitrate was added to oxidise the charred organic material remaining. When the solution was clear it was allowed to cool and diluted to 30 ml. with water. When large samples ($>$ 100 mg.) of tooth were being analysed the solution after digestion was often found to contain precipitated calcium sulphate. When this was observed the solution was centrifuged and the precipitate discarded.

Molybdenum separation. In choosing a chemical separation for activation analysis of molybdenum it was considered that the major problem would be the separation of molybdenum from tungston. Tungsten activates easily under the irradiation conditions for molybdenum analysis - the thermal neutron activation cross-section for W^{187}($T_{\frac{1}{2}}$ - 24.0 hours) production is 9.7 barns[4].

Initial work in separation development aimed at removing tungsten

by a tungsten scavenge. It was known that molybdenum only formed a precipitate with tetraphenylarsonium chloride in neutral solution. It was shown by tracer experiments using Mo^{99} that molybdenum was not extractable from acidic or alkaline solutions by tetraphenylarsonium chloride into chloroform. Table 32 shows the amount of molybdenum extracted from various acid and alkaline solutions by 10 ml. of chloroform when an excess of tetraphenylarsonium chloride was present. 5 mg. of molybdenum carrier were always present. The Mo^{99} was estimated by β-particle detection using a Geiger counter accepting liquid samples.

TABLE 33

EXTRACTION OF MOLYBDENUM BY TETRAPHENYLARSONIUM CHLORIDE INTO CHLOROFORM FROM ACID AND ALKALINE SOLUTIONS

Aqueous conditions		Molybdenum extracted (%)
Hydrochloric acid	4.0 M	2.4
" "	2.0 M	0.3
" "	1.0 M	0.7
" "	0.5 M	1.8
" "	0.1 M	5.0
Sodium hydroxide	0.1 M	1.0
" "	0.05M	1.0
" "	0.01 M	4.3
" "	0.005 M	2.6
" "	0.001 M	4.1

It was also found using Mo^{99} tracer that tungsten carrier could be precipitated from acid solutions containing molybdenum as tungstic acid - H_2WO_4. By warming an acid solution containing molybdenum carrier and tracer and tungsten carrier (10 mg.) it was found that tungstic acid

slowly precipitated as a yellow precipitate and 80-90% of the molybdenum remained in solution. It was hoped to develop a separation as follows:-

(a) Sample digestion in acid solution.

(b) Precipitation of tungstic acid for active tungsten removal.

(c) Extraction by tetraphenylarsonium chloride into chloroform from acid solution. (This was to remove elements extractable by the reagent leaving molybdenum in solution.

(d) Final precipitation of molybdenum from neutral solution by tetraphenylarsonium chloride.

This method was discontinued because of the difficulty of precipitating molybdenum with the reagent from neutral solution. The precipitation was found to be very sensitive to pH and often small precipitates or none at all were obtained.

Use of Tc^{99m} daughter for measurement.

Mo^{99} ($t_{\frac{1}{2}}$ - 67 hours) decays partly via Tc^{99m} ($T_{\frac{1}{2}}$ - 6.0 hours). This parent/daughter relationship was thought suitable for use in a chemical separation using the Tc^{99m} for measurement rather than the Mo^{99} parent. If technetium is completely extracted under conditions where molybdenum is not it may then be re-extracted under the same conditions after a waiting period for Mo^{99}/Tc^{99m} re-equilibration. Since the conditions are unchanged, contaminant isotopes extractable with technetium will be removed with technetium in the initial extract leaving only radiochemically pure technetium in the second extract.

The time taken for equilibration of parent and daughter was estimated by obtaining a growth curve of Tc^{99m} from Mo^{99} from which the Tc^{99m} had been removed. Both rhenium and technetium are completely

removed from molybdenum in basic solution by extraction with tetraphenylarsonium chloride into chloroform[106]. A solution of 0.1 M sodium hydroxide containing Mo^{99} activity was twice extracted by the addition of 2 drops of tetraphenylarsonium chloride solution (2% w/v) and shaking with an equal volume of chloroform in a separating funnel. The Tc^{99m} was extracted leaving the Mo^{99} parent in the aqueous layer. The aqueous solution was placed on a $1\frac{1}{2}$" sodium iodide (Tl) crystal connected to a pulse height analyser. The window of the pulse height analyser was set to cover the 0.14 MeV Tc^{99m} photopeak and the activity over this period was automatically followed for a period of about two days. Figure 45 shows that after about 30 hours the growth of the Tc^{99m} was almost complete and the growth curve of the Tc^{99m} was changing to a decay curve of Mo^{99}. For practical purposes it was convenient, and sufficiently accurate, to allow equilibration to take place overnight. When the growth curve was extrapallated back to the time of extraction it could be seen that at that time there was 13.5% of the pre-separation activity over the Tc^{99m} photopeak region. The pre-separation activity was obtained by extrapallating the part of the curve representing equilibrated Mo^{99} decay back to extraction time. As described earlier (Table 33) no molybdenum is extracted under the conditions used. The residual aqueous activity could be explained in two ways:

(a) Incomplete technetium extraction and

(b) The residual activity after technetium extraction is the contribution by Mo^{99} to the total activity in the 0.14 MeV region. The decay scheme of Mo^{99} shows that of 5 γ-rays, it produces two at 0.140 and 0.181 MeV[102]. It was concluded on the basis of work on molybdenum extraction by cupferron,

described later, that the latter explanation was the correct one.

Initial Molybdenum Separation.

When attempts were made to separate Tc^{99m} from an acid solution of an activated tissue sample, inadequate radiochemical purity resulted. Extraction of Tc^{99m} into chloroform was made from dilute acid solutions, eg. 1 M hydrochloric acid, with tetraphenylarsonium chloride. Despite repeated washings of the chloroform extract, γ-counting did not show decay consistent with Tc^{99m}. From tooth samples little decay was observed over an 8 day counting period. No attempt was made to characterise this activity but it might possibly have been Zn^{65} as this is long lived ($T_{\frac{1}{2}}$ - 245 days), is produced by tooth irradiation in large amounts, and is extractable by the reagent from acid solution[111]. For this reason it was decided to make an initial separation of molybdenum from the bulk of radioactive nuclides produced after sample irradiation.

The reagent used was cupferron since it extracts molybdenum quantitatively from strong acid solutions[38]. Other elements known to extract under the experimental conditions used include titanium, vanadium, iron, copper, zirconium, niobium, tin, antimony, hafnium, tungsten, thorium, protoactinium and uranium[36]. Of these only copper, antimony, tungsten and perhaps iron are present in biological material in amounts which are detectable on activation under the conditions for molybdenum analysis. Using the reagent it is possible to separate molybdenum from the major induced activities. The amount of reagent required for molybdenum extraction was determined using Mo^{99} tracer. Equal amounts of tracer were added to separating funnels each containing 5 mg. of molybdenum carrier in 10 ml. of 1.8 M sulphuric acid. Various amounts of a freshly prepared solution of cupferron (5% w/v) were added.

The reagent which is unstable, was kept in a refrigerator in a dark bottle and was frequently changed. Two extractions using 10 ml. portions of chloroform, were made on each sample and the aqueous layers were counted in a Geiger counter accepting liquid samples. The amount of molybdenum left in the aqueous layer was estimated by comparison with an unextracted aliquot of tracer counted in the same manner. The results obtained are shown in Table 34.

TABLE 34

EXTRACTION OF MOLYBDENUM FROM SULPHURIC ACID SOLUTION BY CUPFERRON

Vol. of cupferron solution used (ml.)	Molybdenum left in aqueous layer (%)
0.25	34.0
0.50	2.6
1.0	1.5
2.0	0.2
3.0	0.3

As is noted by Freiser[36], technetium is not extracted under these conditions. This was confirmed by experiment using Mo^{99} tracer. Equal aliquots of Mo^{99} tracer were added to separating funnels containing 5 mg. molybdenum carrier in 10 ml. of 1.8 M sulphuric acid. 2 ml. of cupferron solution (5% w/v) were added and each solution was extracted with 10 ml. of chloroform to extract the molybdenum. Using a $1\frac{1}{2}$" sodium iodide crystal and a pulse height analyser set over the 0.14 MeV Tc^{99m} photopeak each fraction was counted after separation and compared with an equal volume of unseparated Mo^{99} tracer. The amount of activity found

in each fraction is shown in Table 35. On plotting a decay curve representing the change of activity with time in the acid fractions it was shown that the activity in these fractions was Tc^{99m}($T_{\frac{1}{2}}$ - 6.0 hours). The activities found in each fraction were corrected as shown for Tc^{99m} growth or decay as appropriate. It can then be seen that the total activity in aqueous and organic fractions after separation is in close agreement with the unseparated activity. Also the amount of activity extracted with molybdenum is the same fraction as was found earlier in the Tc^{99m} growth curve experiment for the 0.14 MeV Mo^{99} contribution to the total Mo^{99}/Tc^{99m} activity. This observation supports the conclusion that technetium was fully extracted by tetraphenylarsonium chloride and that only molybdenum was extracted by cupferron.

TABLE 35

CUPFERRON EXTRACTION OF Mo^{99}* FROM SULPHURIC ACID SOLUTION

	Sample 1	Sample 2	Standard
Initial activity c/s	93.7	93.0	94.4
Activity after separation c/s			
Aqueous	76.3 (80.8)	74.9 (79.4)	"
Organic	13.6 (14.4)	15.2 (16.1)	"
Corrected activity c/s			
Aqueous	80.2 (85.0)	78.0 (82.6)	"
Organic	11.5 (12.2)	12.8 (13.6)	"

* The bracketed figures are the percentage of the standard represented by the fraction.

Recovery of molybdenum from chloroform

The simplest and easiest way of recovering the separated molybdenum from chloroform after cupferron extraction was by shaking the chloroform layer with 1 M sodium hydroxide solution in a separating funnel. The molybdenum was extracted into the aqueous layer. It was shown that technetium could be extracted completely from this medium with tetraphenylarsonium chloride/chloroform. Although this was a simple and easy technique it was not used since the cupferron extracted with molybdenum by the sodium hydroxide interfered with the molybdenum recovery estimation at the end of the analysis. Most of the recovery estimations used required acid conditions and cupferron was found to precipitate the molybdenum when the solutions were made acid, so leading to erroneous recovery estimations.

A method which served to recover the molybdenum and destroy the cupferron was developed. The chloroform fraction containing molybdenum cupferrate was heated in a 125 tall conical beaker until all of the chloroform was volatilised. 1 - 2 ml. of 18 M sulphuric acid was added to the charred residue and heated until fumes of sulphur trioxide appeared Solid sodium nitrate was added to oxidise the organic material. When oxidation was complete the solutions were evaporated to dryness. A blue residue, molybdenum blue, was usually observed. On cooling this residue was dissolved in 1.0 M sodium hydroxide giving a clear colourless solution

Final Technetium separation.

Three carriers were considered for technetium for the final separation viz. permanganate, perrhenate, pertechnecate. Two separation conditions were investigated viz. acid and alkaline. Both conditions could be used because molybdenum is not extracted or precipitated by

complete from acid solution it was decided that the extraction would require to be made from alkaline solution. Both rhenium and technetium are completely extracted from alkaline solution by tetraphenylarsonium chloride[106]. It was not possible to use permanganate as a carrier for technetium in alkaline solution as it is only stable in acid solution. Rhenium was preferred as a carrier for technetium rather than ammonium pertechnecate. This was because it was possible to use sufficient rheni carrier to allow a gravimetric separation for recovery and counting estimations. The specific activity of the ammonium pertechnecate is so high that a gravimetric amount would give an unreasonably large count-rate if used for recovery estimations. If, on the other hand, a reasonable amount of Tc^{99} tracer was used as carrier the separation would have required to be by solvent extraction of technetium. While the Tc^{99m} γ-ray could be measured in solution, the recovery measurement of the soft β-particles from Tc^{99} would have presented a problem involvin solvent removal before counting.

The carrier found most suitable was, therefore, rhenium and the extraction conditions were from alkaline solution. After evaporation of the sulphuric acid used for cupferrate residue destruction, the residue was dissolved in 20 ml. of 0.5 M sodium hydroxide solution. 100μg. of rhenium carrier was added and the solution transferred to a separating funnel containing 2 drops of tetraphenylarsonium chloride solution (2% w/v). Extraction of the rhenium carrier and technetium activity was made with 2 volumes (15 ml. each) of chloroform. The organic layers were discarded and the aqueous layer transferred to a 50 ml. centrifuge tube containing 10 mg. of rhenium carrier (sodium perrhenate solution). This extraction step served to remove all con-

taminant activities extractable by the reagent.

The sample was allowed to stand for 18 - 24 hours to allow Mo^{99}/Tc^{99m} equilibration. 1 ml. of tetraphenylarsonium chloride solution (2% w/v) was added to precipitate the rhenium and technetium. Table 36 shows the weight of tetraphenylarsonium perrhenate obtained when varying volumes of reagent were used.

The solution was heated on a water-bath to coagulate the precipitate, centrifuged and the supernate retained for molybdenum recovery estimation. The precipitate was washed with water and, after centrifugation, the washings added to the solution for molybdenum recovery estimation. The precipitate was transferred to weighed aluminium planchets with iso-propanol dried under an infra-red lamp at 100°C and re-weighed. It was found to be soluble in acetone but insoluble in ethanol and isopropanol.

The validity of rhenium as a carrier for technetium was checked using Tc^{99} tracer. Equal amounts of Tc^{99m} tracer were added to a number of solutions of 0.5 M sodium hydroxide each containing 8 mg. of rhenium carrier and 2 drops of hydrogen peroxide solution (100 volumes). The rhenium was precipitated by addition of various volumes of tetra-phenylarsonium chloride solution (2% w/v) and the precipitates were washed with water and transferred to weighed planchets with iso-propanol. After drying the chemical yield of the rhenium was determined gravimetrically and the yield of the Tc^{99} tracer was made by measuring the sample activity. An anthracene β-scintillation crystal was used for measuring the 0.29 MeV Tc^{99} β-particles. The degree of absorption caused by the precipitate was determined by evaporating an equal amount of tracer onto an empty planchet and comparing the activity with a similar sample prepared with the

addition of inactive precipitate. The results obtained are shown in Table 36.

TABLE 36 COMPARISON OF TECHNETIUM AND RHENIUM RECOVERIES

Volume of reagent (ml.)	Precipitate weight (mg)	Precipitate activity (c/s)	Specific activity (c/s/mg.)
0.4	6.8	31.97	4.72
0.6	11.5	50.61	4.38
0.8	12.1	43.97	3.62
1.0	15.1	49.89	3.30
1.5	14.9	47.03	3.15
2	15.7	48.52	3.08
3	15.5	44.67	2.87
4	15.9	48.88	3.07
Tracer alone	-	71.53	-
Tracer plus precipitate	-	52.27	-

It can be seen from the results that the precipitate reduces the detection efficiency to about 72% of that found in the absence of precipitate absorption. It can be seen also that the chemical recoveries of rhenium are comparible with the radiochemical recoveries of technetium showing that rhenium is acting as a proper carrier. On calculating specific activities a self-absorption effect shows up. The precipitates obtained using insufficient reagent gave low chemical yields and hence less self-absorption of the tracer activity. This can be seen by the decrease in specific activity with the increase in chemical yield.

In an identical experiment two other effects were examined; (a) precipitation of Tc^{99} with rhenium in the absence of hydrogen peroxide (b) precipitation of Tc^{99} with rhenium in the presence of residues from molybdenum cupferrate digestion. It is stated[85] that it is advisable to have an oxidising agent present to prevent reduction of TcO_4^- to a lower non-extractable valence state by reagent impurities etc. Since the previous step in the radiochemical separation introduces residues following digestion of molybdenum cupferrate it was necessary to establish that they did not interfere with the precipitation of the technetium. Four samples were prepared as in the full chemical separation for molybdenum and the residues after molybdenum cupferrate digestion added to the solutions containing Tc^{99} tracer and rhenium carrier. Precipitations were made in the presence and absence of hydrogen peroxide. 2 ml. of tetraphenylarsonium chloride were used throughout to precipitate the rhenium and technetium. The results are shown in Table 37.

TABLE 37

EFFECT OF HYDROGEN PEROXIDE AND MOLYBDENUM CUPFERRATE DIGESTION RESIDUES ON TECHNETIUM AND RHENIUM PRECIPITATION

Sample	Presence of peroxide	Precipitate weight (mg.)	Precipitate activity (c/s)
Normal	No	15.5	51.36
"	"	14.5	48.19
"	"	15.0	51.21
"	"	15.2	45.33
Plus residues	Yes	16.5	53.20
" "	"	14.6	50.26
" "	No	16.1	51.16
" "	"	15.7	51.49

It can be seen by comparison with the results in Table 36 obtained in the presence of hydrogen peroxide that under the experimental conditions used here no reduction of recovered technetium has occurred and therefore the presence of peroxide would not seem essential. The reduction of technetium would have resulted in low activity measurements. The presence of molybdenum cupferrate digestion residues does not appear to affect technetium recovery.

Molybdenum recovery estimation

Three methods were tried (a) gravimetric recovery using oxine (b) colorimetric recovery using molybdenum thiocyanate[45] (c) colorimetric recovery using molybdenum ethyl xanthate[82].

(a) Ammonium hydroxide (1 M) was added to acid solutions containing 10 mg. of molybate carrier and bromo-thymol blue indicator until the equivalence point was reached. 0.1 gm. of ammonium acetate was

added as a buffer followed by a solution (5% w/v) of the reagent (oxine) in 1 M acetic acid until the supernate remained yellow. On weighing the separated molybdenum oxinate precipitates recoveries ranging from 100 - 180% were observed. The method was not investigated further as a colorimetric recovery estimation was thought to be a better technique since there were serious dangers of low chemical yields due to mechanical losses in any gravimetric recovery before the final weighing.

(b) It was found possible to produce a red-brown thiocyanate colour in an aqueous solution similar to that left following the final technetium precipitation. 5 ml. of 11M hydrochloric acid, 3 ml. of potassium thiocyanate solution (20% w/v) and 0.25 ml. of stannous chloride solution (40% w/v) in 5 M hydrochloric acid) were added and the mixture diluted to 100 ml. A stable colour was obtained about 1 hour after mixing and it was possible to estimate the molybdenum content of the solution by measuring the absorbance with a colorimeter using a Kodak No. 3 filter. The absorption maximum of the thiocyanate colour was found to be at 525 mμ. Although this was a very easy and convenient method of molybdenum recovery estimation it could not be used due to residual tetraphenylarsonium chloride which caused a precipitate to form with the thiocyanate. This led to cloudy solutions and colour instability.

(c) A violet coloured precipitate was formed in slightly acid solution between molybdenum and potassium ethyl xanthate. This colour was chloroform extractable and had an absorbance maximum at 507 mμ. The visible spectrum of the colour in chloroform solution was measured

using a UNICAM SP 800 spectrometer fitted with a chart recorder and the spectrum is shown in figure 46. The colour appeared stable over 48 hours and repeat absorbance measurements shortly after extraction gave the values shown in Table 38. Absorbance measurements were made in 5 mm. cells in a Spekker colorimeter using a Kodak No. 4 filter (maximum transmittance 522 mμ.).

TABLE 38.

CHANGE OF ABSORBANCE OF XANTHATE/MOLYBDENUM COLOUR WITH TIME

Time from extraction (minutes)	Absorbance at 522 mμ
6	0.551
7	0.561
9	0.568
11	0.567
13	0.569
15	0.567

The method used for colorimetric recovery measurement for molybdenum was as follows:

The aqueous solution after final rhenium precipitation was diluted to 100 ml. and a 10 ml. aliquot taken for recovery measurement. This was placed in a 100 ml. separating funnel containing 10 ml. of chloroform. The pH of the solution was adjusted to about pH2 with 2 M hydrochloric acid solution and 1 ml. of potassium ethyl xanthate solution (10% w/v) added. The red/purple precipitate formed was extracted into the chloroform and the chloroform layer collected in a 20 ml. calibrated stoppered tube. The aqueous layer was extracted

with a further portion of chloroform and this, with washings, was added to the stoppered tube. When no further colour could be extracted the chloroform extracts were diluted to 20 ml. and the absorbance of the solution measured against a chloroform blank in 5 mm. cells using a colorimeter and a Kodak No. 4 filter.

A calibration curve of absorbance against molybdenum content of a 10 ml. aliquot was obtained by taking known amounts of a standard solution of molybdenum, producing and measuring the colour with potassium ethyl xanthate. In figure 47 is shown the calibration curve obtained for the range 0.1 - 1 mg. of molybdenum. It can be seen that Beer's Law is obeyed. The molybdenum content of a solution after analysis was usually found to be around 4 mg.

Technetium activity measurement

Tc^{99m} is a pure γ-emitter with a single photopeak at 0.14 MeV. It was found that detection efficiency using an end-window Geiger counter was only about 10% of the efficiency obtained using a $1\frac{1}{2}$" sodium iodide (Tl) crystal and a spectrometer set over the photopeak region. The γ-counting technique was used as the higher efficiency seemed a greater advantage in analysis than the lower background of the Geiger counter.

RADIOCHEMICAL PURITY

The radiochemical purity of the separated Tc^{99m} from active tissue samples was determined in two ways.

1. By obtaining a γ-spectrum using a 3" sodium iodide (Tl) crystal and a Laben 512 - channel multi-channel analyser.

2. By plotting the decay curve obtained by γ-counting using a single channel analyser with the window set over the 0.14 MeV photo-

peak region.

In figure 48 is shown the γ-spectrum obtained from the Tc^{99m} extract finally obtained after a molybdenum separation from a 30 mg. sample of dry human liver irradiated for 3 days at $10^{12} n/cm^2/sec$. Only a 0.14 MeV Tc^{99m} photopeak appears in the spectrum. In figure 49 is shown the decay curves over the 0.14 MeV part of the γ-energy spectrum obtained from Tc^{99m} activity separated from a 90 mg. sample of liver and a 601 mg. sample of a human tooth after a 3 day irradiation at $10^{12} n/cm^2/sec$. The measured activity was corrected for background radiation. The half-life of the activity from the liver sample was graphically found to be 5.9 hours. This is consistent with Tc^{99m} ($T\frac{1}{2}$ - 6.0 hours) and the decay can be seen to be linear right down to near background level. The amount of activity isolated from the tooth sample can be seen to be much smaller than from the liver but nevertheless it appeared to decay correctly.

PRECISION AND ACCURACY OF ANALYSIS

Analysis of known amounts of molybdenum

Silica ampoules containing known amounts of molybdenum in the form of a solution of 'SPECPURE' molybdenum trioxide were irradiated and analysed with a molybdenum standard. The ampoules were prepared by pipetting various volumes of the molybdenum trioxide solution into weighed ampoules which were re-weighed and then sealed in an oxygen flame. The agreement found between the actual molybdenum used and that found by analysis is shown in Table 39.

ANALYSIS OF KNOWN AMOUNTS OF MOLYBDENUM

Molybdenum content of sample (µg.)	Measured value (µg.)
0.0935	0.106
0.287	0.292
0.589	0.570
0.825	0.828
1.095	1.050
1.320	1.25

The average discrepancy between the actual and the measured value was found to be 3.5%.

Biological Materials. Replicate analyses were made on samples of powdered kale[3] weighing 100 - 150 mg. to test the reproducability and accuracy of the method on a homogeneous sample of biological material. The results obtained are listed in Table 40.

TABLE 40

REPLICATE ANALYSIS ON POWDERED KALE

	Molybdenum content p.p.m.
Analytical results	2.33, 2.48, 2.56, 2.60, 2.67, 2.69, 2.80
Arithmetic mean	2.59 ∓ 0.23
Literature value[10]	2.33 ∓ 0.47

The reproducability is not much better than ∓ 10%. The scatter of values falls within the range of values reported by other workers so the analysis is at least as reproducable as other techniques. The

kale may, of course, not be thoroughly homogeneous with respect to molybdenum content and this could account for the degree of reproducability found.

Some other materials were analysed in addition to kale. These included dried human liver, dried parsley and human teeth. The figures for the teeth are discussed in the section on results of trace element measurement in teeth. The analyses for liver and parsley gave the figures shown in Table 41.

TABLE 41

MOLYBDENUM ANALYSIS OF LIVER AND PARSLEY

Material	Molybdenum content (p.p.m.)
Liver	1.68, 1.87, 1.97, 2.02, 2.03, 2.07, 2.08, 2.49, 2.57
Parsley	1.03, 1.50

The arithmetic means of the analyses for liver and parsley are 2.08 ± 0.5 p.p.m. and 1.27 ± 0.23 p.p.m. respectively. Literature figures available for liver are 5 p.p.m.[101] (dry tissue) and 1.13 $\mu g/gm$[87] (wet tissue). The results obtained by activation are rather lower than these. This may be because the sensitivity of the activation analysis is greater than that of the other techniques, such as colorimetry, used in molybdenum determinations.

HIGH FLUX IRRADIATION

In an attempt to increase the analysis sensitivity some enamel samples were irradiated at Harwell in a thermal neutron flux of $10^{14} n/cm^2/sec.$ for three days. Samples and standard were packed in a silica ampoule 2.5 cm. in length and 3 mm. internal diameter. The molybdenum standard was 0.2 mg. of SPECPURE molybdenum trioxide and was enclosed in the ampoule in a sealed silica capillary. The enamel

samples were separated from each other in the ampoule by layers of silica wool.

The chemical separation and counting procedure used for these samples was the same as that used in irradiations at $10^{12} n/cm^2/sec$. Due to the high dose rate from the active samples the initial part of the chemical procedure was carried out behind lead bricks. The radiochemical purity of the separated samples was checked by decay measurements on the 0.14 MeV photopeak of the separated Tc^{99m} and was found to be satisfactory.

SENSITIVITY OF ANALYSIS

The sensitivity of analysis was taken as being the amount of molybdenum which would give, on irradiation, an amount of activity equal to the background of the detector used. The sensitivity found after an irradiation of 1 week was 0.01 μg. After the high flux irradiation it was found that the sensitivity could be improved to 0.000 μg.

CALCULATION

The measured count-rates over the Tc^{99m} photopeak were corrected for background radiation. A decay correction for Tc^{99m}($T_{\frac{1}{2}}$ - 6.0 hours) was made. As the precipitation of technetium from molybdenum was made at the same time for all the samples no correction for Mo^{99} decay was necessary. Correction for technetium recovery as determined by tetraphenylarsonium perrhenate precipitate weight and molybdenum recovery as determined on the colorimeter were made.

The amount of molybdenum in a sample was made by comparison of the corrected count-rates of sample and standard.

SUMMARY OF ANALYSIS

Samples and standard were irradiated usually for three days at a thermal neutron flux of $10^{12} n/cm^2/sec$. After irradiation a sample was dissolved in 3 ml. 18 M sulphuric acid containing 5 mg. of molybdenum carrier. Organic charring was removed by addition of solid sodium nitrate (20 mg.). On cooling, the solution was diluted to 30 ml. and molybdenum cupferrate extracted into about 15 ml. of chloroform after addition of 2 ml. of a solution of cupferron (5% w/v). The chloroform was evaporated by heating in a 125 ml. tall conical flask and the residue digested as for the primary sample digestion. After digestion was complete the sulphuric acid was evaporated by heating on a hot-plate until a blue residue remained. The residue was dissolved in 10 ml. of 1 M sodium hydroxide and 100 μg. of rhenium carrier added. The solution was transferred to a separatory funnel, 2 drops of tetraphenylarsonium chloride solution (2% w/v) added and shaken with 15 ml. of chloroform. 10 mg. of rhenium carrier was added to the aqueous layer in a 50 ml. centrifuge tube and the solution allowed to stand for at least 18 hours. 2 ml. of tetraphenylarsonium chloride solution (2% w/v) were added and the solution heated on a water-bath. The precipitate was centrifuged off and the supernate plus washings saved for molybdenum recovery estimation. The precipitat was washed and transferred to a weighed aluminium planchet with iso-propanol. It was dried under an infra-red lamp and weighed as tetraphenylarsonium perrhenate to give the chemical recovery of the technetium daughter. The activity of the precipitate was determined by measurement over the 0.14 MeV photopeak of Tc^{99m} using a sodium

iodide (Tl) crystal and a pulse height analyser.

The chemical recovery for molybdenum was determined by taking 10 ml. of the supernate from perrhenate precipitation (after diluting to 100 ml. with water). The aliquot was acidified with 2 M hydrochloric acid to pH2 and 1 ml. of potassium ethyl xanthate solution (10% w/v) was added. The purple compound between the reagent and molybdenum was extracted into 20 ml. of chloroform and the absorbance at 520 mμ. measured using a colorimeter. The measured absorbance was proportional to the chemical recovery of molybdenum.

A standard amount of active molybdenum was processed and counted as for the sample. The measured activities of samples and standard were corrected for background radiation, Tc^{99m} decay, technetium recovery and molybdenum recovery. The amount of molybdenum in a sample was determined by comparison of the corrected count-rates of sample and standard.

C H A P T E R IV

RESULTS

RESULTS

Vanadium

No evidence of vanadium was found in over twenty analyses of sections of sound human teeth varying in sample size from 100 - 500 mg. It was concluded that the level of vanadium in teeth must be $<$0.01 p.p.m. otherwise it should have been observed. The use of larger samples was considered to be of little value and was omitted.

Manganese

Samples for analysis were from sound, human teeth extracted for orthodontic reasons. The teeth were transversely sectioned into an occlusal, a middle and a gingival layer. Using a carborundum cutting disc and a micrometer gauge the enamel from the gingival and middle layers was sectioned into inner and outer sections of equal thickness. The occlusal layer enamel was not sectioned into outer and inner layers but analysed by itself. The results obtained from the analysis of 13 teeth are shown in Table 42.

TABLE 42

MANGANESE CONTENT OF ENAMEL (p.p.m. MANGANESE)

Sample	Tooth	Age	Sex	Occusal Layer	Middle Layer		Gingival Layer	
					Outer Enamel	Inner Enamel	Outer Enamel	Inner Enamel
1	$\lfloor\underline{4}$	12	M	0.85	0.66	0.55	1.32	0.69
2	$\overline{5}\rfloor$	53	F	0.85	0.86	0.56	0.69	0.45
3	$\lceil\overline{8}$	25	F	1.25	2.01	0.60	1.29	0.60
4	$\lceil\underline{4}$	11	F	0.72	0.97	0.66	1.38	0.49
5	$\overline{4}\rfloor$	11	F	0.74	1.12	0.51	1.22	0.54
6	$\lceil\overline{8}$	27	M	1.24	1.29	0.46	0.79	0.76
7	$\overline{8}\rfloor$	26	M	1.74	0.76	0.58	0.92	0.61
8	$\overline{8}\rfloor$	19	M	1.07	0.80	0.64	0.35	0.39
9	$\overline{3}\rfloor$	12	M	1.10	0.76	0.71	Insufficient Material	
10	$\lfloor\underline{3}$	31	M	1.61	1.34	0.79	0.90	0.56
11	$\lfloor\underline{5}$	11	F	0.73	1.10	0.55	0.48	0.49
12	$\lceil\overline{5}$	11	F	0.65	0.91	0.43	0.71	0.30
13	$\lceil\overline{8}$	18	F	0.45	0.90	0.34	1.43	0.42

Samples 4, 5 and 11, 12 are paired teeth i.e. premolars extracted from opposite corners of the same mouth. It can be seen that there is a close relationship between the manganese content of the enamel in these samples. However, repeat analyses made (Table 43) on different specimens of the same sample show that there can be considerable variation in the manganese content of a single sample.

TABLE 43

REPEAT ANALYSES OF ENAMEL FOR MANGANESE

Sample	Manganese Content (p.p.m.)
1	(a) 0.83 (b) 0.89
2	(a) 0.54 (b) 0.42
3	(a) 3.44 (b) 2.31 (c) 2.17 (d) 1.74

In samples 1 and 2 the variation is small enough to be within experimental error but in sample 3 the differences are too large and must represent genuine variations in the manganese content. Sample 9 was an unerupted tooth. There did not appear to be as great a difference between the manganese content of the outer and inner enamel in the middle layer as in the erupted teeth but more samples would require to be analysed to establish whether or not this was a constant feature. If this was found to be the case it would suggest that there was a post-eruptive gain of manganese from sources such as foodstuffs, tooth-pastes or restoration materials.

Cadmium.

Little cadmium activity could be found in any of the samples for which cadmium analysis was made. It was estimated that the amount of cadmium in the teeth analysed was less than 0.03 p.p.m.

ZINC

Analysis of enamel sections from 27 sound teeth was made. The samples were sectioned in the same manner as for manganese analysis. Seven of the teeth were analysed by the method of activation analysis

using Zn^{69} and a full radiochemical separation. The remaining twenty samples were analysed by the non-destructive instrumental technique using Zn^{65}.

In Table 44 are shown the results from both types of analysis. Samples 1 - 20 were analysed by the instrumental method and samples 21 - 27 were analysed by the chemical separation method. Samples 11/12 16/17, 18/19 and 22/23 are paired teeth i.e. from opposite quadrants in the same mouth. Sample 20 is an unerupted tooth. As found in manganese a greater concentration is usually found in the outer layers of enamel than in the inner layers. This gradient of zinc can be seen to occur in 48 samples of outer/inner enamel out of 54 analysed. It can be seen to be present in the unerupted tooth analysed. This does not support the hypothesis of elemental uptake after eruption from food stuffs and etc. suggested as a possible explanation of the higher results in the outer enamel. The close relationship observed on analysis of paired teeth for manganese does not seem to be paralleled in the zinc results. Indeed the overall variation of results found for zinc seems greater than that observed for manganese. (Table 45).

