ACTIVATION ANALYSIS FOR TRACE ELEMENTS IN BIOLOGICAL MATERIAL.

A Thesis for the degree of Ph.D.

by

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CONTENTS

INTRODUCTION

INTRODUCTION

Trace element analysis is becoming increasingly important in chemical, medical and medico-legal practice. In all these fields it is often necessary to apply analysis techniques to very small amounts of sample with the result that, due to working at the extreme limit of sensitivity, the values obtained are inaccurate and only reproducable under strictly controlled conditions. On applying the radioactivation technique to the determination not only is the sensitivity greatly increased, but micro separations are avoided, as it is possible to add relatively large quantities of inactive carrier material, so bringing the separation at least into the semi-micro range. In other words, the analysis no longer required a skilled analyst; the method being easily applied by an unskilled assistant.

This thesis deals with the application of the activation technique to the analysis of arsenic, manganese, and to a lesser extent chromium, in biological material. In the development of the techniques an attempt was made to make them specific and accurate within 1% over a large range of values. This proved possible in both arsenic and manganese analysis though due to the multi-stage separation in the latter element, methods of yield recovery were incorporated for ease of application. A quantitative method was not developed for chromium, but that described shows promise of being as

accurate as the method for manganese. In each case the developed methods were tested against methods already in use, and the possible use of radioactivation without chemical separation was investigated. Thereafter, the new methods of radioactivation analysis methods were applied to medical, medico-legal, and commercial problems.

Among those investigated using the developed methods were the following.

1. The arsenic content of detergents.
2. The effect of washing with detergents on the arsenic content of hair.
3. The arsenic content of lungs from mice which lived in normal and in dust free atmospheres.
4. Arsenic levels and excretion rates in samples from subjects with chronic arsenic poisoning.
5. Detection of arsenic in a case of suspected criminal arsenical poisoning.
6. The arsenic content of tissues from subjects with cancerous skin conditions.
7. A survey of the arsenic content of hair with reference to smoking habits and the incidence of lung cancer.
8. A survey of the arsenic content of teeth to discover whether the arsenic in filling linings caused decay.
9. The manganese content of cigarettes and tea.

REVIEW OF ACTIVATION ANALYSIS

Activation analysis is a technique used for the estimation of certain elements in amounts that are too small for the conventional methods of micro analytical chemistry. Chemical analysis depends on the behaviour of the electrons in the outer part of the atom, while activation analysis uses the properties of the nucleus. In most substances the atomic nuclei have no properties that can be discovered by simple methods, but it is quite a simple matter to detect any ionising particles given out by an unstable atom. Hence if it is possible to make the atom unstable by artificial means it could be easily detected and determined. This was shown to be possible by Curie and Joliot (1) in 1934. The method of activation used at present is largely that of neutron activation. This is performed by placing a stable species in an atomic pile where a flux of neutrons of thermal velocity is available. The reaction which takes place is that of neutron capture; that is a neutron collides with a nucleus and is absorbed becoming part of it. While this is taking place a γ-ray is emitted. The process is said to be an n,γ reaction. Other reactions may take place but with low velocity neutrons the above is more usual. When this reaction takes place the atomic weight increases by one unit but the atomic number is unchanged so that an isotope of the parent species is

formed. The usual method of notation is as follows :-

$$ {}^{A}_{Z}M \quad (n,\gamma) \quad {}^{A+1}_{Z}M $$

Where A is the atomic weight,

and Z is the atomic number.

Unstable isotopes have a characteristic 'half life', that is, the activity falls to half its value in a constant period of time. The activity then, when plotted against time in a graph gives an exponential curve. This phenomenon is useful as a method of identification and has been used to determine the contents of simple mixtures. Generally artificial isotopes decay with the emission of β and/or γ-rays. The detection of these rays is due to their ionising character. They may be detected by using a Geiger counter, a scintillation counter, or instruments of the electrometer type. These different instruments are dealt with in appendix "A".

Artificial radioactivity was discovered by Curie and Joliot early in 1934 (1). They made the important observation that the positrons which were emitted on bombarding aluminium with α particles continued to be emitted after the source of α particles was removed. Following this work many more artificial isotopes were produced by bombarding elements with particles accelerated in high voltage appliances. Some of these workers were Cockcroft, Gilbert & Walton (2)

Lawrence (3) (Cyclotron) and Crane & Lauritsen (4). During 1934-35 a great number of isotopes was produced by Fermi and his associates (5) using neutron irradiation. Ridenour and Yost (6) published a review in Chemical Reviews in 1935 discussing artificial radioactivity and the method of production. By the end of 1935 about 100 artificial radioisotopes were known. A review in 1940 by Seaborg (13) says that using neutron and other particles bombardment 330 radioactive isotopes had been identified. A list was published by Livingood and Seaborg (7) describing their properties. Of special importance among these isotopes, due to their use in tracer techniques are radioactive hydrogen (8) and the long lived isotope of carbon (9). When atomic piles become available as sources of high intensity neutron fluxes, work in the field increased greatly. By 1950 about 700 artificial radioactive isotopes were known (10) and by 1958 about 1,000 (11).

From the medical point of view the discovery of artificial radioactivity opened a vast field for the application of radioactive indicators in biology. Prior to 1939 extensive work was done using radioactive phosphorus, sodium, potassium, carbon, sulphur, the halogens and iron (14). Since that time many more elements have been used and some have been adopted for routine clinical treatments.

The first use of radioactivation analysis in the restricted sense of the term was made in 1936 when a sample

of 'rare earths' was irradiated and some of the contents detected by half-life determination (12). This method of detection was very popular in the earlier work. Activation analysis is to-day proving to be a very versatile tool in chemical practice.

Practical Considerations.

The following conditions are required for an element to be a good subject for activation analysis.

1. A half-life greater than a few hours.
2. The production of a detectable level of activity after a few days irradiation in a pile.
3. Moderately penetrating radiation emitted.

Method.

A few milligrams of the sample to be analysed are sealed into an inert container and placed in a standard aluminium irradiation can along with a few milligrams of a standard comparison material. This material should be very pure and of constant known composition. The element being analysed should be the only one in the compound which becomes radioactive after irradiation. The can is sent to a 'pile' and subjected to irradiation by thermal neutrons until a workable activity is obtained. The sample is returned in a protective container. It is processed chemically after addition of inactive material to act as a carrier. This has the advantage that sub-micro chemical separations need

not be used. After processing, the separated isotope is estimated by its activity and compared with the standard sample. If the chemical process is not 100% complete the recovery is calculated from the ratio of the activity of a processed standard sample, to the activity of a standard sample. For the comparison of samples with the standard it is necessary to find the activity per milligram of the element in the standard. The amount of element in the sample can be calculated by comparison of the activity of the sample per milligram with the activity of the standard per milligram. In carrying out these experiments there is a definite time lapse between the estimation of the activity of the standard and the activity of the sample. The two can only be compared accurately, however, when the activities have been determined at the same instant after leaving the pile. As this is not possible it is necessary to correct the activity of one of the samples so that it would correspond to its value when the other sample was counted. The correcting factor is called the 'decay factor' and is the ratio of the activity obtained at one time to the activity at another time. The value may be obtained from tables or graphs. A further source of error is the dead time of counting devices. This is dealt with in appendix 'B'.

Introduction

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ARSENIC ANALYSIS

There are two main methods:-

1. The wet ashing method, in which the ... oxidised at low temperatures by an a... reagent in liquid form.
2. The dry ashing method, in which the ... ignited in the presence of a solid o...

These processes are complicated by the po... volatility of arsenic compounds. The tempera... should be kept as low as possible and reagents ... more volatile compounds should be excluded. ... also contain chloride groups, which form ... chlorides. It is necessary therefore

THE DIGESTION OF BIOLOGICAL MATERIALS

Introduction

In most micro-analytical techniques the arsenic should be present in the form of a tri-valent inorganic salt. All organic matter should be absent as its presence will reduce the yield of arsenic in the Gutzeit test (15) by about 60%. Generally the sample is destroyed by an oxidising process.

There are two such methods:-

1. The wet ashing method, in which the sample is oxidised at low temperatures by an acid or alkaline reagent in liquid form.
2. The dry ashing method, in which the sample is ignited in the presence of a solid oxidising agent.

These processes are complicated by the generally low volatility of arsenic compounds. The temperature therefore should be kept as low as possible and reagents which form the more volatile compounds should be excluded. Biological material contains chloride groups, which form volatile arsenic chlorides. It is necessary therefore to remove all traces of chloride as early as possible in the digestion.

Comparison of Results for arsenic in Biological tissues (Human)

Normal Subjects
(Arsenic content in parts per million)

Refer -ence	Hair	Skin	Nail	Blood	Liver	Kidney	Muscle	Urine
(39)	12.5	-	-	-	-	-	-	-
(33)	-	-	-	0.6	0.3	0.06	0.14	-
(19)	0.5-2.1	-	0.8-2.8	0.09-0.50	-	-	-	0.01-0.33
(20)	0.6-1.6	-	-	-	-	-	-	-
(21)	1.29	1.5	1.01	-	2	0.2-0.8	-	0.01-0.07
(134)	0.3	-	-	-	-	-	-	-
(135)	0.1-0.3	-	-	-	-	-	-	-
(136)	2.5	-	-	-	-	-	-	-
(137)	0.45-1.69	-	-	-	-	-	-	-

Contaminated subjects

(20)	3 - 10	-	-	-	-	-	-	-
(21)	2.6	19.4	2.5	-	279	188	2.7	-
	-	-	-	-	667	589	-	-
	-	-	-	-	2,968	1,385	-	-
	-	-	-	-	333	217	-	-
	-	-	-	-	372	238	-	-
(68)	65	7	11	-	-	-	-	-
(69)	4.4	-	-	-	-	-	-	-
(137)	8	-	-	-	-	-	-	-
	16	-	-	-	-	-	-	-
	23	-	-	-	-	-	-	-

Nature of the Sample.

It is not definitely established whether arsenic is essential to life. When arsenic is taken internally it is said to be excreted as organic and inorganic compounds. If this is true arsenic may be found in tissue in three different forms.

1. An inorganic salt, when the arsenic is free and easily extractable.
2. An 'included' compound, where the arsenic is held mechanically within the protein structure. This could only be extracted with difficulty.
3. An organic compound, when the arsenic is part of the protein molecule and can only be extracted by destruction of the tissue.

A complication suggested by Satterlee and Blodgett (17) is the production of volatile arsenic alkyls which may escape on dying tissue samples. An excretion of arsenic by volatiles in the breath is also postulated (18) but no proof is given. Young and Rice (16) show that washing hair is as likely to remove arsenic originally in the sample as it is to remove later externally applied contaminations. The arsenic content of the normal human body has been estimated many times (16) (19) (20) (21). The results obtained vary from worker to wroker and from sample to sample. There does not seem to be any fixed relationship. The arsenic in the

contaminated samples depends on the amount and method of administration.

Digestion Methods

Fats and some proteins are difficult to destroy by wet ashing methods (22). These are treated at higher temperatures by dry ashing. Carbonhydrates and many proteins can be digested by the wet ashing technique. Animal tissues are mainly fat or protein so the dry ashing technique must be used sometimes though the elevated temperature makes it unsatisfactory In all processes the purest reagents should be used to prevent contamination.

The Wet Digestion

The usual methods are the following digesting media.

1. Wet Digestion with Sulphuric acid.

   (a) Concentrated sulphuric acid with potassium permanganate.

   This was the earliest digestion mixture and was used for the determination of nitrogen (23). The addition of so many metal ions is not usually preferred.

   (b) Concentrated sulphuric acid with concentrated nitric acid.

   This method is the simplest and easiest to carry out and has been used by many workers (22) (24) (27)

(32). Nitric acid interferes with the Gutzeit test and must be removed. This is accomplished by repeated evaporations with water, or with solutions of ammonium oxalate (22), formaldehyde (29), or urea (29), followed by evaporation with water.

(c) Concentrated sulphuric acid with fuming nitric acid.

This method is sometimes used in conjunction with (b),

(d) Concentrated sulphuric acid with nitric acid and perchloric acid.

This is the most common of all digestion methods and many workers (15) (19) (22) (25) (26) (27) favour it. The method consists of near complete digestion by mixture (b), and completed by the addition of perchloric acid. This acid is dangerous to handle and liable to explode in the presence of organic materials. If care is taken and the acid not added too soon, the method is good. Again all traces of nitric acid must be removed before the Gutzeit test is used. This is accomplished as in (b).

(e) Concentrated sulphuric acid with nitric acid and copper sulphate.

Cassil (15) and Lawson and Scott (28) have used this method. The copper sulphate acts as a catalyst to the digestion but too much inhibits

the evolution of arsenic.

(f) Concentrated sulphuric acid with nitric acid and hydrogen peroxide.

The digestion is carried out as in (b) and hydrogen peroxide added when the digestion mixture becomes yellow (16).

(g) Concentrated sulphuric acid with hydrogen peroxide.

This method and that of digestion with hydrogen peroxide alone has been described by Kahane (27).

(h) Concentrated sulphuric acid with potassium dichromate.

This method proposed by ourselves has the disadvantage of the large excess of added metal ions. The main objection to the sulphuric acid digestion is the formation of stable organic sulphonate compounds. These inhibit the arsenic separation using the Gutzeit method. Chlorides present in a sample may form volatile arsenic chlorides in the presence of sulphuric acid.

2. Wet Digestion with Nitric Acid

(a) Concentrated nitric acid alone.

(1) The original method is that of Carius. In his procedure the sample is heated to 300°C in a sealed, thick walled glass tube with a few drops of fuming nitric acid. The tube however is liable to fracture.

(2) The sample may be heated with nitric acid till a

syrupy mass is obtained. This on further heating undergoes self ignition to give a white ash (22). It is likely that some arsenic may be lost during the period of ignition.

(3) Successful digestion with strong nitric acid at 60°C has been claimed to be the most rapid method and the one with the smallest loss (30).

(b) Concentrated nitric acid and sulphuric acid.

Middleton and Stuckey (31) propose a digestion using nitric acid with a 5% addition of sulphuric acid. Repeated amounts of nitric acid are added and the process completed using fuming nitric acid. For Gutzeit and similar tests all the nitric acid must be removed. This is accomplished by evaporation to dryness or by heating with sulphuric acid.

3. Wet Digestion with Hydrochloric acid.

(a) Dilute hydrochloric acid alone.

This has only been reported once (33). The method appears to be an extraction rather than a digestion.

(b) Concentrated hydrochloric acid and a chlorate.

This method leads to the liberation of free chlorine. Two reports of its use are given (22) (27). It is criticised by Zagumennikova (30) for being lengthy and leading to high arsenic losses.

(c) Aqua regia.

Zagumennikova claims that yields of only 50% are obtainable using this mixture.

4. Wet Digestion with Perchloric Acid

Concentrated perchloric acid with sulphuric acid.

Allcroft and Green (34) report this method but warn that it is quite dangerous.

5. Wet Digestion with Aquous Alkali

(a) Strong alkali.

Stainsby and Taylor (35) use strong alkali for determining the arsenic content of Lewisite contaminated food. They do not recommend the method for other purposes.

(b) Dilute alkali followed by a nitric/sulphuric digestion.

This method of Milton (36) has all the advantages of the wet digestion technique. The sample is boiled with dilute caustic soda solution to fix any free arsenic and then digested.

The Dry Digestion

The method is essentially combustion of the sample at elevated temperatures in the presence of an oxidising agent. The method is unsatisfactory due to the low temperature required to drive off the arsenic. The method has however found some favour. The variations are as follows:

1. Magnesium Nitrate

This method has been used by several workers (15) (16) (37) and consists of a lengthy ignition of the sample with magnesium nitrate.

2. Cerium Nitrate.

This method has been used by Cassil (15), but has not been used since.

3. Silver Nitrate

This method has only been reported once (38). The maximum temperature used in the reaction is 225°C, and can be obtained on an oil bath. This seems to be a useful method but the reaction can be violent if more than 10 mgm. of organic sample is present. It does satisfy the low temperature rule.

Dry ashing has not found the same favour as the wet ashing but it is much simpler. Air is required for the combustion, and could be a source of contamination.

The Oxygen Bomb Method (39)

It is claimed that all arsenic in a compound is recovered by this method. Errors due to air contamination are excluded. The procedure is to burn the dehydrated material in pure oxygen at a pressure of twenty-five atmospheres. The resulting vapour is then washed to absorption bottles by a stream of oxygen from the supply. The method requires experience before use and has not found

much favour. Arsenic may be lost by retention of some oxide in the combustion chamber. However, the method is one of the most efficient.

A selection of the better methods.

For the work which we will undertake using 10 mgm. quantities of sample, the following methods seem to offer the most promise.

1. Milton's Wet Ashing Technique (36)

The sample is boiled with dilute sodium hydroxide solution for a standard time. This process breaks up and fixes in solution any volatile arsenic compound. Following this the sample is digested with nitric acid until all the chlorides are removed. The nitric acid contains 5% sulphuric acid. Then a normal sulphuric/ nitric acid digestion is started and carried on till charring. The char is removed by the addition of a little nitric acid. This is repeated until no further charring is obtained. Dilution and evaporation removes any nitric acid, which may be present as nitrosyl sulphuric acid. This process is carried on until no nitric acid remains. The removal of the acid may be hastened by the addition of ammonium oxalate, formaldehyde or urea, and these in turn may be removed by dilution and evaporation.

2. Middleton and Stuckey's wet ashing method (31)

Five grams of the sample are placed in a one litre lipless beaker, with 20 mls. of water. About 7 mls. of concentrated nitric acid containing 5% sulphuric acid are added. The sulphuric acid acts as a catalyst. The mixture is heated gently until the sample is dispersed. Then evaporate to near dryness, adding capryl alcohol if necessary to stop foaming. This is left on a hot plate (310-350°C) for about fifteen minutes, or till no visible change takes place. Allow the beaker to cool and moisten the residue with nitric acid. The beaker is again allowed to sit on the hot plate for fifteen minutes. The process is repeated until the residue is whitish with dark spots. Continue from here using fuming nitric acid until a white residue is left. The fuming acid should not be added too soon or inflammation will take place. To get the residue into solution, heat with 3 ml. of concentrated sulphuric acid and 20 ml. water. Continue heating until fumes appear, allow to cool and then dilute. No resistant sulphonates are formed by this process.

3. <u>The Silver Nitrate Dry Ashing Method (38)</u>

This method has not been investigated or used much but the low temperature required recommends it above all the other dry ashing techniques. Ten milligrams of sample and three milligrams of silver nitrate are mixed in a tube. This is placed in an oil bath and the temperature is raised

to 225°C. It is necessary to be very careful to prevent violent reactions.

Comparison of Methods of Digestion.

There have been few reports of comparative methods of the suitability of the different methods for various purposes. The following are some of these collected together. No heed has been paid to the sample type used. The figures quoted are percentage yields from the various methods of digestion.

Comparison Table.

Reference Number	15	27	30	33	34	37
H_2SO_4/HNO_3	40%	95%	-	-	57-81%	-
H_2SO_4/H_2O_2	-	95%	-	-	-	-
H_2SO_4/HNO_3 phosphate pptn.	50%	-	-	-	-	-
HNO_3	-	-	used	-	-	-
HNO_3/HCl	-	-	50%	-	-	-
HCl.(conc.)	-	-	-	used	-	poor
HCl.(dil.)	-	-	-	50%	-	-
$HCl/KClO_3$	95%	98%	loss	-	0-49%	-
$HClO_3/HNO_3$	-	-	-	-	63-97%	-
H_2O_2	-	95%	-	-	-	-
$Mg(NO_3)_2$	90%	-	-	-	47-90%	used
$Ce(NO_3)_2$	20%	-	-	-	-	-
K_2SO_4/Cu	-	-	-	-	20-63%	-

As can be seen there is no great agreement in results, and the probable explanation of this is that there is a large personal factor in the carrying out of digestions.

Summary

Many workers have published papers on the analysis of biological materials for arsenic. The results have in many cases shown large variations from person to person as well as the variation of the experimental results themselves. Unfortunately, much of the work, though accurate in other respects, has shown a great indifference to the digestion and preliminary treatment of the sample in question. As the comparison table shows, this is fatal, great losses being incurred by faulty digestions. Some workers have got round the problem by comparing their results with results gained by experiments on known quantities of arsenic. On the surface this seems allowable, but as we do not know the original form of the arsenic present, and as organic and inorganic compounds behave quite differently on digestion, it is not quite correct to follow this relationship too closely. Method number one above or the oxygen bomb procedure seem to come nearest to fulfilling the requirements of being as comprehensive and complete as possible.

Analytical Techniques for Arsenic

Introduction

Due to its poisonous properties arsenic has received considerable attention from biologists and chemists. Injurious effects are shown by both the external and internal applications.

The micro-analysis for this element is important from a medical and medico-legal aspect as well as being an interesting chemical problem. Almost every micro chemical technique has been tried for this analysis. These methods range from the purely physical to the purely chemical. Not all have proved of value and a few are only of academic interest.

This survey is divided into two parts.

I - Dealing with the separation of the arsenic.

II - Dealing with the detection and estimation.

For easy reference an index of these parts is given on the next page.

Index of Methods

PART I

The Gutzeit Test and Variations.

The success of this test depends on the ease with which arsine is liberated from a solution of a trivalent arsenic compound. The arsine is formed by the action of nascent hydrogen on the arsenic salt. The nascent hydrogen is formed by chemical or physical means in the presence of the reaction solution. The different techniques of the Gutzeit test are as follows.

1. The Gutzeit Test using a Zinc/Acid Hydrogen Evolution.

A sample containing arsenic is placed in a generator flask and reduced to the trivalent state by any of the following agents:

(1) Heating with potassium iodide and stannous chloride
(2) Ferrous sulphate.
(3) Hydrazine sulphate.
(4) Ascorbic acid (51).

The solution is then made acid by the addition of a standard volume of sulphuric and/or hydrochloric acid. The zinc is added to this solution and the apparatus quickly resealed. Arsine is produced and is washed into the absorption chamber by the excess hydrogen. The gas is passed through a scrubber of some type which uses

lead acetate to remove hydrogen sulphide. An efficient absorber is required to remove the arsine quantitatively from the excess hydrogen. After this treatment the arsenic is in a state to be determined by micro-analysis. In all these procedures and those following the purest reagents are used. This reduces contamination from external sources. The above test in its simplest form is described in an abstract by British Drug Houses (40).

The Gutzeit test has been investigated many times with the following results:

(a) Acid Criticalness (41) (44)

Results show that the limits of error are large. Cassil (15) found that the addition of 1.5 ml of 60% perchloric acid had no effect on the final recovery of arsenic.

(b) Form of the Zinc

Purity, particle size, methods of activation, and catalysis all affect the results. The zinc has been used in the form of sheet, strip, powder, granules, shot and pellets. At the moment 20 mesh pellets are said to give the steadiest flow of hydrogen (15). It is this steadiness of flow which makes the conditions of the use of zinc so critical (42). Kingsley and Schaffert (33) used granulated zinc. Goldstone (43) prepared pure zinc sticks by casting in pyrex tubes. He coated the walls of the cylinders so obtained, with

wax, leaving a standard end surface exposed. The evolution using this zinc is very lengthy.

(c) Zinc Activaters

Activaters have been used quite extensively. A few are listed below.

(1) Dilute Hydrochloric Acid (17) (35)

The best activater is a mixture of 50 mls. of dilute hydrochloric acid (one acid to three water) with 2 mls. of stannous chloride solution. The zinc is treated with this for five minutes under vacuum followed by washing with distilled water.

(2) Zinc and Copper Alloys.

This is not used much.

(3) Addition of Copper salts to the reaction solution (50)

This method is useful but if too much copper is present the arsenic may be retained in the reaction flask as copper arsenide.

(4) Platinum salts in the reaction solution.

This addition may be of use, but if the platinum deposits on the zinc surface it may absorb some arsenic.

(5) Potassium Iodide in the reaction solution.

The method has been recommended.

(6) Ferrous salts in the reaction solution.

Arguments in favour of and against these compounds have been made.

(d) Interfering substances.

is better than any other as long as the temperature is constant. Thomas and Collier (46) recommend a high temperature with some action taken to condense excess vapours.

(g) The Hydrogen Sulphide Removal.

Lead acetate is generally used absorbed in cotton wool (46) (40) (37), glass wool (15), or pyrex granules (17). It is said that acid attacks cotton wool giving products which absorb arsine (47). Glass wool is also said to decompose arsine. To counter this sprays and scintered glass filters are used.

(h) The apparatus.

Arsenic free glass is recommended. All glass aparatus should be used to prevent leakage and absorption by rubber connections.

2. The Electrolytic Gutzeit Test.

The best method appears to be that of Osterberg and Green (48). A current of one ampere at twelve volts is used. The evolution is allowed to continue for half an hour. Platinum electrodes are used. Among other electrode materials which have been tried are mercury, lead, tin, cadmium, silver and graphite. These have been used with arsenites, but only lead is effective for arsenates. Rogers and Heron (49) used the electrolytic Gutzeit test to avoid the errors of zinc selection but the evolution they recommended lasts for 1½ hours. In these methods the anode and cathode chambers are separated

by a porous substance. Osterberg and Green use a porous glass partition. Other papers on the electrolytic method are shown in the reference (52) (53). The method is criticised by Satterlee and Blodgett (17).

3. Other Gutzeit Methods.

(1) Magnesium and Aluminium have been substituted for zinc.

(2) Sodium amalgam has been used (54) (55)

(3) Zinc and caustic soda (90) have been used but the results are not good. Though antimony does not interfere, there is no recovery from arsenates and the hydrogen evolution is generally difficult.

The Gutzeit method is used by Young and Rice (16) in their hair absorption experiments and by Evans and Bandemar (37) in their experiments on eggs. Several other workers have used the method with varying final methods of detection (56) - (64). Almond (61) describes a field method of analysis.

The Marsh Test

In the early part of the century this was the most popular method. It is not used a great deal now due to the better means of detection used with the Gutzeit test. The actual production of arsine is the same in both tests. The difference is in the method of detection. In the Marsh test this is accomplished by the formation of an arsenic mirror. Nitric acid particularly interferes with the test and must be removed. Fluoride interferes as the resulting arsenic trifluoride is stable to heat. These interferences are not in the separation but in the final determination. A recent paper by Young and Smith (69) has made use the Marsh test with some success. They followed the technique of Lucas (70).

The Arsenic Halide Separation Methods.

These methods depend on the low volatility of the arsenic halides. It is possible to distil them easily from an acid medium. Quite strong acid is necessary due to the ease of hydrolysis of the halides. For example -

$$AsCl_3 + 3H_2O = H_3AsO_3 + 3HCl$$

- the reaction being reversible.

The following methods are used.

1. The Separation of Arsenic Trichloride.

The usual technique is given in a paper by Hubbard (71). The sample used is a part of a digestion in the presence of 10 ml. of concentrated sulphuric acid. To this 5 ml. of 40% hydrobromic acid and 40 ml. of concentrated hydrochloric acid are added. One gram of hydrazine sulphate dissolved in 30 ml. of concentrated hydrochloric acid is mixed with the above. A source of carbon dioxide is connected to the distillation flask so that it bubbles through the reaction mixture at about four bubbles per second. The temperature is allowed to rise to 111°C while the distillate is collected in 40 ml. of well cooled distilled water. The solution so obtained is ready for the final determination. The method is recommended for use when the number of contaminating agents inhibits the proper function of the

Gutzeit test. Workers such as Schaaf and Maurer (72), Treadwell (73) and others (74) have used similar methods.

Variations

1. Sultzaberger (75) uses hydrogen chloride to replace carbon dioxide and absorbs the distillate in nitric acid. This method is reviewed by Klug and Lampson (76).

2. Smales and Pate in three papers (77) (78) (19) used an oxidation followed by a reduction. The distillation of arsenic is from a hydrobromic acid solution in a method similar to Hubbard's (71). A later paper on scintillation counting (79) uses this method.

2. Other Three Valent Halides.

1. Arsenic tribromide

In a report by Klug and Lampson (76) arsenic tribromide is distilled from a mixture of hydrobromic acid and bromine.

2. Arsenic trifluoride.

The behaviour of this compound is very similar to arsine. It is no use for the Marsh test however as it is not decomposed by heat. The method of evolution is to add fluoride to the arsine evolution mixture. It is said that this process enables the separation of three and five valent arsenic. The three valent fluoride is

the one which reacts.

3. The Five Valent Halides.

The method of evolution is described by Magnuson and Watson (80). The digested material is freed from nitric acid. The material is dissolved in 5 ml. of sulphuric acid and a few mls. of water. The solution is brought to boiling and 2 ml. of 30% potassium bromide are washed in. The distillation is allowed to proceed for four minutes and then the receiver is disconnected and washed out. The sample is then ready for the final determination. This method is a development of an earlier one by Chaney and Magnuson (81). In these experiments an oxidising agent is used before distillation and is said to give more repeatable results. A further paper on this method is distillation without oxidation presented by Maren (82) and reviewed by Klug and Lampson (76). Bartlet (83) in his paper on fruit analysis uses the evolution of arsenic pentabromide.

4. Other methods of Gaseous Evolution.

Arsenic has been evolved as tri-methoxy arsine $As(OCH_3)_3$. This is accomplished by adding methanol to the trichloride distillation medium. The method has found no favour in the literature.

Deposition Methods

1. The Reinsch Test

When a solution of an arsenic salt is boiled with copper in the presence of hydrochloric acid, copper arsenide is formed. The sample solution is heated with bright copper foil in the presence of excess hydrochloric acid. The copper becomes stained with a film of copper arsenide. The foil is dried and the arsenic sublimed to form a mirror. A description of the Reinsch Test may be found in the Analyst (84). Two more recent papers are in reference (85), (86). Griffiths (87) carried out some work and claimed a sensitivity of 1 μg. The method is generally thought to be nonstoichiometric. The most recent paper is by Gettler and Kaye (88). In this work the arsenic is deposited on a copper spiral.

Interfering substances include antimony, bismuth, and mercury.

2. Electrolytic deposition.

This has been attempted but has never received much attention.

A Japanese paper on microdeposition has been published (89).

Precipitation Reactions.

Most insoluble arsenic compounds have been used at one time or another for macro-analysis. A few have been applied to micro-analysis with some success. Two of the most favoured are the Bettendorff and Bougault. methods.

1. The Bettendorff Test.

The Bettendorff test is used in a paper by King and Brown (91) with reasonable success. It is useful as the interference of antimony and bismuth is eliminated. Selenium, tellurium, gold, mercury and nitric oxides interfere. This holds also for the Bougault method.

In the Bettendorff method a sample containing three valent arsenic is warmed gently with excess concentrated hydrochloric acid and a little saturated stannous chloride solution. Elementary arsenic is precipitated. This may be separated, dissolved and estimated by the usual techniques, A modification (92) was published in 1952.

2. The Bougault Test.

The Bougault method (93) uses hypophosphite as the precipitant instead of stannous chloride. The method has found some use recently in work by Smales and Pate (19) (77) (78), and in the arsenic/silicon separation (94).

3. Other Similar methods.

A few other reducing agents have been tried, but have found no favour. Milton (36), however, suggests a method using sodium thiosulphate. Sulphur is produced as a bye-product of the reaction. On boiling, the sulphur coagulates and the arsenic is collected in it,

4. Sulphide Precipitation.

Yukito Yosida (95) claims good results for sulphide precipitates. Generally, however, the method is used only with larger quantities of arsenic. Milton (36) suggests the use of ammonium polysulphide as the precipitating reagent. This forms colloidal sulphur which acts as a collector (36). Light metals interfere with this separation.

5. Complex Compounds.

Quinine arsenomolybdate (96) and concaine arsenomolybdate (97) have been used with turbidimetric estimation. An inorganic complex formed by the action of arsenate or phosphate with tellurium acetate and silver nitrate can be produced. The substance is said to be Ag_2TlAsO_4 by the authors (98).

6. Co-precipitation.

Co-precipitation with ferric hydroxide (99) has been used. Lead, antimony, tin and copper interfere (100) Manganese dioxide was used by Luke (101) but antimony and tin interfere.

OTHER METHODS OF SEPARATION

1. Ion Exchange Resin

The method has not received much attention (104) (132).

2. Chromatography.

(a) Column Chromatography.

Chromatography techniques (106) have received some attention. Burstall (102) used a cellulose column method.

(b) Paper chromatography.

Two papers on this application are available (103) (105).

These methods have not been developed very much.

3. Solvent Extraction.

(a) Thiol extraction.

Milton (36) describes the extraction of three valent arsenic from solution by thiols.

(b) Carbon tetrachloride extraction.

Klein and Vorhes (112) extract arsenic ethyl xanthate from suspension in Aquous media using carbon tetrachloride.

4. The Gutzeit test and Chromatography.

Arsine is evolved and absorbed in a suitable medium in which chromatographic separation can be carried out (107).

PART II

Absorbing Agents for Volatile Arsenic Compounds

Before we begin the description of actual techniques of estimation, a foreword is necessary to explain the materials used to collect the gaseous arsine and arsenic halides.

In the Gutzeit test several collecting agents have been used.

1. Mercuric halides.

   Mercuric chloride and bromide are the most important collecting chemicals. They can be used as solutions or as deposits in pieces of material.

2. Silver Nitrate.

   This has been used quite often but it is not so efficient. It is affected more easily by external impurities and light.

3. Gold Chloride.

   The main objection is the price of the reagent and its instability.

4. Others.

   Iodine, caustic soda, oxyhalide and bromide solutions have all been used with some success. In the arsenic halide distillation both distilled water

and nitric acid have been used as collecting agents. The different methods of use will be dealt with along with the estimation methods described below.

Absorption in a liquid followed by Titrimetric estimation.

1. The Method of Thomas and Collier (46) - Absorption in Mercuric Chloride Solution.

In this method the arsine is absorbed in 1 ml. of a 1.6% mercuric chloride solution. The arsine is bubbled through the solution from a 1 mm. capillary tube. After the evolution was complete the equivalent of about 1 ml. of 0.005 N. iodine was accurately added. The iodine is prepared in a 40% potassium iodide solution. A buffer consisting of 2 ml. of a solution containing 5% borax and 5% boric acid is added. The excess iodine is titrated with standard sodium thiosulphate. The indicator is 0.5 ml. of a 1% starch solution. The titration was carried out in the absorber tube using the delivery tube as a stirrer. The disadvantages of this method are the difficult end point and the mercury precipitate. If the quantity of arsenic is greater than 30 µg some mercury arsenide may stick to the glass delivery tube.

2. Cassil and Wichmann (108) (109) - Variations.

They recommend the use of a methyl methacrylate

delivery tube to prevent sticking. They also use a disodium hydrogen phosphate buffer.

3. Other Methods of Arsine Absorption.

(a) The absorption in silver nitrate solution is described by Levy (64). It is used in the same range of determination as the above.

(b) Burrill (110) has a method for the direct iodine titration of arsenic in the hair, but the work is not extensive.

(c) Rogers and Heron (49) in their experiments with electrolytic arsine generation absorb the gas directly in an iodine solution.

4. Arsenic trichloride absorption.

A method has been found for the titration of arsenic trichloride (111). The trichloride after distillation is titrated with a bromate.

Methods involving the use of an impregnated material Absorber

The technique is to pass the arsine carrying gas through an absorbing medium impregnated with a reacting substance. This is usually mercuric bromide or chloride, but silver nitrate and gold chloride (113) have been used. Mercuric chloride and bromide are equally sensitive detecting agents. The great difficulty in this method is to get the material impregnated evenly and accurately. Many elaborate techniques have been devised. An example on the sensitising of filter disks with mercuric bromide by Satterlee and Blodgett (17) is given.

Impregnating paper disks.

A supply of filter papers to last several days is placed in a wide mouthed bottle which is half filled with the mercuric bromide solution. The disks are thoroughly impregnated with the solution and the air is removed from the pores of the paper by evacuation at 100 mm. of mercury for two hours. The mercuric bromide is a 3.5% alcoholic solution. The solution is filtered before use and is not kept beyond two months. The disks in the solution are kept in an amber coloured bottle out of the light for up to ten days. The disks are removed and dried on a filter paper in the air, just before use. The type of paper used considerably affects the results.

Materials used.

1. Paper strips

Paper strips were first used and still are (16) but have some disadvantages.

(a) Unequel stain on opposite sides of the paper.

(b) A slight intensification at the edges.

2. Paper disks

When using this method there is a positive pressure inside the apparatus (40) which can cause leakages at joints. This is overcome by using Satterlee's (17) vacuum method.

The main disadvantage is the fading of the standard comparison spots. This is overcome by preparing photographic standards. This process is quite difficult.

3. String

How (60) introduced impregnated string (mercuric chloride). He placed it in a capillary tube and allowed the gas to flow past it. He plotted a graph of the length of the stain against known arsenic content. The method is quite simple and reasonably successful. It has since been used by Osterberg and Green (48).

4. Thread

Thread was used to replace the string (59) and the test was rendered more sensitive due to the greater stain length.

5. Other fibres and packed tubes have been used but have found no favour.

The yellow stains of the action between arsine and mercury salts have been used as comparisons but it is more usual to darken them with ammonia. The darkening is only temporary.

Colorimetric Determinations.

The colorimetric method has been used with all types of separation. Its main use is in conjunction with the arsine and the arsenic halide distillations. The main drawbacks are colour fading and temperature effects. Use of standard conditions control these to a satisfactory extent. The method is widely used and does well for routine samples.

The Absorption of Arsine in an Iodine Solution followed by Colour development.

Two papers (33) (37) describe techniques for the use of the heteropoly acid blue compound of arsenic. They used different wave lengths for their absorbance curves. It is found that deviation from standard conditions make the absorbance values different.

Colour Development.

Arsine is absorbed in 5 mls. of 0.001 N iodine solution. 0.5 mls. of a 1% ammonium molybdate solution in 5 N sulphuric acid, and 0.2 mls. of a 0.15% hydrazine sulphate solution are added to it. The colour is determined using a spectrophotometer and a graph of absorbance against known arsenic is drawn.

Many workers use the above method of determination (65) but with a number of variations as listed below.

(a) Absorption in bromine water

Morris B. Jacobs (56) absorbs the arsine in 3 mls.

of a mixture of half saturated bromine water and 1 ml. of 0.5 N. sodium hydroxide. The molybdenum blue colour is developed and compared with standards.

(b) The Use of Beer's Law.

Boltz and Mellon (57) use absorbance of 840 mμ light for experiments with quantities in the region of 0-3μg as they find that Beer's Law holds in this range.

(c) Absorption in Silver Nitrate Solution.

McChesney (58) absorbs the arsine in a silver nitrate/ ceric sulphate solution. The ceric sulphate oxidises any metallic silver formed. He then develops the colour and compares with a graph.

(d) Absorption in mercuric bromide impregnated material

Berkhout and Jongen (66) absorb the arsine in 0.5 cm of cellulose powder treated with mercuric bromide. After absorption is complete, the tube of cellulose powder is placed in the mouth of a calibrated flask. It is soaked with 0.5 mls. of sodium hypobromite solution for a few minutes, then washed with 1 ml. of 2 N sulphuric acid and 5 mls. of water. 0.4 mls. of ammonium molybdate solution (2.5% w/v in 2.5 N sulphuric acid) and 0.2 mls. of hydrazine sulphate solution (2.5% w/v) are added to the above solution in the flask. This is heated for five minutes and standardised. It is measured at 720 mμ on a spectrophotometer.

The absorption of Arsenic Halides followed by colour Development.

The absorption of the trichloride in water followed by colour development is popular. Klug and Lampson (76) and Maren (82) both use colour methods with their halide separations. Variations are quite common and a few are shown below.

1. Pentahalides.

Magnuson and Watson (80) use the method of arsenic pentabromide distillation. Colour development is easiest with pentavalent compounds as the blue colour only develops with this valency.