TABLE 44

ZINC CONTENT OF ENAMEL SECTIONS (p.p.m. ZINC).

Sample	Sex	Age	Tooth	Occlusal Layer	Middle Layer		Gingival Layer	
					Outer	Inner	Outer	Inner
1	M	24	$\overline{\lfloor 8}$	773	1070	303	1550	352
2	F	24	$\overline{\lfloor 8}$	475	362	387	425	425
3	M	22	$\overline{8\rfloor}$	406	681	267	775	584
4	M	32	$\overline{8\rfloor}$	359	725	344	626	440
5	M	27	$\overline{\lfloor 8}$	384	774	214	238	247
6	M	18	$\overline{\lfloor 8}$	546	213	251	421	251
7	F	31	$\overline{8\rfloor}$	530	711	196	244	155
8	M	21	$\overline{\lfloor 8}$	762	245	163	392	58
9	F	25	$\overline{\lfloor 8}$	551	496	197	564	201
10	F	27	$\overline{8\rfloor}$	476	301	226	217	267
11	F	21	$\overline{8\rfloor}$	820	387	149	866	151
12	F	21	$\overline{\lfloor 8}$	177	358	167	294	159
13	F	24	$\overline{\lfloor 8}$	385	309	309	471	216
14	F	29	$\underline{8\rfloor}$	407	339	180	332	210
15	M	23	$\overline{8\rfloor}$	352	429	206	441	246
16	M	32	$\underline{3\rfloor}$	322	363	208	493	145
17	M	32	$\underline{\lfloor 3}$	310	441	192	432	192
18	M	17	$\overline{8\rfloor}$	511	492	164	455	206
19	M	17	$\overline{8\rfloor}$	364	291	207	273	182
20	F	22	$\overline{\lfloor 8}$	344	463	183	387	234
21	F	25	$\overline{\lfloor 8}$	327	504	165	655	187
22	F	29	$\overline{\lfloor 8}$	437	566	125	328	192
23	F	29	$\overline{\lfloor 8}$	419	458	435	479	322
24	F	18	$\overline{\lfloor 8}$	371	302	248	580	366
25	F	27	$\overline{\lfloor 8}$	271	520	182	289	110
26	M	15	$\overline{8\rfloor}$	362	800	216	471	131
27	F	27	$\overline{\lfloor 8}$	-	336	231	992	379

TABLE 45

COMPARISON OF RANGE AND MEANS OF ZINC AND MANGANESE ENAMEL FIGURES

	Manganese (p.p.m.)			Zinc (p.p.m.)		
Enamel Section	Mean	Range	Maximum variation from mean (%)	Mean	Range	Maximum variation from mean
Occlusal layer	1.00	0.45-1.74	74	441	177-820	86
Middle (outer)	1.04	0.66-2.01	93	479	213-1070	123
Middle (inner)	0.57	0.34-0.79	40	227	149-387	71
Gingival (outer)	0.96	0.35-1.43	64	507	217-1550	196
Gingival (inner)	0.53	0.30-0.76	43	245	58-584	138

Two experiments were made to try and find an explanation for the unusually high zinc content of outer enamel sections sometimes found. The suggested explanations were:

(a) that the surface of the enamel is exceptionally rich in zinc and hence small samples consisting mostly of surface enamel would give a higher result than usual.

(b) that the samples which gave high results were from a tooth in close contact in the mouth with a tooth containing a zinc oxide based restoration lining.

The sensitivity of the analytical method was sufficiently good to allow analysis of 1 mg. samples. To test the first suggestion transverse sections of a healthy tooth were taken and thin slices at different depths from the enamel surface and different parts of the tooth were obtained by grinding away the unwanted sections. The thickness of the sections was in the range 0.05-0.08 mm. Three depths of enamel were used - an outer, middle and inner section. The results from the zinc analysis are shown

in Table 46.

TABLE 46

SINGLE TOOTH SECTIONAL ANALYSIS FOR ZINC

Section	Zinc content (p.p.m.)
Outer	202, 219, 242
Middle	131
Inner	59, 113, 125

It can be seen that no high outer enamels were observed - in fact the zinc content of the whole tooth can be seen to be generally low on comparison with the results in Table 44.

The second suggested explanation was tested by sectional analysis of two teeth samples which had been situated next to a tooth containing a zinc oxide lining in a filling. Two specimens of each sample were analysed. The results are shown in Table 47.

TABLE 47

ZINC CONTENT OF ENAMEL FROM TEETH ADJACENT TO A TOOTH CONTAINING ZINC OXIDE

Sample	Zinc content (p.p.m.)
Tooth A - Outer Enamel	(a) 219 (b) 220
Tooth A - Inner Enamel	(a) 119 (b) 187
Tooth B - Outer Enamel	(a) 262 (b) 260
Tooth B - Inner Enamel	(a) 288 (b) 271

The replicate analyses of the outer enamel sections show close agreement but not high zinc figures. Only the B sample gave a similar

result for replicate analyses of the inner enamel. The B sample did not show the higher zinc content of the outer enamel usually found. The zinc content of both layers was quite similar.

ANTIMONY

Analysis was made of enamel sections from 10 healthy teeth from patients in the Glasgow area. The teeth were sectioned in the same manner as for zinc and manganese analysis. The results obtained are shown in Table 48.

TABLE 48 ANTIMONY CONTENT OF GLASGOW TEETH ENAMEL (p.p.m.)

Sample	Occlusal Layer	Middle Layer		Gingival Layer	
		Outer Enamel	Inner Enamel	Outer Enamel	Inner Enamel
1	-	0.665	0.037	0.016	0.044
2	-	0.026	0.379	0.079	-
3	-	0.044	0.021	0.065	0.016
4	-	0.037	0.058	-	0.015
5	-	0.011	0.005	0.010	0.020
6	-	0.019	0.048	0.074	0.243
7	0.005	0.019	0.054	0.091	0.099
8	0.139	0.464	0.030	0.282	0.121
9	0.010	0.054	0.034	0.071	0.043
10	0.011	0.019	0.095	0.028	0.060

There does not appear to be any constant differences between the antimony content of the sections analysed. Sample 8 seems generally to have much more antimony in it than the other samples. The mean of all the analyses is 0.085 p.p.m. The median value is 0.044. The latter is probably a more representative figure since the mean is significantly influenced by the few high results.

The reproducability of the antimony analysis was checked by replicate analysis of powdered kale. The results obtained for the antimony content were 0.054, 0.056, 0.060, 0.062, 0.065 p.p.m. The mean is 0.059 and the standard deviation 0.003 (5.1%). These results compare well with those of other workers[10] viz. 0.082 $\pm$ 0.02 p.p.m.

Samples of teeth were obtained from a dental hospital in Alexandria, Egypt. Some of the patients had a history of antimony treatment for the tropical disease bilharziasis prevalent in Egypt and Central African countries. Sections of enamel from the teeth of these patients were analysed for antimony to discover whether the enamel from patients treated with antimony was higher than that of untreated patients. The results are shown in Table 49.

TABLE 49 ANTIMONY CONTENT OF ENAMEL FROM EGYPTIAN TEETH (p.p.m.)

Untreated Patients				Treated patients			
Age	Sex	Tooth	Antimony content	Age	Sex	Tooth	Antimony conte
19	F	$\lfloor 5$	0.055	41	M	$\overline{5}\rceil$	0.080
15	F	$\underline{6}\rceil$	0.027	30	F	$\overline{6}\rceil$	0.091
15	F	$\overline{6}\rceil$	0.012	15	M	$\lfloor \underline{5}$	0.19
23	F	$\lfloor \underline{6}$	0.018	50	M	$\overline{7}\rceil$	0.058
23	F	$\lceil \overline{6}$	0.096	30	-	$\overline{6}\rceil$	0.19
28	F	$\underline{8}\rceil$	0.026	22	M	$\overline{6}\rceil$	0.043
20	F	$\lceil \overline{6}$	0.039	27	M	$\overline{7}\rceil$	0.034
22	M	$\overline{6}\rceil$	0.022	40	F	$\overline{6}\rceil$	0.025
50	F	$\overline{6}\rceil$	0.027	25	M	$\lceil \overline{8}$	0.024
45	F	$\underline{6}\rceil$	0.017				
Mean			0.034	Mean			0.082

No consistent difference between treated and untreated patients was

observed. The treated patients' results show overall a higher trend but further samples would require to be analysed to show if this was significant. Greater control of the experiment would be required with respect to the number of injections given during treatment, - the doses an drugs used and the period of treatment relative to the age of the patient when the tooth was extracted. The results of the enamel analysis of these teeth do not seem to differ greatly from the results obtained for British teeth shown in Table 48. The mean of the untreated samples is 0.034 p.p.m. and the median is 0.026 p.p.m. This is slightly lower than the corresponding British figures and may be attributable to a higher antimony dietary intake due to the higher degree of industrial contamination found in Glasgow relative to Alexandria.

MOLYBDENUM

The sensitivity obtainable at the normal neutron flux of 10^{12}n/cm^2/se was not good enough to permit analysis of enamel samples from individual teeth. Instead whole teeth minus the root or samples of the crown were analysed at this flux to find the general level at which molybdenum occurs in teeth. Some analysis of enamel from a single tooth were made using fluxes of 4×10^{12}n/cm^2/sec and 10^{14}n/cm^2/sec. Smaller samples could be analysed at the higher fluxes because the sensitivity was increased. The results found for molybdenum are summarised in table 50.

TABLE 50

MOLYBDENUM CONTENT OF HUMAN TEETH

Sample	Weight (mg)	Molybdenum content (p.p.m.)	Thermal Neutron Flux ($n/cm^2/sec$)
Crown	378	0.042	1.2×10^{12}
"	265	0.028	" "
"	56	0.065	" "
"	601	0.026	" "
Outer Enamel	68	0.043	4×10^{12}
Inner Enamel	34	0.12	" "
Enamel	23.0	0.073	10^{14}
"	21.4	0.049	"
"	17.3	0.054	"
"	11.8	0.063	"
"	11.8	0.075	"
Powdered Tooth	250	0.14	1.2×10^{12}

The mean of the results for the samples of enamel and crown analysed is 0.048 p.p.m. The powdered tooth result may be slightly high due to contamination of the material by the steel mortar and pestle used in grinding the powder. The results from the high flux determinations are slightly higher than those obtained at a flux of $1.2 \times 10^{12} n/cm^2/sec$. It is possible that some molybdenum may have been introduced into the sample by the cutting process using a carborundum disc. The samples analysed at the lower flux were obtained by chipping the specimens from

whole teeth. The molybdenum content of carborundum has been estimated by neutron activation analysis to be around 1 - 2 p.p.m.[68]

COPPER

Analyses were made on samples of calculus obtained from dental patient whose teeth had deposits on them. The samples were divided where possible into a sub-gingival and a supra-gingival specimen. The results of copper analyses are shown in Table 51.

TABLE 51

COPPER CONTENT OF CALCULUS SAMPLES

Sample	Copper Content (p.p.m.) Sub-Gingival	Supra-Gingival
1	15.0	31.1
2	8.40	12.1
3	19.3	4.1
4	35.7	8.01
5	9.64	4.76
6	8.46	14.4
7	14.0	13.1
8	20.9	8.59
9	16.4	24.5
10	14.0	3.39

There does not appear to be any correlation between the copper content of the calculus samples in contact with the saliva (supra) and those below the gum margin (sub). The copper content of saliva has been estimated as being in the range 10.0 - 47.5 mg/100 ml.[33]

Copper content of rat's teeth

A number of rats were selected belonging to a strain having a fast rate of incisor growth. They were divided into four groups of three rats each, Group 1 being a control group. The aim of the experiment was to demonstrate copper/molybdenum antagonism. Daily injections of solutions of copper and molybdenum salts were given peritoneally to the rats for a five week period. The doses given to the rats were varied as follows:

Group 1. 0.0002 mg. molybdenum daily.

Group 2. 0.0002 mg. molybdenum and 0.0005 mg. copper daily.

Group 3. Weekly increasing daily doses of molybdenum from 0.0002 - 0.0016 mg. and daily doses of copper 0.0005.

Group 4. Weekly increasing daily doses of copper from 0.0002 - 0.016 mg. and daily doses of molybdenum 0.0002 mg.

A sample weighing about 1 mg. was taken from the tip of both incisor teeth of each rat every week and saved for analysis. The results from copper analysis are shown in Table 52.

TABLE 52. COPPER CONTENT OF RAT'S TEETH (Results p.p.m. copper)

	Group 1			Group 2			Group 3			Group 4		
Rat No.	1	2	3	1	2	3	1	2	3	1	2	3
Week 1	8.5	-	8.5	9.2	4.4	-	6.8	13.7	13.4	18.1	7.4	7.7
Week 2	7.7	8.3	18.6	18.2	16.5	6.5	14.9	7.8	9.3	14.3	18.4	6.6
Week 3	3.8	2.6	1.1	1.0	1.0	-	0.84	1.3	-	1.9	0.82	1.8
Week 4	3.1	2.2	3.4	2.6	2.8	2.7	1.4	1.5	1.5	2.9	2.4	4.4
Week 5	-	0.83	1.6	1.6	1.2	1.6	1.4	1.2	1.6	1.7	2.8	4.3
Week 6	2.0	1.8	1.8	1.7	2.3	1.0	1.7	2.8	3.4	2.2	2.2	2.6

The results on samples taken during weeks 3 - 6 can be seen to lie in the range 1 - 3 p.p.m. Those from week 1 and 2 (in particular, week 2) can be seen to be several times higher. Group 3, Rat 1 shows an exceptionally high result on the sample taken in week 2. This result and the higher ones raises the question whether the early high results represent a general rise in the copper level of the rat's enamel following injection or whether the samples have been contaminated with copper prior to analysis. The experiment was repeated in duplicate but results were only obtained from the samples taken during weeks 1 and 2 because of the accidental presence of P^{32} contamination in the final copper quinaldate precipitate containing the Cu^{64} being measured. The results are shown in Table 53.

TABLE 53.

COPPER CONTENT OF RATS TEETH (DUPLICATE EXPERIMENT)

copper p.p.m.

	Group 1			Group 2			Group 3			Group 4		
Rat No.	1	2	3	1	2	3	1	2	3	1	2	3
Week 1	3.2	1.7	-	5.9	1.9	1.6	-	2.0	3.2	3.0	2.9	3.1
Week 2	2.2	4.5	4.1	1.6	8.9	4.1	4.4	5.9	4.5	6.6	6.4	4.2

These results show a less marked increase in the copper levels during week 2 than found in the first experiment but the rise still seems significant. Analysis of the samples taken for molybdenum determination was not possible as the sensitivity of the analytical method was not enough to allow measurement.

Mercury content of urine.

Some urine samples from patient's given dental treatment involving mercury amalgams were collected for a period after treatment. Analysis was made of these samples by two methods: (a) by drying the samples in a dessicator and analysing the residue and (b) by irradiating and analysing the wet urine. Table 54 shows the mercury content found in the samples of dry residue analysed.

TABLE 54

MERCURY CONTENT OF URINE RESIDUES

Time from treatment (days)	Mercury content (p.p.m.)
1	0.028
2	8.75
3	0.029
4	0.034
5	0.015
6	0.088
7	0.013
8	0.046
9	0.218
10	0.017

The interesting observation in these results was the high figure obtained on analysis of the urine sample on day 2 after treatment. In Table 55 are shown the results of urine analyses from two other patients. The samples were not dried but aliquots of the liquid taken for irradiation

and analysis. No mercury could be detected in some of the samples at the flux of 6 x 10^{11} $n/cm^2/sec$ used. A higher flux irradiation would probably have given more useful results.

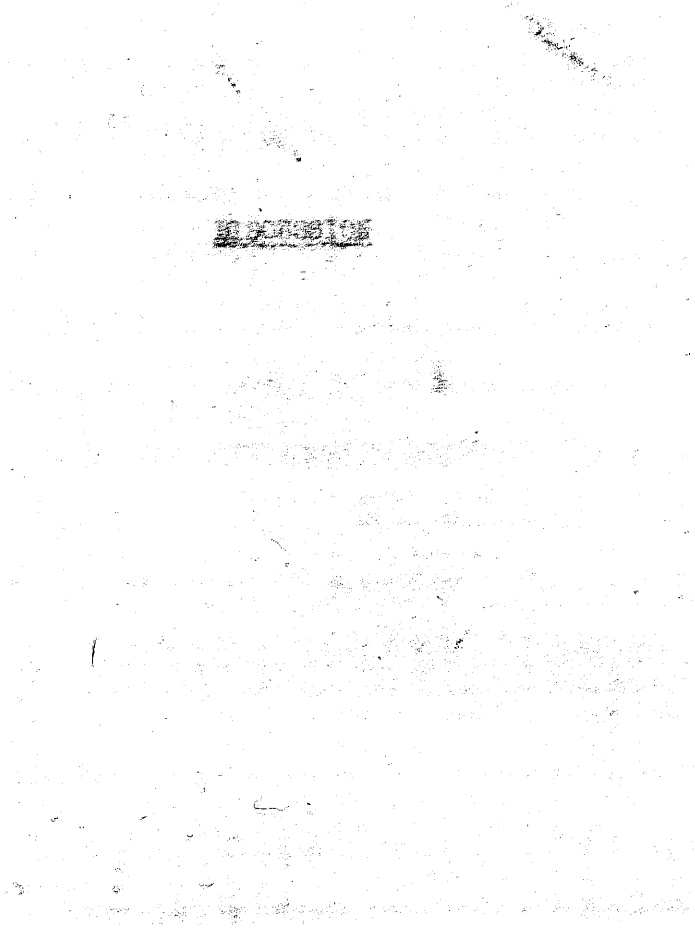
TABLE 55 MERCURY CONTENT OF URINE FROM DENTAL PATIENTS

	Time from treatment	Mercury content (µg/ml.)
Patient 1	After treatment	0.08
	4 hours	N.D
	3 days	N.D
	4 days	0.14
	5 days	N.D
	6 days	0.10
Patient 2	Day 0 (evening)	0.06
	Day 1 (morning)	0.14
	Day 1 (evening)	N.D
	Day 2 (morning)	N.D.
	Day 2 (evening)	0.07
	Day 3 (morning)	N.D

N.D. - not detected.

It is difficult to relate the results from Tables 54 and 55. The residue left from urine is highly variable and the mercury present may not be homogeneously distributed throughout the residue. It would appear that the mercury output in the urine is higher following mercury amalgam

treatment when these results are compared with the normal mercury content of urine. The mean of 10 determinations of normal urine was 0.014 µg/ml which is considerably less than many of the results in Table 55. The ra of normal values was from 0.007 - 0.039 µg./ml.



C H A P T E R V

DISCUSSION

DISCUSSION

Application of the technique of activation analysis provides an opportunity for the study of minor elements in teeth and other biological materials. A routine and reliable chemical analysis for trace elements is an invaluable tool for a large number of research workers, biochemists, dentists, doctors, biologists, forensic scientists, nutritionists and etc. Activation analysis meets the requirements of most workers.

It has the chief attraction of being a highly sensitive analytical technique which requires only a tiny sample for analysis. It lacks the accompanying disadvantage of micro-separations. Since chemical losses are taken into account a high degree of manipulative skill is not required to produce an accurate result. This means that an inexperienced worker can quickly acquire a working knowledge of the technique. It is suited to the analysis of a large number of analyses at the one time. The analysis of up to fifty samples (excluding preparation and irradiation can often be made in one day). With the exception of vanadium the analyses developed in the course of this Thesis are all suitable for measuring quickly and accurately a large number of samples.

A further advantage of this method of analysis is that the identity of the detected element can be confirmed by decay or γ-spectra studies. This provides a check against the interference in a measurement by another element. Once the technique has been established there is seldom any difficulty with interferences.

The levels at which over thirty elements occur in enamel from human teeth have now been measured. In Table 56 is shown an mean figure for

each element measured. Some figures have been obtained from published work and others from the work described in this Thesis. The source of each figure can be found by consulting the reference listed. Oxygen, hydrogen and carbon occur in combination with other elements eg. oxygen in phosphate and carbonate, hydrogen in water and organic compounds and carbon in carbonate and organic compounds. No figure for the sulphur content of teeth or enamel could be obtained.

The carbonate content of enamel has been estimated as 2.5% and the organic content as 0.6%.[48] The moisture content of enamel has been measured by Burnett and Zenewitz[24] and found to be 2.2%.

In general there is good agreement between the results obtained by activation analysis in this work and other determinations made by other workers using both activation and other techniques. Apart from genuine differences between the analytical methods used, results may vary on account of other factors. If the samples being analysed are dried before analysis the concentration of trace elements found will be higher than if undried samples were used. As the distribution of many elements varies throughout a single tooth and even throughout the enamel from a single tooth, results will vary according to the method by which samples are chosen for analysis. Pooled total enamel samples will give an average element concentration while pooled enamel layers will give average variations between layers. Individual sections or samples of unseparated enamel will show variations not detectable when pooled samples are used.

Soremark and Samsahl[96] found an average figure for the manganese content of whole enamel samples (dry weight basis) to be 0.54 p.p.m. In this work the outer enamel from single samples was shown to have a mean

TABLE 56

INORGANIC CONSTITUENTS OF HUMAN ENAMEL

Macro-elements (>0.1%)			Trace Elements (<0.1%)					
Element	Mean (%) Concentration	Reference	Element	Mean (p.p.m.) Concentration	Reference	Element	Mean (p.p.m.) Concentration	Reference
Hydrogen	0.3		Zinc	400	*	Selenium	0.9	41
Carbon	0.5		Fluorine	500	20	Antimony	0.04	*
Nitrogen	0.058	6	Iron	135	16	Manganese	0.8	*
Oxygen	41.7		Silicon	240	16	Cadmium	0.01	*
Sodium	1.2	96	Lead	90	18	Vanadium	0.01	*
Magnesium	0.4	48	Strontium	94	96	Molybdenum	0.05	*
Phosphorus	18.3	96	Aluminium	130	16	Mercury	2.6	79
Sulphur	-		Copper	10	78	Bromine	0.5	96
Chlorine	0.65	96	Silver	11	16	Tungsten	0.2	96
Potassium	0.1	49	Tin	7	19	Gold	0.02	96
Calcium	37.4	96				Arsenic	0.06	80

* From results in this Thesis, calculated from phosphorus, carbonate, and moisture figures.

manganese level of 1.0 p.p.m. while the inner enamel had a mean figure of 0.55 p.p.m. Brudevold et al.[16] report much higher manganese levels by spectrography. Soremark and Samsahl found a zinc figure of 276 p.p.m. In this work the average zinc content of outer enamel was 476 p.p.m. while the inner enamel was 236 p.p.m. These figures all agree with those obtained by workers using methods other than activation analysis. Cruickshank[30] found an average value of the zinc content of human teeth to be about 200 p.p.m. using a micro-analytical technique. Brudevold et al.[21] analysed pooled samples of enamel layers from the enamel surface to the dentino-enamel boundary using both spectrographic and spectrophotometric methods. They found surface zinc levels in the range 1000-2000 p.p.m. decreasing steadily down to around 200 p.p.m. near to the dentine boundary.

Although the element molybdenum has been suggested as reducing caries incidence[69] little information on its enamel levels are available due to the difficulties in its estimation at the low concentrations present. Healy and Ludwig[70] analysed pooled crown samples, containing enamel and dentine, by chemical separation of the molybdenum followed by colorimetric measurement. They found levels of 0.069 and 0.046 p.p.m in deciduous teeth of children from Napier and Hastings, New Zealand. Permanent teeth levels were 0.034 p.p.m. (Napier) and 0.032 p.p.m. (Hastings). It was suggested that the higher incidence of molybdenum in the Napier teeth was an explanation for the low rate of caries found in Napier relative to the rest of New Zealand. The mean figure of 0.048 p.p.m. found for analysis of both crown and enamel samples in this Thesis (Table 50) would appear to agree with the above results.

No previous determinations of antimony can be found but the mean arsenic level in enamel of 0.06 p.p.m.[91] is similar to the average antimony level found viz. 0.044 p.p.m. It would be expected that the levels would be similar since the elements are chemically alike and are found together throughout the environment.

No detectable amounts of vanadium or cadmium were found in the teeth analysed in this work. Lowater and Murray[67] reported the spectrographical detection of a number of trace elements in teeth. This work was only qualitative and no figures for the levels present were given. Ag, Al, Ba, Cu, Fe, Mg, Ni, Pb, Si, Sr, Ti, V and Zn were detected in most or all of the teeth studied. Cr, K, Li, Mn and Sn were found in some but not all of the samples analysed. Quantitative information is now available for all the elements mentioned excepting Ba, Ni, Ti, V, Cr and Li. Soremark[95] suggested that the vanadium content of teeth must be less than 10^{-5} p.p.m. but this seems a rather low estimate from the neutron flux of $10^{12} n/cm^2/sec.$ which he used.

The mean copper content of the calculus samples analysed in this Thesis was 22.4 p.p.m. (Table 51). This is much higher than the mean value of 3.9 p.p.m. reported by Samsahl and Soremark[97] for the copper contect of calculus samples. Enamel samples analysed for copper by the method used in this Thesis gave a mean value of 10.4 p.p.m.[80,78] Enamel copper levels as determined by Samsahl and Soremark gave a mean value of 0.26 p.p.m.[96] The discrepancy between the results obtained by the two methods would suggest that there is not a genuine difference in the copper content of the samples analysed but that the difference is due to one or both of the analysis methods giving invalid results.

Other workers measuring the copper content of enamel by spectrographic means have found levels of 12 - 30 p.p.m. for total enamel[99] and a mean level of 20 p.p.m. for external enamel[17].

In figure 50 are shown histograms displaying the distributions of the trace element content found in enamel analysis for copper, zinc, antimony, manganese, mercury and arsenic. The zinc, manganese and antimony figures were compiled from data given earlier in this Thesis, while the copper, arsenic and mercury figures are from data accumulated in earlier work in this laboratory.[78,79,80]

The observations made on these figures are as follows:

1. The concentrations over which a given element occurs in enamel varies from a narrow range eg. manganese, to a broad one eg. antimony and mercury.

2. The elements which are found present in a narrow range eg. zinc and manganese, are those considered as essential to humans and are present in most body tissues in amounts which do not vary widely.

3. The elements present in a broad range eg. antimony and mercury are those considered toxic and non-essential to humans so that their presence can be regarded as the chance incorporation of the element into the enamel.

4. The copper and arsenic ranges are intermediate ones; copper, though an essential element falls in a fairly broad range. Arsenic, though chemically similar to antimony and occurring at about the same

concentration level is not so widely distributed compared with antimony.

Of the elements determined only manganese and zinc were found to occur in higher concentrations in the outer enamel layers. Other elements which have been shown to occur in greater amounts in the outer enamel are fluorine, lead, iron, silver, silicon and tin[22]. In the same work it was found that sodium, magnesium and carbonate follow the reverse pattern ie. a higher element concentration in the inner enamel while copper and strontium are found in about the same concentrations.

It can be seen from Table 45 that the zinc and manganese content of inner enamel samples shows less variation than outer enamel samples. In the analyses made for manganese and zinc an unerupted tooth was analysed. As described in the results the outer enamel content of both manganese and zinc was also found to be greater than the inner enamel in these teeth. This observation supports the view that the higher outer enamel levels are not only formed post-eruptively. It has been shown that outer enamel fluorine content increases post-eruptively with age and dietary fluoride intake, while inner enamel fluorine content remains unchanged[16]. A similar situation has been shown to exist for zinc with respect to age increases of outer enamel zinc levels[22]. Both zinc and fluorine uptake from solution were shown to take place on a synthetic hydroxyapatite. The presence of either element increased the resistance of the hydroxyapatite to acid solution. It may be that zinc is as important as fluorine in producing a caries resistant tooth.

The wide range of values found in outer enamel samples on trace element analysis can be explained by several theories. All involve post-eruptive uptake of the given element from an external source. It has been shown that enamel from teeth containing a silver/mercury amalgam filling contains high mercury levels[79]. Soremark and Samsahl[98] have shown that enamel from teeth adjacent to teeth fitted with gold, cobalt/chromium or platinum clasps, or containing gold crowns or silver, mercury or zinc containing restorations have elevated levels of the appropriate element. Also Steadman, Brudevold[17] et al. showed that teeth exposed to the soil for thousands of years contained high surface concentrations of several trace elements.

The results shown in Table 47 do not support the suggestion that zinc from a zinc oxide based restoration lining can lead to elevated zinc levels in the outer enamel layers of neighbouring teeth. Further work to investigate this possibility should be carried out to examine this theory more fully. The results in Table 46 seem to eliminate the hypothesis that the high enamel surface zinc figures were caused by sampling only surface enamel in those samples where high figures were found.

In conclusion, it would appear that using the technique of neutron activation analysis the determination of a greater number of trace constituents of teeth than those previously determined, can be made. Since very small samples are required individual variations can be studied in much more detail than was formerly possible.