2. Trihalides.

Sultzaberger (75) distills arsenic as the trihalide and collects it in nitric acid. As this nitric acid interferes with the molybdenum blue colour formation the solution is evaporated to dryness to remove it. All trivalent compounds must be oxidised to pentavalent compounds before the blue colour will form.

A Direct Colour Method.

Gullstrom and Mellon (114) have developed a method whereby arsenic is determined in the mother liquid as the yellow molybdo-vanado-arsenic acid. Interfering substances should be in low concentration. The method is not so sensitive as the molybdenum blue.

Other Detection Techniques

1. Photometric comparison

This has been carried out by a few workers. Stringer (62) for example.

2. Densitometric determinations.

These are used with the metallic mirrors of the Marsh test. The mirror is produced by heating the tube through which the arsine passes. The older method of the Marsh test was to weigh the deposit. The density of the mirrors can be made standard and drawn into a graph. The method is not sensitive.

3. Nephelometric (115) or Turbidimetric measurements.

The nephelometric estimation of arsenic is described by Milton (116). The sample is distilled as the trichloride and collected in N sodium hydroxide. The resulting arsenite is oxidised to the arsenate by hydrogen peroxide. The distillate is neutralised and evaporated to small bulk (5 ml.). The nephelometric reagent is added in equal volume and the cloud compared with standard solutions.

The nephelometric agent consists of 1 vol. of potassium molybdate (1% solution). 2 vols. of N hydrochloric acid, and to this is added with shaking 1 vol. of cocaine hydrochloride (2% solution).

4. Fluorimetry

This has been tried, but found of little interest.

5. Polarography

In polarography a dropping mercury electrode is used with a large pool electrode. A gradually increasing voltage is applied across the electrodes and the current flowing through the solution is measured continuously. A polarogram is drawn. That is a current-voltage graph. The position of the wave in the voltage axis is characteristic of any particular substance and the height of the wave is proportional to the concentration. A method (117) has been used to determine the arsenic content of a sample after arsenic distillation. The method is as good as the photometric or colourimetric techniques. Three further papers are given in reference (118) (119) (120).

6. Potentiometric methods

This method is used to detect end points in titration experiments. The actual working mechanism is based on the abrupt changes of ionic concentration found at the end points of reactions of the titration types.

7. Amperometric Methods.

Amperometric methods indicate end points by an abrupt change in the graph of current plotted against the volume of added titrant. A paper (121) has been published on the method.

8. Coulometric Methods.

A given quantity of electricity will liberate a fixed amount of a substance. An indicator is added to the reaction and electrolysis carried out until the end-point is reached. The quantity of current used can be measured on a coulometer which is connected in series with the reaction cell. Everett and Reilly (122) in their method liberate iodine which oxidises the arsenic. The end point is detected by photometric means due to the appearance of the iodine colour. It was not found necessary to use starch as an indicator. Ramsey (123) using pure arsenic compounds used the method with some success. Oxidation of three valent arsenic by four valent cerium has been used (124). The cerium is in I N sulphuric acid. The method however required great care and accuracy in the lower range estimation and blanks were found to be unrepeatable, making the use of arbitrary blanks necessary.

9. Spectrography

Spark discharge methods have been used by Milbourn and Hartley (125). A more recent method (99) uses coprecipitation of arsenic with ferric hydroxide. This is then absorbed on carbon electrodes and sparked. In both cases the method of line comparison is used.

10. Activation Analysis.

All chemical methods of analysis are dependant on

the electron configuration, which represents the chemical behaviour of the element. In activation analysis the nucleus itself is the part which is detected. This is made possible by the fact that most elements when placed in a 'pile' become radioactive, emitting β and/or γ rays. Arsenic is a suitable element for this procedure. A snag is found in the fact that other elements which are found in biological material may become radioactive. A chemical separation in then required. Smales and Pate (19) (77) (78) use a combination of the arsenic halide distillation method and the Bougault precipitation method. Other workers have followed this process (94) (79). Scintillation spectrometry in analysis has been used (79) and the use of the instrument is described (126). Griffon and Barbaud (127) (128) count 2mm. sections along a hair and plot a graph of count against distance. Any peak deviation from linearity can be related to abnormally high concentrations of radioactivity in the hair. Half life measurements can show this to be arsenic. Goyanes (98) uses labelled precipitates which contain active silver.

Three reviews (129) (130) (131) have been published recently on the use of radioactive tracer and analytical techniques.

Comparison of Methods

Reference	Concentration(μg/g)	error %	Type of Estimation
(109)	5	2	Iodine Titration
(46)	5	1	Iodine Titration
(43)	5 - 10	3	Strip Stain length
(48)	0.1 - 100	-	String Stain length
(59)	0.5	10	Thread Stain length
(60)	0.1	7.4	String Stain length
(17)	0.02 - 0.9	3	Spot Stains with Photo standard
(37)	5	3	Molybdenum blue colour
(33)	0.1	10	do.
(80)	1	10	do.
(71)	10	2	do.
(66)	1.5	-	do.
(67)	3	-	do.
(133)	0.1*	-	do.
(112)	100	3.5	Extraction (Liquid)
(124)	59	1.4	Coulometric method
(123)	60	0.1	do.
(122)	10-50	2	do.
(117)	1*	-	Polarograph
-	200	0.57	Potentiometry
(133)	0.4*	-	Amperometric Method

Reference	Concentration(μg/g)	Error %	Type of Estimation
(99)	0.02	-	Spectrography
(133)	5*	-	Copper Spark Spectrograph
(133)	10*	-	D.C.Arc. Spectrograph
(79)	0.001	13	Scintillation Spectrograph
(133)	0.0001*	-	Activation Analysis
(19)	0.0001	10	Activation Analysis

* Results marked this way mean they are only of the approximate order of magnitude shown.

The table shows on inspection that the sensitivity range is as follows.

1. Chemical separation and activation scintillation spectrometry.
2. Chemical methods with colourimetric determination.
3. Amperometric determination.
4. Purely chemical means.

The recommended method is therefore the best chemical methods of separation combined with detection by activation analysis.

CONCLUSION

The simples and quickest way of separating arsenic from digested biological material is to carry out a Gutzeit reaction. The arsenic may be absorbed quantitatively in a mercuric chloride solution. The best method of detection and estimation is that of counting radioactive disintegrations from irradiated samples. If these two methods could be combined, and there is no reason they should not, an easy and very accurate method of arsenic estimation could be evolved. A digestion method is required which will give the best yield in the simplest manner. Such a method is proposed in the section on digestion. The great advantage of the above proposed method lies in the fact that blanks are completely removed, apart from the standardising experiment with each new batch of samples. The estimation, due to its great sensitivity, can be used with amounts of material in the region of 2 or 3 mgm. and hence the difficulty of obtaining large samples of hair, etc. can be avoided.

The Initial Investigation of the Arsenic Analysis Method

After a careful consideration of all the information reviewed above, it was decided to use one of the methods which depend on the evolution of arsenic as a gas. The determining factor here was the greater selectivity provided by the gas evolution methods. A number of trials were made and it was decided to use the Gutzeit method. Its accuracy was at least equal to that of the halide evolution methods and the technique was simpler.

It was considered that of the various Gutzeit methods, that of Thomas and Collier (46) was the best and it was used as a starting point in the investigation. The method is as follows.

> Arsenic as an inorganic salt is placed in the 200 ml. reaction flask (appendix C) with additional acid so that about 5 ml. of concentrated sulphuric acid and 3 ml. of concentrated hydrochloric acid are present. Then 5 ml. of a 15% potassium iodide solution and 0.4 ml. of a 40% stannous chloride solution in 50% hydrochloric acid are added to reduce the arsenic to the trivalent state. The solution is then diluted to 100 ml. and the flask placed in a slowly boiling water bath. The receiver system containing 1 ml. of 1.6% mercuric chloride in the absorber is connected. The arsine is

liberated by adding 7g. of granulated zinc to the solution in the flask, The reaction is continued for ten to fifteen minutes. After the evolution is complete the absorber system is removed and 1 ml. (accurately measured) of 0.005N iodine solution in 40% potassium iodide is added to the mercuric chloride solution. The resulting solution is stirred until any deposit is completely dissolved. Then 2 ml. of a buffer containing 5% borax and 5% boric acid are added. The excess iodine is titrated with thiosulphate using 0.5 ml. of a 1% starch solution as indicator. The titration is carried out in the absorber tube using the delivery tube as a stirring rod, and viewing the solution through the length of the absorber tube.

The digestion method used for the trial samples was that of Milton (36) and is as follows:

The sample is boiled with dilute sodium hydroxide solution; to break up and fix in solution any volatile arsenic compounds. This is followed by digestion with concentrated nitric acid containing 5% sulphuric acid until all the chlorides present are displaced, followed by a normal sulphuric/nitric digestion.

This method gave disappointing results and the following modifications were made.

1. The apparatus was redesigned to an all glass form

as shown in appendix C. This eliminated any reaction with or leakage through rubber joints.

2. The concentration of the iodine solution was reduced to 0.001 N. This increased the accuracy and enabled a larger volume of 40% potassium iodide solution to be used.

3. A 1% sodium starch glycollate indicator was used in place of starch.

Using the method with these modifications, a large number of blank and standard experiments were carried out from which the following results were obtained.

1. The Standardisation of the Iodine solution.

This was found to be unchanged by the presence or absence of the buffer solution. The maximum deviation between the standardising readings was 0.02 ml. in a 5 ml. titration. This gave a miximum error of 0.4%.

2. The Blank Distillation.

The blank includes the arsenic in the absorbing medium and the evolved gases. It was found that the value varied from batch to batch as shown below. No reason was found for this until a series of standardising experiments were completed as described below.

Number of Determinations.	Arsenic found (µg.)
4	1.78
2	0.93
2	0.83

Number of Determinations.	Arsenic found (µg.)
2	0.92
2	1.76
2	1.70
2	1.41
2	1.82

3. The Standardising Distillation.

In these experiments 10 µg. of arsenic were added to each. It was found that the recovery varied from 64 - 97% and that samples done at the same time were approximately the same, as was the case in 2 above. From these experiments it appeared that not all the arsenic was being evolved in the time given for the experiments, and not at the same rate in different batches.

It was decided to modify the experimental conditions to counteract these results. The following changes were made.

(a) The water bath was kept at a more constant temperature.

(b) The reaction mixture was allowed to reach the temperature of the water bath.

(c) The form of the zinc was standardised to 16-22 mesh.

(d) The standards were carefully checked before each batch.

(e) The reaction time was increased to 30 mins.

Using these changes the results became reproducable to within ± 4% of the total added arsenic.

4. The Blank Digestion and Standardising Distillation.

Using the method described above it was found that the digestion gave a recovery of 100%(± 4%). This value, however, tended to fall when the digestion was prolonged.

Conclusion.

It was decided that a method had now been evolved of sufficient accuracy to justify the undertaking of a preliminary set of analyses. It was hoped that during the course of this it would be possible to deal with any further difficulties which might arise. In fact, only one further point arose: that of acid concentration. This will be dealt with later.

Arsenic in Detergents (138)

During an investigation of the arsenic content of human hair by Lenihan et Al. (137) it was discovered by accident that a well-known household detergent contained an appreciable proportion of arsenic . It was decided to obtain some samples of common detergents and analyse them using the above method. The detergents were bought at random and were as follows.

Sample	Type	Producer
A	liquid (household)	1
B	liquid (commercial)	1
C	liquid (household)	2
D	solid (household)	3
E	blue solid (household)	3
F	solid (household)	3
G	solid (household)	4
H	blue solid (household)	5

The containers on examination showed no deterioration, each being in perfect condition and sealed. Sample B was taken from a 1 gallon container.

Experimental Details.

The digestion was carried out on 10 gm. samples for each detergent using the method described above. Following the digestion, the solutions were made up to a standard volume (100 ml.) and portions (about 10 ml.) of this were

taken and subjected to analysis using the above modified Gutzeit method. All the portions from the same detergent were not analysed together, but in different batches so that the results would not be influenced by any one peculiar set of circumstances. As a further check, some of the analysis were made in duplicate. Corrections were made for the blank and for the recovery in some cases when the conditions varied. Two of the analysis were spoiled by losses caused by splashing and an order of magnitude only has been given. The results obtained were as follows:

Table of Arsenic in Detergents

Detergent	Results (µg/g)	Volume of sample(ml.)	Duplicate (µg/g)	Volume of sample(ml.)	Average Results (µg/g)
A	60.1	10	59.9	2	
	60.1	10	59.9	2	60
	58.5	2	60.2	2	
B	73.6	5	same order	5	74
	74.5	2	-	-	
C	0.65	30	same order	30	0.5
	0.48	60	-	-	

Detergent	Results (µg/g)	Volume of sample(ml.)	Duplicate (µg/g)	Volume of sample(ml.)	Average Results (µg/g
D	8.5	10	9.5	10	8.8
	8.3	10	8.8	10	
E	6.8	10	-	-	7.1
	7.4	10	-	-	
F	1.68	10	1.18	20	1.2
	1.12	10	1.22	20	
G	8.3	10	-	-	
	9.0	10	-	-	8.8
	9.0	10	-	-	
H	5.3	10	-	-	
	5.0	10	-	-	5.1
	5.0	10	-	-	

The results of the samples A and B were very high and this was in keeping with Lenihan's view of the matter (138). It was decided that the arsenic content of these two detergents was worth further investigation, but that the analysis method was still not sufficiently accurate. Examination of the

results showed that differences of up to 12% existed. The accuracy which was required was set at 1%, and to this end every step in the complete analysis was subjected to a thorough investigation. The following section describes this work.

Tracer Investigation of the Method using Arsenic-74

Introduction.

The technique of tracer analysis is simple and efficient. A known amount of activity is added to a standard inactive sample and the progress of the sample followed by tracing the activity. This is possible and accurate as the radioactive isotope follows the stable isotope exactly in any normal reaction. In the following experiments, arsenic-74 was the active isotope added. This isotope emits β and γ rays and is, therefore, easy to detect using a Geiger or Scintillation counter (appendix A). Arsenic-76, the isotope which will be detected in the actual analysis, was not used because of its short half life of 26.8 hours compared with that of arsenic-74, which is 16.8 days. The arsenic-74 was extracted from germanium, which had been bombarded with deuterons, by Smales (19) method. The nuclear reaction is

$$^{74}_{32}\mathrm{Ge}\ (d,n)\ ^{74}_{33}\mathrm{As}$$

In the quantitative analysis for arsenic in biological tissue, there are three problems.

1. The necessity of destroying the tissue and at the same time keeping the arsenic in the reaction medium.
2. The separation of the arsenic. This was done by the modified Gutzeit method described before, but a

detailed investigation of all the steps will now be given.

3. The final determination of the arsenic. In the present investigation, this will be done by electronic methods.

These problems will be dealt with in turn in the following pages.

The Investigation of the Wet Digestion Method

The activation method which we propose to use is very sensitive and we hope to be able to analyse samples weighing only a few mgm. Due to this, therefore, the digestion experiments were made on a piece of filter paper weighing only 20 mgm., to which a known quantity of arsenic was added.

A few experiments were made using Milton's method, as described before, and the results were found to be reproducable to within 4%. The experiments were repeated using the method of concentrated sulphuric/nitric acid mixture digestion and it was found that the results were in the same region of accuracy. It was then decided to investigate the latter digestion technique more completely. The standard sample used in the digestions again consisted of a piece of filter paper to which radioactive arsenic had been added. This gives approximately the same digestion conditions as in actual practice. The following series of experiments were made.

(a) The standard samples were placed in pyrex test tubes measuring 6 x $\frac{5}{8}$ inches and of 25 ml. capacity. Eight ml. of a mixture of concentrated sulphuric and nitric acids in the proportions of 3 to 5 were added to each. The tubes were placed on a digestion stand and heated to boiling, using fine carborundum as bubbler. The boiling was continued until the paper was digested

and the nitric acid evaporated. The vessels were allowed to cool, the contents diluted to a standard volume and the activity estimated by counting in an M-6 Geiger tube accepting liquid samples. Four further samples were made up and digested in the same manner, with the addition of 10 µg. of inactive arsenic as carrier. The following results were obtained-

Sample	Carrier Arsenic (µg)	% Recovery
1	-	97.3
2	-	94.0
3	-	96.6
4	-	94.8
5	-	93.0
6	-	93.0
7	10	90.6
8	10	93.8
9	10	91.1
10	10	94.0

The digestion was completed in under one hour. Some splashing from the tubes was noticed and this accounted for the loss. It was decided to use a flask of a greater capacity to prevent these physical losses.

(b) A further set of digestions was carried out in 50 ml. Kjeldahl digestion flasks. The results

obtained were as follows.

Sample	Carrier Arsenic (µg)	% Recovery
11	-	81
12	-	84
13	-	84
14	-	83
15	-	87
16	-	81

The flasks were too large and the digestions took three hours due to the acid refluxing in the necks of the flasks. The results show a large loss due to the lengthy digestion and the high temperature required to remove the nitric acid from the flasks.

(c) A third set of digestions were carried out in conical bottom flasks (appendix F), designed by the author. These had six inch necks and were of 25 ml. capacity. The results were as follows.

Sample	Carrier Arsenic (µg)	% Recovery
17	-	99.8
18	-	99.3
19	-	99.9
20	-	99.7
21	-	100.3
22	-	99.5

Sample	Carrier Arsenic (μg)	% Recovery
23	10	100.1
24	10	99.7
25	10	99.1
26	10	99.6
27	10	100.1

This digestion took less then one hour and all the samples show 100% recovery within the statistical counting error (1%). It was decided on these grounds to use this method in all subsequent investigations.

Summary of the Digestion Method.

The sample is irradiated and returned for processing. Three ml. of concentrated sulphuric acid and five ml. of concentrated nitric acid are placed in the special flask. The sample is added to the flask and then heat is applied until complete removal of the nitric acid: this in the flask described, takes about 45 mins. The reaction mixture is then allowed to cool and is diluted. It is then ready to undergo the next stage of the analysis. Inactive arsenic as a carrier need not be added until the next stage is being prepared.

The Method of Separation of the Arsenic

The method used was the Gutzeit modified as described earlier. In order to attempt to increase the accuracy, further consideration was given to the following points during the course of the analysis.

1. The Evolution Time.

A standard solution was prepared in the following manner. Ten micrograms of inactive arsenic were placed in the evolution flask (appendix C) and enough active arsenic added to give a count rate of 10,000 counts per minute. Five ml. of concentrated sulphuric acid, 4 ml. of concentrated hydrochloric acid, 5 ml. of a 15% sodium iodide solution and 0.4 ml. of a 40% solution of stannous chloride in 50% hydrochloric acid, were also added and the resulting mixture made up to 150 ml. Each sample was allowed to heat in the water bath for five minutes before the evolution of hydrogen was started. During this time the temperature of the flask contents attained that of the water bath. In this experiment, the water was boiling. Ten grams of 16-22 mesh zinc pellets were added and the evolution allowed to continue for varying times. The following results were obtained.

Time (mins.)	% Recovery
5	95
10	99.3

Time (mins.)	% Recovery.
15	100.1
20	99.8 and 100.1
25	100.2

From this it was deduced that at the boiling point the time required for the total recovery of the arsenic was about fourteen minutes. In order to confirm this the following experiments were made.

2. The Reaction Mixture Temperature.

These experiments were conducted using a similar reaction mixture to that described above, and the same procedure, but in this case the time of evolution was kept constant at fifteen minutes and the temperature of the water bath, and hence the reaction mixture varied. The results obtained were as follows.

Temperature(^{o}C)	% Recovery
17	33
32	59
50	90
70	98.5
90	99.8
99.5(boiling)	100

From the results of the two experiments, it was concluded that 100% recovery would be obtained when the solution was given 5 minutes to reach the water bath temperature

followed by 15 minutes evolution: the process taking place in a boiling water bath. This method was used throughout all the subsequent experiments.

3. The Form of the Zinc.

The form of the zinc used in the Gutzeit test has received much attention. It has been used as sheet, strip, powder, granules, shot and pellets. Cassil (15) recommends 20 mesh zinc pellets. They give a steady rapid flow of gas which is useful for paper strip and disc absorption techniques.

In the method under investigation, steadiness of flow is not essential, but speed is. Several forms of zinc were used in standard experiments of the above type. The results were as follows:-

Type of Zinc	% Recovery
granulated	81
4 mesh	73
4-8 mesh	87
8-16 mesh	90-97
16-22 mesh	100
20-30 mesh	100

Ten grams of 16-22 mesh were used in all the subsequent standardising and routine experiments.

4. The Acid Concentration.

The standard solutions were prepared as in series

1, but for this series the volumes of the acids were changed. The evolution took place at the boiling point for fifteen minutes as described in series 1 and 2. The results obtained were as follows.

Experiment number	Volume of Concentrated Sulphuric acid (ml)	Volume of Concentrated Hydrochloric acid (ml)	Recovery (%)
1	3	-	82.6
2	4	-	92.2
3	5	-	97.0
4	5	1	97.8
5	5	2	99.1
6	5	3	100
7	5	4	100
8	9	-	100

The results showed that the acid concentration was not critical, a variation of 1ml. of concentrated acid in the reaction mixture having no effect. It was decided to maintain the acid at a total of 5 ml. of concentrated sulphuric acid and 4 ml. of concentrated hydrochloric acid.

5. The Use of Sodium Iodide.

Sodium iodide was used in the evolution flask

instead of potassium iodide for two reasons. The first, because it acts as a hold back carrier. This means that it effectively dilutes the radioisotope of sodium which is formed when biological material is irradiated. This dilution means practically that no active sodium-24 is likely to be carried over in the distillation process. The second, because the potassium of potassium iodide contains a naturally radioactive species which could be carried over during the reaction. This activity is demonstrated easily; a 10 ml. volume of 40% potassium iodide gives a count rate of about four times the background when placed in an M-6 Geiger tube. Sodium iodide is also used to make the iodine solution described in 7. (below).

6. The Lead Acetate Filter.

Small amounts of hydrogen sulphide are liberated from the reaction mixture. This is removed by passing the evolved gases through a filter of cotton wool impregnated with lead acetate. Though the cotton wool was said to react with arsine, no activity due to arsenic could be detected in the filter. The method is, therefore, safe to use.

7. Collecting the arsine.

The arsenic in the form of arsine is removed from the hydrogen by passing it through a trap containing 1 ml. of a 1.6% solution of mercuric chloride.

Experiments were made to detect loss of arsine from the trap. Only a few showed positive results and of these all were below 0.1%. To help solution of the arsenic, 5 ml. of 0.001N iodine in 40% sodium iodide was added (see 5 above).

Summary of the Separation Method.

The active sample from the digestion is washed into the 200 ml. reaction flask with 10 µg of inactive carrier arsenic. Acid is added so that finally 5 ml. of concentrated sulphuric acid and 4 ml. of concentrated hydrochloric acid are present. Then 5 ml. of a 15% solution of sodium iodide and 0.4 ml. of a 40% solution of stannous chloride in 50% hydrochloric acid are added. By this means, the arsenic is brought to the three valent state. The volume is made up to 150 ml. and the flask is placed on a boiling water bath for five minutes when the reaction mixture will have reached the temperature of the bath. Ten grams of 16-22 mesh zinc pellets are added, the system sealed and the evolution allowed to run for 15 minutes before removing the trap. After removal, 5 ml. of 0.001N iodine solution in 40% sodium iodide is added to aid solution of the arsenic and the resulting solution is made up to a standard volume, 10 ml. of which is counted in a liquid counter, as described in the next section.

The Detection of the Radioactive Arsenic

In this part of the investigation, it was necessary to use arsenic -76 as this is the isotope which is produced when normal arsenic is irradiated in the pile. Both β and γ rays are emitted as with arsenic -74 but the energies of the radiations are different.

Two types of counter were available, and both were tested. They were as follows.

1. An M-6 Geiger tube accepting liquid samples. This tube was operated at 350 V.
2. A 3 ins. well type scintillation crystal. The E.H.T, was 1300 V and the discrimination 20 V.

Using these two units identical samples were measured in both. The following results were obtained.

Background of Geiger counter	-	555 counts/22 mins.
	-	25 c/m.
Total count with sample	-	20854counts/21 mins.
	-	993 c/m.
Net sample count	-	968 c/m
Background of Scintillation counter	-	440 counts/100 secs.
	-	264 c/m.
Total count with sample	-	20898 counts/1000 secs.
	-	1254 c/m.
Net sample count	-	990 c/m.

Therefore, the counting efficiency is apparently equal in both cases, but the relatively high background of the scintillation counter in fact makes the Geiger counter the more efficient, especially at low count rates. This is shown as follows.

	Geiger	Scintillation
Background	25c/m	261c/m
Sample count	500c/10m	1415c/300 secs.
	50c/m	283c/m
∴ Net sample count	25c/m	22c/m

In this low range of counting, the sample count is only a small fraction of the background count and is, therefore, inaccurate. The count on the Geiger counter is much more accurate, being twice as great as the background.

Summary.

In all the experiments, this method of counting in an M-6 Geiger tube accepting liquid samples (10 ml.) was used and it was decided to continue this method for the remainder of the work.

Conclusion of the Tracer Investigation

The information gained in the above investigation has shown that the chemical processing of the radioactive samples can result in the complete and rapid separation of arsenic from biological materials and in yields about the 100% mark. As the yields are reproducible within 1%, it is not necessary to carry out further experiments to determine the yield in each case. It was felt at this stage that test experiments of the full analysis method on known samples could be made. This is dealt with in the following section.

Testing the Analysis Method.

Introduction.

This section of the work deals with three methods of testing the accuracy of the analytical method which has previously been described.

1. The recovery of known added activity after digestion and separation.
2. The estimation of samples containing known amounts of arsenic by the full method of activation analysis. This also deals with the sending of samples for irradiation in the pile, and the treatment of the standard afterwards.
3. The analysis of detergents by the method in 2 above and the comparison of the results with two methods of chemical analysis.

Method 1. - Added known activity.

A known activity of arsenic-74 was absorbed on filter paper in the digestion flasks as described in the blank digestion experiments and digested by the sulphuric/nitric method described in the summary of the digestion experiments. After this process, 10µg. of inactive carrier arsenic was added and the separation carried out as described in the summary to the separation experiments. Thereafter, the recoveries were estimated in a Geiger tube.. On investigation

of the results, it was found that the recoveries were all within 1% of the total activity added. This was the result indicated by the tracer experiments above.

Method 2. - Activation analysis of known samples.

a. The sending of the samples for irradiation in an atomic pile.

A few mgm. of the sample accurately weighed are placed in a polythene ampoule or bag and this then carefully sealed. A standard is prepared by placing a mgm. of the pure material to be estimated in a polythene container and sealing very carefully, or by sealing a few µg. of the material in standard solution form in pure silica ampoules. In these experiments, the standard was a mgm. of arsenious oxide sealed in polythene. The sealed samples are placed in a standard aluminium can and sent to Harwell where it is placed in a pile (BEPO usually) for a suitable time (1 day for arsenic). After irradiation, the can is sent back in a protective container. The samples are removed and digested as described in the summary to the digestion experiments. The standard is removed and dissolved in sodium hydroxide (in this case). The solution obtained is diluted to give, on counting, the same order of count rate as the samples. From this rate, the count rate of the arsenic in counts/minutes/mgm.

of the element can be obtained.

b. Activation analysis.

In activation analysis, the count rate of the arsenic in the sample is compared with the count rate of the standard and hence the amount of arsenic in the sample is obtained.

A series of samples with different known amounts of arsenic were prepared and sent for irradiation. On returning radioactive, the samples were digested (see page 68) and separated (see page 74). This was followed by radioactive assay and comparison with the standard as above. The results obtained were as follows.

Calculated Arsenic (p.p.m.)	Arsenic found (p.p.m.)	Error (%)
0.56	0.55	1.3
1.78	1.75	1.7
1.96	1.99	1.5
4.82	4.69	2.7
9.58	9.33	2.3
19.6	19.0	3.0
105.0	108.5	3.5

Investigation of the table shows that the method is more accurate in the region below 4 p.p.m. falling off as the concentration rises. The larger error in the higher concentration regions is probably due to errors in the

counting equipment at the high count rate obtained.

The experiment was repeated carefully and portions taken so that reasonable count rates could be used. Full corrections were made throughout. The following results were obtained.

Calculated Arsenic (p.p.m.)	Arsenic found (p.p.m.)	Error (%)
0.415	0.420	1.1
1.70	1.68	1.2
1.91	1.90	0.5
4.76	4.68	1.6
9.05	8.98	0.8
20.5	20.2	1.5
110.0	108.3	1.2

Method 3. - Comparison analysis.

Using samples of detergents for the analysis, three different methods were compared.

a. A Gutzeit separation and titrimetric estimation using the method of Thomas and Collier (46).

b. The Gutzeit separation and British Drug Houses stain comparison method (40).

c. Activation analysis as described in 2 above.

The results obtained were as follows.

A.		B.		C.	
Arsenic (p.p.m.)	Sample weight (gm)	Arsenic (p.p.m.)	Sample weight (gm)	Arsenic (p.p.m.)	Sample weight (gm)
31.0	1.0	30	0.10	35.9	0.00467
25.0	1.0	25	0.10	24.8	0.00839
13.5	1.0	14	0.25	13.8	0.00454
18.5	1.0	17.5	0.25	18.2	0.00889
1.5	1.0	1.2	0.25	1.87	0.00768
0.85	1.0	1	0.25	0.67	0.00540
1.6	1.0	-	-	1.64	0.00762
0.5	6.0	-	-	0.57	0.00381

The table shows the close agreement between the results. It also demonstrates the use of very small weights of sample in the activation method. This is very important when samples of tissue from a living subject are to be analysed, and in medico-legal cases where the amount of available material is often limited.

Conclusion.

The method is now satisfactory for accurate work where the concentration of arsenic and the sample weight are low. In these regions, the expected error is in the region of 1% and the method is, therefore, usable for analysis. It was hoped that it would be of particular value in dealing with very small amounts of tissue such as hair, and the next part of this study contains extensive investigations into the

application of the method to the determination of arsenic in tissue, hair and organic material.

[illegible]

Immediately the analysis method was proven satisfactory it was applied to cases which [illegible] investigation of poisoning. Cases A and B [illegible] in the preliminary investigation before, were [illegible] special attention. On chemical investigation, following values had been obtained.

A - [illegible] p.p.m. sample 1

B - [illegible] p.p.m.

The arsenic analysis method in which [illegible] of the liquid nitrogen were employed [illegible] Whitman filter paper gave the following results

A - 68 p.p.m. sample 1

- 81 p.p.m. sample 2

- 93 p.p.m. sample 3

On further investigation, it was found the

The Applications of the Analysis (139).

The method developed in the preceeding section was used for several practical investigations of commercial and medical importance. They are discussed below under the appropriate headings, and in the following sections.

1. Detergent Analysis (138)

Immediately the analysis method was proved to be satisfactory, it was decided to carry on with the investigation of detergents. Types A and B as described in the preliminary investigation before, were chosen for special attention. On chemical investigation, the following values had been obtained.

A — 60 p.p.m. sample 1

B - 74 p.p.m.

The activation analysis method in which a few mgm. of the liquid detergent were absorbed into No.1, Whatman filter paper gave the following results.

A - 88 p.p.m. sample 1

- 81 p.p.m. sample 2

- 93 p.p.m. sample 3

On further investigation, it was found that on standing and taking samples from the top of the bottles, the arsenic content decreased with time as follows.

Sample 2 of type A was used.

Detergent A		Arsenic content (p.p.m.)
Samples from well shaken bottle	-	81
After standing one month	-	65
After standing three months	-	47

The concentrations of arsenic in detergents A and B are surprisingly high, but probably not dangerous. The analyses were made on the undiluted materials; solutions used in the laboratory or in the home would normally contain one part of detergent in 200-300 parts of water.

Many detergents include components made by the sulphation or sulphonation of hydrocarbons, alcohols or other organic substances; arsenic might be expected in the final product if the sulphuric acid used in the manufacturing process is made by the lead-chamber process from pyrites, which often contains arsenic as an impurity. Sulphuric acid is now generally made by the contact process in which sulphur dioxide and air are passed over a catalyst to form sulphur trioxide. The sulphur dioxide may be obtained by burning pyrites, but is carefully purified since any residual arsenic may poison the catalyst. Sulphur is, however, the more usual source of sulphur dioxide in the contact process of sulphuric acid manufacture. Sulphuric acid made by the contact process is almost free from arsenic; if made by the lead chamber method it may contain 0.1% or more.

The findings and interpretation were reported to the manufacturer of detergents A and B, who took prompt and effective action to eliminate the arsenic from their products. This was done by replacing the arsenical sulphuric acid, used in the sulphation process, by acid derived wholly from sulphur.

These measures were successful, as the analyses in the following table shows.

Source of Sample	Date	Arsenic content (p.p.m.) Detergent A	Detergent B
Hospital laboratory	20/10/56	60	74
Manufacturer	21/8/57	13.8	18.2
Hospital laboratory	29/8/57	35.9	24.8
Manufacturer	10/10/57	-	1.87
Manufacturer	25/10/57	-	0.61
Hospital laboratory	15/1/58	-	1.64

As sample C when subjected to chemical analysis earlier had shown the very low value of 0.5 p.p.m. a sample was analysed by activation analysis. The value obtained was in agreement being 0.57 p.p.m.

2. Contamination of Hair by Detergents - Analysis of Hair.

This section of work which had received some investigation by Lenihan et al.(137) (138) really lead

up to the investigation of the arsenic content of detergents. Lenihan had found that female laboratory workers had been washing their hair with detergent B (74 p.p.m. described above) undiluted and then not rinsing properly with the result that their hair had a high arsenic content. To follow up this work, it was arranged for some volunteers to wash their hair with a 10 ml. sample of detergent A (81 p.p.m.) in the normal manner with rinsing. The results obtained are listed below.

Subject	Arsenic content (p.p.m.)	
	before wash	After wash
Female	0.66	0.44
Male	3.20	0.86
Male	1.46	0.96
Male	0.31	0.34

The results show a decrease in the arsenic content of the hair after washing so it appears that no contamination of the hair takes place in these circumstances.

It was decided to analyse the hair of several people to see what affect the washing of their hair had, when done in their usual manner. The results obtained were as follows.

Last washed (days)	Oils, sets etc.	Soap or Shampoo	Result (p.p.m.) Before wash	After wash
5	-	Beer Shampoo	0.19	0.11.
7	Brylcream	Silvikrin	0.55	0.57
7	-	White Rain	0.37	0.42
7	-	Gloria	0.41	0.24
11	Vaseline hair tonic	Derbac soap.	0.67	0.96
14	-	Gloria	0.80	1.09
7	-	Pears transparent	0.72	0.46
7	Creme Rinse and set	Sunsilk	0.29	0.32
7	Vaseline hair tonic	Lifebuoy soap	0.90	0.46
6	-	Lux soap	0.36	0.41
1	-	Toilet soap	0.74	0.61
1	-	do.	0.49	0.96
14	-	Vaseline powder	0.54	0.35
6	-	Knights Castile	0.38	0.40
6	-	Vaseline	0.30	0.38

The results show no particular trend one way or the other. The mean value is the same (0.48 p.p.m.) for before and after.

For people with uncontaminated hair, washing does not appreciably alter the arsenic content.

3. Chronic Arsenic Poisoning - Analysis of Tissue Samples.

Arsenic is used in the manufacture of insecticides (lead arsenate), weed-killer (sodium arsenite) and sheep-dip (arsenious oxide, sodium arsenite). Ingestion and external contamination may be avoided by the use of protective clothing and other simple hygienic measures which are, however, not always observed scrupulously.

A worker from a sheep-dip factory was admitted to hospital suffering from squamous carcinoma of the scrotum, attributed to chronic irritation by arsenical dust. Samples of tissue were examined by activation analysis with the following results.

Sample	Arsenic (p.p.m.)
Skin	1.86
Nail	117
Hair (head)	329

The nail and hair samples were probably contaminated externally with arsenical dust.

Later, samples of beard hair, obtained with an electric razor at intervals after the patients admission to hospital, were found to contain the following amounts of arsenic.

Date of Sample.	Arsenic (p.p.m.)
7/6/57	3.12
14/6/57	1.79
28/6/57	0.84
5/7/57	0.94

The results show the rapid return to a normal level.

A similar experiment carried out by Dewar and Lenihan (68) gave the following results.

Sample	Arsenic (p.p.m.)
Head Hair	65
Finger Nail	11
Skin	7
Beard Hair	8
Beard Hair (8 days later)	2

About 18 months later the subject of this experiment was again admitted to hospital. Tissue samples were taken and showed the following results.

Sample	Arsenic (p.p.m.)
Hair	0.89
Nail	0.47
Skin	0.27

4. Suspected criminal arsenical poisoning - Tissue analysis.

An elderly person repeatedly became ill after food, with vomiting and abdominal pain. Extensive medical

and surgical investigations, including laparatomy, revealed no abnormality. Food was taken without incident in hospital but the illness recurred on the patients return home. Arsenical poisoning was then suspected. Samples of vomitus were sent to two laboratories for chemical examination by conventional methods. The results were inconclusive, one analyst reporting slight traces of arsenic and the other none. Samples examined by activation analysis gave the following results.

Sample	Arsenic (p.p.m.)
Hair (head)	0.77
Nail	0.20
Vomitus	0.15

On the basis of these findings, which are all within normal limits, enquiries into the possibility of arsenical poisoning were discontinued.

5. The Arsenical Contamination of Mouse Lungs - Tissue Analysis

A cancer research laboratory submitted ten samples of complete dried mouse lung for examination. Five of the mice had lived since birth in a dust-free atmosphere and the other five had inhaled the normal air of Glasgow, containing about 3×10^{-12}gm. of arsenic per litre. The diet and other living conditions were the same for both groups. The arsenic contents of the ten

samples were found by activation analysis to be as follows.

Sample	Atmosphere	Arsenic content (p.p.m.)
1	clean	0.18
2	clean	0.32
7	clean	0.44
8	clean	0.42
9	clean	0.43
3	normal	0.80
4	normal	0.72
5	normal	0.71
6	normal	0.88
10	normal	1.01

The results show the decidedly higher arsenic content of the lungs exposed to the normal atmosphere: being 0.82 p.p.m. mean as against the 0.36 p.p.m. mean of the clean lungs.

6. Analysis of Cancerous Tissues.

Arsenic is known to cause concerous conditions in the skin. The Department of Dermatology of Stobhill General Hospital has taken many samples of tissue (skin, nail, hair and wet tissue) from patients suffering from skin diseases. These were analysed to see if the arsenic content was abnormal and so to relate the disease with arsenic irritation. In all the cases presented -

with two exceptions - none showed any abnormal arsenic content and so far as the investigation has gone the results have been negative. The two exceptions were accounted for as follows.

a) This subject had in the recent past been treated for 'specific' disease by means of organic arsenicals. Only in the slowly growing nails was this apparent.

b) This subject had been treated with arsenic over the past six months for psoriasis.

The details of the experiments are shown below.