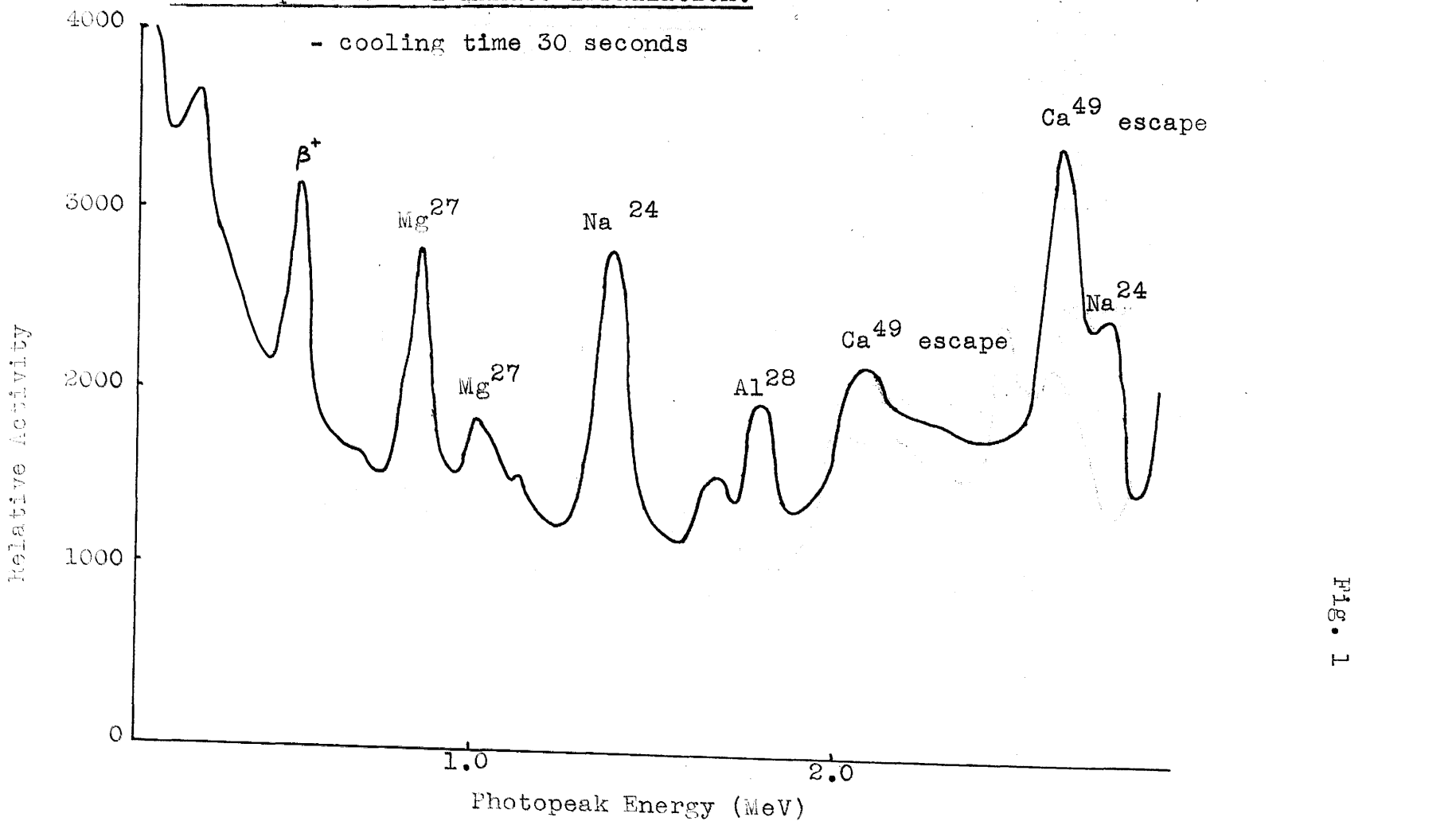

Tooth spectrum - 1 minute irradiation.
- cooling time 30 seconds
4000
3000
2000
1000
0
Relative Activity
β+
Mg27
Mg27
Na 24
Al28
Ca49 escape
Ca49 escape
Na24
1.0
2.0
Photopeak Energy (MeV)

Fig. 1

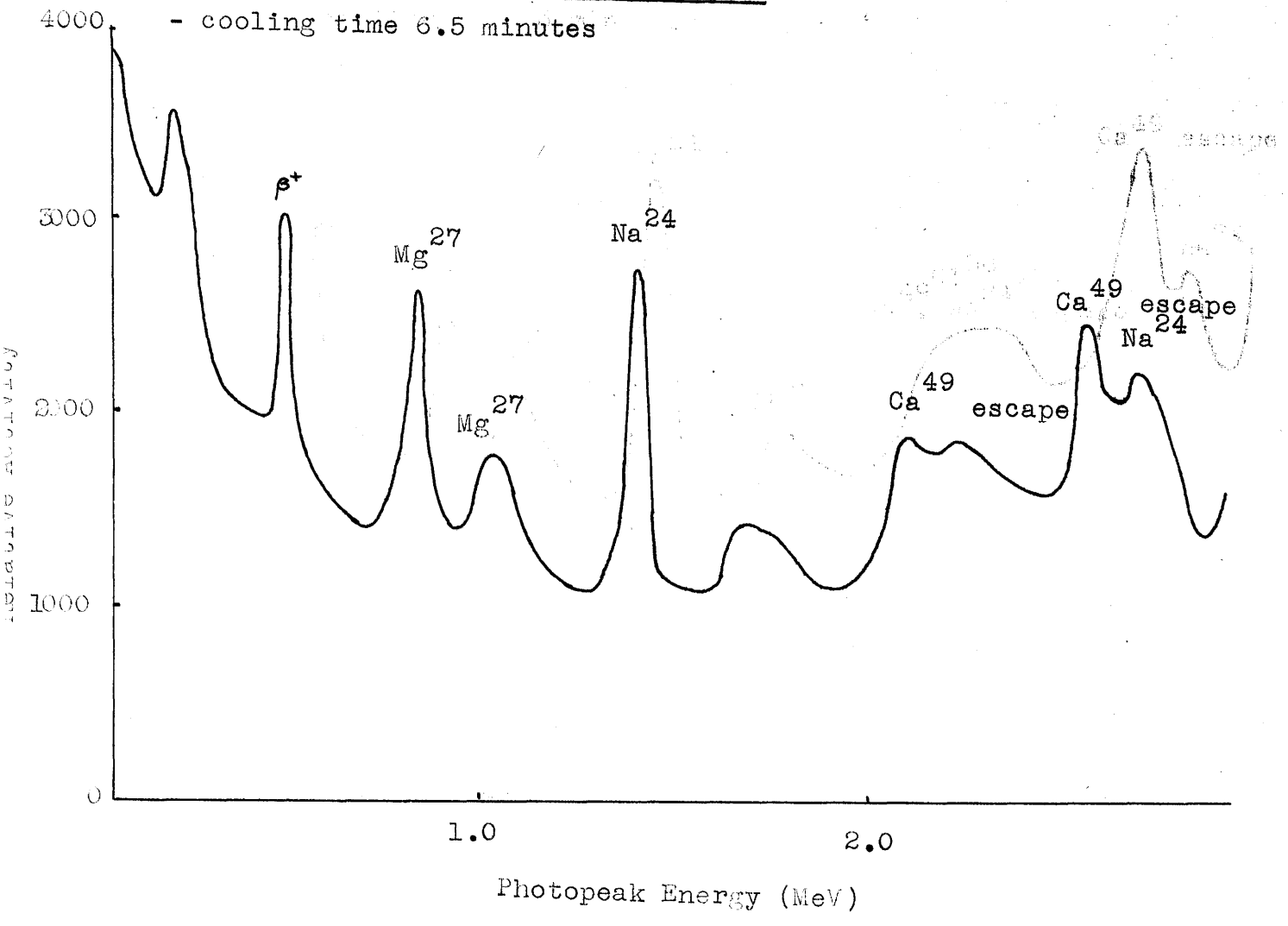

Tooth spectrum - 1 minute irradiation.
- cooling time 6.5 minutes
4000
3000
2000
1000
0
β+
Mg 27
Mg 27
Na 24
Ca 49
escape
Ca 49
escape
Na 24
1.0
2.0
Photopeak Energy (MeV)

Fig. 2

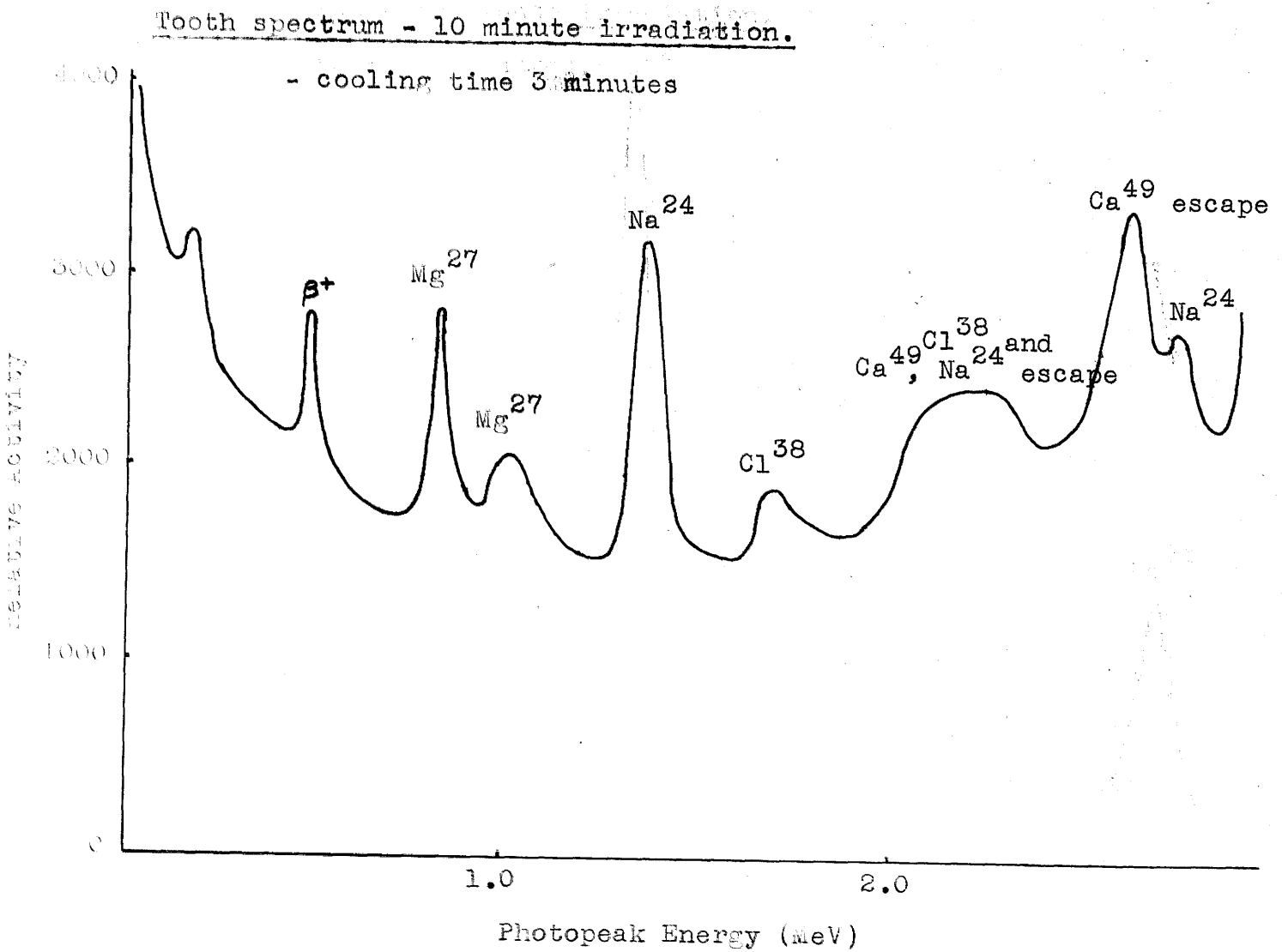

Tooth spectrum - 10 minute irradiation.
- cooling time 3 minutes
Relative activity
3000
2000
1000
0
β+
Mg27
Mg27
Na24
Cl38
Ca49, Cl38 and Na24 escape
Ca49 escape
Na24
1.0
2.0
Photopeak Energy (MeV)

Fig. 3

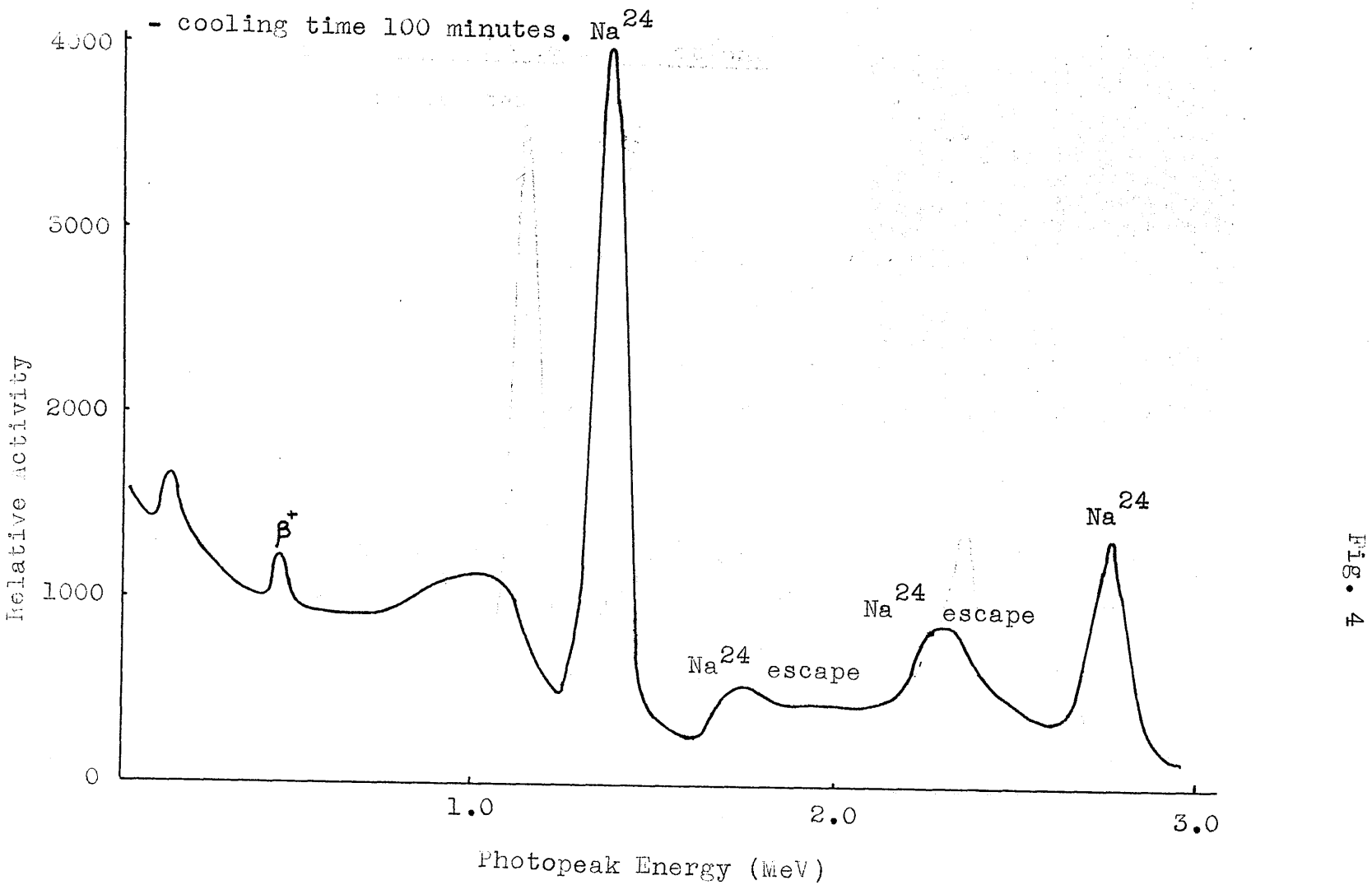

Tooth spectrum - 10 minute irradiation.
- cooling time 100 minutes. Na24
4000
3000
2000
1000
0
Relative activity
β+
Na24 escape
Na24 escape
Na24
1.0
2.0
3.0
Photopeak Energy (MeV)

Fig. 4

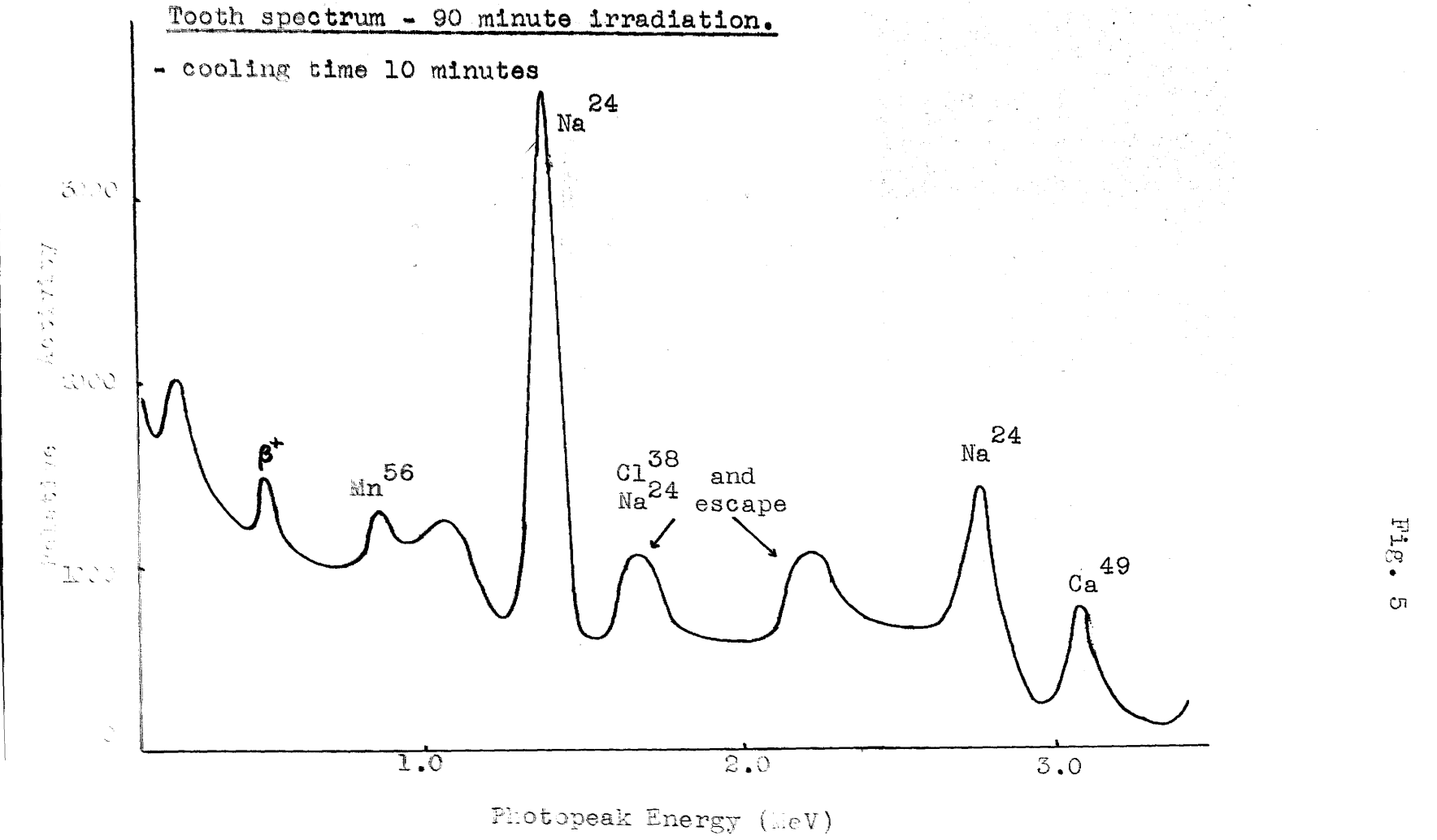

Tooth spectrum - 90 minute irradiation.
- cooling time 10 minutes
Na 24
β+
Mn 56
Cl 38
Na 24
and
escape
Na 24
Ca 49
1.0
2.0
3.0
Photopeak Energy (MeV)

Fig. 5

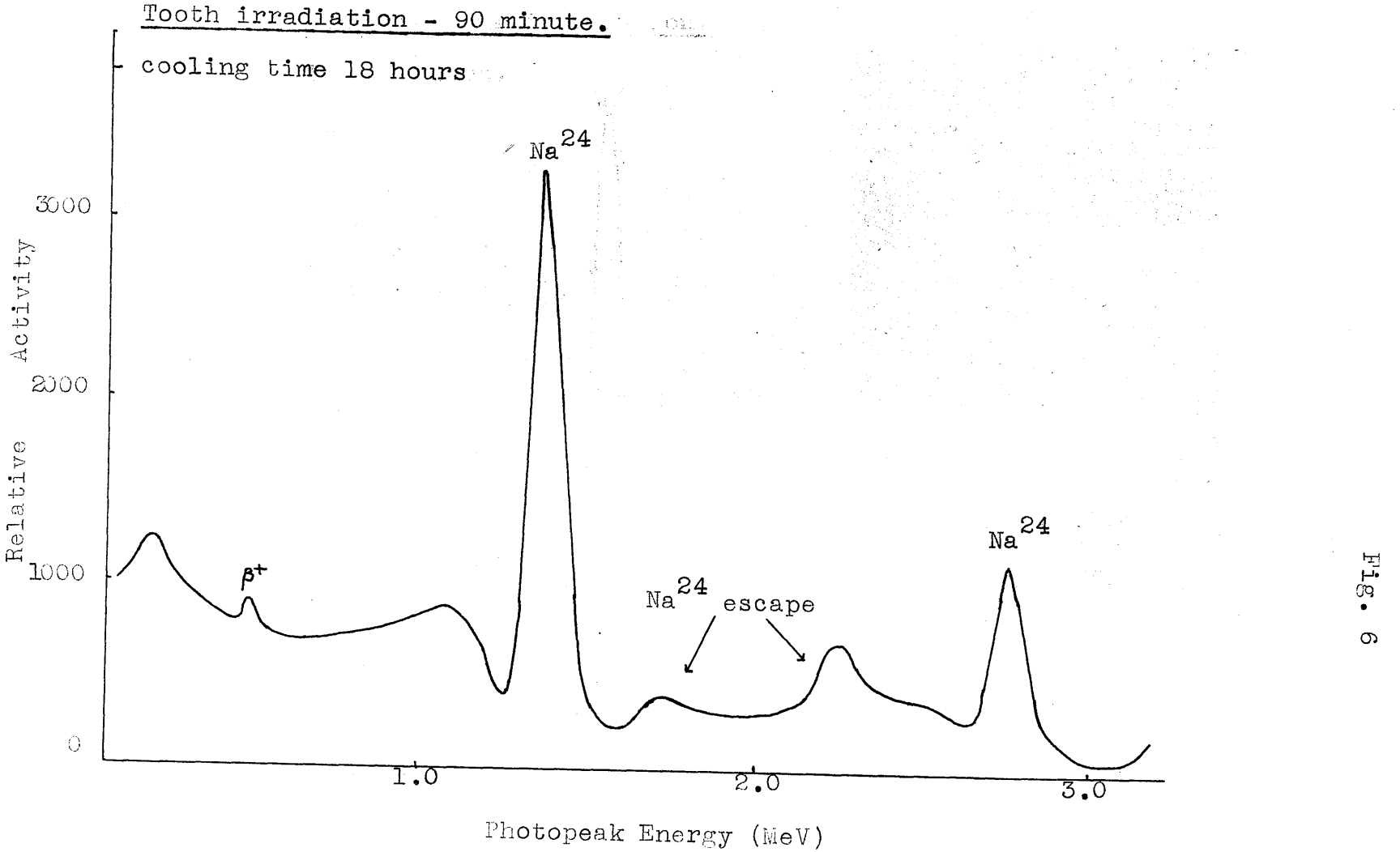

Tooth irradiation - 90 minute.
cooling time 18 hours
Na24
3000
2000
1000
0
Relative Activity
β+
Na24 escape
Na24
1.0
2.0
3.0
Photopeak Energy (MeV)

Fig. 6

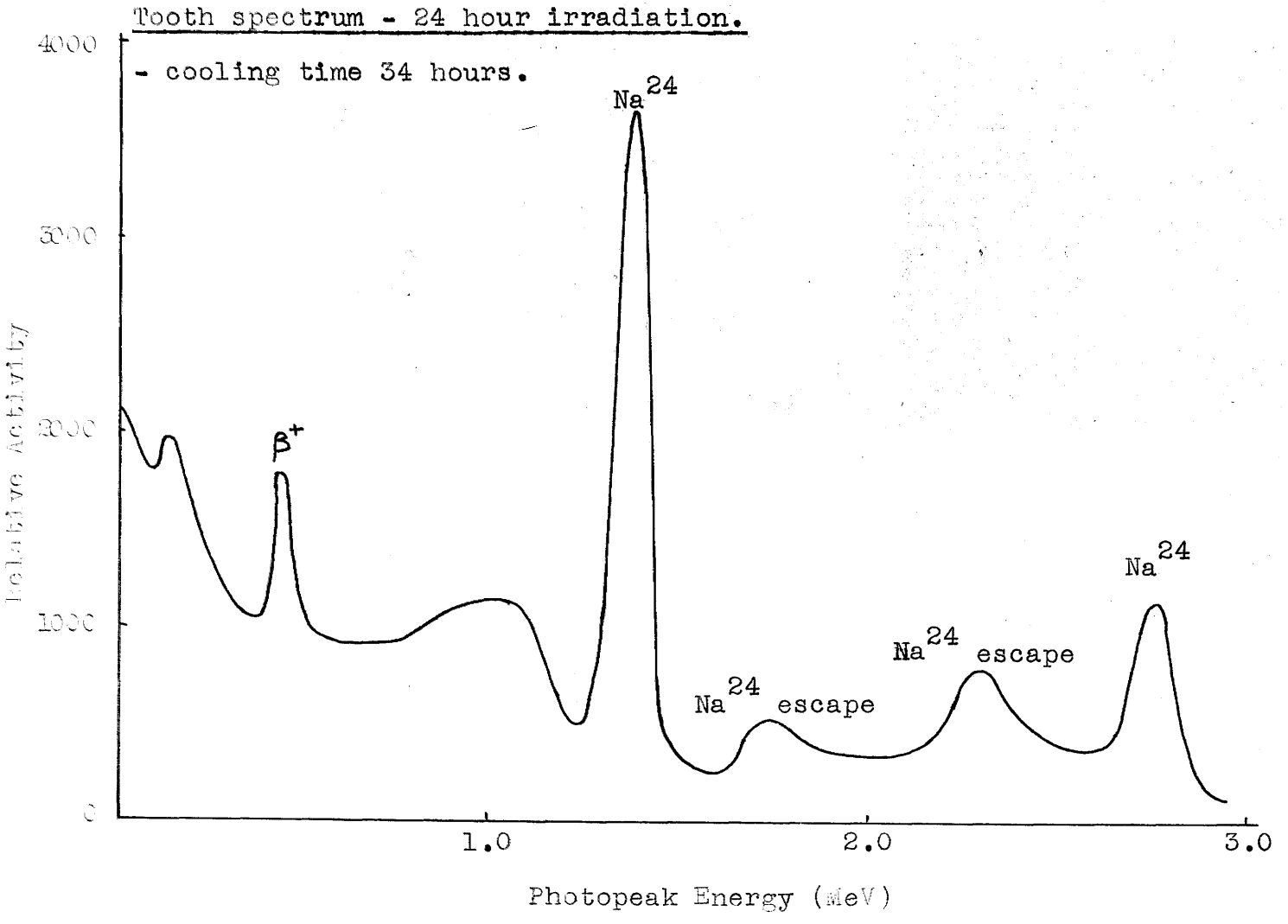

Tooth spectrum - 24 hour irradiation.
- cooling time 34 hours.
Na24
β+
Na24 escape
Na24 escape
Na24
4000
3000
2000
1000
0
Relative Activity
1.0
2.0
3.0
Photopeak Energy (MeV)

Fig. 7

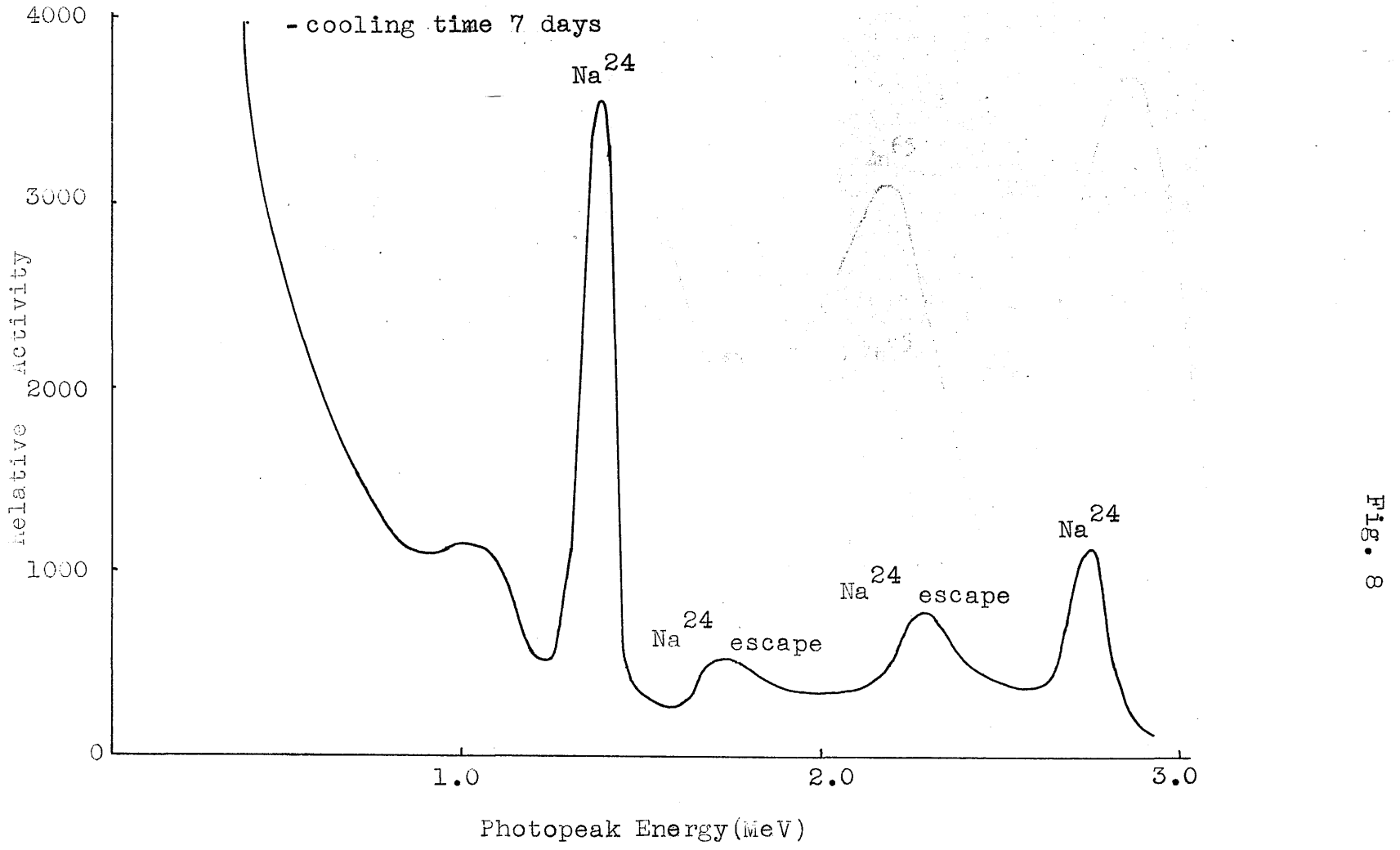
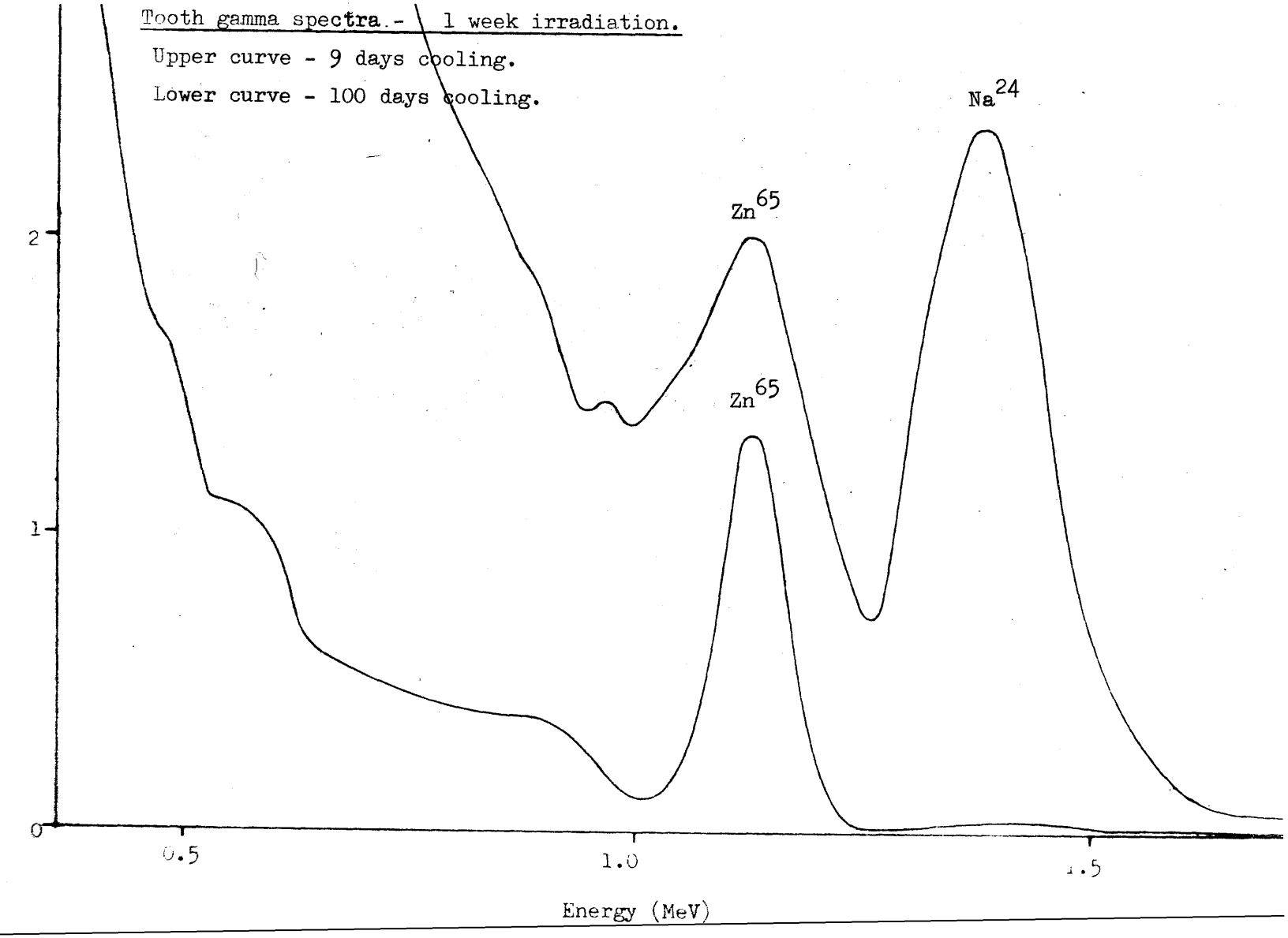

Tooth spectrum - 24 hour irradiation.
- cooling time 7 days
Na24
Na24 escape
Na24 escape
Na24
4000
3000
2000
1000
0
Relative Activity
1.0
2.0
3.0
Photopeak Energy(MeV)

Fig. 8


Tooth gamma spectra.- 1 week irradiation.
Upper curve - 9 days cooling.
Lower curve - 100 days cooling.
Na24
Zn65
Zn65
2
1
0
0.5
1.0
1.5
Energy (MeV)

Fe^{59} standard spectrum.