Subject	Diagnosis	Results (Arsenic in p.p.m.) Hair	Nail	Skin	Wet Tissue
Female	Eczema and pigmentation	0.11	0.42	-	-
Male	Psoriasis & Keratitic lesions on palms	0.49	0.35	0.15	-
Female	Keratoderma Climactericum	0.38	0.39	0.11	-
Male	Squamous Carcinoma of hand	0.60	0.29	-	-
Male	Keratoses of feet	0.18	0.39	0.14	-
Male	Psoriasis & follicular keratoses of palms & soles	0.51	0.22	0.16	-
Male	Bowen's disease	0.57	0.72	0.18	0.04
Male	Pigmentation (confluent)	0.54	0.78	-	-
Male	Bowen's precancerous dermatosis	-	-	-	0.026

Subject	Diagnosis	Results (Arsenic in p.p.m.) Hair	Nail	Skin	Wet Tissue
Male	Exfoliative Erythroderma	0.34	0.37	-	-
Male	Psoriasis	0.48	0.32	0.17	-
Male	Arsenical Keratoses	0.89	0.47	0.27	-
Female	Ichthyosis	0.45	0.32	-	-
Female	Keratoses of palms & soles	0.46	0.48	0.26	-
Female	Carcinomata	0.90	1.14	-	-
Male	Senile Keratoses	0.14	-	-	-
Female	Hyperkeratoses	0.12	-	-	0.06
Male	Bowen's Disease	0.12	0.54Y	0.27	-
Male	Psoriasis	0.36	0.37	0.22	-
Female (a)	Scarring from 'Specific' infection	0.83	1.88	0.62	-
Female (b)	Psoriasis	10.5	27.4	33.5	-

The Measurement of Arsenic Levels in Normal Hair and the Correlation with Smoking Habits

Arsenic is a known carcinogen, though not a very powerful one. It has been suggested that its ingestion might contribute to the increased incidence of lung cancer in smokers. This possibility arises from the fact that cigarettes contain appreciable amounts of arsenic, presumably from the insecticides used to spray the tobacco plants.

Below are some results on the arsenic content of tobacco obtained by other workers.

1. Bailey, Kennaway and Urquhart (140) investigated the arsenic content of cigarettes of many countries with the following results.

Country	Arsenic Content of Different Cigarette Brands (p.p.m.)
Austria	1.5
Bulgaria	0.6, 0.36
Canada	48, 13.6, 55.7, 47.9, 57.0
Denmark	81.0
England	54.2, 53.0, 1.6, 1.85, 1.4
Finland	5.3
France	1.0
Germany	1.55
Greece	0.5
Italy	10.3, 1.5, 0.0

Country	Arsenic Content of Different Cigarette Brands (p.p.m.)
Norway	70.2
Poland	2.0
Rhodesia	56.0
Spain	3.2
Switzerland	4.2
Turkey	0.0
U.S.A.	44.0, 27.9, 46.5, 51.0

2. Thomas and Collier (46) made an investigation of the arsenic content of tobacco with the following results.

Tobacco	Arsenic Content (p.p.m.)
Cigarettes	35.4 - 114
Cigars	13.7 - 29.5
Pipe Tobacco	22.7 - 42.8

3. Investigations have been made on the amount of arsenic which is in the smoke, ash and butts of cigarettes etc., with the results shown as follows.

Reference	Material	Arsenic content (%) Smoke	Ash	Butt
(140)	Cigarette	15	60	25
(141)	Tobacco	50%	-	-
(142)	Cigarette	23.3	48.5	28.1
	Cigar	11.1	60.6	28.3
	Pipe Tobacco	30.5	69.5	-
(143)	Cigarette	15.8	-	-

No great agreement is shown in the above tables but this may be accounted for by the discovery that the arsenic content of cigarettes is variable, this even along the same cigarette (144). Another contribution to this variation is that the arsenic content of some popular brands of cigarettes fell (140) from 25-100 p.p.m. in 1948 to 1-2 p.p.m. in 1956.

If smokers do indeed absorb greater amounts of arsenic (from the smoke) than non-smokers, it should be possible to demonstrate higher concentrations in the hair, which is one of the main routes of excretion for this element. With these considerations in mind, the following experiment was made.

Samples of hair from more than 1000 subjects were examined by the activation analysis technique. Information on the smoking habits of each subject was recorded when the sample was obtained. These data were concealed from the analyst until the work was completed. A summary of the findings on the first 1000 samples and of the information supplied by each subject, was transferred to punched cards and analysed.

The subjects were drawn from patients and staff in various hospitals and from members of Glasgow University. Patients known to have been treated with arsenical drugs were excluded but otherwise nothing was done to influence the choice of subjects by the physicians, surgeons and university teachers who obtained the samples. No attempt was made to select a group of subjects representative of the whole population in regard to age, sex or smoking habits; it was desired only to obtain a large number of samples which would include some from smokers and some from non-smokers.

After the analysis of the 1000 samples had been completed, the individual records were examined to find the constitution of the group, which was as follows:

1. Constitution of the 1000-subject group.

Category	Age under 25	Age 25-50	Age over 50	Total
Male smokers	94	117	130	341
Male non-smokers	165	49	49	263
Female smokers	58	82	16	156
Female non-smokers	113	70	57	240

Totals:

	Smokers	Non-Smokers	
Male	341	263	604
Female	156	240	396
Total	497	503	1000

2. Distribution.

Category	Arsenic content of hair, parts per million				
	0-0.49	0.5-0.99	1.0-1.49	1.5-2.49	2.5 -
Male smokers					
age under 25	29	33	17	8	7
25-50	46	46	12	9	4
over 50	56	49	12	5	8
Total	131	128	41	22	19
Male Non-smokers					
under 25	36	65	34	19	11
25 - 50	33	12	1	2	1
Over 50	19	25	3	1	1
Total	88	102	38	22	13
Female smokers					
under 25	38	15	4	1	0
25 - 50	62	11	6	1	2
Over 50	6	6	3	1	0
Total	106	32	13	3	2
Female Non-smokers					
under 25	66	37	5	5,	0
25 - 50	53	13	4	0	0
Over 50	40	14	3	0	0
Total	159	64	12	5	0

3. Summary of Distribution.

Category	0-0.49	0.5-0.99	1.0-1.49	1.5-2.49	2.5
Males	219	230	79	44	34
Females	265	96	25	8	2
Smokers	237	160	54	25	21
Non-Smokers	247	166	50	27	13

4. The Median Values.

Category	Male As (p.p.m.)	Male Number of Samples	Female As (p.p.m.)	Female Number of Samples	Total As (p.p.m.)	Total Number of Samples
All subjects	0.62	604	0.365	396	0.51	1000
Non-Smokers	0.62	263	0.36	240	0.50	503
Smokers	0.61	341	0.375	156	0.52	497
Subjects under 25 years	0.76	259	0.43	171	0.58	430
Subject 25 – 50 years	0.525	166	0.32	152	0.41	318
Subjects over 50 years	0.58	179	0.38	73	0.52	252
Smokers of 20 cig/day or more	0.49	45	0.44	5	0.49	50
Smokers of 10-20 cig./day	0.625	160	0.37	60	0.57	220
Smokers of 10 cig./day or less	0.645	96	0.37	91	0.47	187
Pipe Smokers	0.575	40	-	-	-	-

SUMMARY.

On plotting the full distribution curve (appendix D.1.), it was seen that the distribution over the 1000 samples was decidedly skew. This is obvious also when the mean value of 0.81 p.p.m. is compared with the median value of 0.51 p.p.m. The true mean value for normal hair is less than 0.81 p.p.m. as some of the values obtained showed obvious arsenic contamination. As an example of this one value of 74 p.p.m. was obtained. When the values of over 4 p.p.m. are ignored, the mean is found to be 0.66 p.p.m. Two other values of interest were the 90% and 95% values; being 1.39 p.p.m. and 2.05 p.p.m. respectively.

From this work and the abstract which is represented graphically in appendix D.2., it is clear that there is no observable difference in the arsenic content of hair between smokers and non-smokers in the group examined. There is, however, a markedly greater arsenic content in the hair of the males than of the females. No obvious explanation of this phenomenon has presented itself. It has been said that arsenic can be removed from the hair by washing with solvents and acid or alkali solutions (16). In this case the average arsenic content of longer hair, the ends being more washed, would be lower than that of shorter hair which is common in males. However, hair washing experiments (see page 86) have shown little or no difference in the arsenic content before and after.

Measurement of the arsenic content of long hair (20 c.m.) from women gave the following results.

Arsenic content (p.p.m.)

Bulb quarter	2nd quarter	3rd quarter	End quarter
0.05	0.10	0.12	0.08
0.30	0.21	0.19	0.15
0.07	0.08	0.04	0.05
0.14	0.14	0.17	0.23

The table shows some tendency of the arsenic concentration to fall off with the distance from the bulb, but not in every case.

CONCLUSION.

The results, therefore, failed to demonstrate any relationship between smoking habits and the arsenic content of hair, and hence a possible relationship with the greater incidence of lung cancer in smokers. Neglecting smoking habits female hair generally has a lower arsenic content and surveys have shown that they have a lower lung cancer incidence, but no reasonable relationship between the two can be drawn. Expert opinion (217) (218) (219) on this point, is that the difference is related to occupational exposure.

[illegible]

[illegible] teeth the [illegible] were recorded before [illegible] the results obtained were as follows.

Smoker	[illegible] Arsenic Teeth.
Healthy Teeth	0.050
Filled Teeth	0.050

The values ranged from 0.01 to 0.10 p.p.m. [illegible] mean values differed, being 0.050 and 0.076 for healthy and filled teeth respectively. This w[illegible] by the presence in the filled teeth batch of se[illegible]

The Measurement of Arsenic Levels in Teeth

It has been thought for some time that the arsenic content of the zinc phosphate cement, used for lining the fillings in teeth, might be responsible for the subsequent decay of the pulp. It was found that the average value for the arsenic content of zinc phosphate cement, as supplied for use with fillings, was 0.27 parts per million. In order to discover if the arsenic content of filled teeth was greater the following experiment was made.

A number of sections were taken of healthy teeth and of teeth which had been filled. The sections were analysed by the activation method, and in the case of the bad teeth the fillings were removed before analysis. The results obtained were as follows.

Sample	Median Arsenic Content p.p.m.
Healthy Teeth	0.050
Filled Teeth	0.050

The values ranged from 0.01 to 0.10 p.p.m. The mean values differed, being 0.058 and 0.076 for the healthy and filled teeth respectively. This was caused by the presence in the filled teeth batch of sample with a very high arsenic content; being 0.635 p.p.m. No explanation for this high level was found.

The conclusion reached was that there was no apparent

increase of the arsenic content of filled teeth and that destruction of the pulp was not caused by the arsenic from the cement.

Physical Analysis Methods

The object of the following experiments was to discover if arsenic in biological material could be determined directly in the irradiated material without the use of chemical separations.

Throughout the experiments it was assumed that the activity of the samples was due only to the radiations from sodium-24 and arsenic-76, other possible activities being neglected.

The following experiments were made.

Estimation by the use of Solid Filters.

In this experiment the property being used was that of the absorption of the radiation from active sources when passing through a material body.

Using an M-4 end window Geiger tube, the transmission curves for the radiation from pure arsenic-76 and sodium-24 through varying densities of solid material were found and plotted on a graph (appendix E.1.). The values obtained were as follows.

Density of Filter ($mgm./cm^2$)	sodium -24 (% transmission)	arsenic -76 (% transmission)
0	100	100
13.8	86.54	95.62
27	75.05	90.10
70	51.81	78.64
100	39.22	69.96
200	14.34	47.54
300	7.19	34.65
400	5.09	22.83
500	4.30	14.66
600	4.23	9.27
700	4.23	5.42
800	4.12	3.55
1000	3.71	1.07
1200	3.64	0.44
1500	3.16	0.28
1800	3.16	0.25
3000	2.39	0.21

In the above table, the accuracy of the value in the 100-200 $mgm./cm^2$ region was 1% for both isotopes. Taking this region, it was found that there the widest separation in the values of the curve occurred. This set of values was therefore, the basis of the solid filter separation investigation

Method: The sample to be analysed was counted three times as follows.

a. Without any filter.

b. With a filter of 100 mgm./cm^2.

c. With a filter of 200 mgm./cm^2.

Hence, using these values and a quadratic equation obtained from the table above, it was possible to find the relative amounts of sodium and arsenic present in the mixture. A typical example is shown below.

Total count of ^{76}As and ^{24}Na mixture	7828 c/m.
Count using 100 mgm./cm^2 filter	4158 c/m.
Count using 200 mgm./cm^2 filter	2291 c/m.

With the 100 mgm./cm^2 filter in place, 0.700 of the radiation from the arsenic and 0.392 of the radiation from the sodium is registered.

With the 200 mgm./cm^2 filter in place, 0.475 of the radiation from the arsenic and 0.143 of the radiation from the sodium is registered.

Hence, the following equations were obtained.

$$0.700\ As + 0.392\ Na = 4158\ c/m.$$
$$0.475\ As + 0.143\ Na = 2291\ c/m.$$

Therefore, the arsenic gives 3491/ c/m.

and the sodium gives 4395 c/m.

Total count of the mixture; calculated - 7886 c/m.

This agrees within the statistical counting error with the original experimental estimation of the mixture.

For accuracy the count rate of one isotope in the mixture should not be near the counting error of the other constituent. This is demonstrated below.

Activity of arsenic in mixture	644 ± 18 c/m
Activity of sodium in mixture	36 ± 6 c/m
Activity of mixture	695 ± 25 c/m
Activity of mixture through 100 mgm./cm^2 filter	372 ± 20 c/m
Activity of mixture through 200 mgm./cm^2 filter	269 ± 15 c/m

From an equation similar to that used above -

Activity of arsenic 619 c/m

This has a difference in activity of only 3.8% from the known added activity and is, therefore, quite accurate.

On attempting to find a result for sodium, a negative value was obtained. This means, that at such a relative activity, sodium could not be estimated.

The greatest accuracy is obtained when the count rates of the constituents are of the same order of magnitute.

The technique was carried out on a hair sample which had a very high arsenic content. The values obtained were as follows.

Total count of digested sample	4474 c/m
Count with 100 mgm./cm^2 filter	2701 c/m
Count with 200 mgm./cm^2 filter	1686 c/m
From equations:	
Sodium activity	1194 c/m
Arsenic activity	3190 c/m
Total activity	4384 c/m

The total activity shows only a 2% error from the experimental value. On calculating the arsenic content of the hair it was found to be within 5% of the value obtained by activation analysis.

The method is reasonably accurate for large arsenic contents. In normal biological material the lowest value that could be used with any accuracy would be about 20 p.p.m. and this concentration is not often obtained.

Separation by Electronic devices.

The property used in this set of experiments is the varying acceptance of pulses by the scaler with varying E.H.T. and discriminator voltages .

Using a three inch well type scintillation crystal, the radiation per cent registered at varying discriminator voltages and varying E.H.T. was found and plotted (appendix E.2.). The standard conditions of reference were arbitrarily fixed at an E.H.T. of 1300 V. and a discrimination of 5 V. The percentage variation was calculated as if the standard condition was

100%. The values found were as follows.

E.H.T. (volts)	Disc. Bias (volts)	Sodium -24 (% registered)	Arsenic -76 (% registered)
1300	5	100	100
1300	10	91.26	97.70
1300	15	89.74	95.43
1300	20	88.70	92.39
1300	25	86.96	88.45
1300	30	85.92	86.42
1300	35	84.37	82.14
1300	40	82.34	78.53
1300	45	80.79	76.10
1300	50	79.52	73.86
800	5	73.69	63.54
800	10	59.82	39.11
800	15	49.64	10.34
800	20	38.14	4.76
800	25	26.73	1.49
800	30	18.13	0.44
800	35	11.71	-
800	40	4.20	-
800	45	1.595	-
800	50	0.847	-

The region around 800 V. E.H.T. and 15 V. discriminator

bias has a good separation, but it was found that the resulting values could not be reproduced accurately. This is possibly due to the rapid change of value in this region in conjunction with change of discriminator voltage, the setting of which is manual and not easy to reproduce. The method is, therefore, unsatisfactory.

Separation by Decay Time.

The property used in these experiments is that different isotopes have different half lives. The ruling factor is that the half lives should be different enough to show a reasonable separation after a reasonable time. The difference between sodium -24 and arsenic -76 is not great, being less than a factor of two, but after 1-2 days there is a great enough separation. From experimental data and from the decay curves of the two isotopes (appendix E.3.) it is possible to find the proportion of each in a mixture. A typical example is as follows.

Activity of digested tissue at 14.06 hrs. on the 19th May - 4474 c/m

Activity of digested tissue at 11.46 hrs. on the 22nd May - 672 c/m

hence

$$As + Na \quad - \quad 4474 \text{ c/m}$$

$$0.165\ As + 0.0413\ Na \quad - \quad 672 \text{ c/m}$$

where 0.165 is the factor to which the arsenic has decayed and 0.0413 is the factor to which the sodium has decayed.

From this equation:

Arsenic Activity	3130 c/m
Sodium Activity	1344 c/m

The recovery of counts is accurate and on calculating the arsenic content of the tissue (hair) it was found to be exactly similar to the result gained by using the solid filters, and the same sample was used in both cases.

SUMMARY

The methods using filters and decay time show promise when relatively large concentrations of arsenic are present, but not for normal tissue analysis. The electronic method due to its lack of reproducability is valueless.

The Distribution of Trace Elements in Hair

Introduction.

When a hair is placed in a flux of thermal neutrons, such as are available in a graphite moderated reactor like 'BEPO', the stable elements in the hair may be rendered articicially radioactive. The activity is due to the activation of the trace elements found in the hair (mostly sodium, sulphur and arsenic) and not due to the organic material of which the hair is composed. This means that any variation in the excretion of one of the trace elements would result in a variation in the activity of a section of an irradiated hair. If a normal hair, that is one in which the trace element distribution is uniform, is rendered active and the activity of the small lengths along the hair is plotted against the distance from the bulb, it is possible to obtain a graph which should be approximately linear. It is found however that, due to the reduction in the mass of the hair and the decay during the period of measurement, the graph slopes toward the axis (128). This phenomenon can be seen in some of the graphs in appendix G.

If the hair is 'abnormal', that is one in which the trace element varies appreciably from the normal concentration, the normal hair graph is broken by a peak or a trough, sharp if the condition is acute and continuous if chronic. The troughs are small being due generally to the variation in

sodium excretion. The peaks may be large and are due to the appearance in the hair of trace elements not usually there in any great quantity. Arsenic is such an element and the condition has been investigated by Griffon and others (128) (145) (146). In some of this work attempts, apparently successful, have been made to tell the time when the arsenic was administered from the distance from the bulb at which the activity peak, due to the increased arsenic content, occurred. The identity of the arsenic was established by half life measurements.

It was decided that this work should be repeated to see if it could be confirmed and to find out how efficient the method was. To this end the following set of experiments was made.

1. Normal Hair Samples.

Two samples of normal hair were irradiated. A single hair from each sample was fixed to a piece of graph paper with self adhesive tape. The hair was then cut into 5 mm. lengths and counted with an end window counter. Curves 1 and 2, as shown in appendix G, were obtained. These proved to be roughly linear and in agreement with Griffon's results. The maximum variation found from the graph was 20% from the mean represented by the straight line.

2. Hair Contaminated with Arsenic.

A volunteer had taken 5 minims of Liquor Arsenicalis (about 3 mgm. of arsenious oxide) and four months later a sample of his hair was taken and treated in the above manner. The graph was plotted and a small peak was found at 6 cm. from the bulb (see appendix G.3.). This distance is the same as that calculated, the growth rate being taken as 15 mm. per month. The deviation from the mean was found to be about 50% - appreciably greater than for the normal hair. By chemical separation, the arsenic content of the peak area was found to be about 36 p.p.m., the remainder of the hair being 0.89 p.p.m. approximately. Apparently, therefore, it would be useless to use this method when the concentration was much under 30 p.p.m.

Conclusion

As the results above agreed well enough with Griffons work, and the sensitivity has been established, it was decided to carry on to a new series of experiments using photographic measuring techniques and this is described in the following section.

Autoradiography.

Introduction

Autoradiography is a technique whereby a substance which emits ionising radiations produces an image of itself on a photographic emulsion. The closer the contact between the emulsion and the active sample the more definite is the resulting radiograph. As this work deals with a simple qualitative method, the special techniques and emulsions necessary for the production of the sharpest images have not been used. All that was said in the introduction to the distribution of trace elements in the hair, applies to this section also. The advantage of autoradiography is that it supplies a permanent photographic record, but as will be seen, the method is insensitive.

Procedure and Materials.

A. Types of hair used:

1. Normal Hair. This was hair obtained from a subject who had no known exposure to arsenic. A normal hair contains some arsenic in traces, but as it is distributed evenly it does not interfere with the smoothness of the normal activity of the hair.

2. Contaminated hair. This was hair in which arsenic was deposited by taking it internally. The concentration reached in the section of hair concerned was about 36 p.p.m.

3. Artificially contaminated hair. This was normal hair as in 1 above, but a small section of it had been soaked in a solution of arsenic. On analysis it was found that the local concentration of arsenic was in the region of 300 p.p.m. It was also discovered that 1 mgm. of hair would absorb 0.27 µg of arsenic from 1 ml. of a solution containing 10 µg of arsenic.

B. Irradiation.

The hair was sealed in polythene containers, placed in a standard can and sent for irradiation in 'BEPO' for 24 hrs. at pile factor 14.

C. The preparation of the Autoradiographs.

1. Film. The film used was 'Kodirex' X-ray type. Other emulsions tried were - Ilford contact paper, Kodak bromide paper and Kodak lantern slides.

2. Fixing the hair to the Emulsion. This was accomplished by sticking the hair to a strip of self adhesive tape and then fixing the tape as closely as possible to the emulsion. If the tape was not fixed down properly the clearness and value of the autoradiograph was lost.

3. Exposure. An exposure of 16 hrs. was used for the X-ray film and 3 days for the other emulsions.

4. Removal of the hair. The tape with the hair was removed from the film by slowly and steadily pulling the tape and film apart under water. If the tape was

removed carelessly, or not in the water, bad fogging resulted, due to static electricity.

5. Development. Both X-ray and 1D -2 developers gave satisfactory results.

6. Printing. The prints were made on Kodak reflex paper using 1D -2 developer.

The Measurement of the Autoradiographs.

Four techniques for measuring the activity of the hair from the autoradiographs were used. They are described below.

1. The measurement of the relative optical density of the exposed emulsion at short intervals along the length of the hair by means of a densitometer.

2. The measurement of the width of fogging of the emulsion at short intervals along the length of the hair. This was carried out by the use of a microscope with a superimposed scale. In this case it was often found difficult to establish the border of fogging exactly. The width between the areas of most rapid change of emulsion density was chosen as the easiest to measure.

3. The measurement of the width of a print of the autoradiograph at small intervals along its length. The first was made on extra hard paper (Kodak reflex paper) and, therefore, the contrast was greatly emphasised, with the result that the edges of the hair image became very sharp. This made measurement by the

scaled microscope relatively easy.

4. The measurement of the width of a projected image of the autoradiograph which was printed on a lantern slide. The edges of the image were unsharp but the magnification made the method usable.

RESULTS.

General.

It was found that all the emulsion except the X-ray emulsion were of no use due to the exposure required being too long. Three days exposure was not enough to give a reasonable autoradiograph.

The measuring techniques were found to agree reasonably well with each other, although the prints tended to lose the small details. The reproducability, however, was good.

Normal Hair.

An example of the graphed results obtained for a normal hair using the methods 1, 3 and 4 above is shown in appendix H.1. As can be seen, the three graphs are very much alike. The deviation calculated showed a maximum 90% variation from the mean and this was true for five other normal samples.

Contaminated Hair.

Using the hair described in 'materials' above, it was discovered that no variation which would distinguish it from normal hair could be found. The method, therefore, is not sensitive to arsenic concentrations of about 36 p.p.m.

Artificially contaminated hair.

Two graphs are shown in appendix as H.2. and H.3. Number two shows a facsimilie of a chronic arsenic contamination case. Number three shows a facsimilie of an acute contamination case. Again it is seen that the measuring techniques give similar graphs. Method 1, using the densitometer is more sensitive than the others.

In all the samples investigated, it was found that the variation using the most sensitive method was about 600%; this being for an arsenic content of about 300 p.p.m. In order to be demonstratable against the background variation of the normal hair, the minimum arsenic content must be somewhere over 50 p.p.m.

Conclusion.

The graphs show very well that hair contaminated with arsenic may be activated and autoradiographed, when the excess of the element will show itself. Unfortunately, in this investigation the minimum concentration of arsenic required - i.e. over 50 p.p.m. - is somewhat higher than is normally encountered even in poisoning cases. The method, therefore, although attractive is not sufficiently sensitive for use in the majority of cases.

Conclusion of Arsenic Analysis.

The method of activation analysis was applied to the determination of arsenic in biological materials with very satisfactory results. The sensitivity of detection was increased by a factor of 200, the accuracy being maintained at $\pm$ 1% and this on milligram amounts of sample. This method was then applied successfully to large numbers of biological samples; in one case 1000 samples of hair were analysed with the object of co-relating arsenic content with smoking habits. The result obtained was negative thus eliminating arsenic absorbed (as indicated by hair content) from cigarette smoke as a primary cause of lung cancer. This does not eliminate the possible effect of arsenic absorbed in other ways. A definite difference between the arsenic content of the hair of males and females was discovered. This may be of interest to those who are investigating the causes of the difference in incidence of lung cancer between males and females.

Some purely physical methods of estimation by induced radioactivity were also developed and evaluated. They proved to be much less sensitive and less accurate than the combined chemical and activation estimation of activation analysis, though in circumstances where the arsenic concentration was high they proved satisfactory.

MANGANESE ANALYSIS

A Survey of Manganese Analysis Methods

Introduction.

As in the investigation of arsenic, there are three steps in the analysis for manganese in biological materials.

1. The digestion, in which the manganese is separated from any organic material.
2. The separation of the manganese from other elements.
3. The estimation of the amount of manganese present.

The digestion is not critical as is the case with arsenic: no investigation, therefore, of methods of digestion will be included. Manganese forms no volatile compounds and as a wet digestion is simpler and easier than incineration, this method was adopted in the final analysis method.

The survey will, therefore, deal only with separation and estimation methods, these being dealt with together under the common heading below.

Colorimetry.

This analytical method for manganese is the most popular in use; its sensitivity depends on the technique of the final estimation. It may, in fact, be said that apart from research laboratories this is the only method used for quantitative analysis. Most of the methods depend on forming the permanganate ion and its reactions. These methods are explained below.

Colorimetry using the permanganate ion.

Several reactions may be used to oxidise the manganous ion to the permanganate ion. The most common are as follows.

1. Lead dioxide. On boiling a solution of manganous ions, acidified with nitric acid, in the presence of lead dioxide, the red-violet permanganate ion is formed. Chlorides should be absent as the chlorine liberated destroys the permanganate again. The method is seldom used quantitatively due to the necessity of filtering off the lead compound.

2. Sodium bismuthate. The permanganate ion is formed when this is added to a cold solution of manganous ions in the presence of nitric or sulphuric acid. Chlorides interfere and again the method is not much used due to the necessity of filtering off the solid residue.

3. Potassium or Ammonium persulphate. When this solid is boiled with a manganous solution in the presence of a silver catalyst, the permanganate is formed. This is perhaps the most widely used method but at the same time it is the one most open to errors, due to the reaction not always being complete. A selection of papers using this method is given in the reference (147) (148) (149) (150). The unstable intermediate silver peroxide is the oxidising agent of this reaction and one paper (151) deals with its preparation and use in place of the catalysed persulphate reaction.

4. Potassium periodate. This solid, on boiling with

acidified manganous ions gives permanganate ions. Chloride interferes. The method, however, is very good and is often used. A description may he found in 'Vogel' (152) or in a paper which uses the method (153). Generally no separation is used and the manganese is estimated by the density of the colour due to the permanganate ion. The density is usually measured photometrically and compared with values of standard concentrations measured in the same manner.

Sometimes other coloured ions interfere and to surmount this, further reactions of the permanganate ion are used as follows.

Colorimitry using reactions of the permanganate ion.

The permanganate ion can react with many organic substances to form intensely coloured compounds or decomposition products. These are either measured directly in the reaction solution or may be extracted into some other solvent and measured in that. An example is as follows.

4,4' - Tetramethyldiaminotriphenylmethane. In a paper by Gates and Ellis (154), the manganous ion is oxidised by periodate to give the permanganate ion which in turn is reacted with the reagent to give a yellow colour which is extractable into organic solvents, giving a blue solution in most cases. This is then measured photometrically.

Colorimetry using the Manganous ion.

The manganous ion in certain conditions will react with organic compounds to give coloured complexes which are measurable photometrically. Two such complexes are as follows.

1. 8 - Hydroxyquinoline (155)
2. Diethylammonium diethyldithiocarbamate (156)

In both cases the complex is formed with the manganous ion and in both cases they can be extracted into organic solvents and measured photometrically.

Colour comparison.

J. Hoffmann (157) used sodium fluoride beads with manganese in them as a colour comparison method.

Precipitation Methods.

Many precipitation methods are available for manganese analysis. A few of the more common and a few 'interesting' methods are given below.

Inorganic precipitates.

1. Phosphates. Manganous ions may be removed completely from solution by precipitation with di-ammonium hydrogen phosphate in the presence of ammonia, but the product is not of constant composition (158). Barcia-Goyanes and Serrano (159) use a similar method to precipitate the manganese as the orthophosphate and estimate the amount of precipitate by using radioactive phosphorus

in the precipitant.

2. Oxides. Manganese dioxide may be precipitated using several reagents, e.g. potassium persulphate (160) or potassium chlorate in concentrate nitric acid (161).

3. Bismuth complexes. Hein and Holzapfel (162) describe a method where a solution of bismuthyl perchlorate ($BiOClO_4$) treated with alkali permanganates yield a difficultly soluble crystalline precipitate of basic bismuth permanganates. The formula for this is approximately $(Bi_2O_2OH)\ MnO_4\ 1.5\ H_2O$. These small hexagonal crystals may, on heating, be transformed to a black even less soluble amorphous compound of formula $Bi_3O_5H_2MnO_4$.

Coprecipitation.

Coprecipitation is a method whereby a compound is precipitated along with some other precipitate in circumstances when precipitation would not normally occur. An example of this occurs when manganese is precipitated together with aluminium hydroxide (163) or magnesium hydroxide (164) or both (165) by means of sodium hydroxide solution. A paper by Gordon, Teicher and Burtt (166) describes the precipitation of manganous ions with stannic ions using manganese-54 as a tracer.

Organic complex precipitates.

The most common precipitate of this type is the oxine

(8 - hydroxyquinoline) complex. Generally this is prepared by adding oxine to an acid solution of manganous ions and then precipitating the complex by making just alkaline with ammonia. A selection of four papers are given in reference (167) (168) (169) (170). Among other complexing agents used for precipitation are, diethyldithiocarbamate (171), 5,6 - benzoquinaldinic acid (172) and picrolonic acid (173).

Generally, precipitates are unsatisfactory for micro separations due to inclusion and absorption of impurities and to the inconstancy of composition that is often found.

Other methods of analysis.

1. Spectroscopic analysis.

This is a frequently used method in its different forms. The use of the spectroscope for biochemical analysis is described by Bell (174) and others (175) and the methods and sensitivity are given by Bell (174) and by Hess (176). The Manganese line 2576.17Å was used, but was insensitive below 50 p.p.m. Other papers on spectrographic results are in reference (177) (178). Underwater excitation methods in salt solutions in water cooled sells have been used (179) and the sensitivity quoted is 50 μg/ml ± 20%.

Flame colorimetry has been used by a Japanese worker (180) and flame spectrography by British workers (181). This latter method detects about 4×10^{-9}gm.

2. Polarography.

A few papers have been published on this subject. The sensitivity is reasonable in micro analysis. Dole, al and Adam (182) detect manganese in the region of 10^{-3} to 10^{-4}molar. Other papers deal with special mixed ion solutions (183) (184) and polarography in general (185) (120).

3. Chromatography.

Valuable information on the theory and practice of chromatography for a whole series of elements is given in a review by Carleson (186). Among the special techniques the three following are of interest.

a. Partition chromatography on granular cellulose acetate using dithizone in carbon tetrachloride as the eluant (187).

b. A paper by Strain on two and three way electrochromatography (188).

c. Microchromatography where the amounts of manganese used are 0.15 to 0.20 µg (189).

4. Ion Exchange Chromatography.

There is a valuable paper on the separation of manganese from the transition elements by Kraus and Moore (190) but it was originally decided not to use this method.

5. Biological Analysis.

A method has been devised (191) whereby the amount of manganese in a solution is estimated by the extent

of bacteriological growth it promotes.

6. Complexometric titration analysis.

Hara and West (192) describe a method of complexometric titration using E.D.T.A. in which the end point is detected by high frequency oscillator methods. The range of analysis is in the region of 0.001 for for .0002 molar. A further paper (193) describes the estimation in the region 60 to 600 µg of manganese.

7. Electrical End point determinations.

Sample papers on potentiometric (194) and amperometric (195) titration methods are referred to.

8. Gas evolution analysis.

A method has been developed (196) whereby manganese in the region of 20 to 200 µg can be detected by measuring the oxygen liberated from hydrogen peroxide.

Radioactivation Analytical techniques.

This latest method of ultra sensitive analysis has been investigated previously with respect to the determination of manganese. Several techniques have been used from chemical separations to purely physical separations. Among the best activation analysis methods is that of Bowen (197). The method is separation by a series of precipitations followed by yield calculation on the difference in weight given by samples to which the same weight of carrier was added. Another precipitation method is that of Fischer and Beydon (198). They precipitate manganese dioxide by means of potassium

bromate. Heinke (133) gives a table of comparisons of different methods. Scintillation spectrometry has also been used (199) (200).

Summary.

Using the methods described above, many workers have investigated the manganese content of tissue. Below is set out a selection of these results and the sources from which they came.

The Manganese content of tissue.

Tissue (Human)	Manganese content (p.p.m.)	Reference
Blood (wet)	0.16	(201)
	0.02(μg/100ml)	(202)
	0.14 do.	(203)
	0.15 do.	(204)
Brain (wet)	0.28	(201)
	0.35	(203)(204)
(dry)	1.32	(175)
Heart (wet)	0.38	(201)
(dry)	1.47	(175)

Tissue (Human)	Manganese content (p.p.m.)	Reference
Hair	1.65	(201)
Nail	6.03	(201)
Liver (wet)	1.61	(201)
	1.7	(203)
	0.38	(154)
(dry)	5.32	(175)
	7.0	(205)
Kidney (wet)	1.02	(201)
	0.61	(203)(204)
(dry)	3.01	(174)
Stomach (wet)	0.33	(201)
	0.36	(203)(204)
Lung (dry)	1.77	(175)
Spleen (dry)	1.35	(175)

Tissue (Human)	Manganese content (p.p.m.)	Reference
Bone	3.0	(204)
	2-14	(154)
Urine	0	(206)(207)
	0.01mgm/litre	(204)
Milk	7 µg/litre	(208)

Conclusion.

In general, the most successful method of separation of an element is by the evolution of a gaseous compound of it. In the case of manganese it is found that none of its compounds, which can be prepared by ordinary methods, are volatile. This then leaves us with a choice of the methods described above. The next choice of methods is for precipitation and complex formation with solvent extraction. Precipitation requires several steps to produce a pure substance, due to inclusion absorption and coprecipitation of impurities, and there is a loss in yield with each step. Complex formation with solvent extraction can be much more specific and can be 100% efficient.

The Development of the Analysis.

Introduction.

Originally it was decided to try the use of 8-hydroxyquinoline as the complexing agent. A large number of experiments were carried out to this effect but the method was finally described as unsatisfactory (see later). A new complexing agent, tetraphenylarsonium chloride was investigated and a satisfactory method for its use developed. In the following experiments manganese-54 was used as the tracer. The half-life is 310 days and the main radiations are γ-rays and X-rays. The radiation was detected by Geiger counters. Although this was very inefficient it was used, as this was the method to be employed in the detection of the normally produced manganese-56 ($\beta.\gamma$ and $t_{\frac{1}{2}}$ - 2.6 hours).

Solvent Extraction using the Oxine (8-hydroxyquinoline) Complex.

It was decided to find out if the oxine complex would extract under the conditions laid down by Gentry and Sherrington (155). The method used, however, was different, their method consisting of extraction into a solution of oxine in chloroform.

The method used for the investigation consisted of adding 1 ml. of 0.5% oxine in 2N acetic acid to a solution containing 100 µg of manganese, this being adjusted to sufficient activity to count well on a Geiger counter. The resulting solution was then adjusted to the required pH value by adding 2N ammonia solution and using 'Universal Indicator' as the indicator. The oxine complex so formed was extracted by shaking twice with 5 ml. of chloroform. The activity of the combined extractions was then measured and compared with a standard unprocessed sample. The following results were obtained and compared with previous work (155).

pH	Recovery (%)	Recovery % (155)
6	2.5	4
7	46	93
8	63	100
9	78	100
10	90	100
11	100	100
12	100	100

This shows a gradual increase in extractability compared with the sudden increase shown by the values of Gentry and Sherrington.

A further set of experiments were made under similar conditions to the above and this time the pH was adjusted to

11 using sodium hydroxide solution. The results in most cases showed 100% recovery. It was also found that the time between forming the complex and extracting it had no effect on the results.

The ease of extraction of the Oxine complex.

A series of experiments were carried out with a view to finding how complete the extraction of the oxine was. In each case the mother liquor was extracted with 5 ml. of chloroform and made up to 10 ml. for counting, this was repeated three times. The following results were obtained.

Extraction	% Recovery (average).
1st 5 ml.	99
2nd 5 ml.	1
3rd 5 ml.	0

The Complete Method.

Using the digestion method as described for arsenic, it was found that 2 ml. of concentrated sulphuric acid remained, in which was the 100 µg. of active manganese. The digestion was succcesful as was proved by comparison with the standard sample. The pH required was 11 so the acid was partially neutralised by strong sodium hydroxide solution, the oxine solution added and the sodium hydroxide again added till a pH of 11 was reached. The extraction was made in the normal manner. Large quantities of sodium sulphate crystallised out of the solutions and in some cases the oxine as well.

On estimating the yield, it was found to vary from 5 to 30%. When sufficient water was added to prevent precipitation it was found that the yield improved to about 90 to 100% but was not constant.

To surmount the excess acid problem described above, the digestions were made with nitric acid alone or with only 0.2 ml. of concentrated sulphuric acid present. Using this method, the digestion could be continued until all the acid was driven off. The results obtained on extraction gave very good results but the digestion took a long time due to very careful heating being necessary to prevent bumping and physical loss.

Summary.

As the digestion was not considered to be satisfactory without the sulphuric acid and the time taken was appreciably near one half life of manganese-56, it was decided to try another method which would be more specific and not so sensitive to variations in acid concentration.

Solvent Extraction using Tetraphenylarsonium Chloride $(C_6H_5)_4AsCl$

On investigating the literature, references were found in two (209) (210) which claimed the interference of permanganate ions in the determination of perrhenates by tetraphenylarsonium chloride. It was decided after investigation of the other available papers (211) (212) and by analogy with the rhenium method to investigate the use of the method with permanganates.

The proposed method of analysis was activation determination of the manganese combined with yield checks on the added carrier manganese by means of colorimetry. The following experiments were carried out with that purpose.

The Investigation of the possible use of Colorimetry.

When tetraphenylarsonium chloride solution is added to an acidified solution of permanganate ions, a magenta precipitate of tetraphenylarsonium permanganate, $(C_6H_5)_4AsMnO_4$, is formed. The compound is slightly soluble in water and acid solutions, and very soluble in organic solvents (e.g. acetone or chloroform). In chloroform solution, the characteristic permanganate colour is shown and it is this which provides the basis for colorimetric measurements. However, the compound is unstable in 'Analar' chloroform solution and after about 30 mins. the colour changes slowly to a dirty pink and finally the whole of the

manganese precipitates as the dioxide. The time taken for the colour to change was reasonably long so it was decided to carry on with the method.

A few experiments were carried out in the following manner to determine the type of filter to be used. Solutions of permanganate containing approximately known weights of manganese were prepared, a few ml. of 3N sulphuric acid were added and the solution made up to 20 ml. To this were added 5 drops of the reagent solution (2.5 gm of tetraphenylarsonium chloride hydrochloride in 75 ml. of distilled water). Some of the solutions gave precipitates and some did not. In general, under these conditions the solubility was about 6 to 7 μg/ml. The complex was extracted twice by shaking with 8 ml. of chloroform. The bulked chloroform solution was then made up to 20 ml. Portions of this were taken and the transmission measured on a Hilger Absorptiometer using matched glass tubes. The following results were obtained using different filters. The wavelength quoted is the peak wavelength.