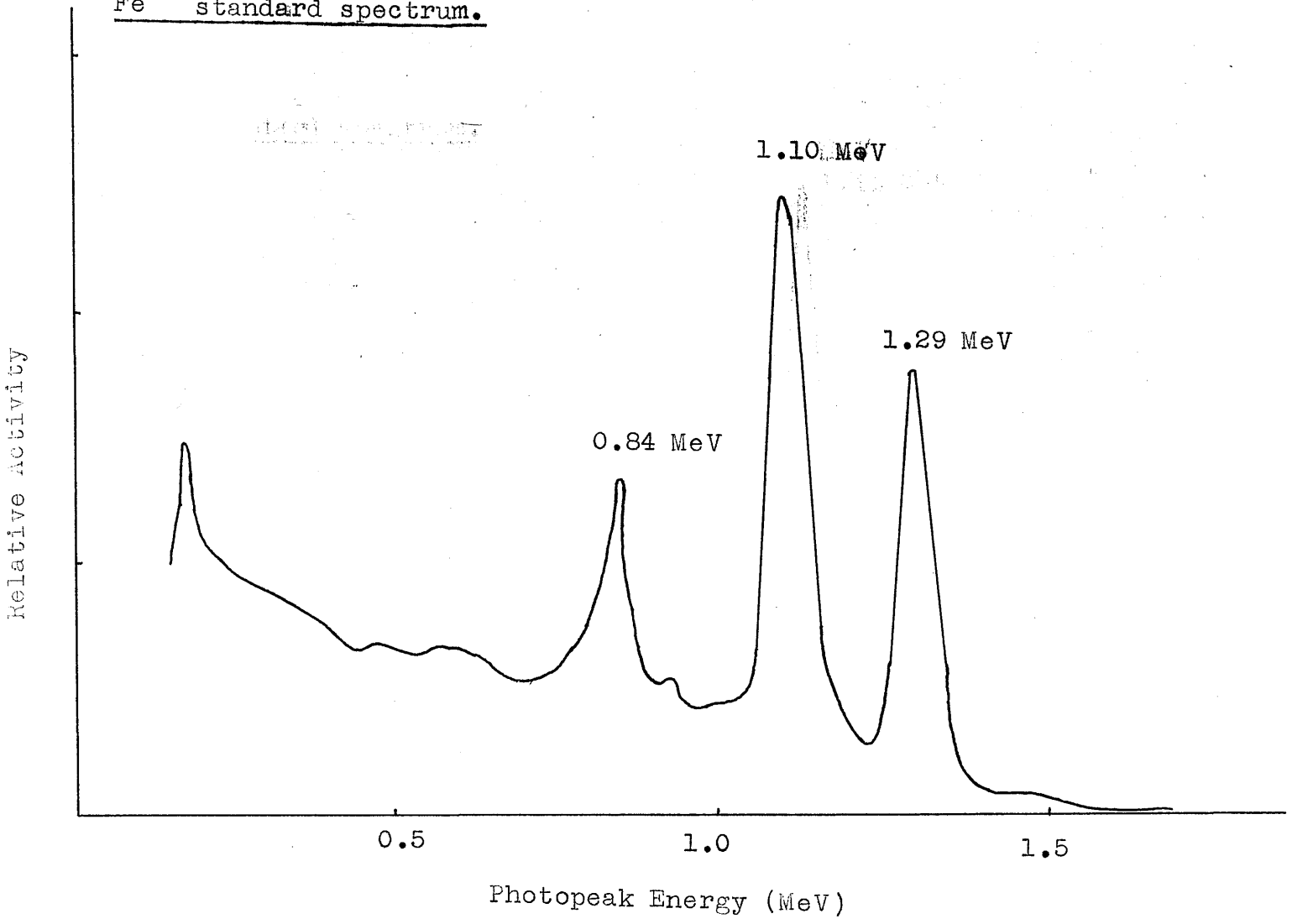


Fig. 10

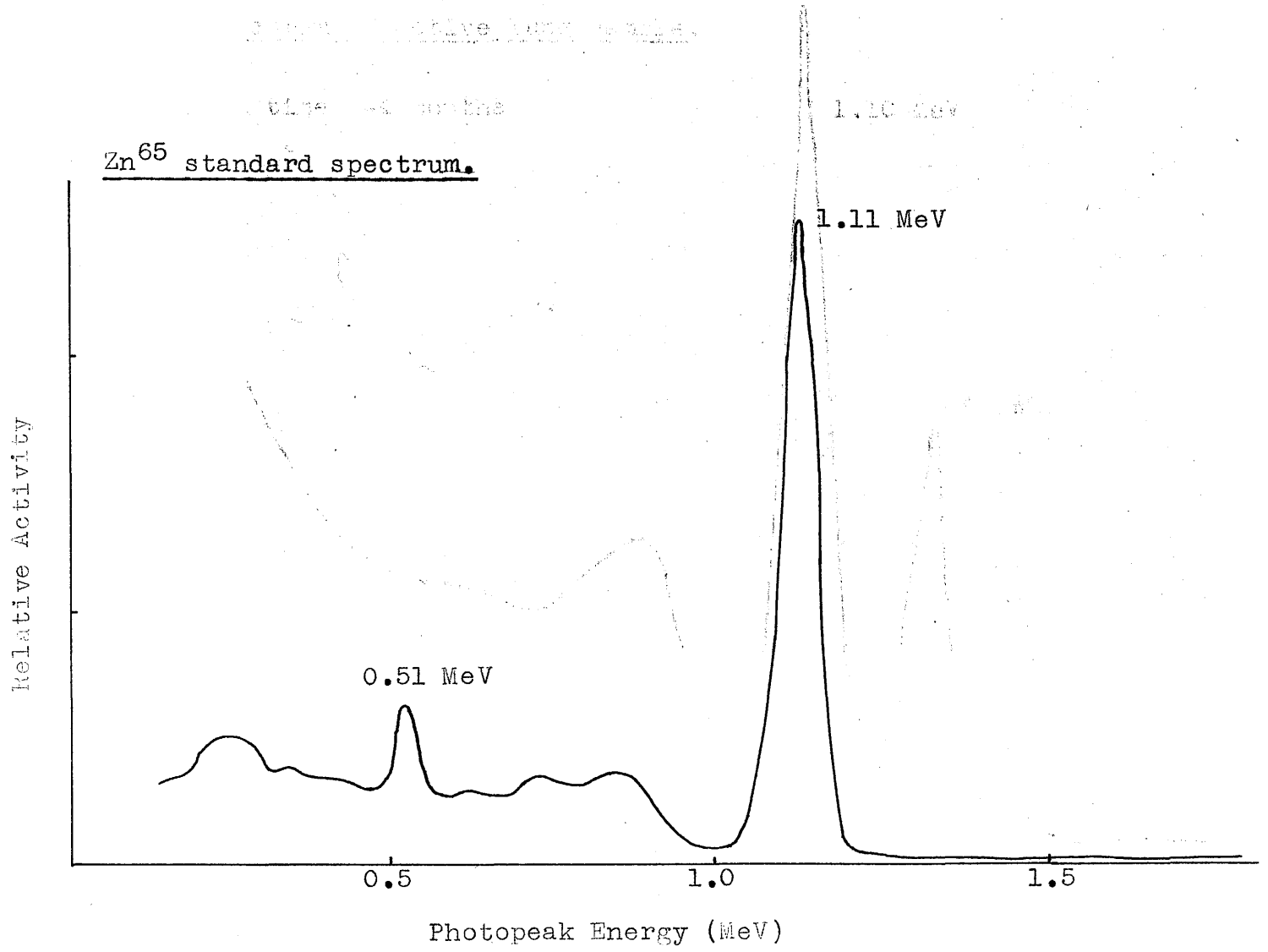

Zn^{65} standard spectrum.
1.11 MeV
0.51 MeV
Relative Activity
0.5
1.0
1.5
Photopeak Energy (MeV)

Fig. 11

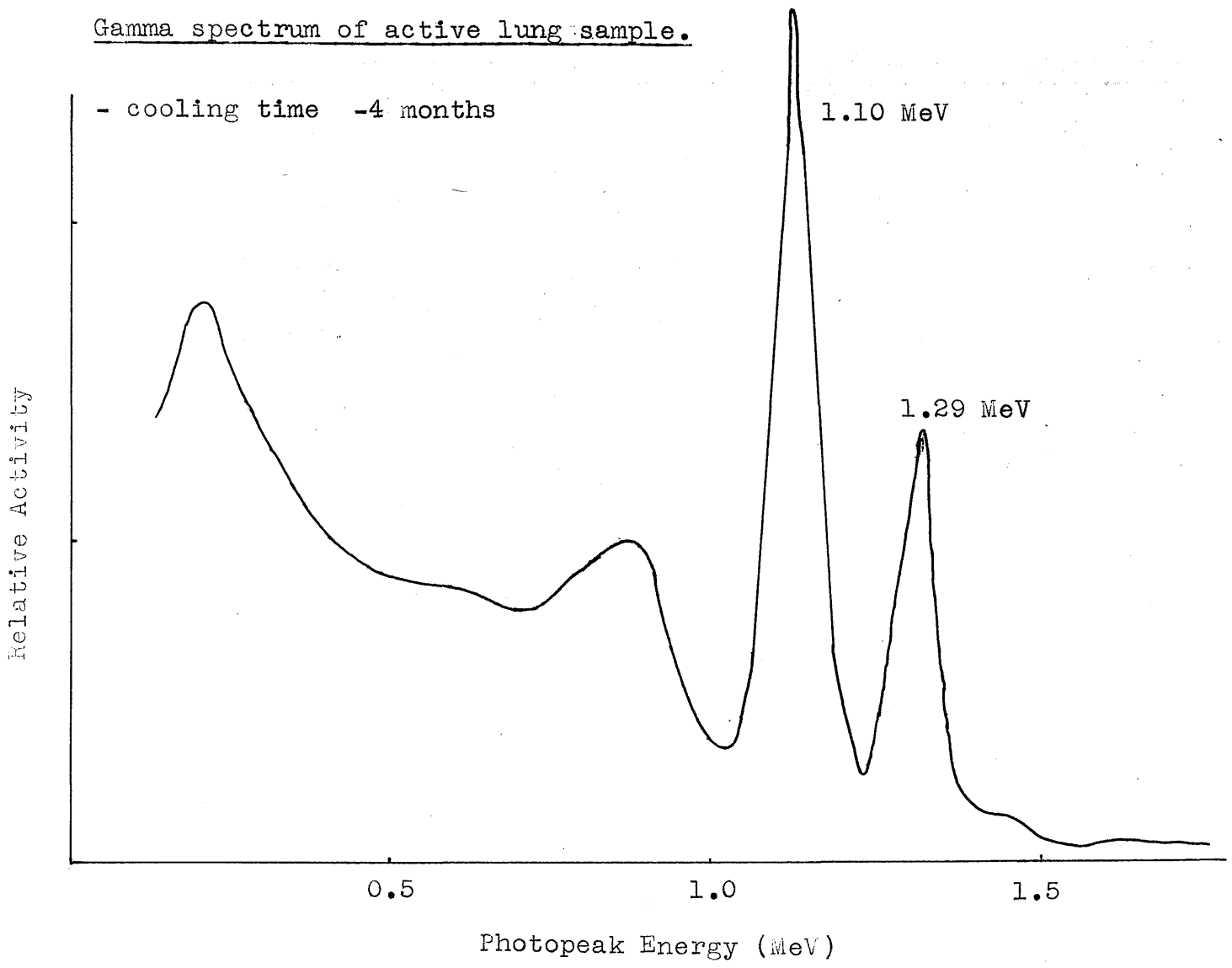

Gamma spectrum of active lung sample.
- cooling time -4 months
1.10 MeV
1.29 MeV
Relative Activity
0.5
1.0
1.5
Photopeak Energy (MeV)

Fig. 12

Change of Absorbance at 400 mμ. of Vanadium Cupferrate in Chloroform.

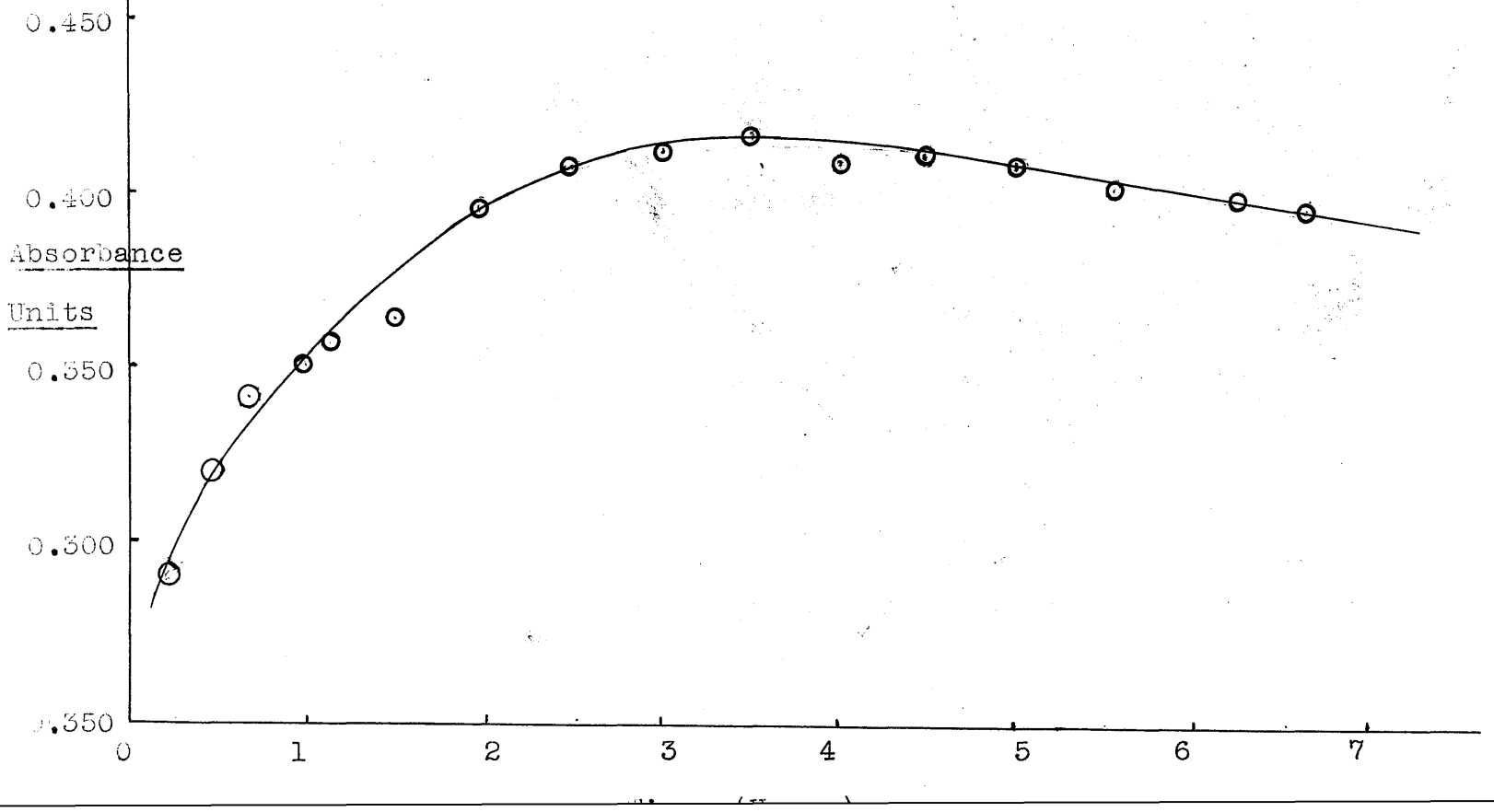


Fig. 13

Visible spectra of vanadium cupferrate/chloroform solutions.

a) red solution

b) yellow solution

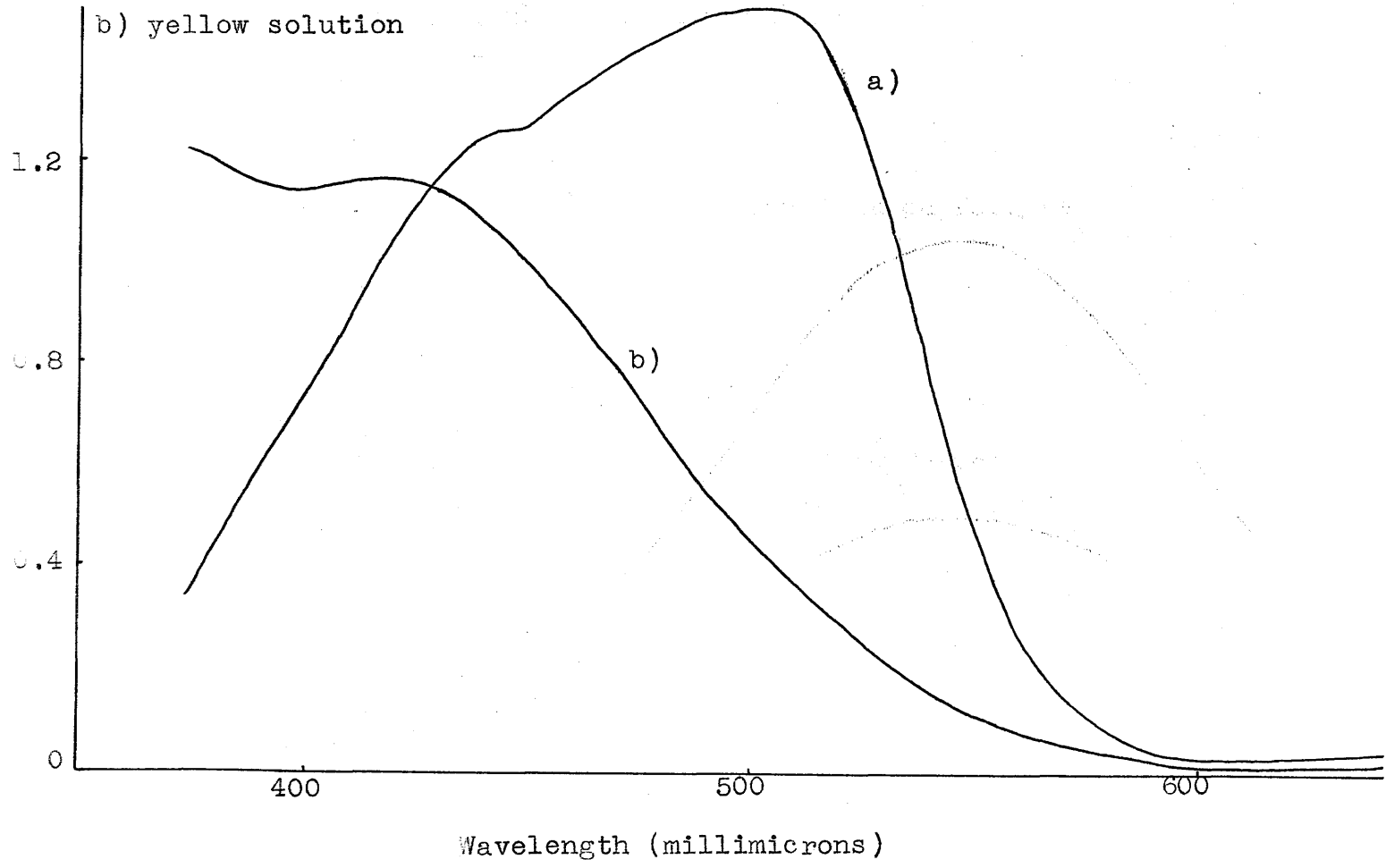


Fig. 14

Visible spectra of cupferron and vanadium cupferrate in chloroform after standing for 24 hours.

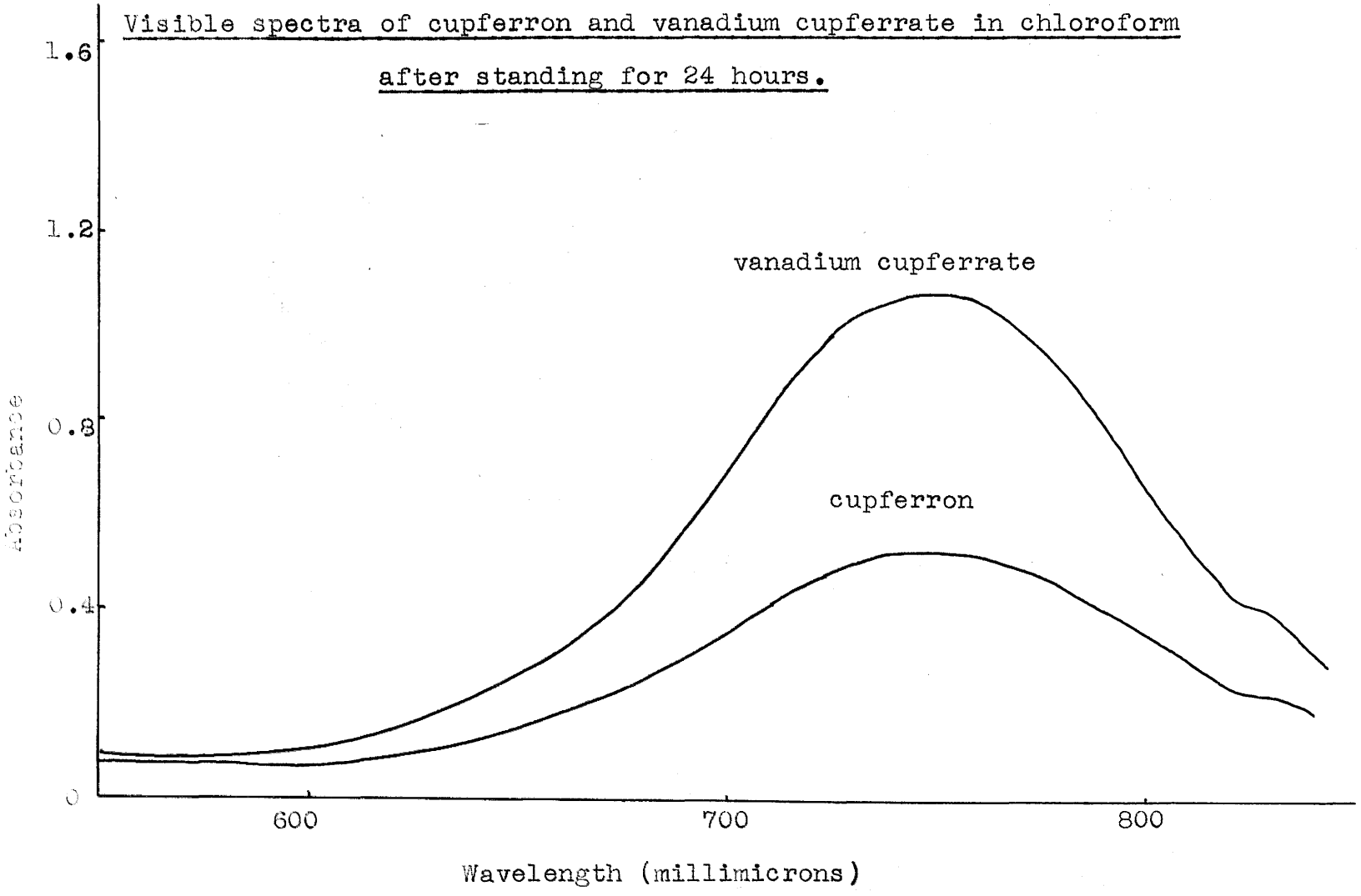


Fig. 15

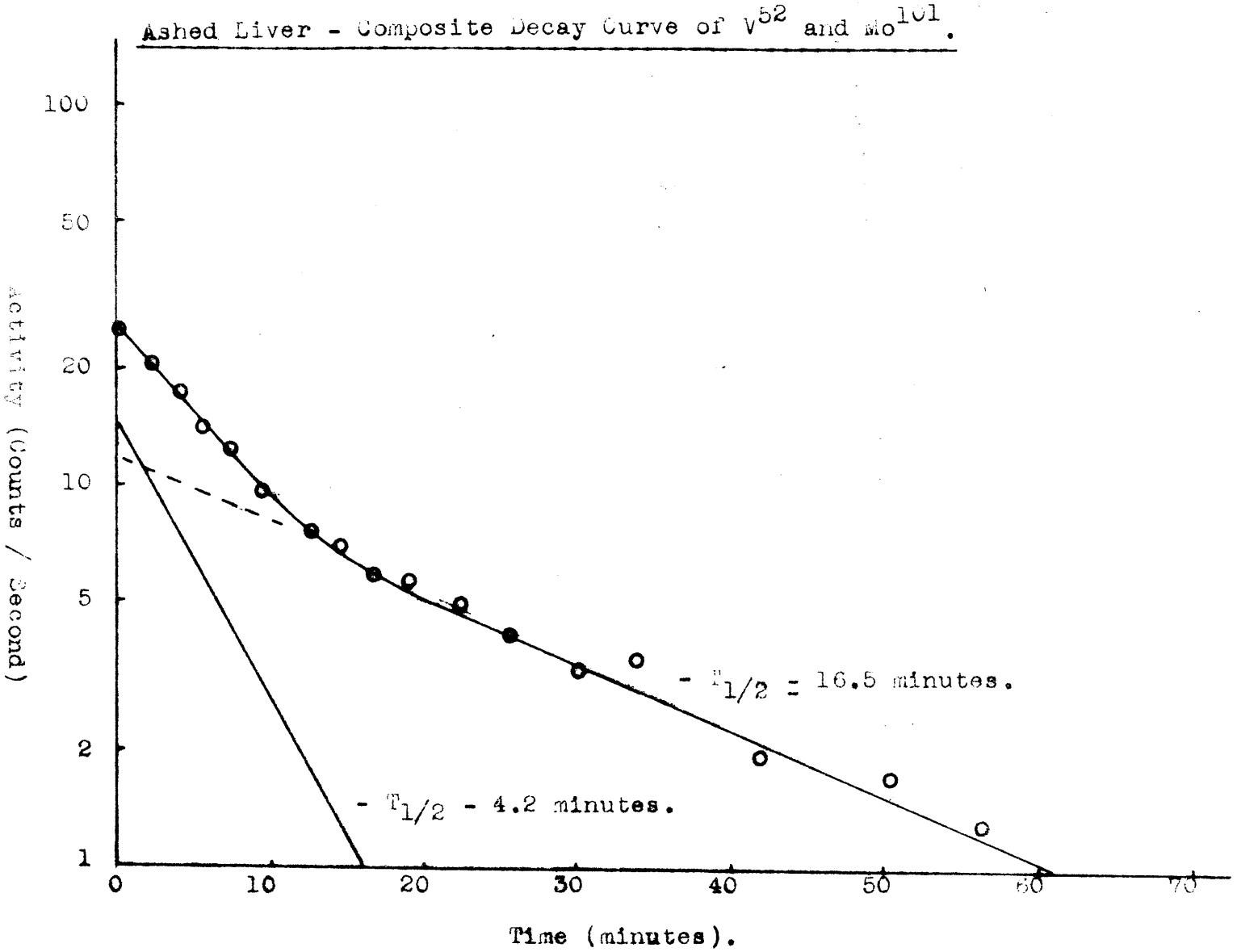
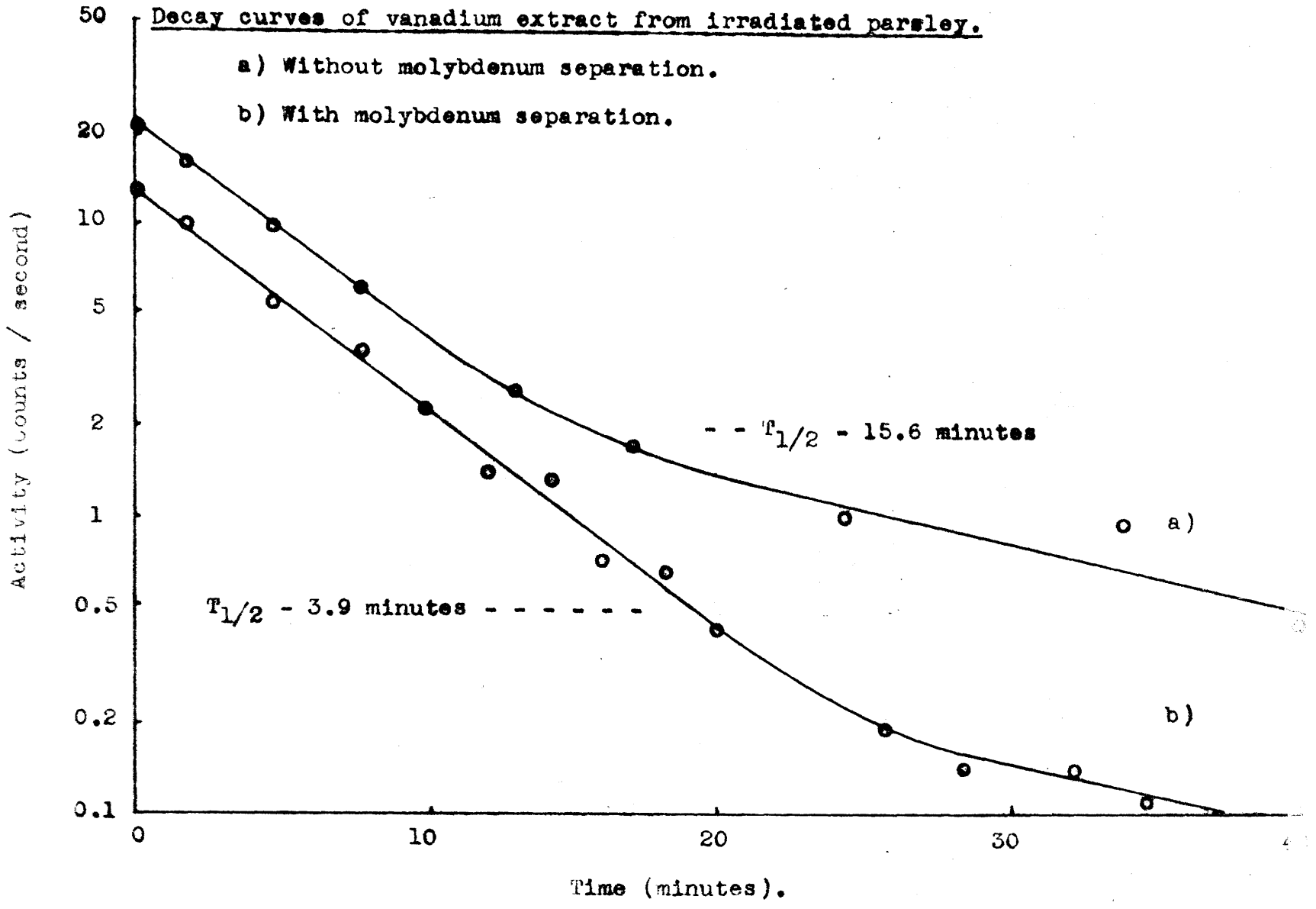

Ashed Liver - Composite Decay Curve of V^{52} and Mo^{101}.
100
50
20
10
5
2
1
0
10
20
30
40
50
60
70
Activity (Counts / Second)
Time (minutes).
- $T_{1/2}$ = 16.5 minutes.
- $T_{1/2}$ - 4.2 minutes.

Fig. 16.


Decay curves of vanadium extract from irradiated parsley.
a) Without molybdenum separation.
b) With molybdenum separation.
50
20
10
5
2
1
0.5
0.2
0.1
Activity (counts / second)
- - $T_{1/2}$ - 15.6 minutes
a)
$T_{1/2}$ - 3.9 minutes - - - - - -
b)
0
10
20
30
Time (minutes).