Total Mn. (μg)	Concentration measured (μg/ml)	Transmission (%) 5800A		5450A		5200A	4900A
200	10	73	38	38.5	36	32.6	67
180	9	74.3	44	40.7	40	38	72
160	8	-	-	43	-	40	-
140	7	78	50.5	52.6	49	47	75
120	6	81	59	56.5	54.4	51.3	79.6
100	5	87	66	63.5	65	62	85
80	4	90	70	71.8	71	70	85
60	3	94	78	77.1	75.5	75.3	89.5
40	2	96.5	84.5	85.5	88	81	90
20	1	91.5	91.8	91	92.8	89.8	96

It can be seen in the table that the filters with peak wavelengths 5450A and 5200A are both satisfactory. Repeat experiments gave values which varied appreciably from the others. This was found to be due to inaccuracies in the matched tubes used and from this point, optical cells of 1 cm. width were used. The filter chosen was that of 5450A peak wavelength, due to difficulties in setting the instrument when using the 5200A filter.

Repeating the experiment using the filter and cell chosen above, the following results were obtained.

Total Mn. (μg)	Conc. Measured (μg/ml)	Transmission (%)	
152.2	7.61	51.0	51.5
136.8	6.84	54.4	54.2
121.6	6.08	59.0	59.1
106.4	5.32	63.6	63.2
91.4	4.57	68.6	68.0
76.2	3.81	73.5	73.4
60.8	3.04	78.0	78.5
45.6	2.28	83.3	83.3
30.4	1.52	89.0	89.1
15.2	0.76	96.0	96.0
0.0	0.00	100.0	100.0

These results were then plotted on a graph (appendix I) and used as the standard recovery graph. The reproducability of the results is very much better and accurate concentrations have been used.

The method appeared to be satisfactory and so it was decided to carry on with the investigation as described below.

The Development of the Analysis Method.

Having decided on the solvent extraction method described above, it was necessary to find out how sensitive the method was under varying conditions. The most important consideration was the effect of varying acid concentrations as it was hoped to work from a digestion residue containing about 2 ml. of concentrated sulphuric acid. Using the method described above and varying the acid content as shown, the following results were obtained.

Acid content of 20 ml. of solution.	Transmission (%)
No Acid	73.7
3 ml. of 3N sulphuric acid	73.8
6 ml. of 3N sulphuric acid	73.6
10 ml. of 3N sulphuric acid	73.2
1 ml. concentrated sulphuric acid	73.6
2 ml. concentrated sulphuric acid	81.3
2½ ml. concentrated sulphuric acid	87.0
2 ml. concentrated sulphuric acid partially neutralised	74.7

The table shows that the working limit of the method is

reached when a concentration of 1 ml. concentrated sulphuric acid to 20 ml. of water is used. This is not unreasonable for a working condition and indeed when the full method is described a concentration of one in forty is used.

It was noticed that when nitric acid was used with sulphuric acid, the complex tended to remain in the aquous solvent. In order to investigate this, a further series of acid concentration experiments was carried out. The method was the same as before, but a different standard was used.

Acid Conditions in 20 ml.	Transmission (%)
3 ml. 3N sulphuric acid	70.0
10 ml. 3N sulphuric acid	70.1
3 ml. 3N sulphuric acid and 1 ml. 3N nitric acid.	71.0
3 ml. 3N sulphuric acid and 3 ml. 3N nitric acid	75.1

Small amounts of nitric acid have an appreciable effect on the extractability of the complex so it is necessary, as with the arsenic estimation, to remove the nitric acid after digestion. This can be accomplished in this case by continuing the digestion till the sulphuric acid fumes.

As the problem of the acid concentration had now been solved, attention was turned to the amount of complexing agent used and its effect. This problem was important because of the great scarcity of the reagent. Using the

usual conditions and varying the amount of reagent, the following results were obtained.

Vol. of reagent used (drops of conc. $2\frac{1}{2}$ gm/75 ml)	Transmission (%)	
15	72.5	
10	71.8	
5	70.0	70.0
2	70.0	
1	70.0	69.9

It was decided to carry on using five drops as before, but this later gave complications.

At this stage of the development it was thought that the method was advanced enough to attempt some analysis starting from the manganous state. In order to do this, standard amounts of permanganate were taken and reduced by sulphur dioxide to the manganous state. The excess sulphur dioxide was boiled out and the solution made up to 40 ml. 2 ml. of concentrated sulphuric acid having been added. About 0.3 gm. of sodium bismuthate was added to the solution and well shaken up. The resulting suspension was centrifuged and the supernatant solution of permanganate poured into a separating funnel. The deposit in the tube was washed with 40 ml. of acidified (1 ml. 3N sulphuric acid) water and added to the contents of the separating funnel. The volume was such that there was 40 ml. of water to 1 ml.

of concentrated acid. Five drops of the reagent were added to the solution and shaken up. The solution was then extracted twice with 8 ml. portions of chloroform. The bulked chloroform extracts were then made up to 20 ml. The transmission was measured in the usual way and found to give a value of 70.0% which agrees with former values.

A further set of experiments were made starting with a solution of manganous ions and following the proceedure above. The results obtained agreed very well, being transmissions of 64.1%, 64.0% and 64.6%.

These results being satisfactory, it was decided to attempt the proposed full analysis method. This was as follows. After the digestion of the organic sample and carrier in the nitric/sulphuric acid mixture (as for arsenic) and continuing till fumes of sulphuric acid appear, the cooled acid (about 2 ml.) was diluted to 40 ml., 5 drops of the reagent were added and the mixture extracted thoroughly with chloroform to remove any interfering ions. The aquous layer containing the manganese in the manganous state was then evaporated to small bulk in a beaker and treated with the bismuthate, followed by the processing described above The resulting transmission measurements should have given a value of 64% but in fact a whole series of experiments gave transmissions varying from 69 to 100% i.e., from no recovery to almost complete recovery.

On close investigation of this method by radioactive

tracer techniques using manganese -54, the following results were obtained.

Activity at beginning of experiment	-	100%
Activity extracted by 1st $CHCl_3$ process	-	0%
Activity remaining	-	100%
Activity after boiling to low bulk	-	100%
Activity extracted as complex	-	0-30%
Activity not in complex form	-	5-20%

From these values it is obvious that some of the manganous ions are not being oxidised to the permanganate state. The reason proposed for this is that the chloride of the complexing reagent and traces from the chloroform were interfering with the completeness of the oxidation process. It was also found that the high percentage of lost activity was retained among the bismuthate in the form of the precipitated complex which had persisted from the first extraction.

To surmount these difficulties, it was decided to reduce the amount of reagent in the first extraction to one drop and instead of boiling to small bulk, continuing till sulphuric acid fumes appear so that any chloride would be removed. In the final extraction of the permanganate, only two drops of reagent were used.

The application of these modifications resulted in transmission values of 64% and 64.2% being obtained. This

is in keeping with 100% recoveries. Complete recovery of manganese was not what we aimed at but with care, this can be achieved every time to within 1%. An example of this is given below.

<u>The determination of the concentration of manganese in an active solution.</u>

1. Activity of solution per ml. - 2511 c/m

Activity recovered after analysis - 2575 c/m

This represents 100% recovery.

Added inactive manganese - 98.6 µg.

transmission of 98.6 µg of manganese - 65.2%

transmission of this + Mn from active solution. - 53.5%

Therefore.

98.6 µg of manganese give 98.6 µg manganese (Graph appendix I)

98.6 + unknown µg manganese give 144 µg manganese (do.)

∴ Manganese in active solution - 45.4 µg/ml.

2. Activity of solution per ml. - 2511 c/m

Activity recovered after analysis - 2588 c/m

This represents 100% recovery.

Transmission of complex from active solution - 82%

This corresponds to 45.6 µg. manganese (Graph, appendix I)

Hence by two methods it is possible to get a good agreement between values.

In order to test if the method is useful when less than 100% recovery is obtained, the recovery was deliberately depressed by allowing chloride to be present. The following results were obtained.

Transmission	56%	64.2%
Recovered activity	2236c/m	1690c/m
Recovered activity	89%	67%
Recovery by Colour	92%	69%

The results agree fairly well and show the method to be useful in this circumstance also.

As an example of the use of the method in an analysis of an organic specimen, the manganese content of tea has been determined and is as follows.

The Manganese content of tea leaves (unused).

A sample of tea sealed in a polythene capsule and a sample of a manganous solution sealed in a silica ampoule were irradiated for an hour in 'BEPO'. The irradiated samples were removed to beakers and 98.6 μg of manganese were added to each followed by 2 ml. concentrated sulphuric acid and 8 ml. of concentrated nitric acid. The beakers were covered and the contents heated to fumes of sulphuric acid. The usual method was then followed when the following results were obtained.

Results calculated from the count rate and assuming 100% recovery

	Tea Sample	Standard
Weight (gm)	0.0449	0.2415g of solution i.e. 23.8 µg. Mn.
Count rate (c/m)	12,200	17,400
Manganese content (µg) assuming 100% recovery	16.8	23.8
Manganese (p.p.m.)	374	-

Results calculated from colorimetry. (The standard colour solution was made up to 25ml. in this case).

	Tea Sample	Standard
Weight (gm)	0.0449	0.2415g of solution i.e. 23.8 µg. Mn.
Manganese added (µg)	98.6	98.6
Total Mn. content (µg)	98.6+	122.4
Transmission %	67.2	65.2
Hence concentration(µg/ml)	4.61	4.92
Total Mn.content (µg)	115.5	123.0
Mn.content - carrier (µg)	16.9	24.4
Values from count rate experiments	16.8	23.8 (known)

The agreement is good and shows that even with a multiplication factor of 25, accuracy can be obtained. Some difficulty would be experienced in the calculation of

the true value if the recovery were not 100% and the amount of manganese in the sample appreciable when compared with the weight of added manganese. The result can, however, be determined using the method of approximations. For samples which contain less than 1 μg, the recovery could be taken directly from the manganese content as found from the transmission value. An example of this is as follows.

Manganese in Finger Nails.

Manganese found by activity measurements	-	0.34 p.p.m.
Manganese added (μg)	-	98.6
Manganese recovered (μg) from colorimetry	-	92.2.
Hence actual Manganese content	-	0.36 p.p.m.

A Further Development of the Method.

The same proceedure may be used as in the method described above using 10 mgm. of manganese as carrier. The final separation, however, is not by solvent extraction but by precipitation of the complex on adding an excess of the precipitating agent. The yield is reasonably good, being about 70 to 80%. However, only two cases were tried and it is probable that the yield could be increased to 80 to 90% by careful washing and transfer of the permanganate precipitate. If 10 mgm. of manganese were present the theoretical yield would be 91.6 mgm.

In order to determine if the tetraphenylarsonium permanganate precipitate was stable to the temperature of the

oven which was used for drying, a sample was held at a temperature of 105°C for a length of time and the weight checked at intervals. The following results were obtained.

Time of heating (hours)	Weight (mgm.)	Loss (%)
½	44.0	0
1	44.0	0
1½	44.0	0
2	44.0	0
3	43.0	2.5
26	41.4	5.5
90	41.0	7.0

The complex shows good stability at the drying temperature, and in practise 15 mins. is enough. The colour of the complex changes from magenta to a light purple brown after two hours.

Interfering substances.

The only interfering substance which would follow the manganese through the full method as described was chromium. It is an easy matter, however, to determine manganese -56 in the presence of chromium -51 by physical methods. There are three methods available as described below.

1. Separation by Discrimination using a Scintillation counter.

A graph is shown in appendix J.1. in which the activity of a mixture of chromium -51 and manganese -54 is

plotted against the discriminator voltage. Manganese -54 was used in place of manganese -56 because of its longer half life. It was allowable in this case as the γ-ray energies are almost the same. On the same graph is shown the traces of the two constituents of the mixture by themselves. The photopeaks of the manganese and chromium are 0.82 Mev. and 0.32 Mev. respectively and the separation by discrimination is very distinct. The scintillation counter was operated at 900 volts and the discrimination varied from 5 to 60 volts. This method is very good where a scintillation counter is available, it being necessary only to set the discrimination at a value which cuts out all the activity from the chromium; about 25 volts in the experiment described above.

2. Separation by Scintillation Spectrometry.

Scintillation spectrometry is a method whereby the activity over a small range of discrimination is measured. For example, it is possible out of the 60 volt range of discrimination to measure only the activity which has the correct energy to appear in the region below 30 volts and above 29 volts. The same energy of radiation does not always appear in this region as it is also dependent on the amplification applied to the pulses from the counter. In any case it is possible to set the 'channel width', (the space between discrimination voltages, where the activity registers) and the amplification, constant and hence, by examining the full range of discrimination with the small

detecting channel width, to obtain a plot of activity against energy of radiation. As each isotope has characteristic energies of radiation which appear on the graph as peaks of activity, a means of identification is possible. Such a trace from a mixture of manganese -54 and chromium -51 is shown in appendix J.2.

In order to use this refinement of method, 1 above, it is necessary to set the instrument on the peak of the manganese radiation and open the channel width to include the whole peak. The method is much more useful when the method described in 1. is not usable,

3. Separation by Decay.

If using the analysis method as developed, the final product contains, or is suspected to contain, both manganese -56 and chromium -51 and a scintillation counter is not available then it is possible to separate the activity of the two isotopes by decay. The activity measured immediately contains activity from both, but if left for a short period (one or two days) the activity from the manganese will have decayed almost completely and the remaining chromium activity can be corelated to its activity when the mixture was counted and hence corrected for.

Manganese -56 has a half life of 2.6 hours.

Chromium -51 has a half life of 26.5 days.

The Efficiency of the Radio-Chemical Separation.

In order to test the completeness of the separation of the active isotopes, the activity of the sample, extracted in the tea analysis above, was followed and plotted until it merged with the background activity. In all the activity decay was traced over 42 hours. On tracing the results on semilogarithmic graph paper, the sample gave a straight line decay until it became unmeasurable against the background, i.e. until it fell below one count per minute above background. This shows a very complete separation.

In order to compare this method with other radiochemical methods, a similar sample of tea was analysed using Bowen's method (197), which is described below. A portion of the finally separated manganese of the same activity as the sample above was subjected to the same investigation. In this case, the straight line graph began to deviate at about eight counts per minute above background and became more pronounced until the count rate here, also became indistinguishable from the background. Both methods are good, however, as the error due to impurities was less than 0.1% of the original count rate. A graph comparing the two decay traces is shown in appendix K.

Comparison of Methods.

To check the results obtained in the use of the above developed method, it was decided to use the radiochemical

separation of Bowen (197) as follows.

The biological samples and the standard were added to 20 ml. portions of fuming nitric acid containing equal weights of manganese carrier (about 50 mgm.). The solutions were then heated until digestion was complete and thereafter the manganese was precipitated as the dioxide from concentrated nitric acid by the addition of potassium chlorate. The precipitate was then centrifuged and washed with distilled water. Then followed two 'scavenging' processes; one for iron (hydroxide) and one for copper (thiocyanate). The manganese was finally taken into solution in concentrated nitric acid and precipitated as the dioxide as before and washed. The precipitate was then removed as a slurry in acetone to counting trays. The efficiency of this separation is described above.

Using Bowen's method, the results, using the extraction method, were checked and the following comparison obtained.

Sample	New Method Mn(p.p.m.)	Bowen's method Mn(p.p.m.)
Nail	0.36	0.32
Tea(unused)	374	359
Cigarette	93	95

Unfortunately, the analysis could not be made on the same sample of nail or cigarette, but the results still agree well and the method is successful.

Further work on the cigarette samples has been done and is in agreement with the values above. Some analyses were done by scintillation spectrometry on cigarettes and the values obtained also agree with those above. These results will be dealt with in due course in the following pages.

A Modification of the recovery estimation.

In Bowen's method of separation, the chemical yield of the separation is calculated on the weight and activity of manganese dioxide recovered in each case compared with the standard activity. In a series of experiments, a known activity of manganese-54 was added to each sample and the recovery calculated from the recovery of the long lived isotope. The activity of this isotope was found by allowing the other isotope to decay. A typical curve of such a decay is shown in appendix L. Results of the following nature were obtained.

Property	Samples 1	Samples 2	Standards A	Standards B
Weight (gm)	0.0225	0.1788	0.2201	0.2375
Recovery of weight (gm)	0.0515	0.0486	0.0973	0.0975
Count Rate (c/m)	10900	13000	31800	34500
Count Rate (c/m) Correcting recovery to A	20600	26000	31800	-
Count Rate (c/m) Correcting recovery to B	20600	26000	-	34500
Known Mn content (μg)	-	-	21.7	23.5
Mn content calculated on A (μg)	14.0	17.7	-	-
Mn content calculated on B (μg)	14.0	17.7	-	-
Mn content (p.p.m.)	622	99	-	-
Recovery of ^{54}Mn (c/m)	1002	980	1953	1934
Count Rate of samples(c/m) correcting recovery to A	21300	26000	-	-
Count Rate of samples(c/m) correcting recovery to B	21000	25700	-	-
Mn content calculated on A (μg)	14.5	17.7	-	-
Mn content calculated on B (μg)	14.3	17.5	-	-
Mn content (p.p.m.)	640	99	-	-

The results of the two methods used in the one experiment show good agreement. When considered with the similar methods described in the comparison of yields by colorimetry and activity, the use of a long lived isotope for recovery

calculation shows itself to be a very convenient method. This becomes more evident in the case of Bowen's method when for some reason the weight of carrier must be restricted or in the case of the new method, if it is desired to avoid the use of delicate colorimetric methods. In this latter case, the complex must be obtained in the form of a solid as the solution in chloroform is unstable.

A Physical Analysis Method.

Due to the high manganese content of cigarettes, it was possible to analyse them by a physical method, i.e. directly on an active sample without a radiochemical separation. The method used was that of comparative scintillation spectrometry and consists simply of tracing the peak energy of a manganese standard, followed by tracing the manganese peak of a known weight of sample, followed at an equal interval of time by another trace of the same standard. On averaging the standard peak heights, the result can be compared with the sample peak height and hence the manganese content found. The peak height of a peak is from the top of the peak to a line drawn across the base of the peak. An example of this is shown in appendix M.1. using a spectrograph of manganese-54. An example of the analysis traces is shown in appendix M.2.

The results obtained for this method in the concentration region of about 100 p.p.m. agree well with results from activation analysis methods. The results are given in the following section.

The Manganese content of Cigarettes.

As manganese is a poison and an industrial hazard, and the manganese content of tobacco is high, it was decided to investigate the concentration levels met with in cigarettes and to find out if any manganese was ingested from the smoke.

The first step in the investigation was a survey of the manganese content of six common brands of cigarettes. A sample of about 0.5 cm. of cigarette was used in each case. The estimation was carried out by the peak height comparison method, using a γ-ray scintillation spectrometer. The following results were obtained.

Tobacco sample	1	2	3	4	5	6
Manganese (p.p.m.)	95	106	102	93	85	129

These results agree well among themselves in the order of magnitude and are in keeping with Thomson's and Askew's (213) statement that sound tobacco leaves should contain under 130 p.p.m.

In order to see how consistent the manganese concentration was, one cigarette was tested from each of ten different packets. The following results were obtained by activation analysis and radiochemical separation. All the cigarettes were of type 1 above.

Sample	1A	1B	1C	1D	1E	1F	1G	1H	1J	1K
Manganese (p.p.m.)	95	91	92	94	93	93	97	95	96	102

The manganese content is about 95% ± 5% and, therefore, can be said to be reasonably consistent.

The average content of the cigarette being established, a series of experiments were made to discover the manganese content of the products of smoking the cigarettes. In order to do this the following materials were collected and tested.

A. Cigarette ash. All the ash from on cigarette was collected and weighed. A portion was analysed by the radiochemical method.