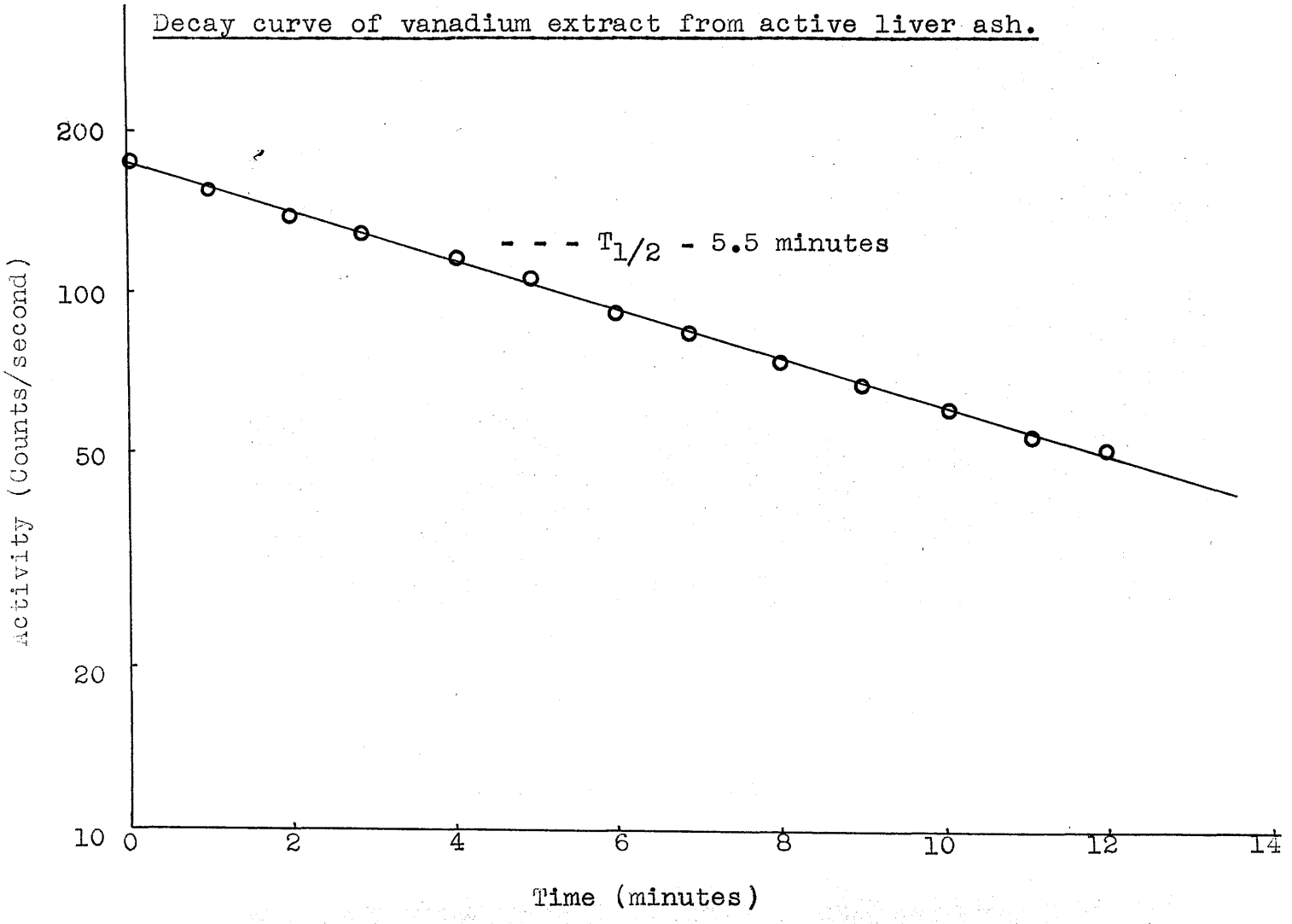
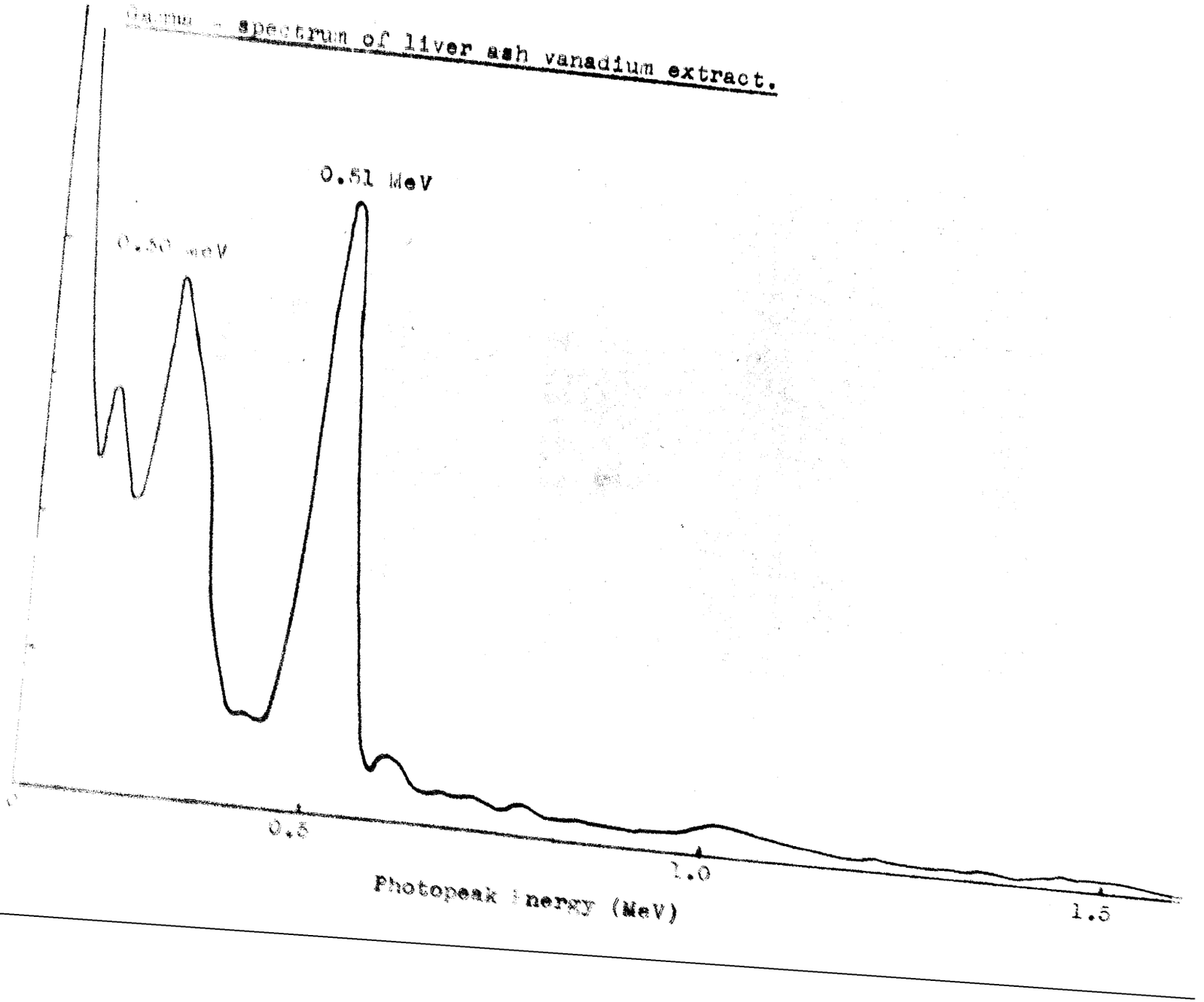
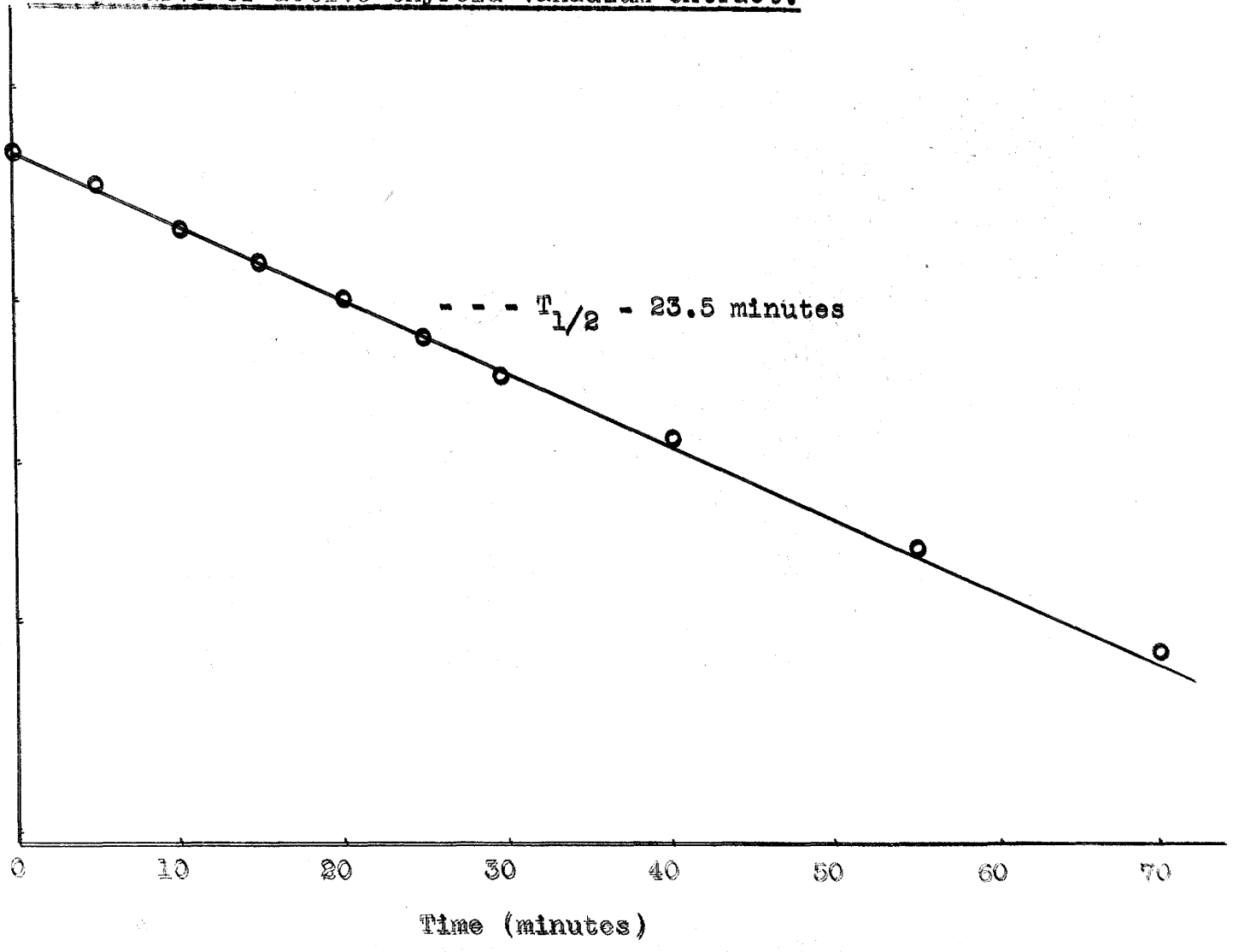

Decay curve of vanadium extract from active liver ash.
200
100
50
20
10
Activity (Counts/second)
- - - $T_{1/2}$ - 5.5 minutes
0
2
4
6
8
10
12
14
Time (minutes)

Fig. 18

Gamma - spectrum of liver ash vanadium extract.



Decay curve of active thyroid vanadium extract.



Gamma spectrum of V^{52} standard.

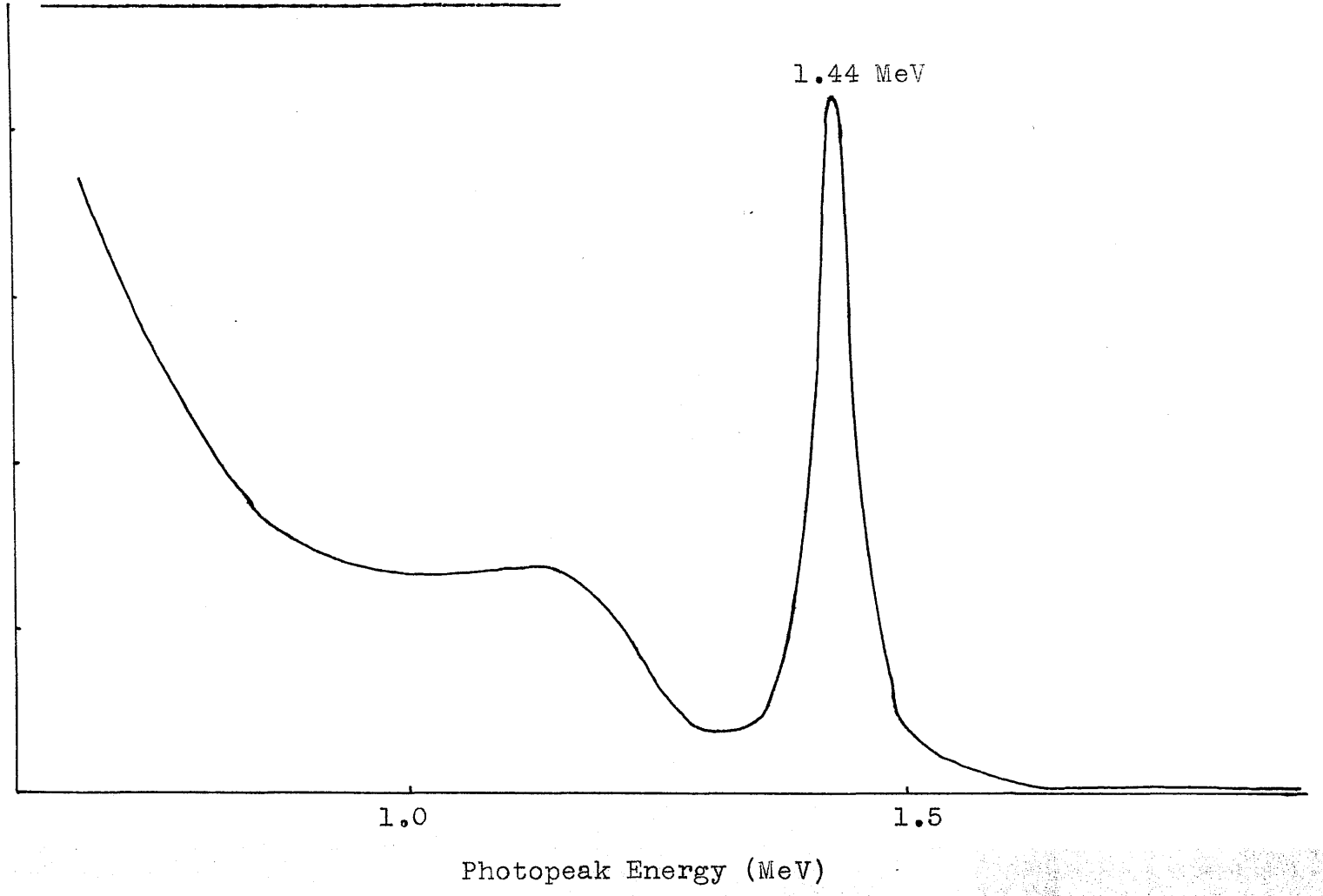


Fig. 21

Gamma spectra of parsley extract for vanadium.

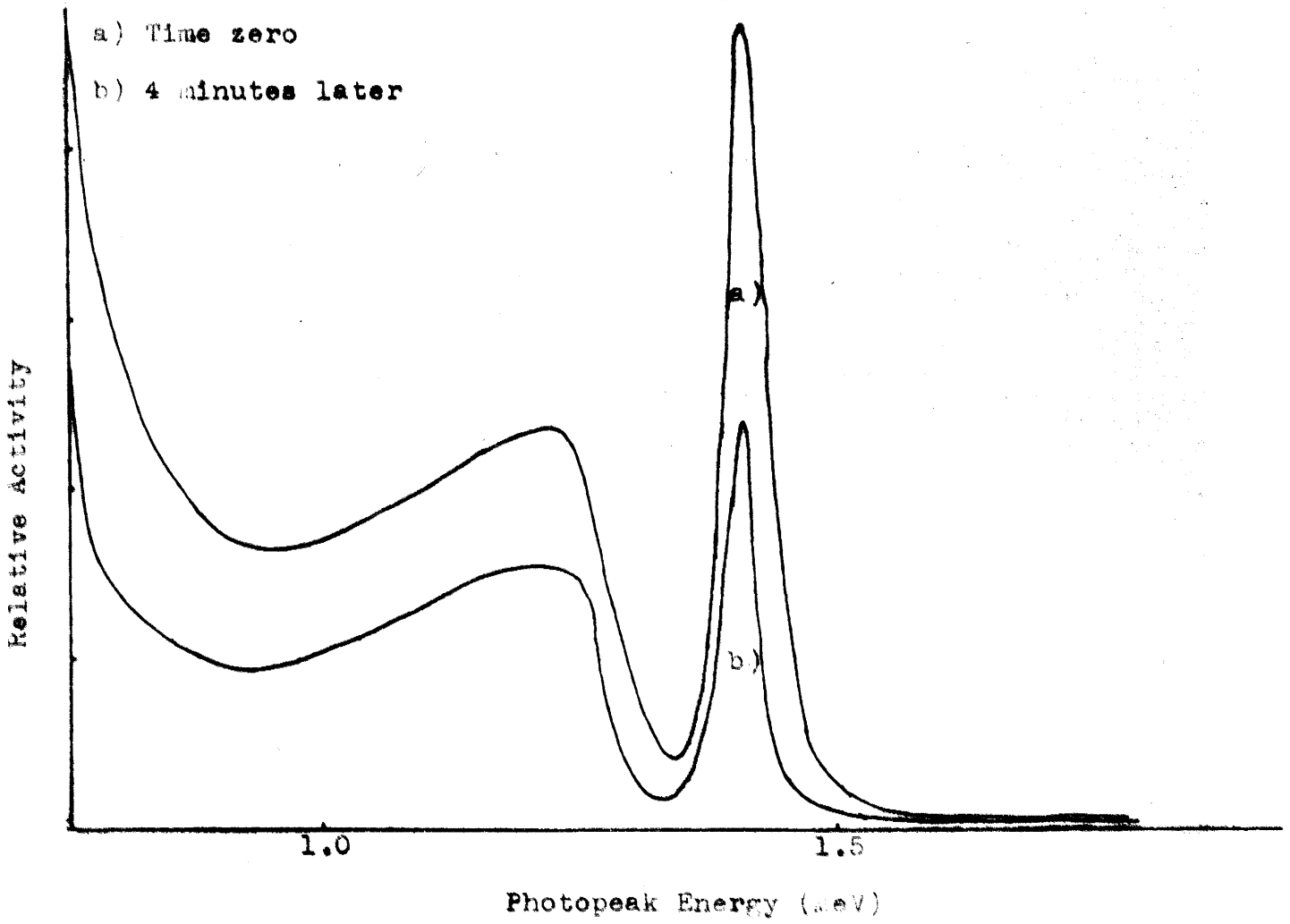
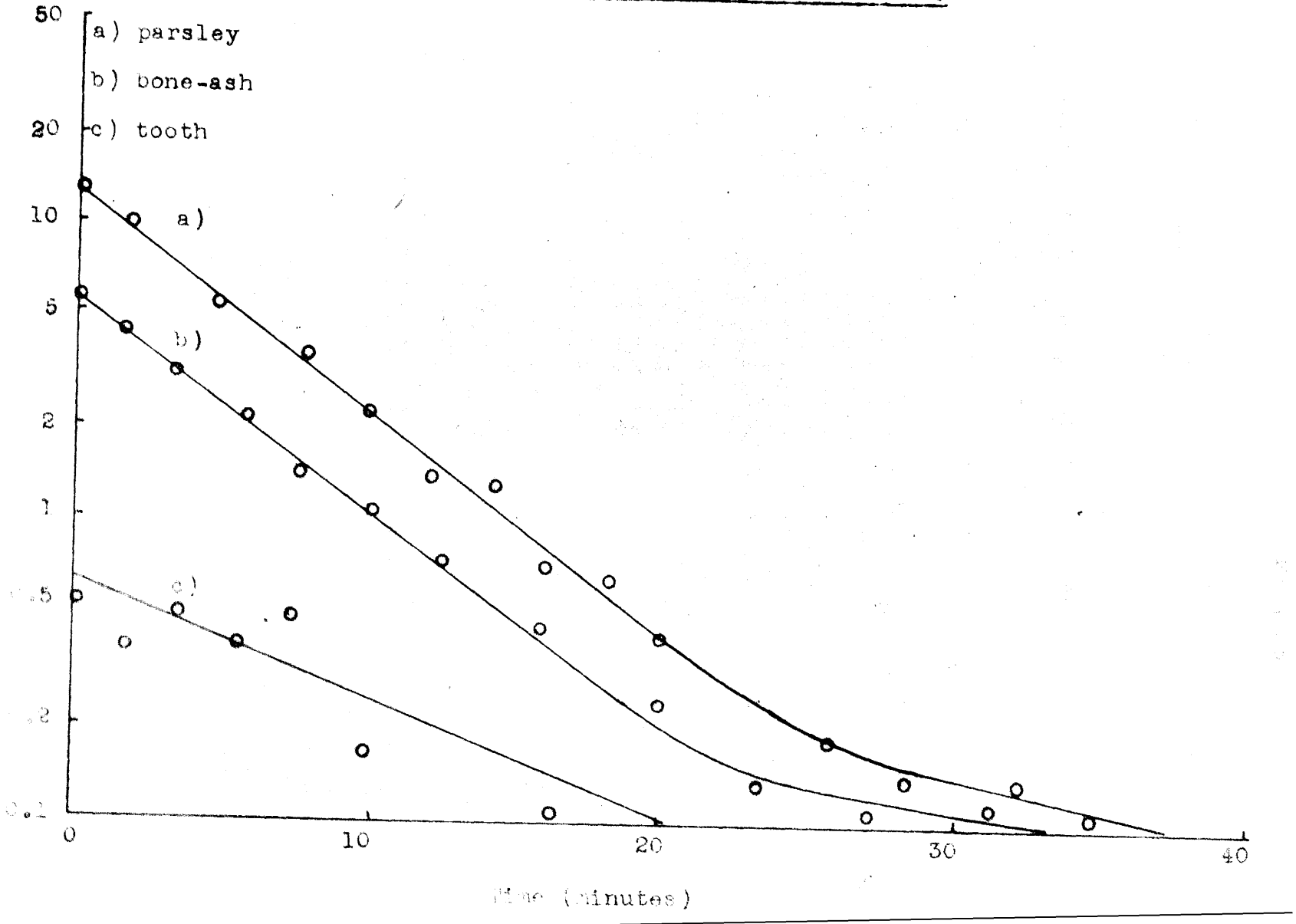


Fig. 22

Vanadium extract decay from various biological materials.



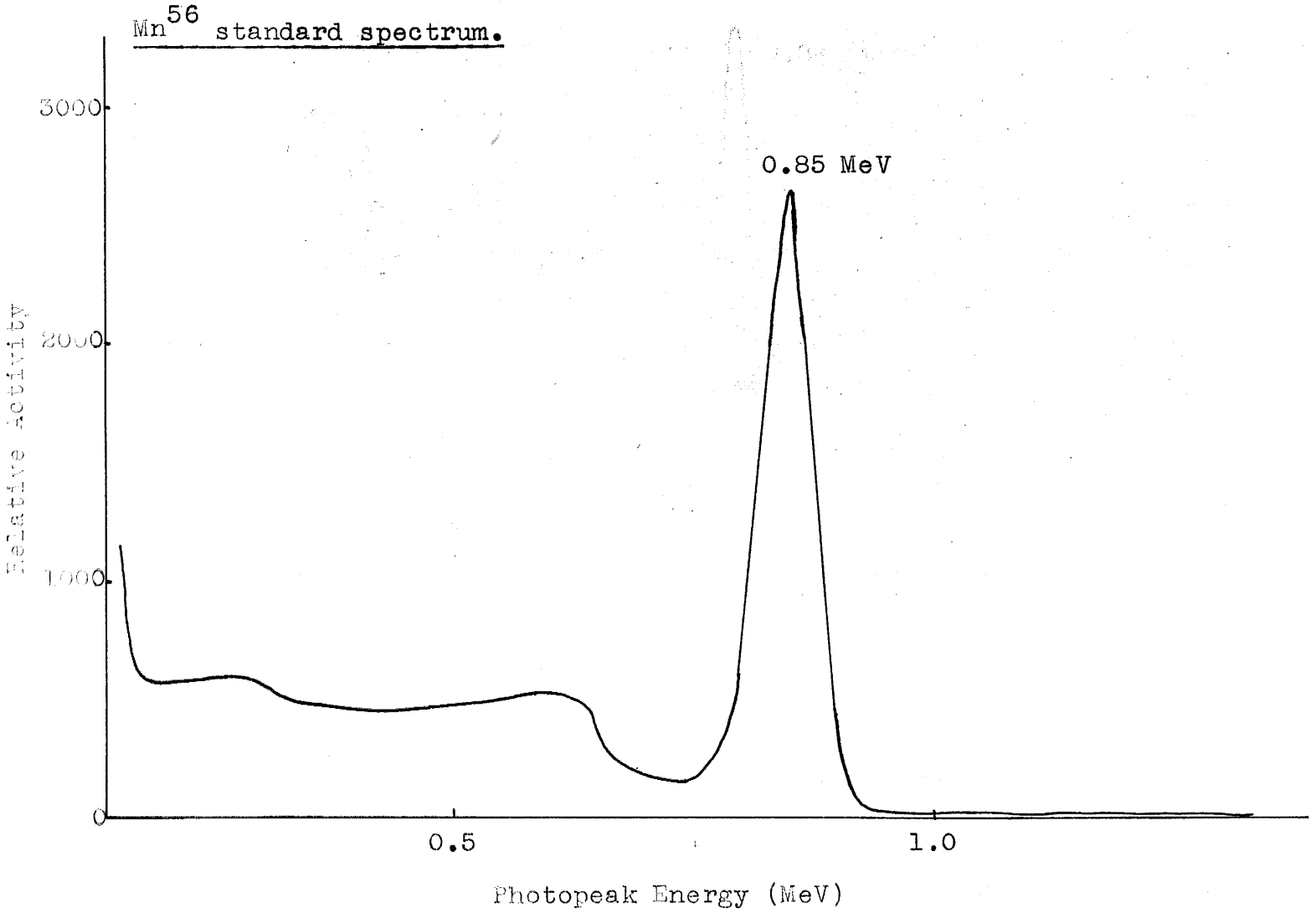

Mn56 standard spectrum.
3000
2000
1000
0
Relative Activity
0.85 MeV
0.5
1.0
Photopeak Energy (MeV)

Fig. 24

Spectrum of manganese dioxide precipitate from active enamel.

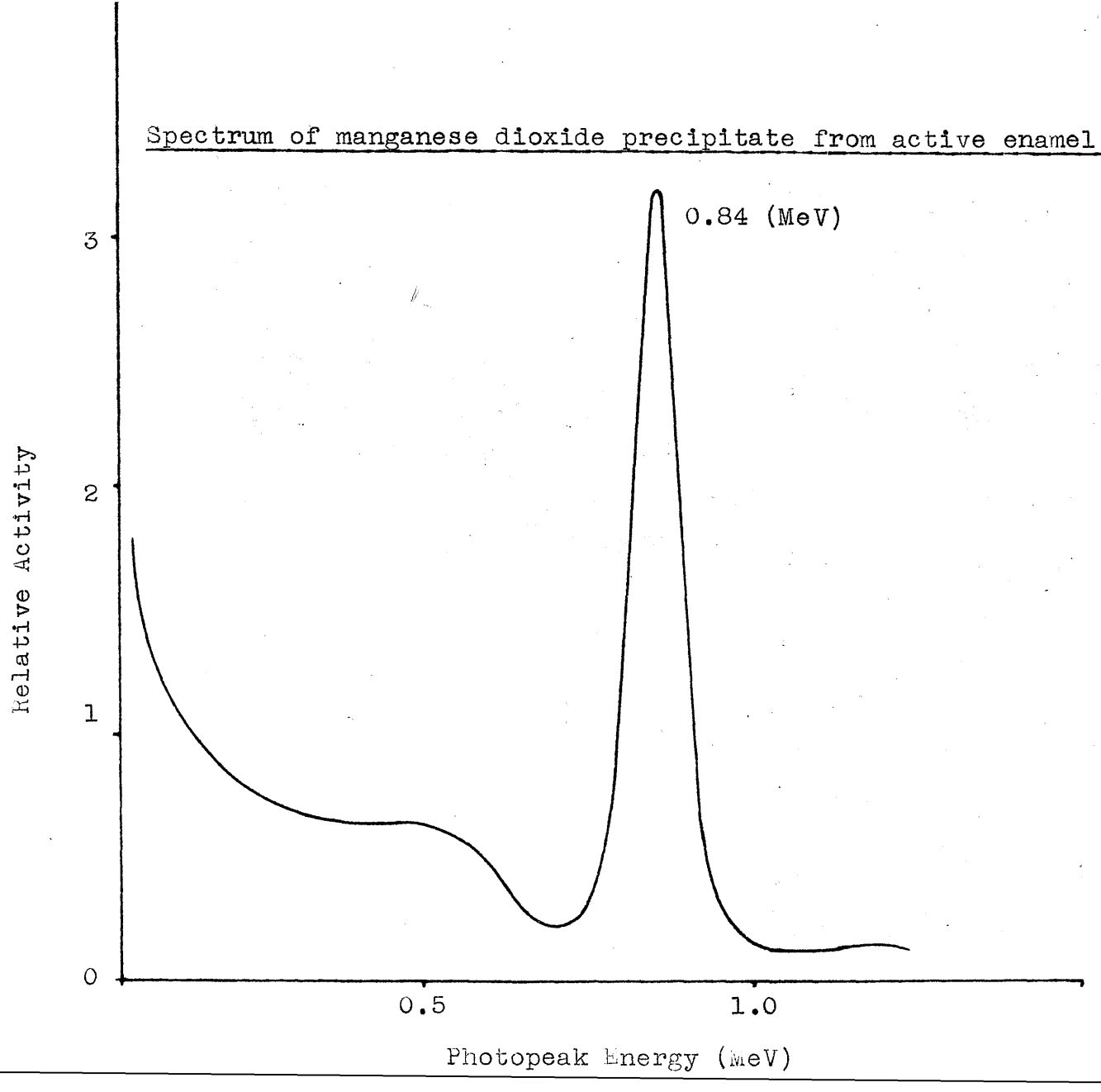


Fig. 25

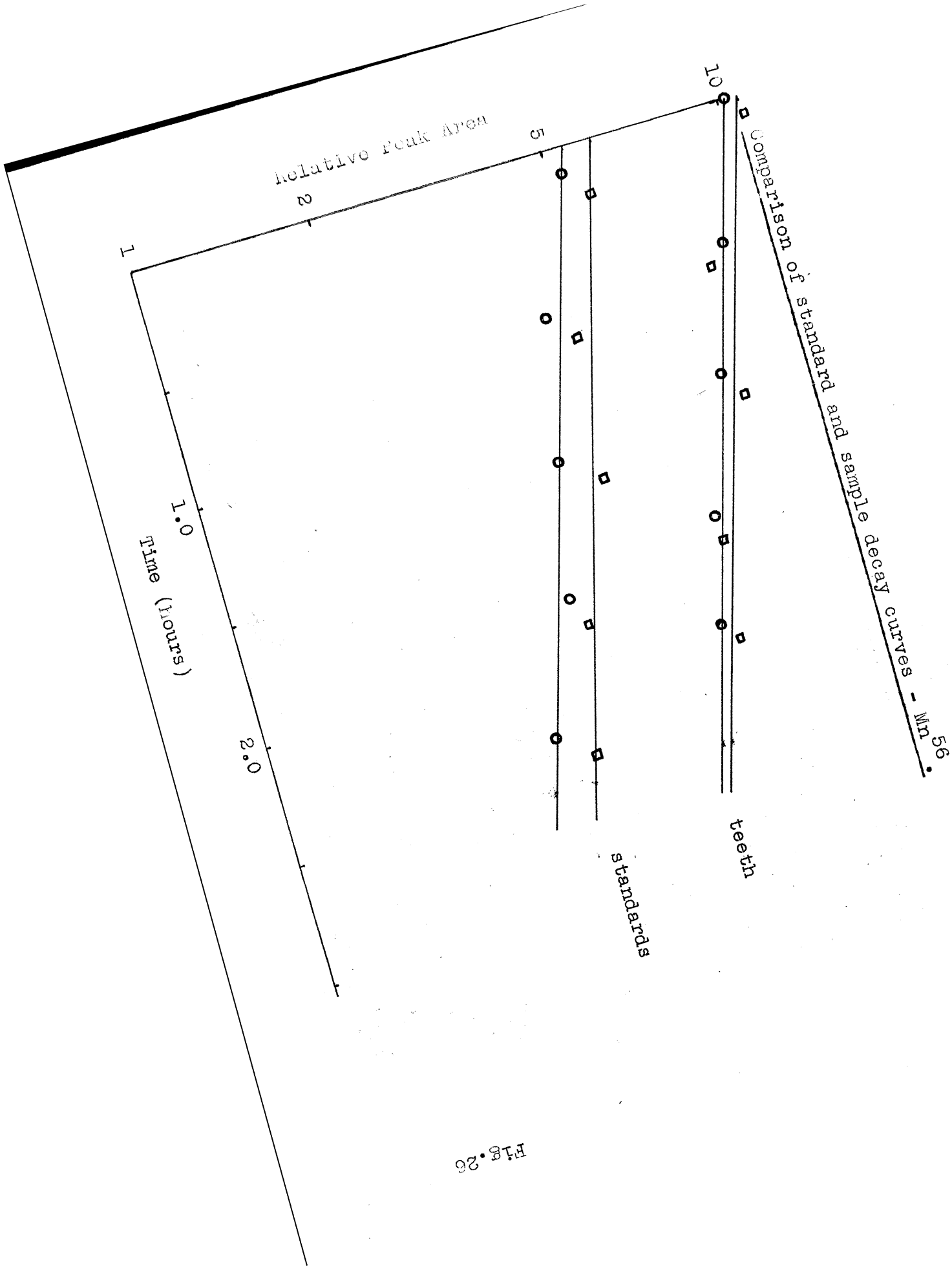

Comparison of standard and sample decay curves - Mn^{56}.
teeth
standards
Relative Peak Area
10
5
2
1
1.0
2.0
Time (hours)

Fig.26

Barium contamination of manganese activity from enamel.

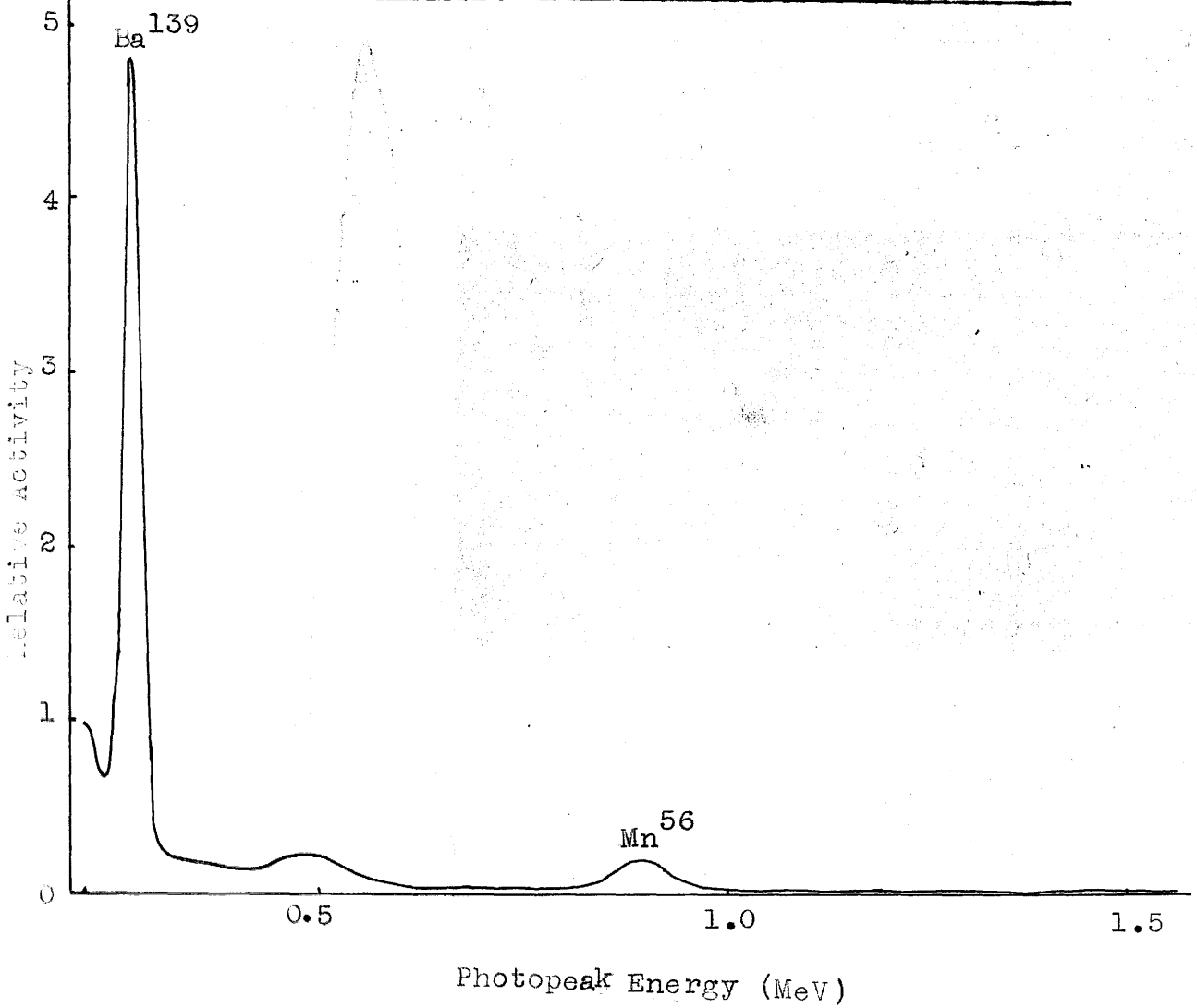


Fig. 27

Barium chromate precipitate spectra.

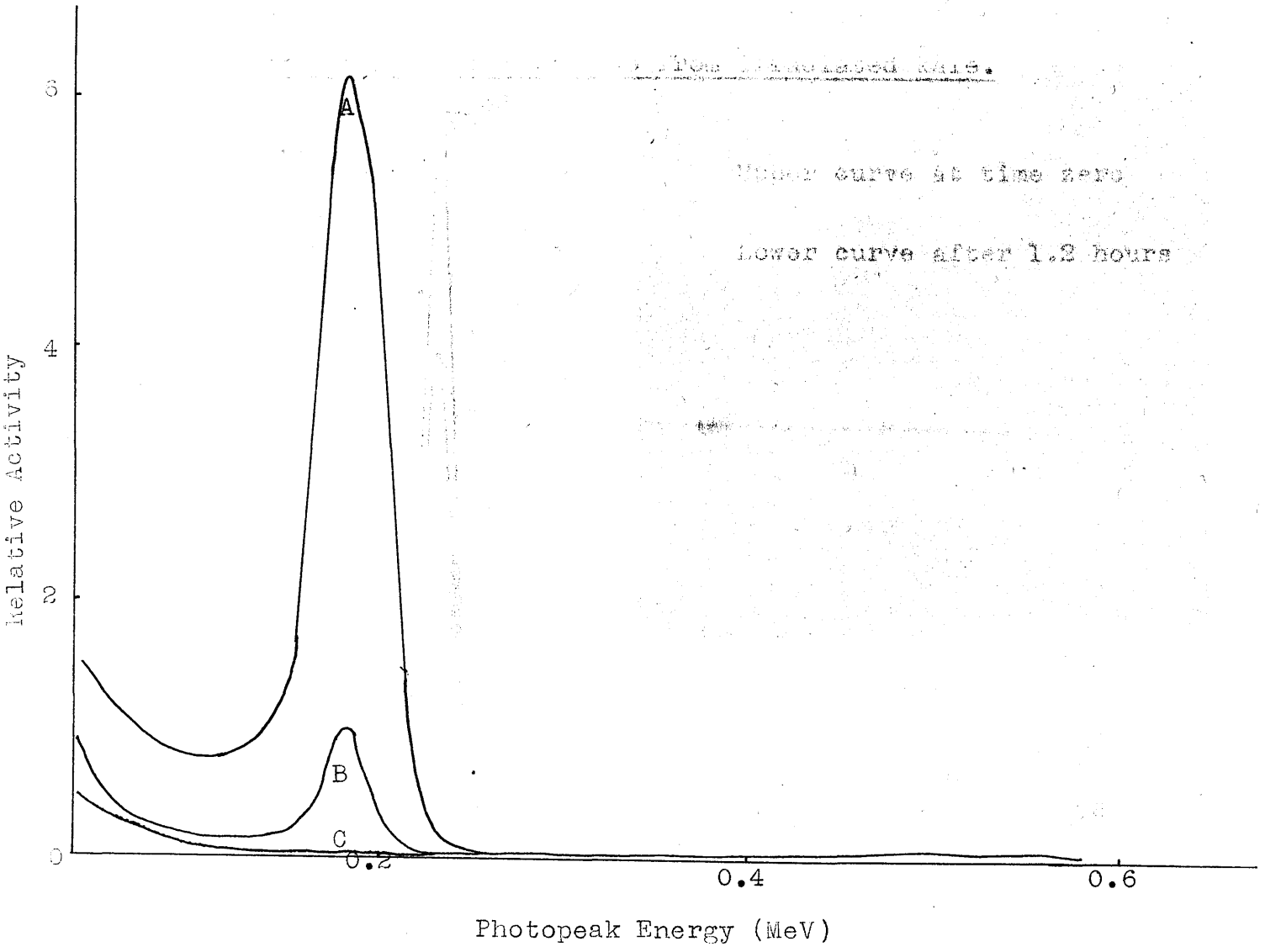


Fig. 28

Spectrum of manganous ferrocyanide from irradiated kale.

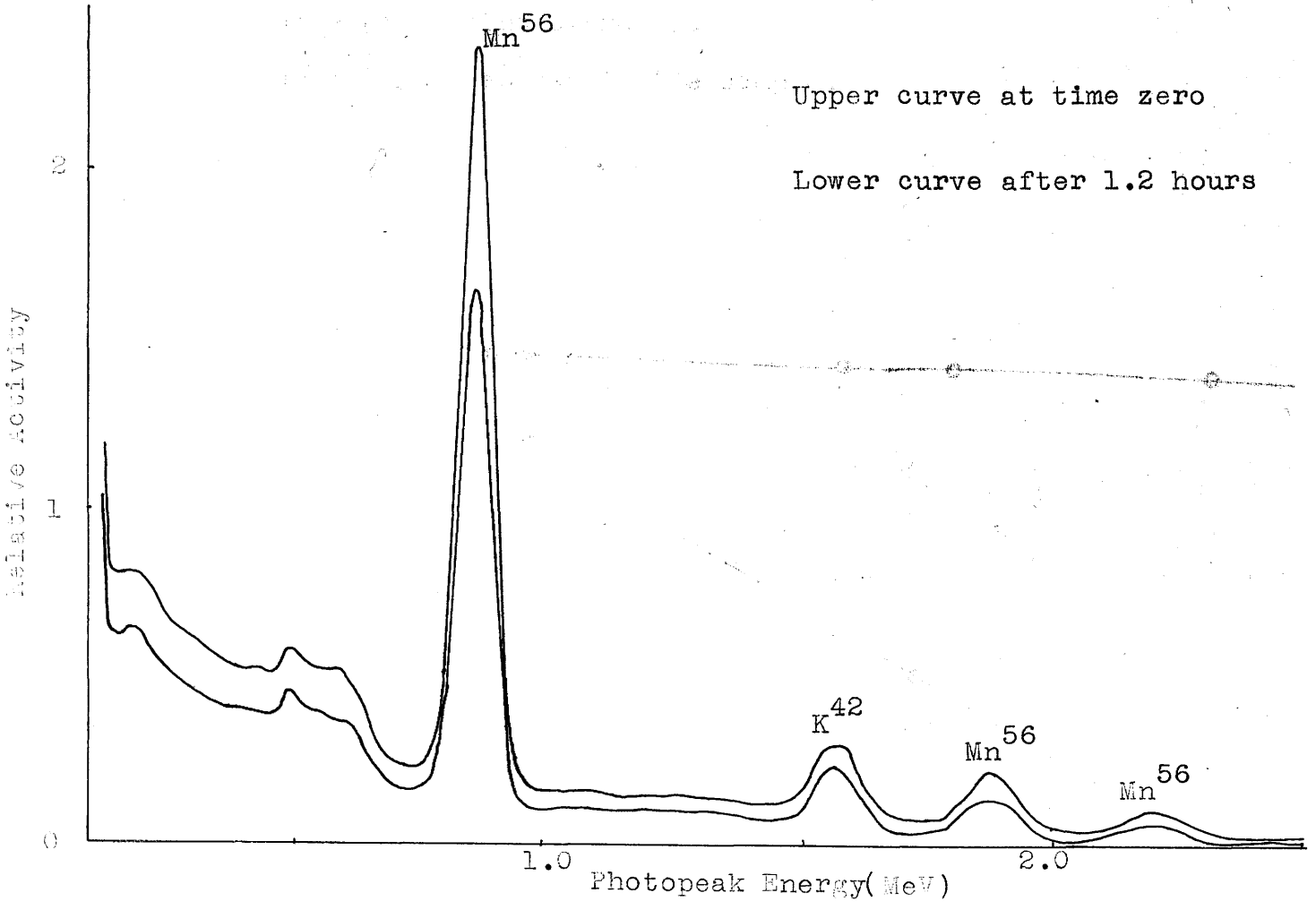
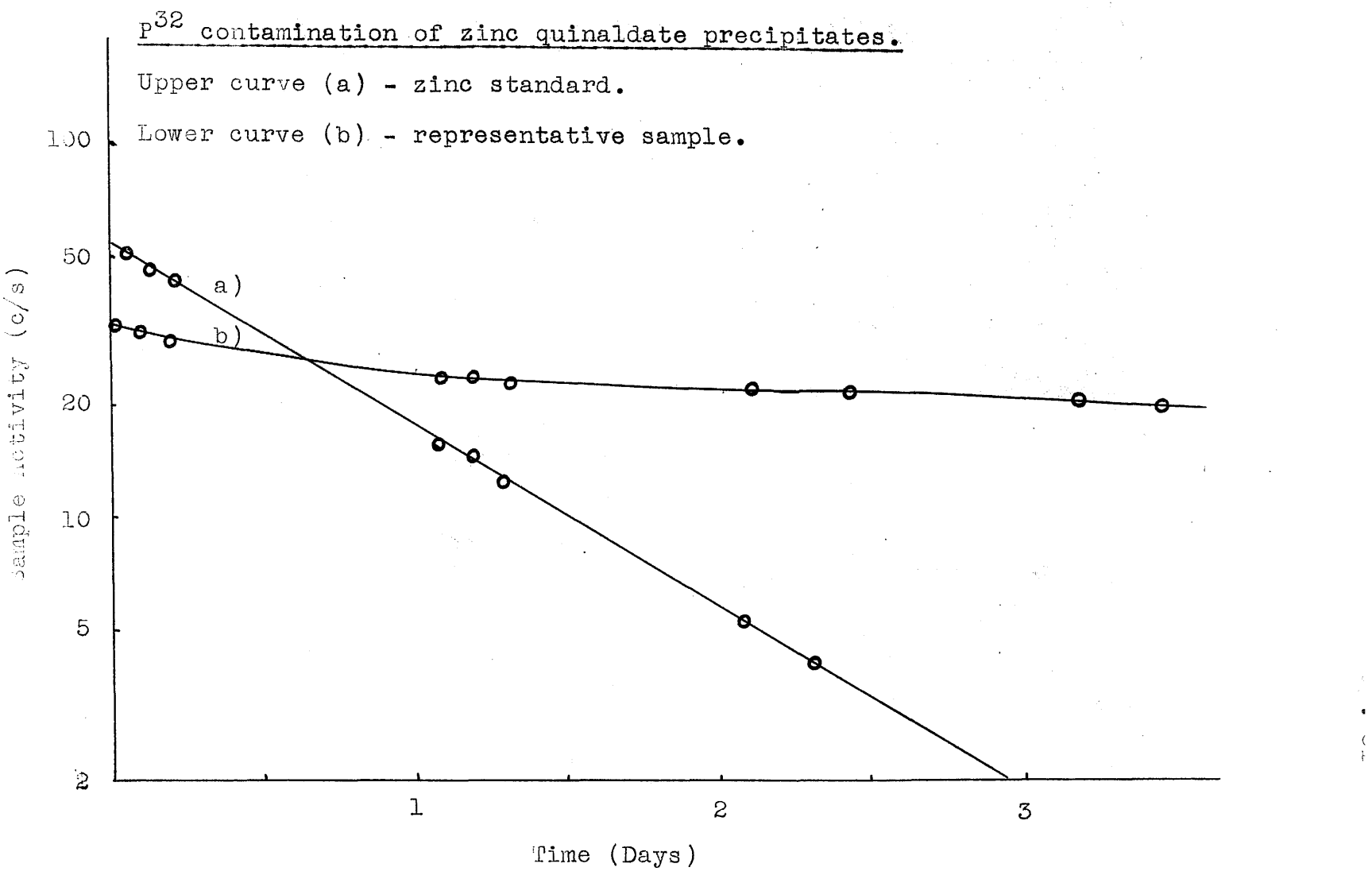
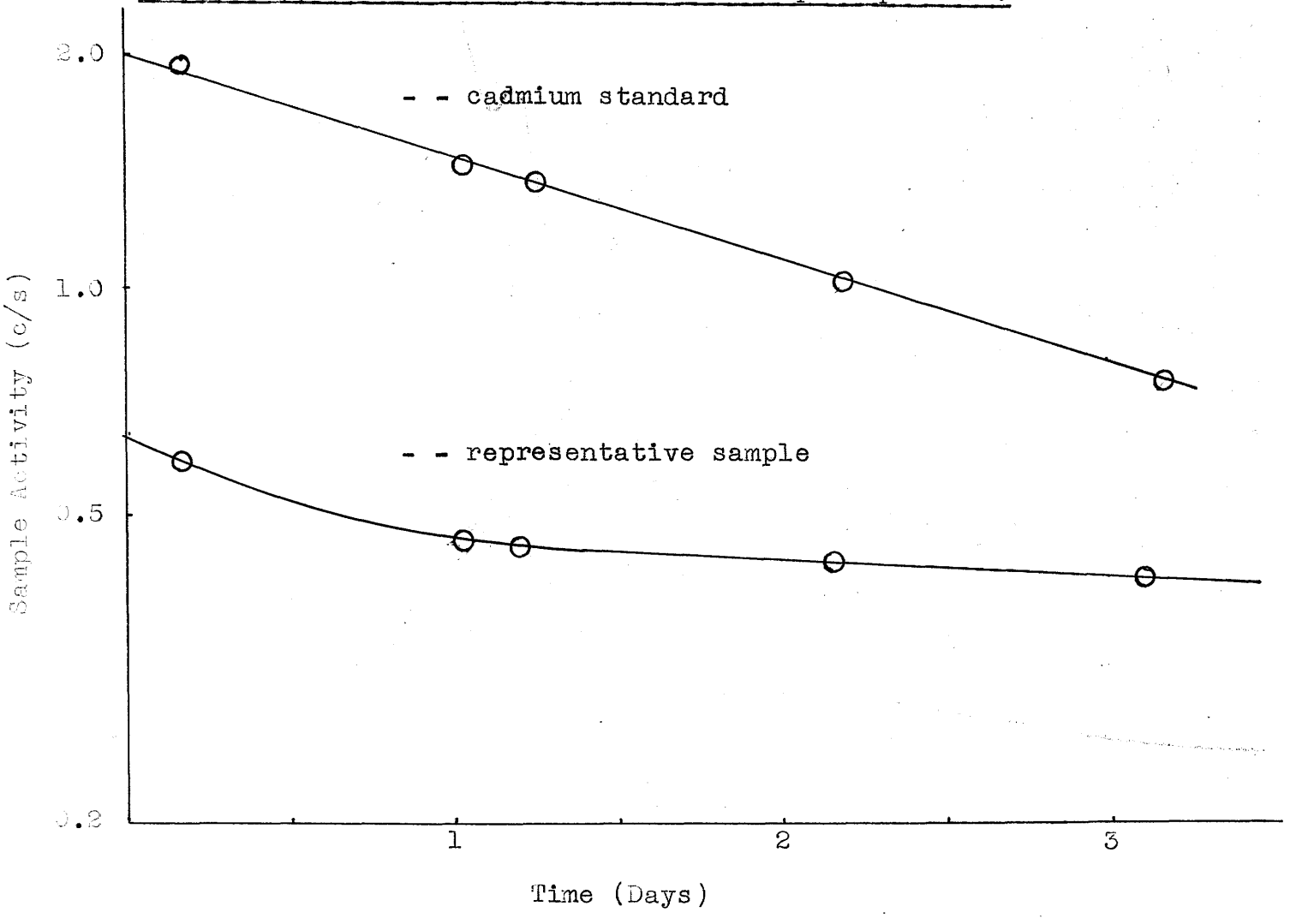


Fig. 29


P32 contamination of zinc quinaldate precipitates.
Upper curve (a) - zinc standard.
Lower curve (b) - representative sample.
100
50
20
10
5
2
a)
b)
Sample activity (c/s)
1
2
3
Time (Days)

P^{32} contamination of cadmium reineckate precipitates.



Gamma spectrum of cadmium reineckate precipitate.

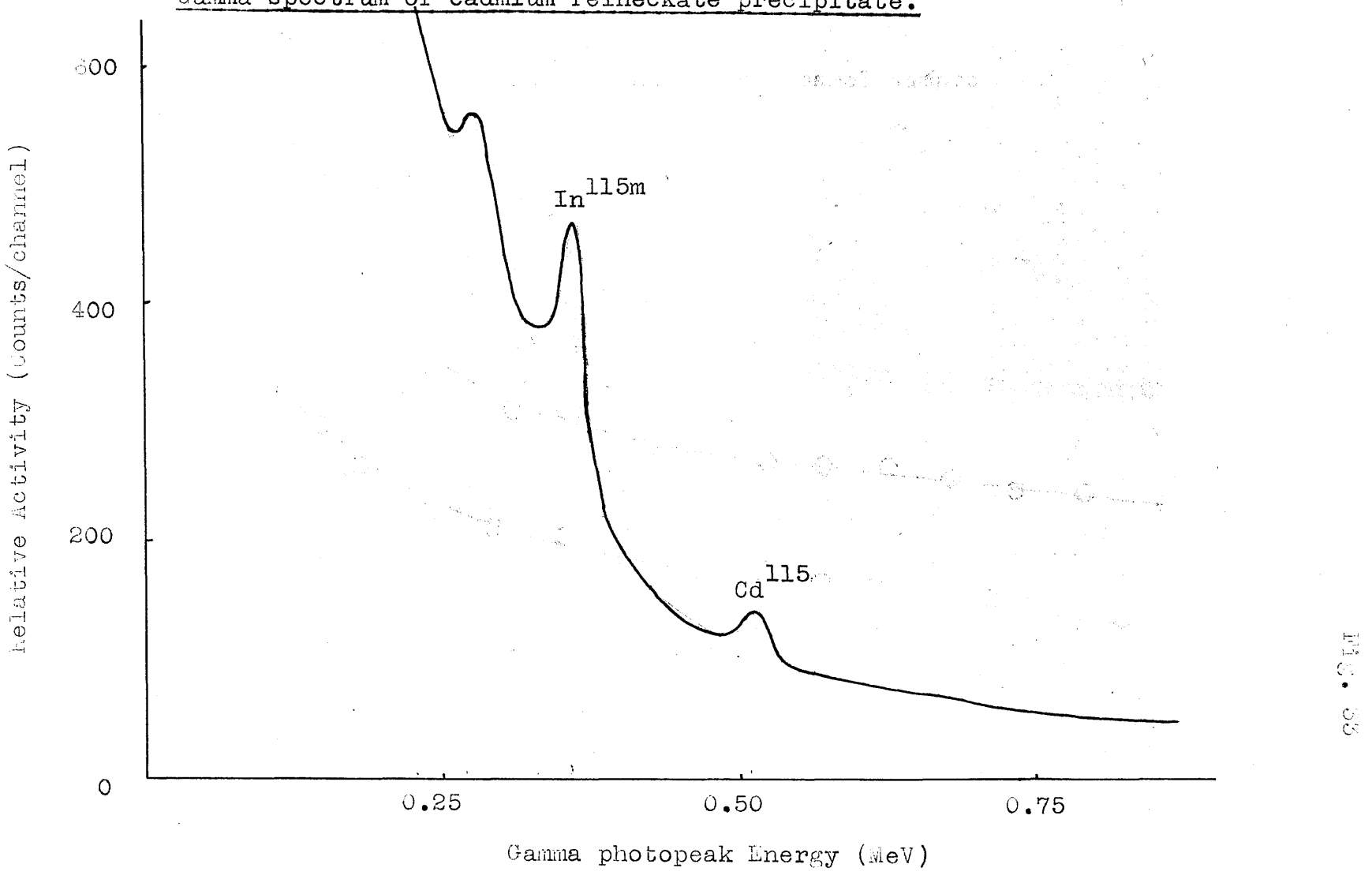
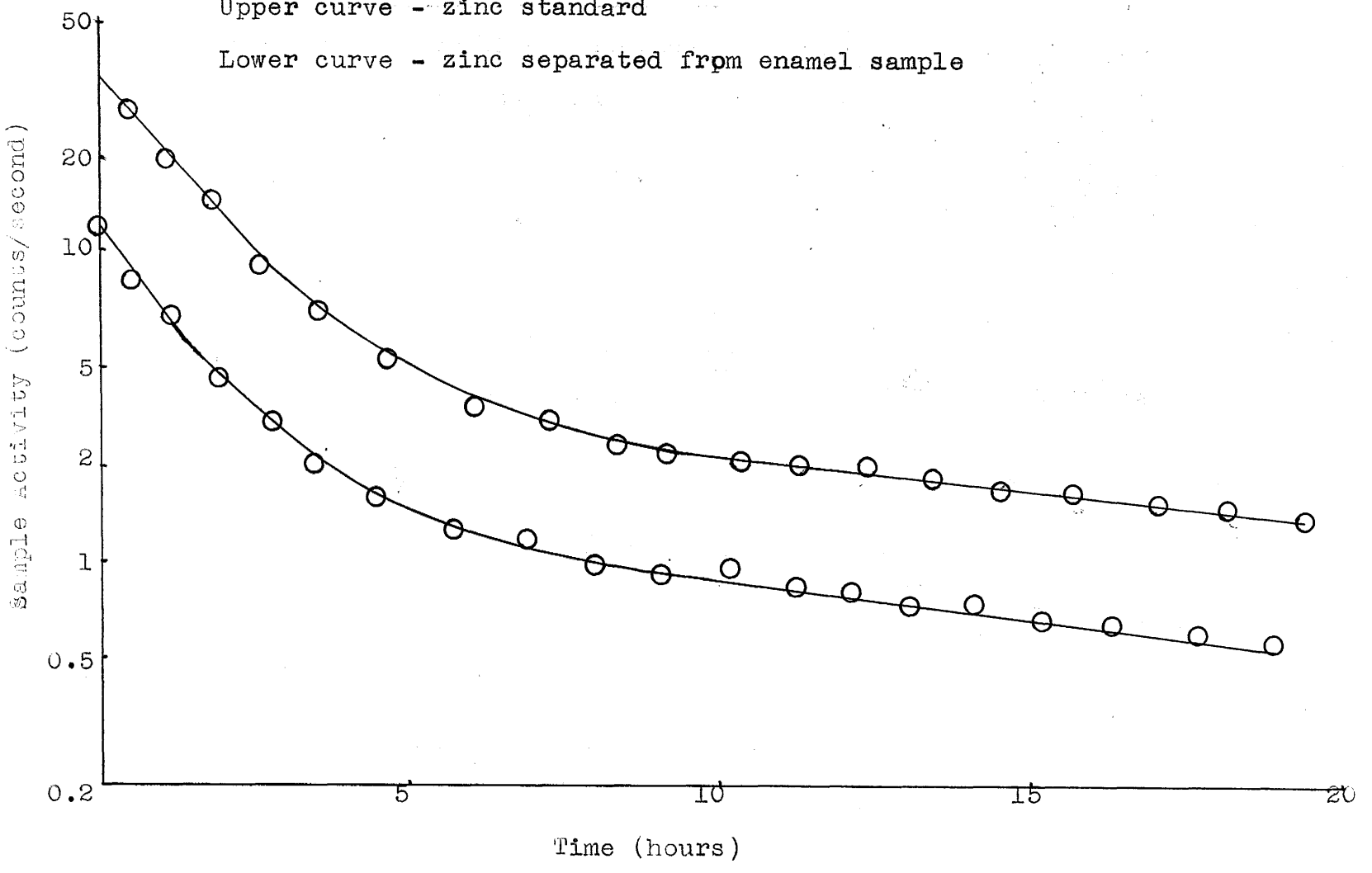


Fig. 33

$Zn^{69/69m}$ decay curves - zinc quinaldate precipitates.



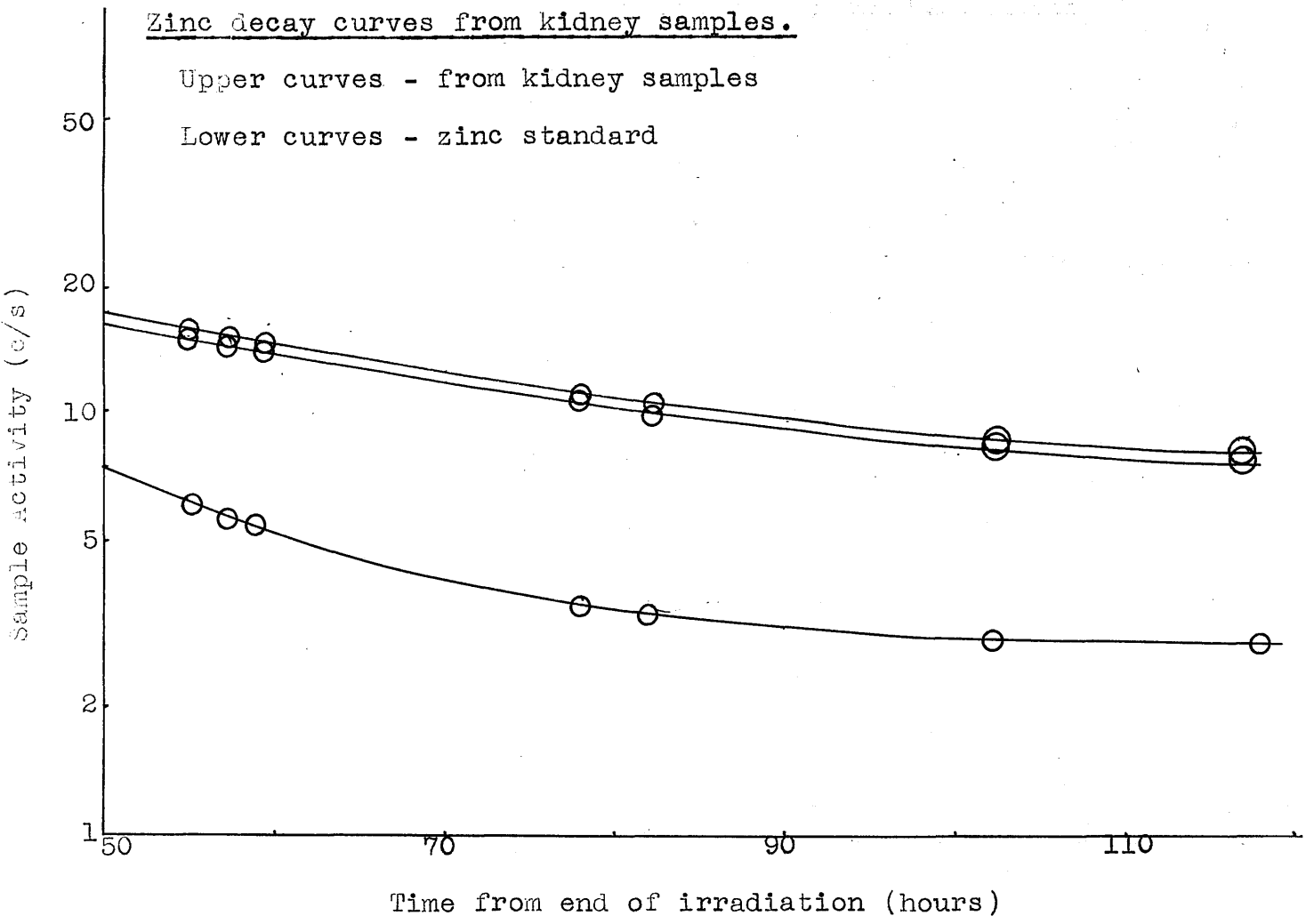
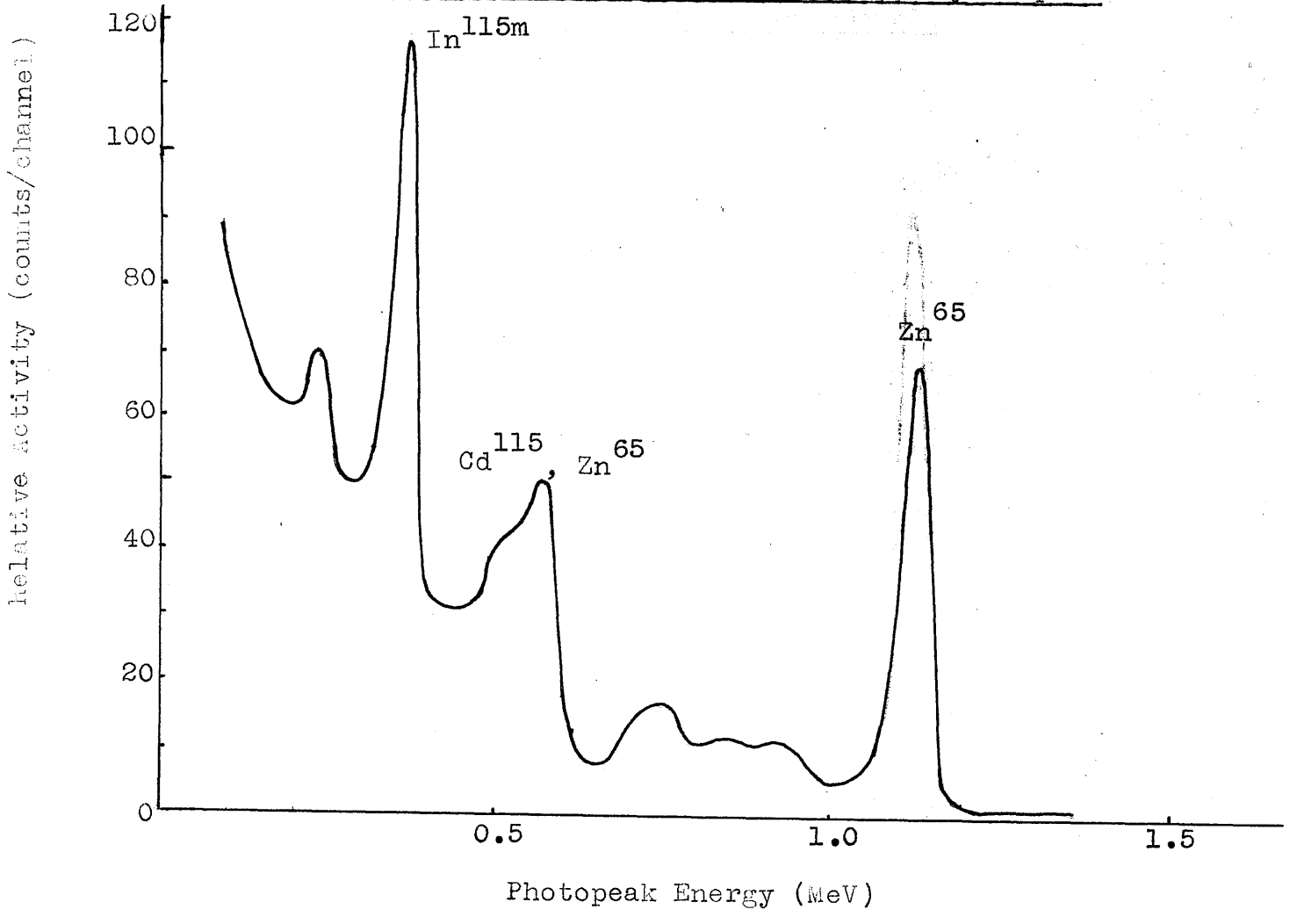
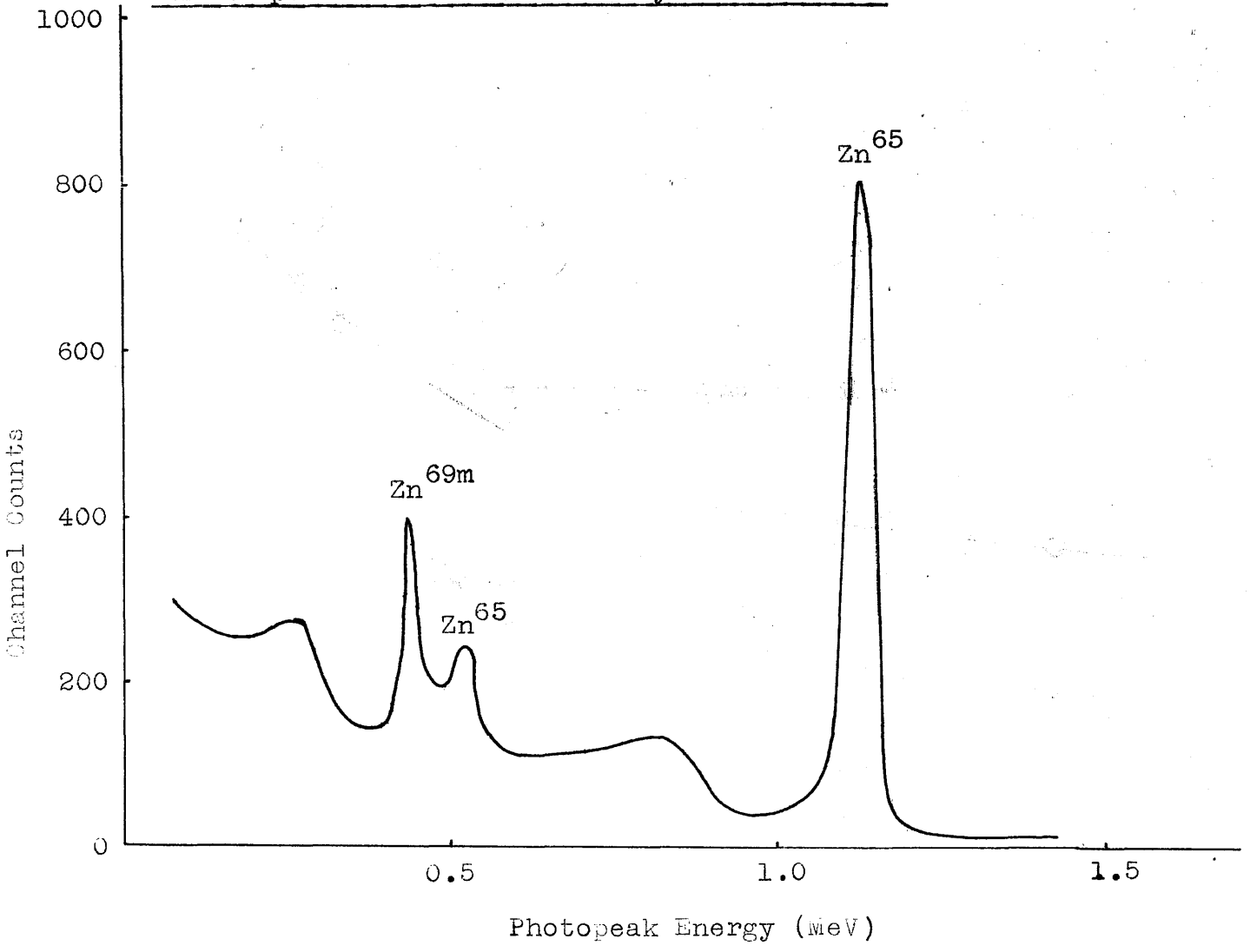

Zinc decay curves from kidney samples.
Upper curves - from kidney samples
Lower curves - zinc standard
Sample activity (c/s)
50
20
10
5
2
1
50
70
90
110
Time from end of irradiation (hours)

Fig. 65

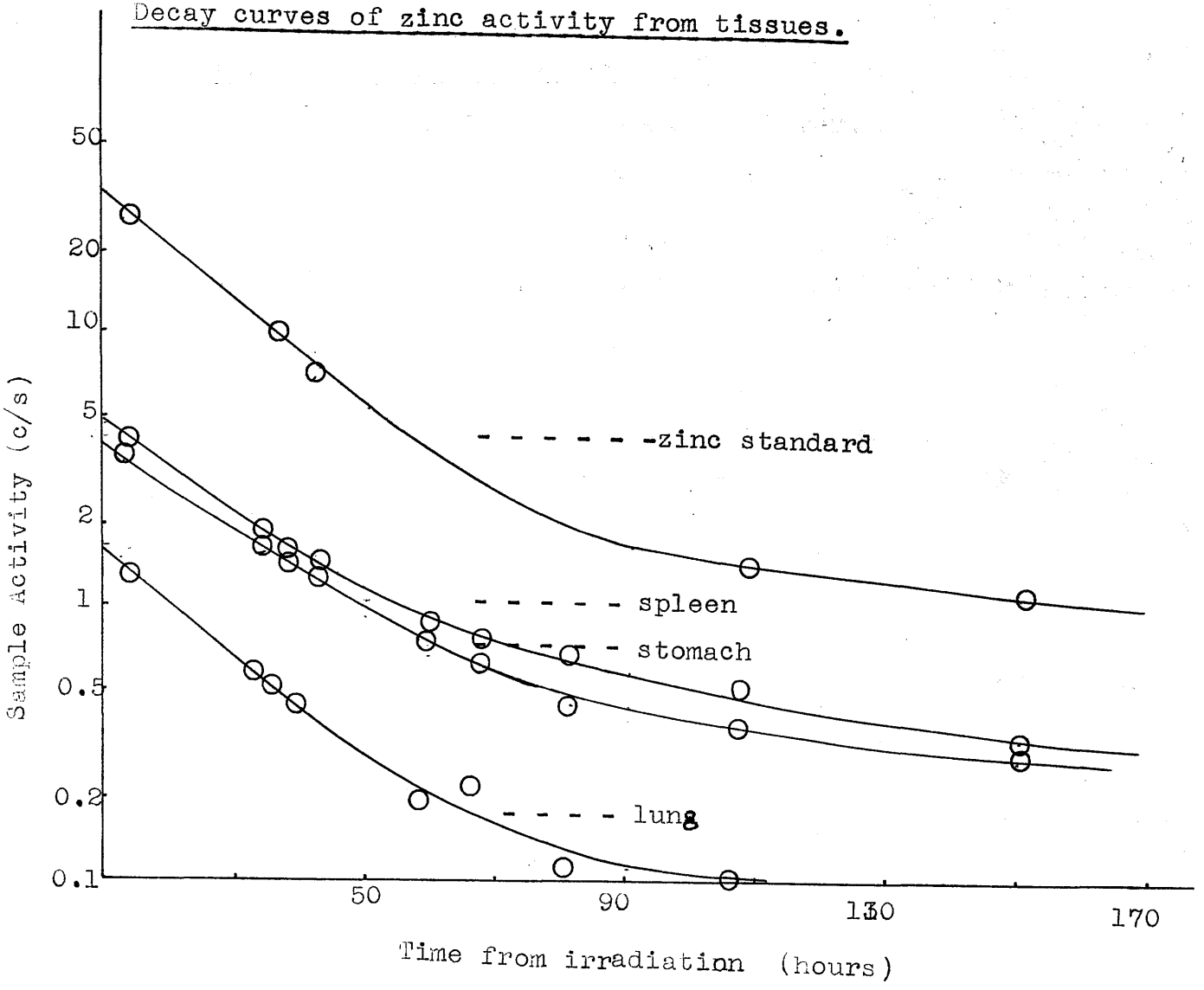
Gamma spectrum of zinc activity from a kidney sample.



Gamma spectrum of zinc activity from enamel.



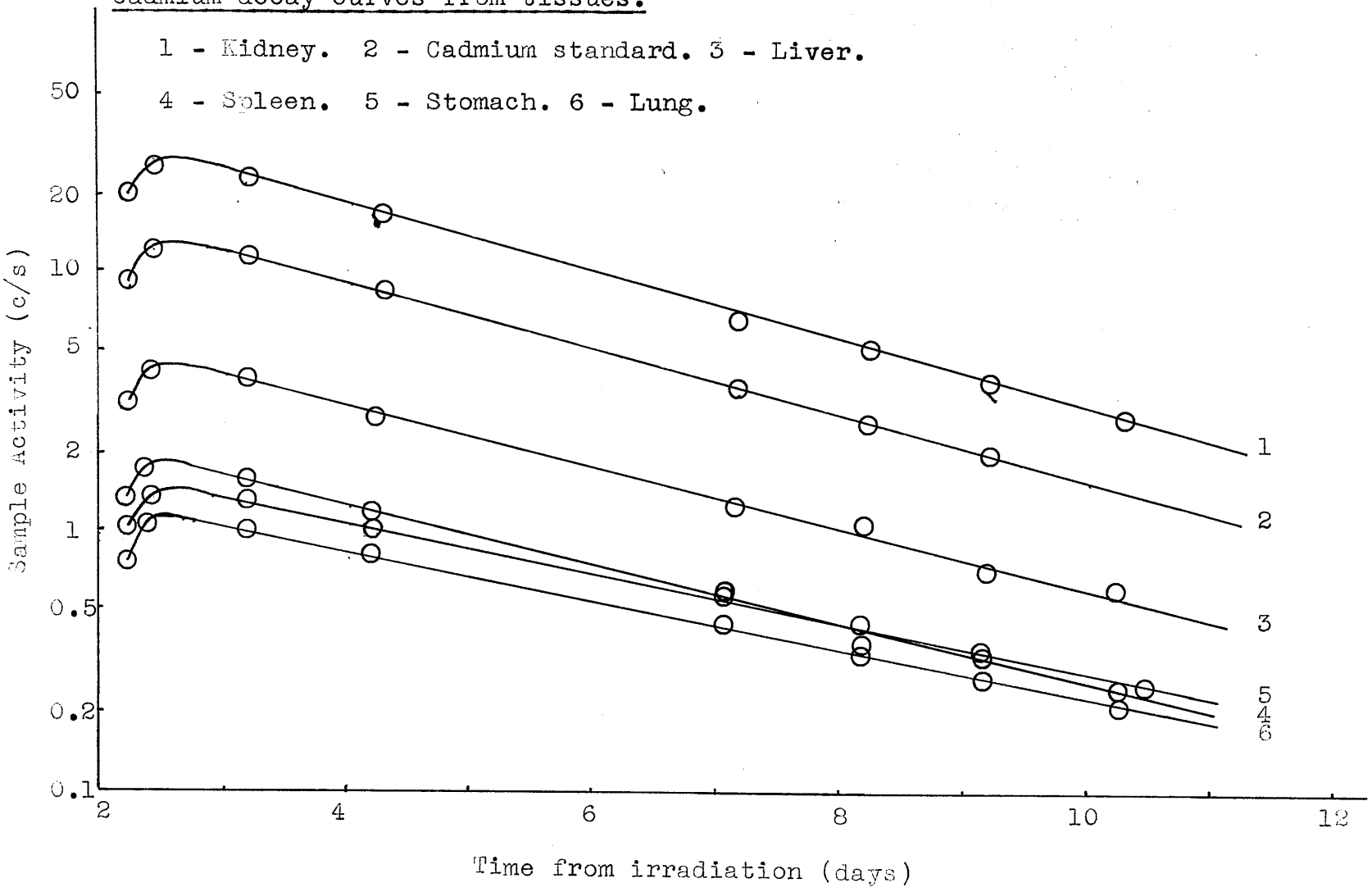
Decay curves of zinc activity from tissues.



Cadmium decay curves from tissues.

1 - Kidney. 2 - Cadmium standard. 3 - Liver.

4 - Spleen. 5 - Stomach. 6 - Lung.



Gamma spectrum showing cadmium activity from kidney tissue.

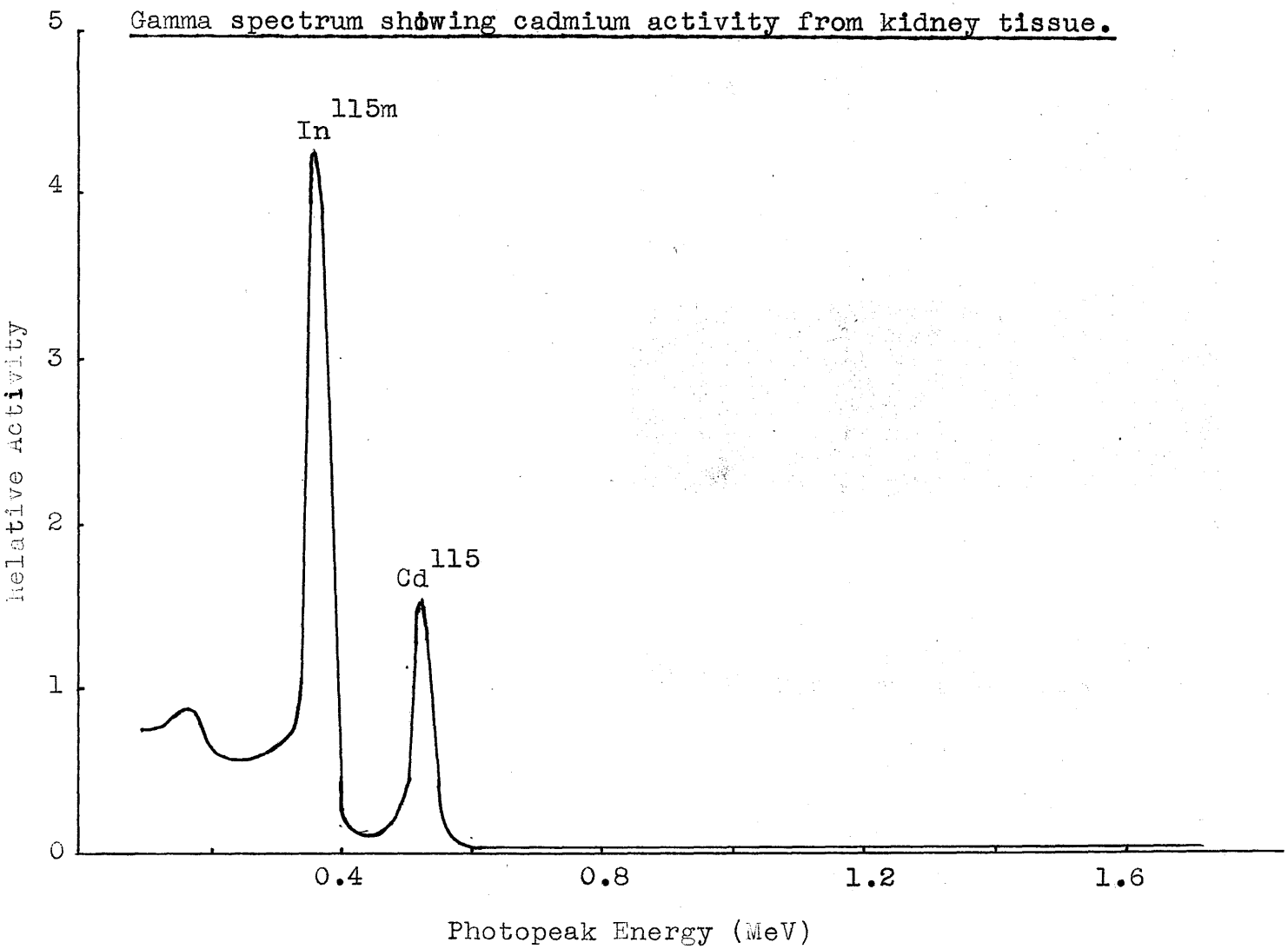


Fig. 40

Decay curve analysis of cadmium contaminant.

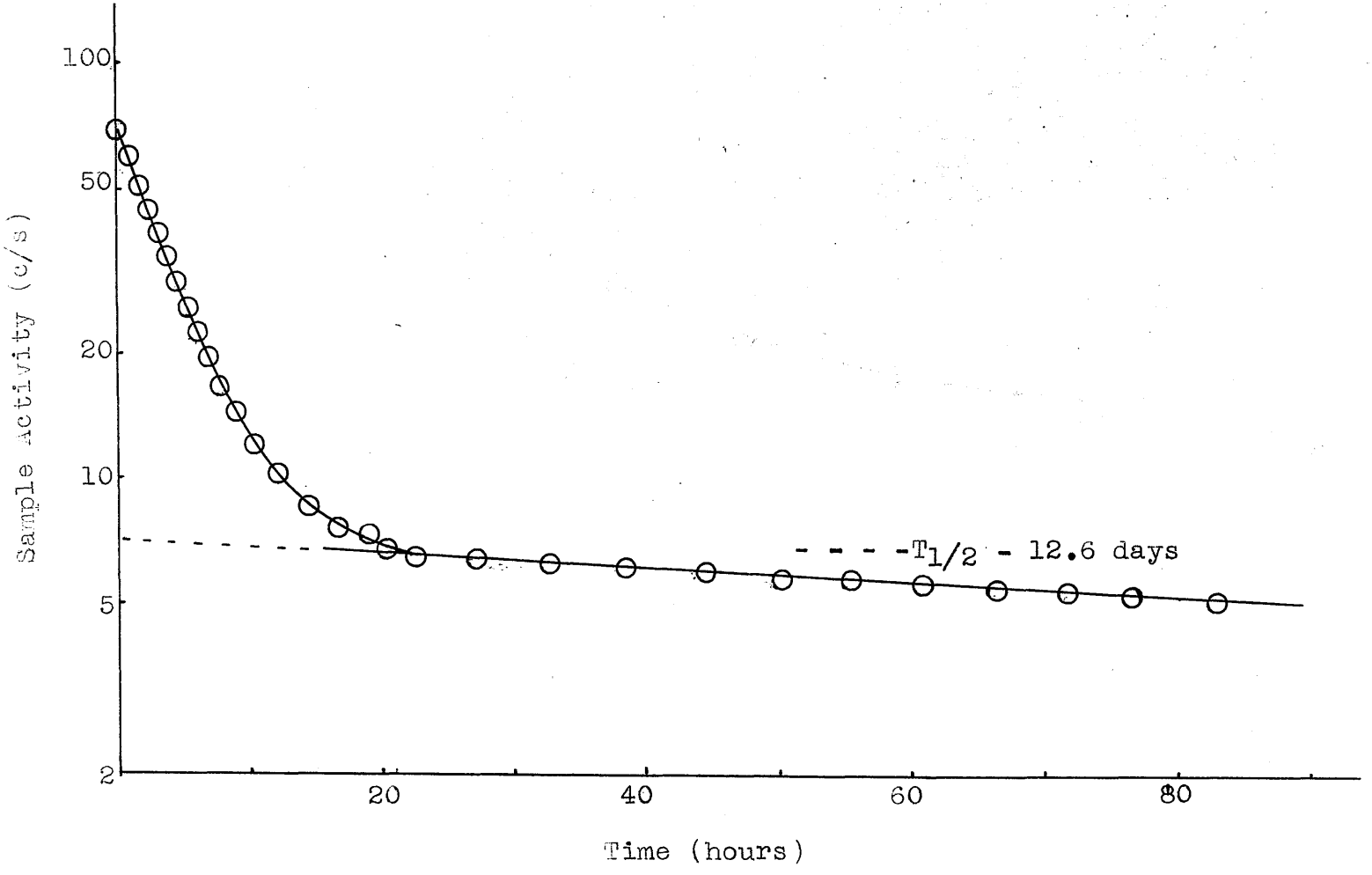
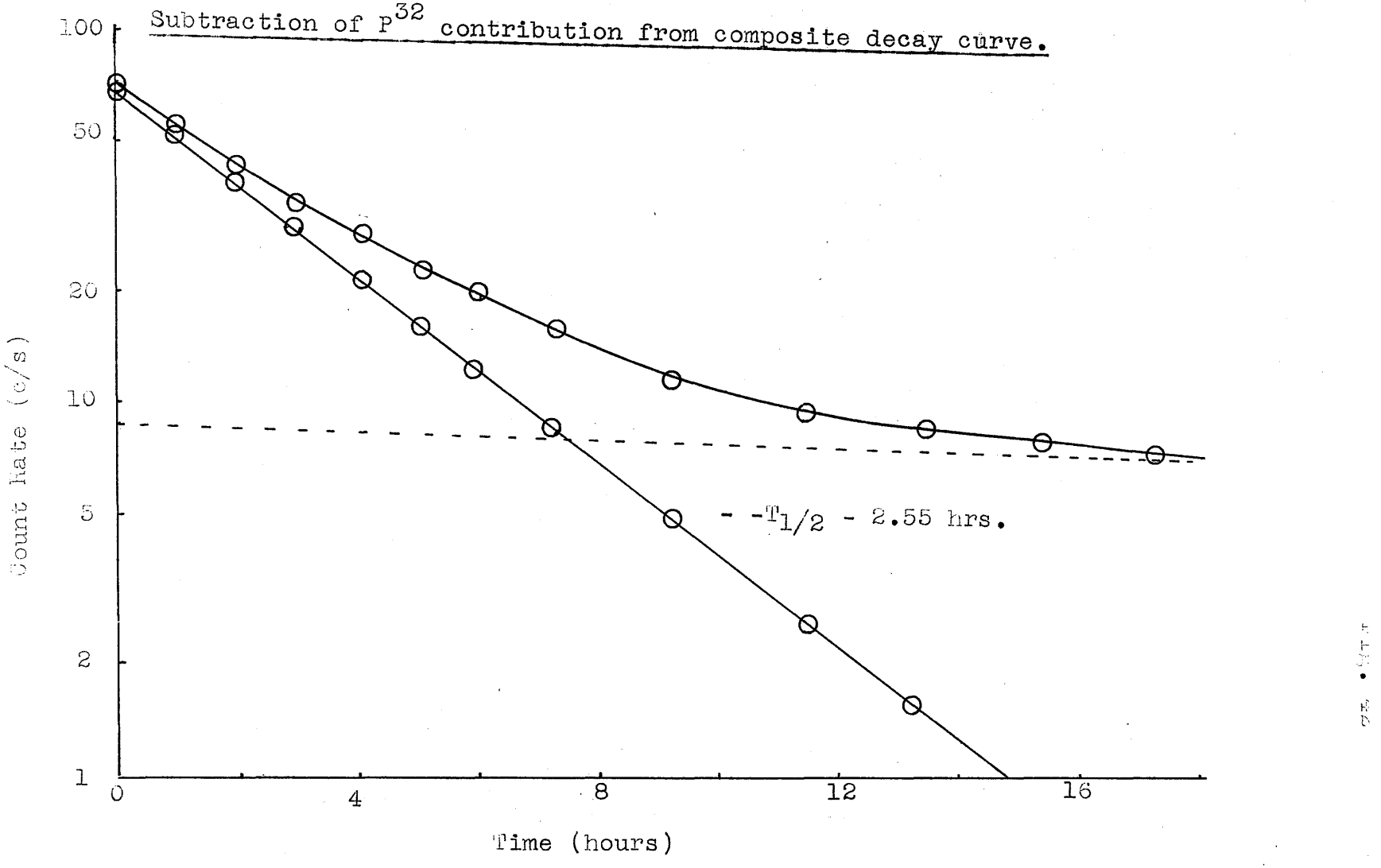
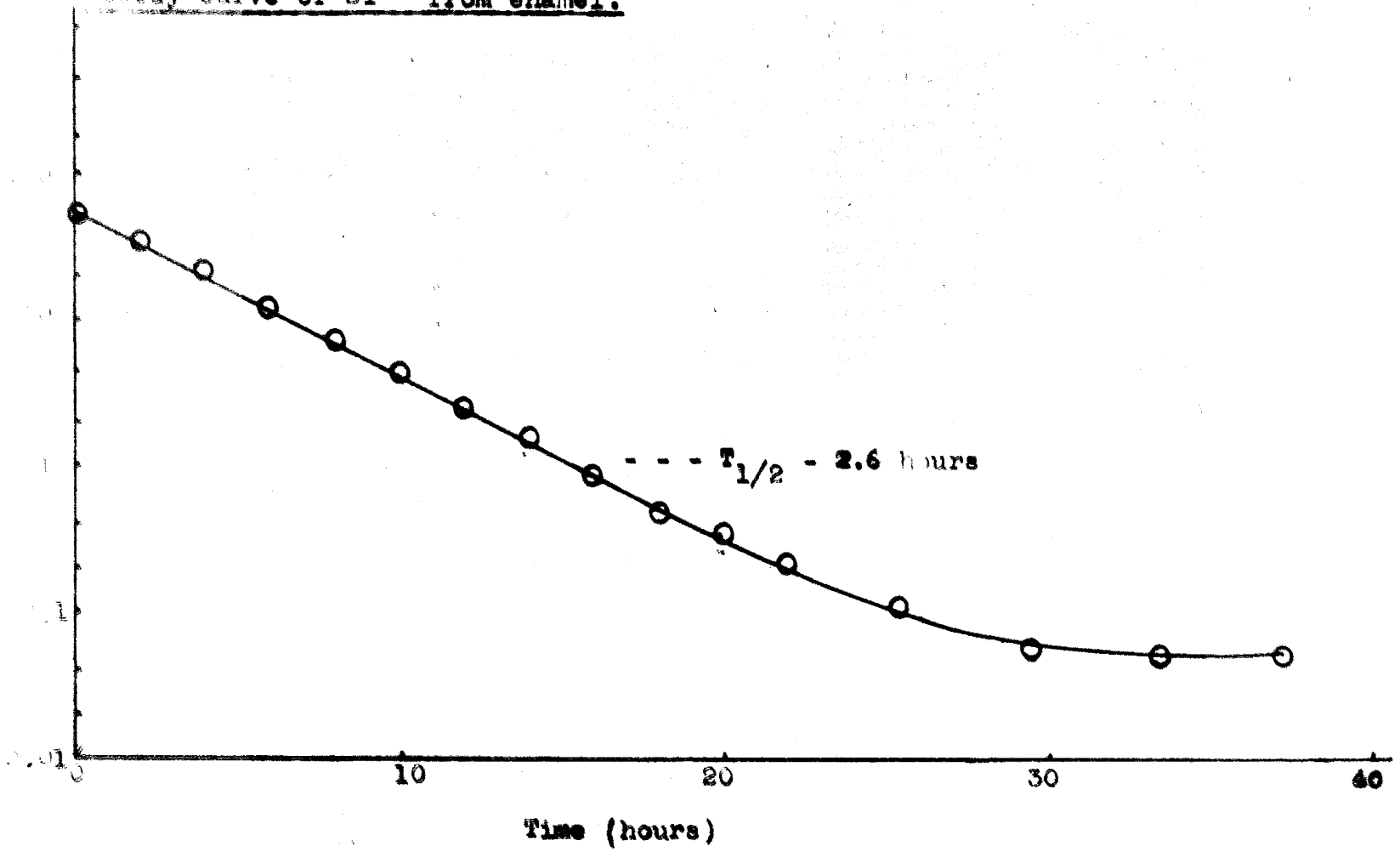


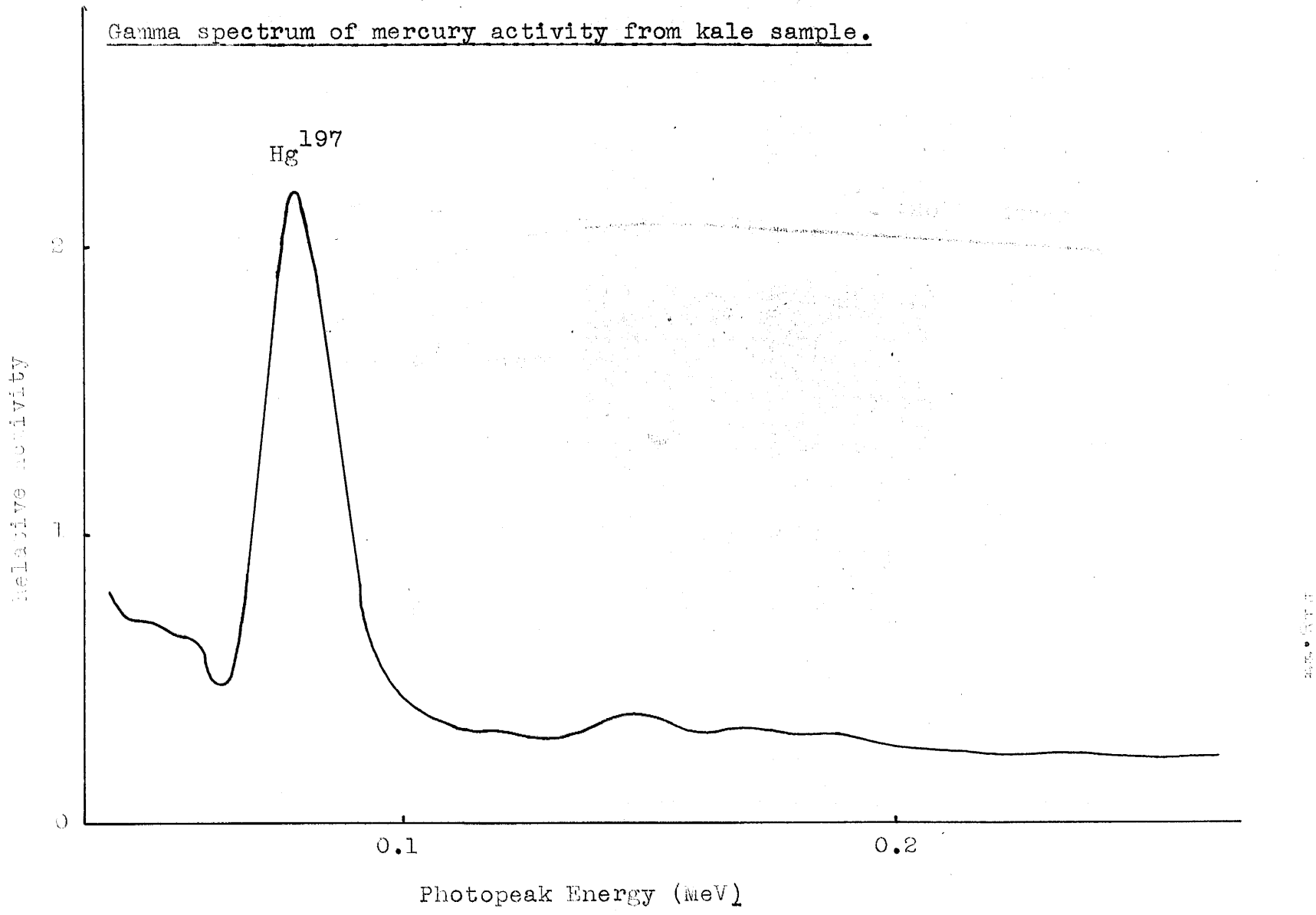
Fig. 41

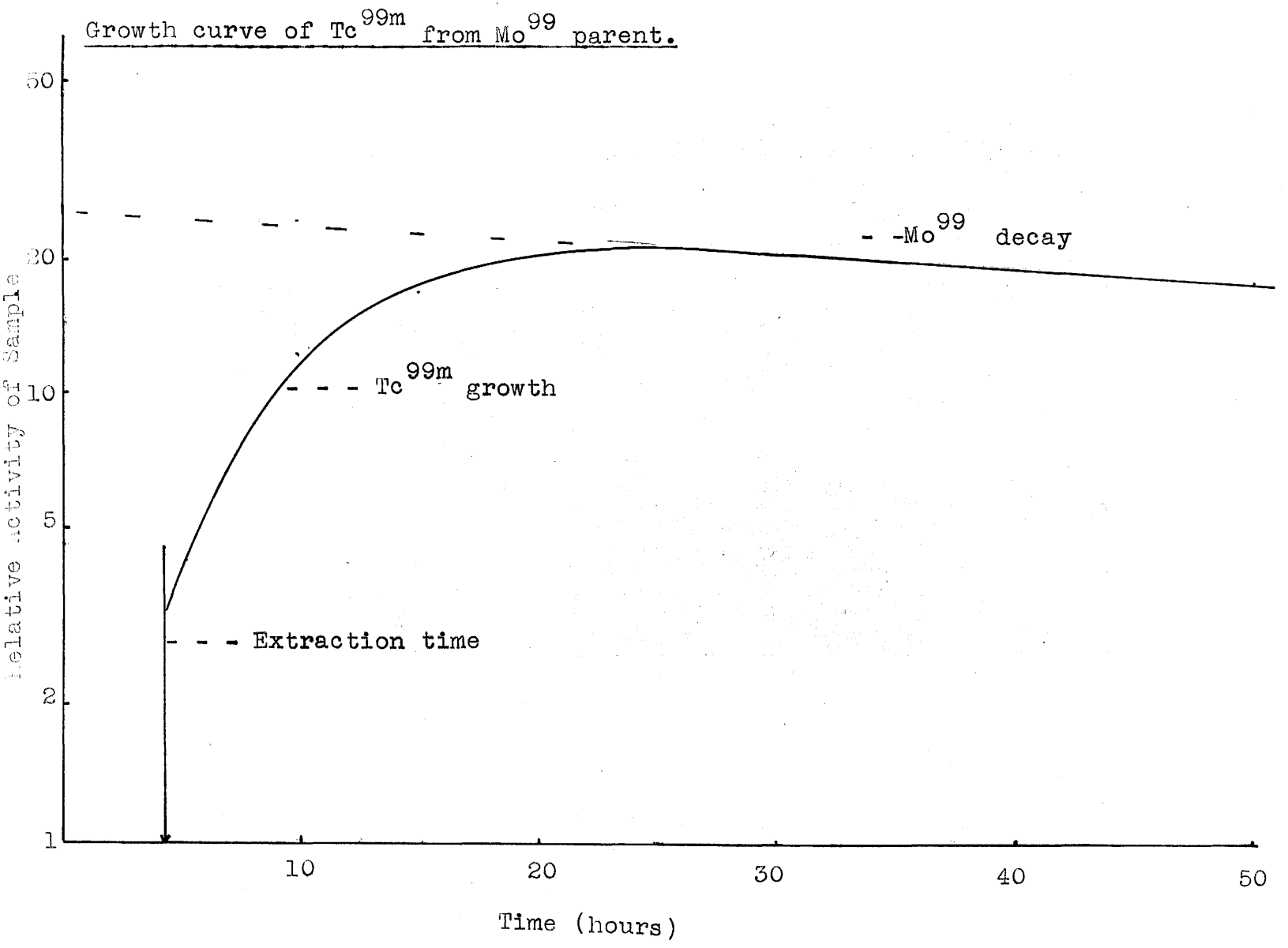

Subtraction of P^{32} contribution from composite decay curve.
100
50
20
10
5
2
1
Count rate (c/s)
0
4
8
12
16
Time (hours)
- - $T_{1/2}$ - 2.55 hrs.

Decay curve of Si^{31} from enamel.

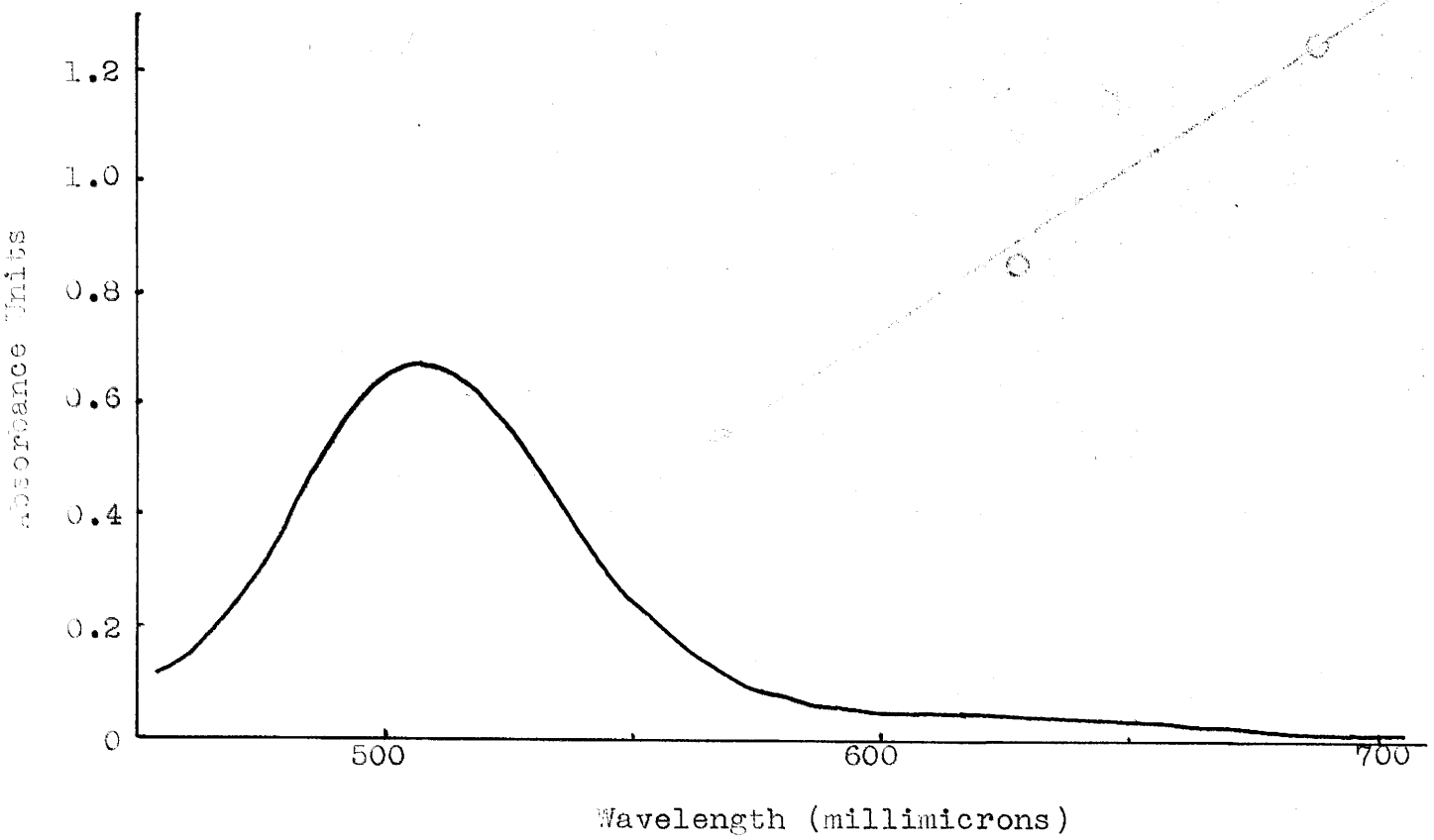


Gamma spectrum of mercury activity from kale sample.

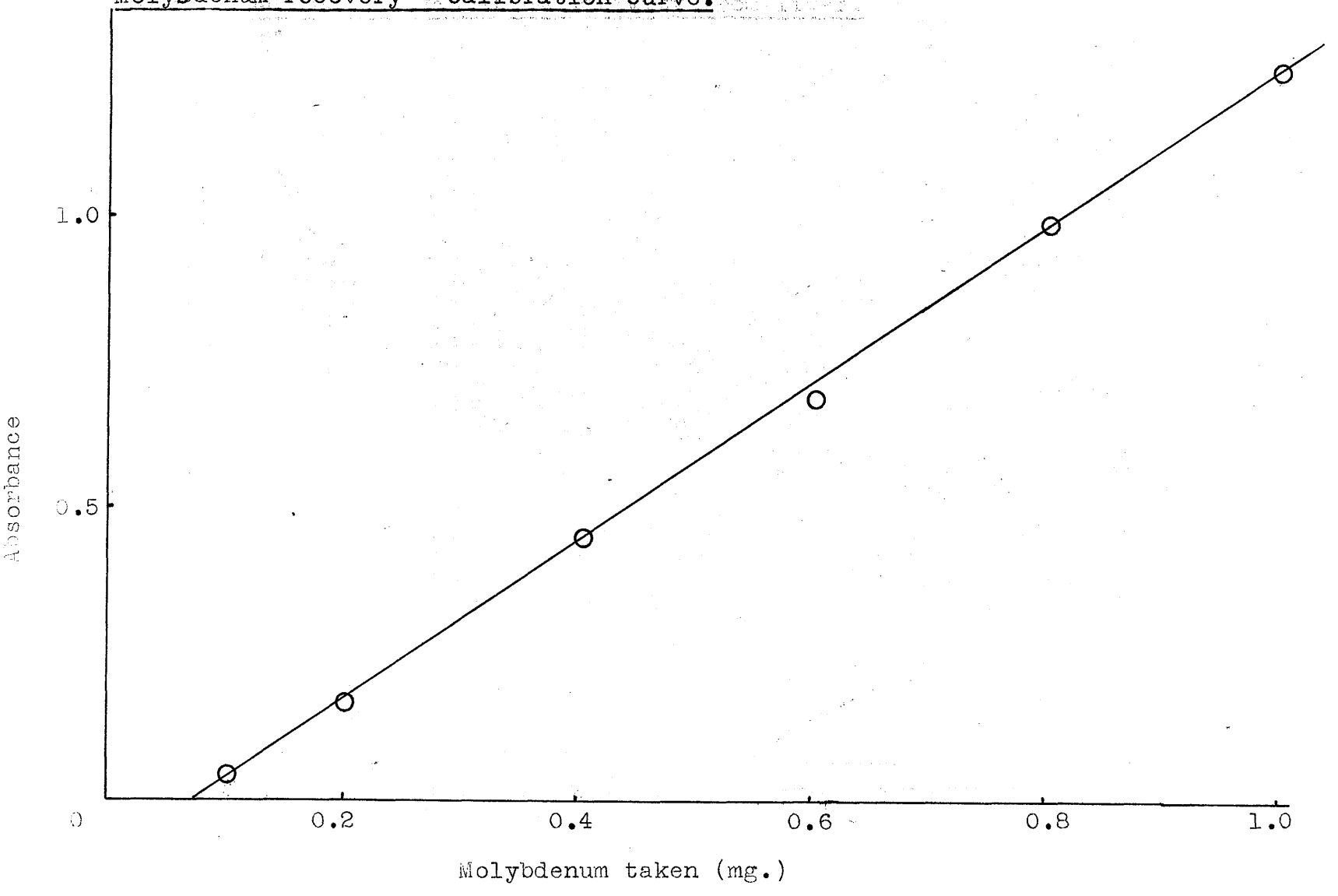



Growth curve of Tc99m from Mo99 parent.
50
20
10
5
2
1
Relative activity of Sample
- - - - - - - - - - -Mo99 decay
- - - Tc99m growth
- - - Extraction time
10
20
30
40
50
Time (hours)

Visible spectrum of complex between ethyl potassium xanthate and molybdenum in chloroform.



Molybdenum recovery - calibration curve.



Gamma spectrum of technetium activity from liver.

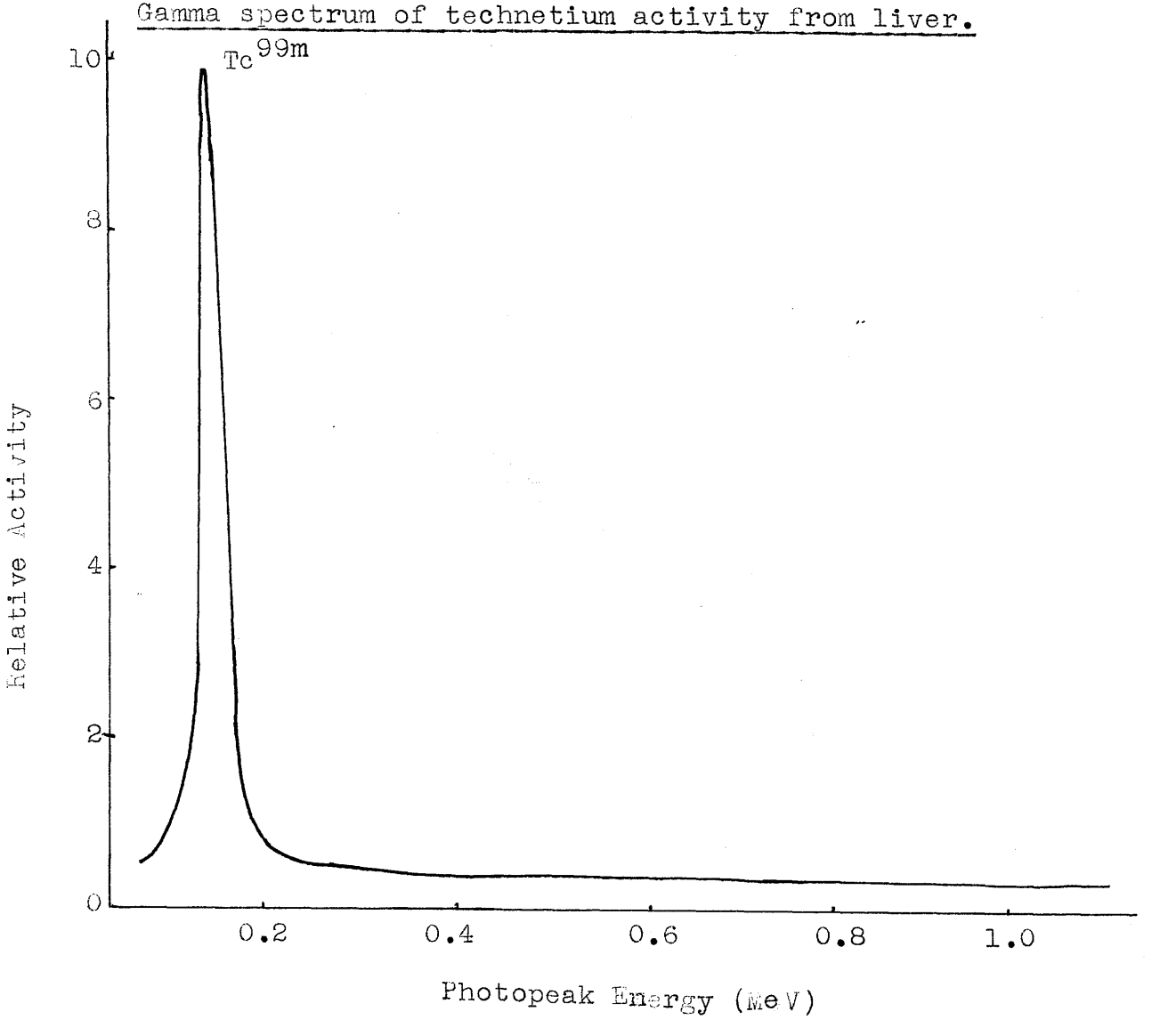
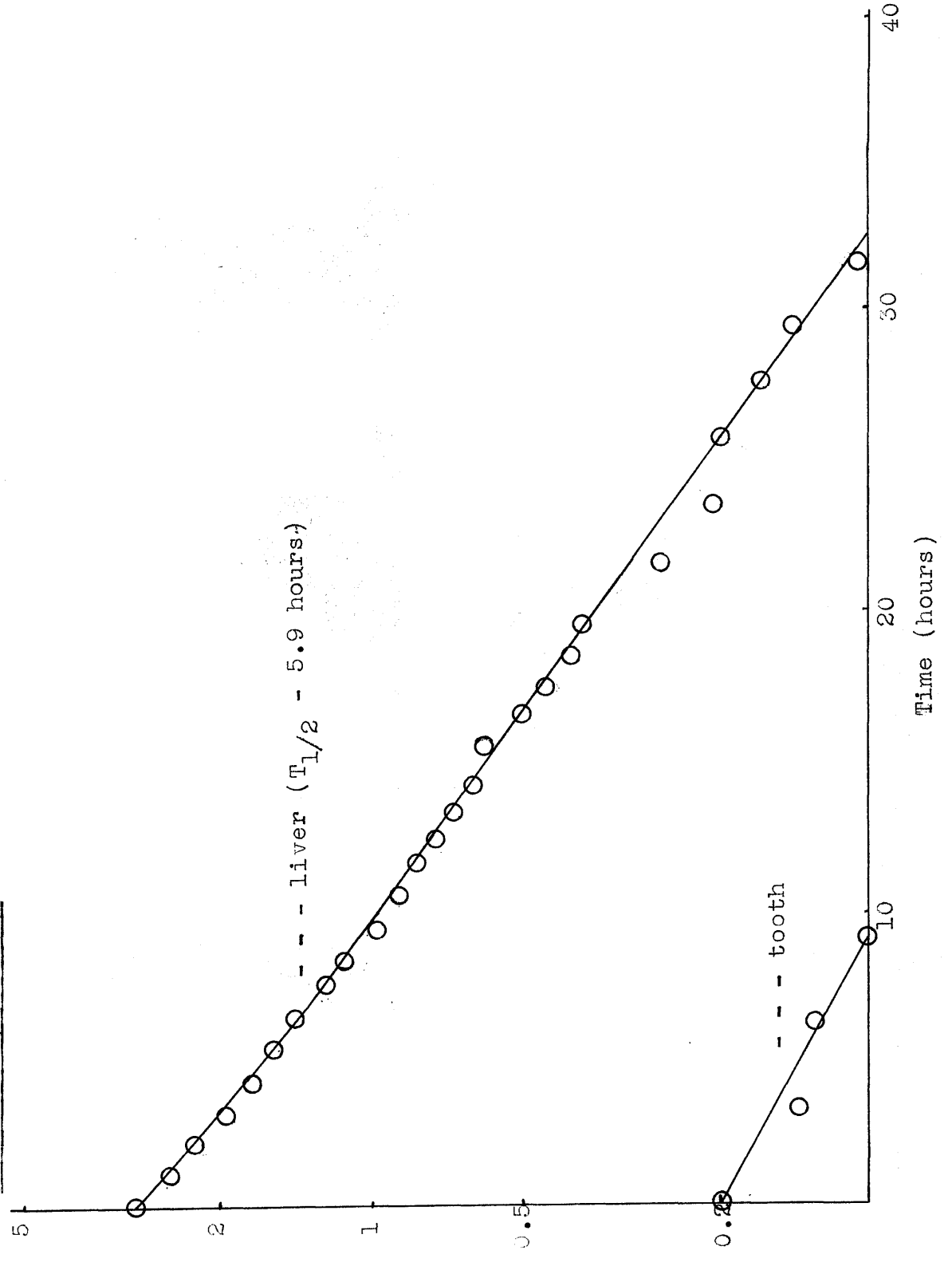
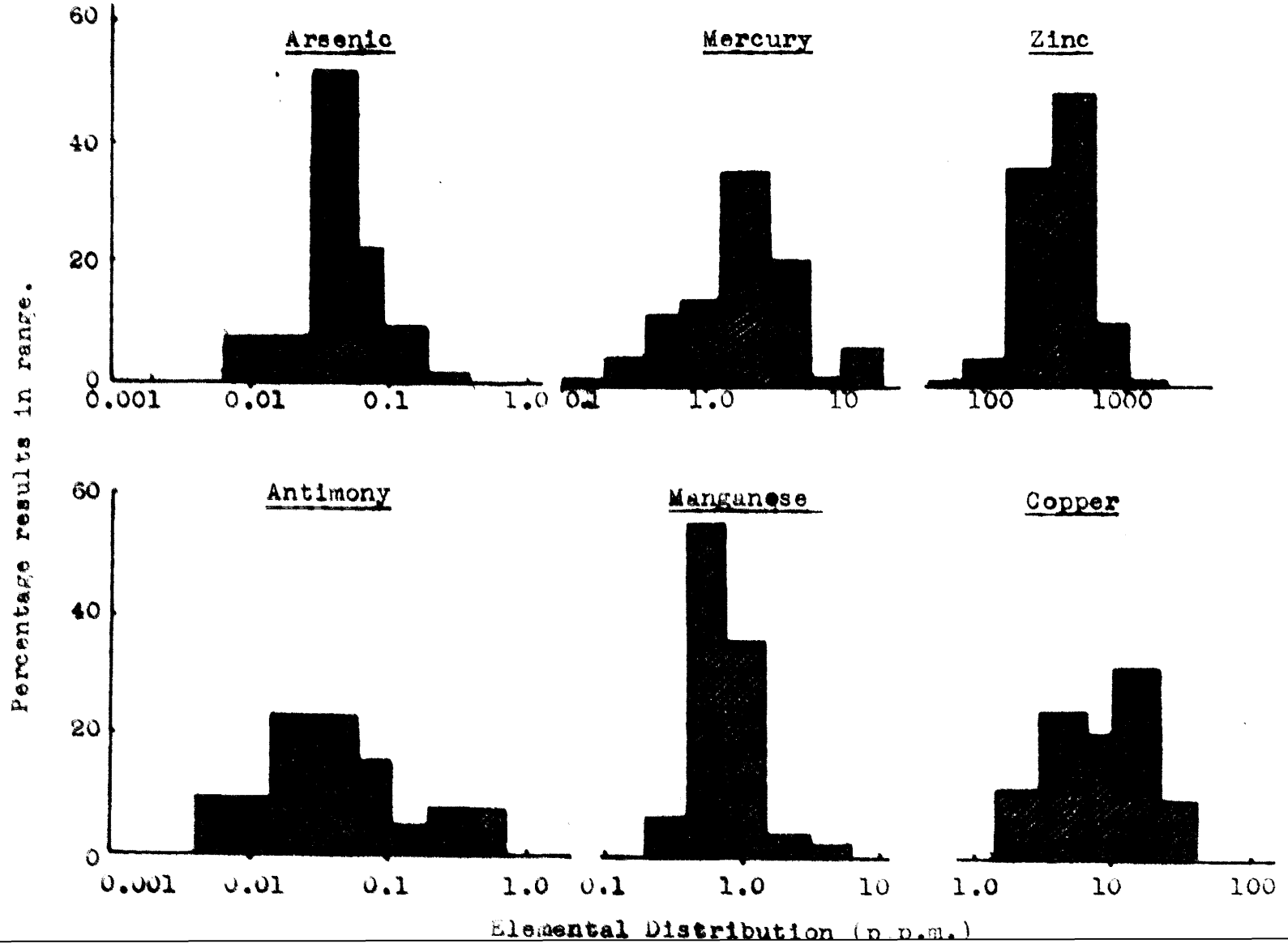


Fig. 48

Fig. 49


Tc^{99m} decay curves.
- - - liver ($T_{1/2}$ - 5.9 hours)
- - - tooth
Corrected sample activity (c/s)
Time (hours)
5
2
1
0.5
0.25
10
20
30
40

Distribution of elements in human enamel.



BIBLIOGRAPHY

1. Activation Analysis - Principles and Applications. Edited by J.M.A. Lenihan and S.J. Thomson (Academic Press: London and New York 1965) Ch. 10-11.

2. Ibid. Ch. 21.

3. Ibid. Ch. 24.

4. Allen, R.A., Smith, D.B., and Hiscott, J.E., U.K.A.E.A. Report AERE-R 2938 (1961)

5. Allen, S.H., and Hamilton, M.B., Anal. Chim. Acta, 7, 483 (1952).

6. Bhussry, B.R., I.A.D.R. Preps. Abst., 91, (1957)

7. Bibby, B.G., J. Canad. Dent. Assoc., 14, 3, (1948).

8. Bowen, H.J.M., and Cawse, P.A., U.K.A.E.A. Report AERE-R 4309 (1963).

9. Bowen, H.J.M., J. Nuclear Energy, 3, 18, (1956).

10. Bowen, H.J.M., Dept. of Chemistry, University of Reading, England (private communication).

11. Bowen, H.J.M., U.K.A.E.A. Report AERE R/4196 (1963).

12. Bowen, H.J.M., Int. J. Appl. Rad. Isot., 5, 227, (1959).

13. Bowen, H.J.M., and Gibbons, D., Radioactivation Analysis.(Oxford University Press 1963).

14. Bowie, S.H.U., et al., Bull. Inst. Min. Met. Trans. 69, 329, (1960).

15. Brislin, J.F., and Cox, G.J., Survey of the Literature of Dental Caries 1948-1960 (University of Pittsburgh Press 1964).

16. Brudevold, F. et al., Ann. N.Y. Acad. Sci., 85, Article 1, 110, (1960).

17. Brudevold, F. and Steadman, L.T., J. Dent. Res., 34, 209, (1955).

18. Brudevold, F., and Steadman, L.T., J. Dent. Res., 35, 430, (1956).

19. " " " " Ibid., 749.

20. Brudevold, F., and Steadman, L.T., Ibid., 35, 420 (1956).

21. Brudevold, F. et al., Arch. Oral. Biol., 8, 135, (1963).

22. Brudevold, F., J. Dent. Res., 39, 1083, (1960).

23. Brues, A.M., and Robertson, O.H., U.S.A.E.C. Report AECD, 2009, (1947).

24. Burnett, G.W., and Zenewitz, J., J. Dent. Res., 37, 581, (1958).

25. Cook et al., Radioactivation Analysis Symposium, Butterworth, London, (1960).

26. Covell, D.F., Anal. Chem., 31, 1785, (1959).

27. Cremer, H.D., and Voelkner, W., Biochem. Z., 324 (2), 89, (1953).

28. Crowther, P., and Kemp, D.M., Anal. Chim. Acta., 29, 97 (1963).

29. Cruickshank, D.B., Analyst, 73, 444, (1948).

30. Cruickshank, D.B., Brit. Dent. J., 68, 257, (1940).

31. Davies, O.L., Statistical Methods in Research and Production, p 147 (Oliver and Boyd 1957).

32. DeVoe, J.R., and Meinke, W.W., Anal. Chem., 31, 1428, (1959).

33. Driezen, S., Spies, H.A., and Spies, T.D., J. Dent. Res., 31, 137, (1952).

34. Editorial, Brit. Dent. J., 96, 189, (1954).

35. Eichoff, H.J., Dittmann, G., and Cremer, H.D., Biochem. Z, 324 (1), 32, (1953).

36. Freiser, H., Chemist-Analyst, 51, 62, 64, (1962).

37. Fukai, R., and Meinke, W.W., Limnology and Oceanography, 7, 186, (1962).

38. Furman, N.H., Mason, W.B., and Pekola, J.S., Anal. Chem., 21, 1325 (1949).

39. Gilbert, T.W., Jr., Ph.D. Thesis, University of Minnesota, (1956).

40. Guide to Activation Analysis, Ed. by W.S. Lyon, Jr., (Van Nostrand, 1964).

41. Hadjimarkos, D.M., and Bohhurst, C.W., Oral. Surgery, 12, 113, (1959).

42. Hamaguchi, H. et al., J. Atom. Energy Soc. Japan, 2, 317 (1960).

43. Hoste, J., et al., Int. J. App. Rad. and Isotopes, 10, 149, (1961).

44. Howie, R.A., Malokhia, M.M. and Smith, H., Anal. Chem., 37, 1059 (1965).

45. Hurd, G., and Allen, M., Ind. Eng. Chem., Anal. Ed., 1, 396, (1935).

46. Jenkins, E.N., and Smales, A.A., Quart. Rev., 10, 83, (1956).

47. Jenkins, G.N., Brit. Dent. J., 99, 249, (1955).

48. Jenkins, G.N., The Physiology of the Mouth (Blackwell 1960) p. 41, 46.

49. Jenkins, G.N. Ibid., p. 43.

50. Jenkins, G.N. Ibid. p.53.

51. Johnson, C.M., and Arkley, T.H., Anal. Chem., 26, 572, (1954).

52. Jowanowitz, L.S. et al., Anal. Chem., 32, 1270 (1960).

53. Kaiser, D.G., and Meinke, W.W., Anal. Chim. Acta., 29, 211, (1963).

54. Kallmann, S., Steele, C.G., and Chu, N.Y., Anal. Chem., 28, 230 (1956).

55. Kamemoto, Y. and Yamagishi, S., Talanta, 11, 27, (1964).

56. Kemp, D.M. and Smales, A.A., Anal. Chim. Acta, 23, 397, (1960).

57. Keyes, P.H., J. Dent. Res., 35, 95, (1956).

58. Koch, R.C., Activation Analysis Handbook, (Academic Press: London and New York 1960).

59. Krikos, A., Penderson, P.O., and Marshall-Day, C.D., Internat. Dent. J., 3, 386, (1953).

60. Kraus, K.A., and Nelson, F., Proc. of the Int. Conf. on the Peaceful Uses of Atomic Energy, Geneva, (1955), Paper No. 837.

61. Kraus, K.A., and Nelson, F., Ann. Rev. Nucl. Sci., 7, 31, (1957).

62. Leddicotte, G.W. et al., Proc. 2nd U.N. Conf. on Peaceful Uses of Atomic Energy, Geneva (1958), 28, 478, (1959).

63. Leliart, G., Hoste, J., and Eeckhant, J., Anal. Chim. Acta., 19, 100, (1958).

64. Livingston, H.D. and Smith, H., Anal. Chem., 37, 1285, (1965).

65. Losee, F.L., I.A.D.R., Prepr. Abst., 66, Abst. No. 170 (1957).

66. Loveridge, B.A., and Smales, A.A., Methods of Biochemical Analysis, Ed. Glick, O.S., 225, Interscience, (1957).

67. Lowater, F. and Murray, M.M., Biochem. J., 31, 837, (1937).

68. Lowe, L.F., et al. Anal. Chem., 31, 1951, (1959).

69. Ludwig, T.G., Healy, W.B., and Losee, F.L., Nature, 186, 695 (1960)

70. Ludwig, T.G., and Healy, W.B., I.A.D.R. 41, 130, (1963), Abst.

71. Meinke, W.W., Conf. on uses of radioisotopes in physical sciences and industry, paper 283, Copenhagen (1960).

72. Meinke, W.W., University of Michigan, Progress Report No. 7, (1957-58) p. 71-2.

73. Meinke, W.W. Nucleonics, 17, No. 9, 86, (1959).

74. Meinke, W.W., Science, 121, No. 3137, 177, (1955).

75. Morrison, G.H. and Cosgrove, J.F., Anal. Chem., 27, 810 (1955).

76. Muhler, J.C., J. Dent. Res., 36, 787, (1957).

77. Nixon, G.S., Livingston, H.D., and Smith, H., Arch. Oral Biol. 11, 247 (1966).

78. Nixon, G.S. and Smith, H., J. Dent. Res. 41, 1013, (1962).

79. Nixon, G.S. Paxton, G.D., and Smith, H., J. Dent. Res. 44, 654 (1965).

80. Nixon, G.S. and Smith, H., J. Dent. Res., 39, 514, (1960).

81. Papavasiliou, P.S., and Cotzias, G.C., J. Biol. Chem., 236, 2365, (1961).

82. Pavelka, F. and Laghi, A., Mikrochemie ver. Mikrochim. Acta. 31, 138, (1943).

83. Perkons, A.K., Hummel, R.L., and Jervis, R.E., 2nd National Meeting Society for Applied Spectroscopy, San Diego (1963).

84. Radiochemistry of Silicon, National Academy of Sciences Publicatio U.S.A.E.C., NAS/NS 3049, p 21.

85. Radiochemistry of Technetium, National Academy of Sciences Publication, U.S.A.E.C. NAS/NS 3021, p 15.

86. Report of Committee Two, Health Physics, 3, 146 (1960).

87. Sandell, E.B., Colorimetric Determination of Traces of Metals, p.653 (Interscience 1959).

88. Sidgwick, N.V., The Chemical Elements and their Compounds, 2, 1272 (Clarendon Press, Oxford 1952).

89. Smales, A.A., and Wager, L.R., Methods in geochemistry. (Interscience 1960).

90. Smith, H., Anal. Chem., 35, 635, (1963).

91. Smith, H., J. Forensic Medicine, 9, No. 4, (1962).

92. Smith, H., Anal. Chem., 34, 190, (1962).

93. Sognnaes, R.F., and Shaw, J.H., J. Nutrit., 53, 195, (1954).

94. Sognnaes, R.F., Ibid., 207.

95. Soremark, R., 'L'Analyse par Radioactivation', Proceedings of the III[e] Colloque Internationale de Biologie de Saclay, 223, (1963).

96. Soremark, R., and Samsahl, K., Arch. Oral. Biol. 6, 275, (1961).

97. " " " J. Dent. Res., 41, No. 3, 596, (1962).

98. " " " Acta. Odontol Scand., 20, 215 (1962).

99. Steadman, L.T. et al., J. Dent. Res., 38, No. 2, 285 (1959).

100. Stephan, R.M., I.A.D.R. Prepr. Abst., p 67, Abst. 173, (1957).

101. Stitch, S.R., A.E.R.E., (Harwell) Report, MRC/R 1952 (1956).

102. Strominger, D., Hollander, J.M., and Seaborg, G.T., Rev. Mod. Phys., 30, No. 2, Part II, (1958).

103. Sunderman, D.H., and Meinke, W.W., Anal. Chem., 29, 1578, (1959).

104. Syrrist, A., Odont. Revy., 3, 291 (1952).

105. Tobias, C.A., et al., Unio. Int. Contra Cancrum Acta, 1, 894 (1952).

106. Tribalat, S., and Beydon, J., Anal. Chim. Acta., 8, 22, (1953).

107. Vogel, A.I., Quantitative Inorganic Analysis, p 133, (Longmans Green 1961).

108. Wainerdi, R.E., and Du Beau, N.P., Science, 139, 1027, (1963).

109. Westermark, T., and Bjostrand, B. Int. J. App. Rad. and Isotopes, 9, 78, (1960).

110. Willard, H.H., Martin, E.L., and Feltham, R., Anal. Chem. 25, 1863, (1953).

111. Willard, H.H., and Smith, G.M., Ind. and Eng. Chem. (Anal. Ed.) 11, 269, (1939).

112. Winchester, J.W., Progress in Inorganic Chemistry - edited by Cotton, F.A.Z.I. (Interscience 1960).

113. Wolfe, R., Dunn, R.W., and Tobias, C.A. U.S.A.E.C. Report UCRL 480, (1949).

114. Zanten, B. van, Decat. D., and Leliarert, G., Talanta, 9, 213, (1962).