B. Cigarette butt. The butt including the scorched end was weighed and analysed as in A.

C. Cigarette smoke. The smoke from the combustion of a cigarette was drawn into a trap containing concentrated nitric acid absorbed in silica wool. No smoke passed this absorbing medium. The resulting solution was filtered and made to standard volume. A known weight of samples was analysed as in A.

The following are a typical set of results obtained for this experiment.

Weight of one cigarette	-	1.14 gm.
Absolute Manganese content at 95 p.p.m.	-	109 µg.
Weight of butt	-	0.1788 gm.
Manganese content	-	99 p.p.m.
Absolute content	-	17.7 µg.
Weight of ash	-	0.1461 gm.
Manganese content	-	630 p.p.m.
Absolute content	-	93 µg.
Total Manganese (butt + ash)	-	110.7 µg.

This is in good agreement with the estimated total content.

In all cases where the manganese content of smoke was determined, it was found that the solution in acid content was indistinguishable from the normal acid content. It was concluded that no manganese was lost in the smoke from cigarettes.

Radioactivity of Normal Cigarettes.

It was considered possible that manganese-54 (310 day half life) might be a 'full out' product of the explosion of nuclear or thermo-nuclear weapons. As manganese occurs in high concentrations in tobacco, it was decided to separate the manganese and attempt to find any activity in it. To this end the manganese was extracted from one cigarette using Bowen's method (187). An identical blank was carried out at the same time. The following results were obtained using

an 'end window' Geiger counter.

Sample c/m	Standard c/m	Difference c/m
12.49	11.83	+0.66
13.03	11.83	+1.20

A further sample was counted on a 'end window' Geiger counter with an anticoincidense shield and the sample was again found to be slightly higher than the standard.

The results, however, were inconclusive and the experiments were discontinued due to the lack of suitable samples and equipment. It would be more suitable and much more efficient to count the X-ray and γ-ray activity of the manganese-54 by means of a large volume proportional counter.

Conclusion to Manganese Analysis.

The application of activation analysis to manganese micro estimation, allows amounts of the order of 10^{-9} gm. to be determined with ease. The method developed is accurate to within 1%, and this can be assumed without checking. If, however, it is thought necessary to check each recovery, the colorimetric gravimetric or activity recovery method described, may be used. Unfortunately, due to the short half life of manganese-56, the sensitivity claimed above may not be obtainable unless a suitable pile is near at hand. Application of the method to the manganese content of plant material and tissue showed satisfactory behaviour under normal working conditions.

The purely physical method of γ-ray scintillation spectrometry was investigated and for the concentration conditions found in plant material, proved to be a useful tool. It was not, however, as accurate as the activation analysis method.

CHROMIUM ANALYSIS

the dichromate state under the same conditions as manganese oxidation by sodium bismuthate. It was that the dichromate formed tetraphenylarsonium di when treated with the complexing agent. The comp found to be extractable, under the same conditions permanganate complex, giving a characteristic orang coloured solution in chloroform. The solution, h was unstable though less so than the corresponding solution. In this instance, the complex decompos with the formation of a chromic salt.

In order to test the radiochemical separation of an impure chromium salt (chromic nitrate) was i in a suitable atomic pile (BEPO) for 30 mins. Th mixture of isotopes was investigated using a scint spectrometer. A trace showing the presence of so with no evidence of the chromium-51, was obtained On separating the chromium by the method described was obtained from the scintillation spectrum

Chromium Analysis.

In the course of the analysis development for manganese, it was thought that chromium might interfere. It was found that chromium could be oxidised from the chromic state to the dichromate state under the same conditions as the manganese oxidation by sodium bismuthate. It was also found that the dichromate formed tetraphenylarsonium dichromate when treated with the complexing agent. The complex was found to be extractable, under the same conditions as the permanganate complex, giving a characteristic orange coloured solution in chloroform. The solution, however, was unstable though less so than the corresponding permanganate solution. In this instance, the complex decomposed overnight with the formation of a chromic salt.

In order to test the radiochemical separation, a sample of an impure chromium salt (chromic nitrate) was irradiated in a suitable atomic pile (BEPO) for 30 mins. The resulting mixture of isotopes was investigated using a scintillation spectrometer. A trace showing the presence of sodium, but with no evidence of the chromium-51, was obtained (appendix N.1) On separating the chromium by the method described above, a trace was obtained from the scintillation spectrometer which showed the presence of chromium-51 only (appendix N.2).

Hence, the separation of chromium by this method is efficient, at least qualitatively. A further set of

experiments were made as described below.

Subtractive Scintillation Spectrometry.

Peirson (214)(215) has shown that the spectrum of recoil electrons can be eliminated by observing a source simultaneously with two scintillation counters, using sodium iodide (thallium activated) and anthracene as phosphors. By suitable matching, the output of the anthracene counter (which records the recoil spectrum only) can be subtracted from that of the sodium iodide (Tl) counter, to leave only the photoelectric responses of the latter. Putman and Taylor (216) have shown that if two samples, one of which had an additive, were irradiated and measured simultaneously with two sodium iodide (Tl) scintillation counters of equal resolution, then the responses of the counters could be subtracted exactly as described by Peirson (214) to leave only the spectrum of the contaminant.

Two experiments were made using the above method of Putman and Taylor. These were as follows.

1. A sample of irradiated chromic nitrate was placed in one scintillation counter and a sample of chromium-51 obtained by the above separation was placed in another. The activity of the pure chromium sample was varied, by varying its distance from the crystal, until the small peak due to the chromium-51 in the mixture was removed using the subtraction method described above. The trace obtained with and without subtraction is shown in

appendix P. On tracing the area where this peak was, over three days, a perfect decay curve for sodium-24 was obtained.

2. In another experiment, the activity contaminating the chromium was taken to be only sodium-24 and the peak given by this being free from any chromium activity (no activity due to chromium-51 remains above 0.32MeV whereas sodium-24 has peaks at 1.38 and 2.76MeV) it was relatively easy to remove it by the subtraction method. The remaining activity was a trace of chromium-51.

Conclusion.

The separation of the tetraphenylarsonium dichromate complex is efficient and has every sign of being capable of development into an excellent quantitative method. The method of subtractive scintillation spectrometry, as described above, appears to have great use as a rapid and effective method of analysis when the activity sought after is not too near the count variation of the background mixture.

APPENDIX

lows.

The electroscope - This is useful for detecti activity sources and depends on the discharge charged electroscope. The method is not use now but was once very popular.

The Geiger-Muller counter - The counter is an tube along the axis of which is an insulated The tube is gas filled and usually at lower t atmospheric pressure. A high potential (300 depending on the gas filling is applied acro When an ionising particle enters the tube and ions by a type of chain reaction, a cascade o flows to the anode and so produces a 'pulse' be registered on a scaler after suitable ampl If the operating voltage of the tube is too l cascades are formed, if it is too high the tu down' and a continuous discharge occurs. Th

Instruments for the detection of β and γ radiations

Measurement of β-radiation.

There are four methods of estimating β-activity as follows.

1. The electroscope - This is useful for detecting high activity sources and depends on the discharge of a charged electroscope. The method is not used much now but was once very popular.
2. The Geiger-Muller counter - The counter is an earthed tube along the axis of which is an insulated wire anode. The tube is gas filled and usually at lower than atmospheric pressure. A high potential (300-1300 volts) depending on the gas filling is applied across the tube. When an ionising particle enters the tube and forms ions by a type of chain reaction, a cascade of electrons flows to the anode and so produces a 'pulse' which may be registered on a scaler after suitable amplification. If the operating voltage of the tube is too low no cascades are formed, if it is too high the tube 'breaks down' and a continuous discharge occurs. This means of detection is the most popular at the moment and has been used extensively in the work for this thesis. However, it has the disadvantages of a relatively long

'dead time' (about 300 μs) and the necessity of counting a sample which is outside the tube through a low density 'window'.

3. The Proportional Counter - This counter has a similar design to the Geiger tube but usually has a means of placing the sample inside the tube itself. The operating voltage is higher than the Geiger counter and the pressure is atmospheric, it being usual to pass the gas through the counter continously. The pulses obtained are proportional to the energy of the particles and the dead time is very low. This apparatus was not available for use by the author.

4. The β-Scintillation Counter - This counter is similar to the γ-counter, but there is only a thin metal shield between the source and the scintillation crystal. When very weak β-activity has to be measured, it is usual to mix the active agent with a liquid scintillation medium. The method is used mostly in the latter manner and has not been used by the author.

Measurement of γ-radiation.

The only efficient way of measuring γ-radiation is by the scintillation method. The other methods as used in β-counting may be used, but they are very inefficient.

The most efficient scintillation crystal is sodium iodide, activated with thallium. The γ-rays produce, in the crystal, a pulse of light which is amplified proportionally by a

photomultiplier and again amplified before being registered on a scaler. Due to the final pulse being in proportion to the energy of the radiation, it is possible by use of discriminators or pulse analysers ('kick-sorters') to obtain the activity of any given energy of γ-ray or to trace a spectrum of the full range of energies. The different ways in which this can be used have been described in the main body of the work.

Scalers.

A scaler is an instrument which registers the total number of pulses reaching it from the amplifier and counter.

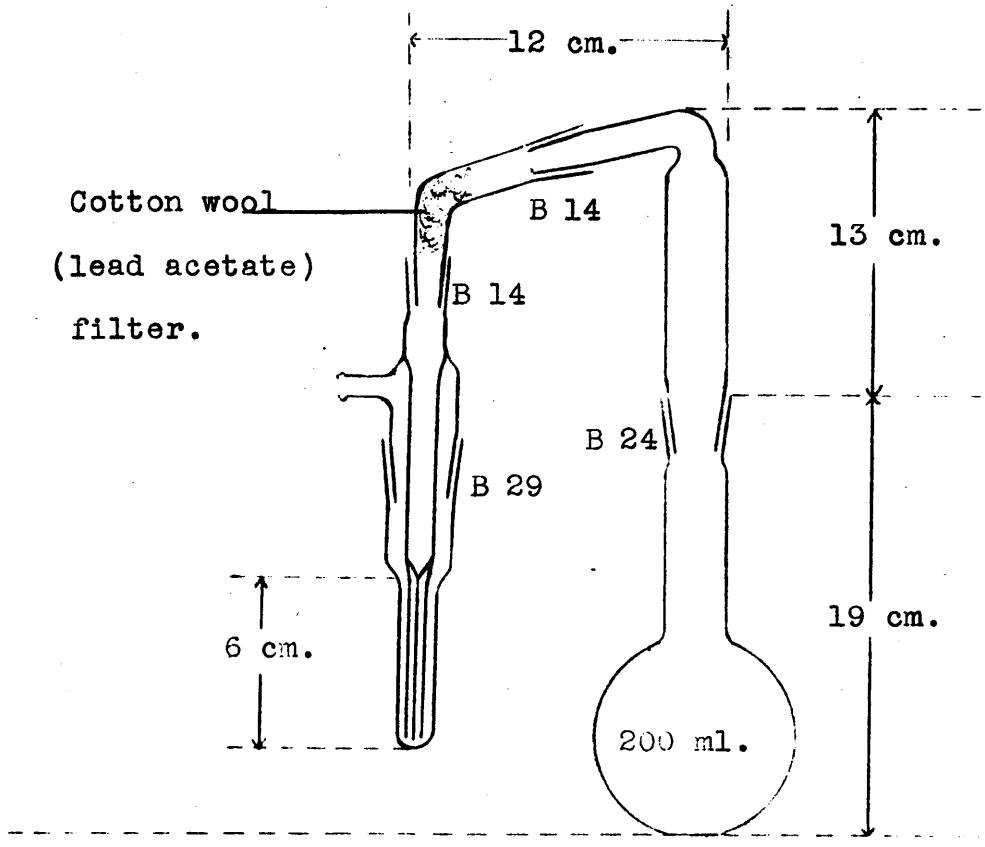
Dead Time.

Dead time is a function of a counter or electronic circuit which is characterised by the systems inability to repeat its function above a certain number of times per second. In scintillation counters, when the dead time is only a few microseconds, it is not very important, being negligible in most circumstances. In Geiger counter, however, the dead time is about 200-400 μs and is a very important factor. If, for example, we take a dead time of 400 μs and a count rate of 300c/m, the actual count rate would be 300c/m, but if the count rate was 30,000c/m, the actual count rate would be 37,500c/m. This shows a loss of 20% of the counts.

A Geiger counter becomes inoperative after a discharge because of the upset potential field due to the presence of positive ions which are being neutralised by the quenching gas. When the tube has returned to its normal working state, a further cascade pulse may form, the time taken is the dead time.

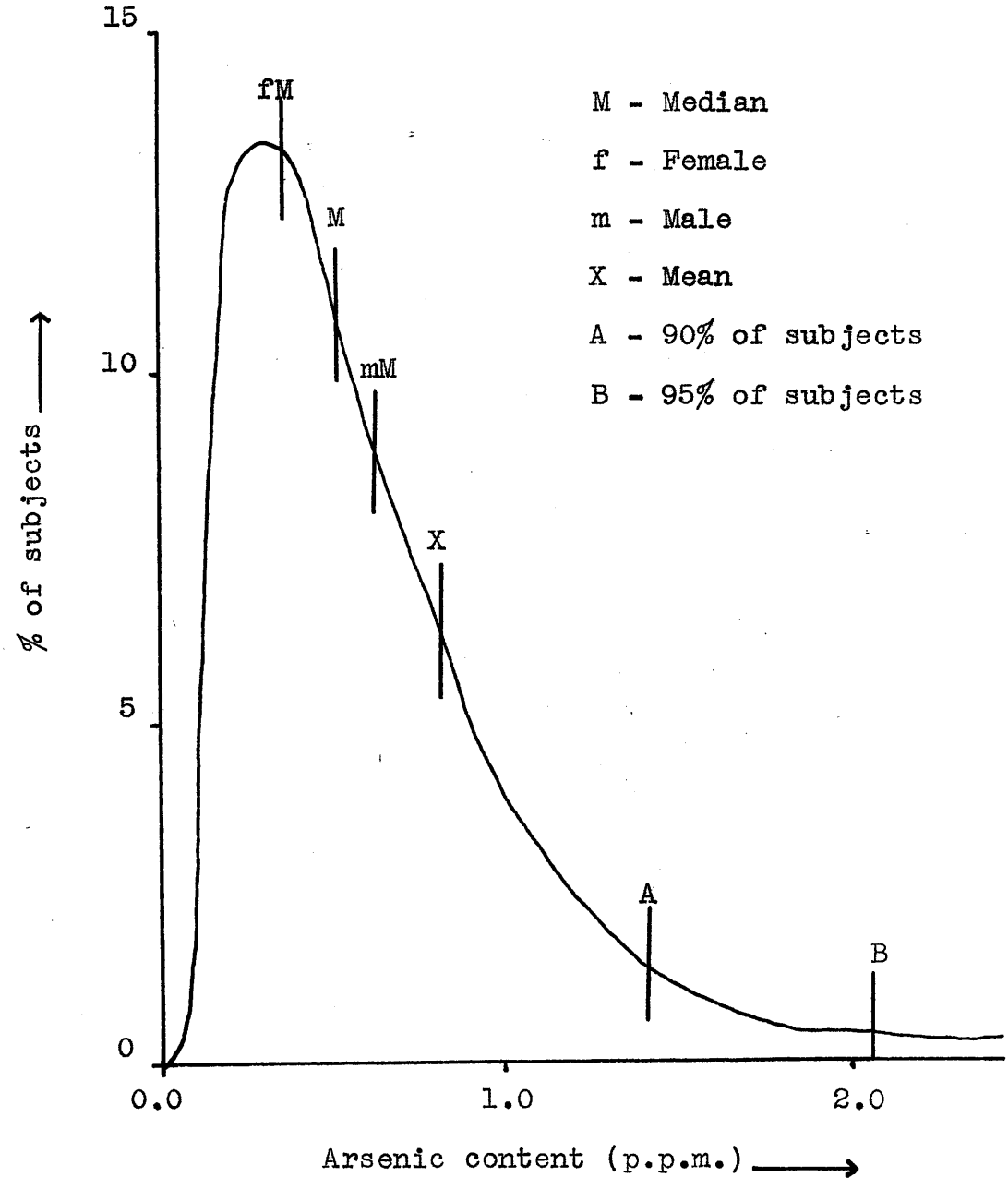
The correction for the dead time may be calculated as follows.

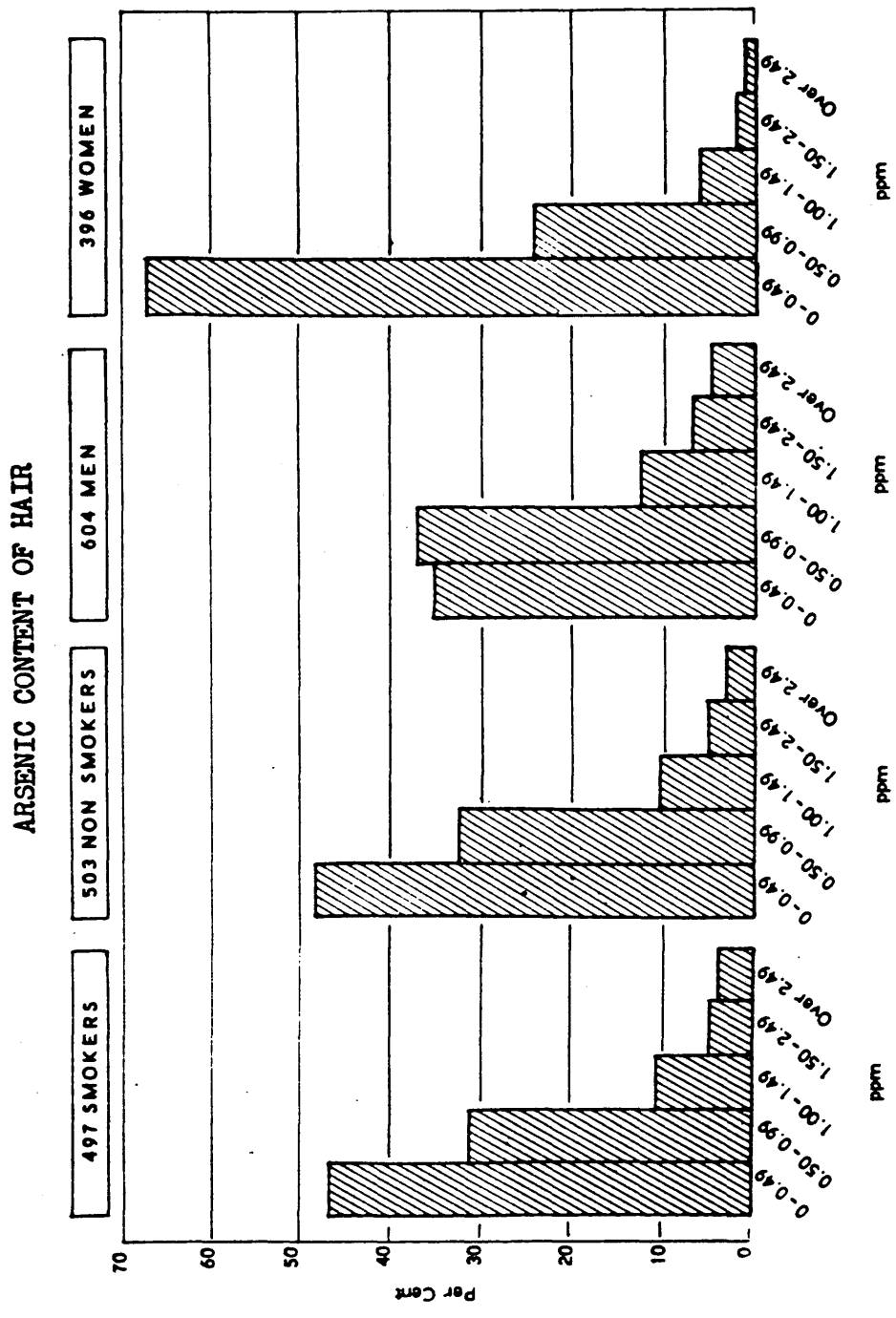
Arsine evolution apparatus



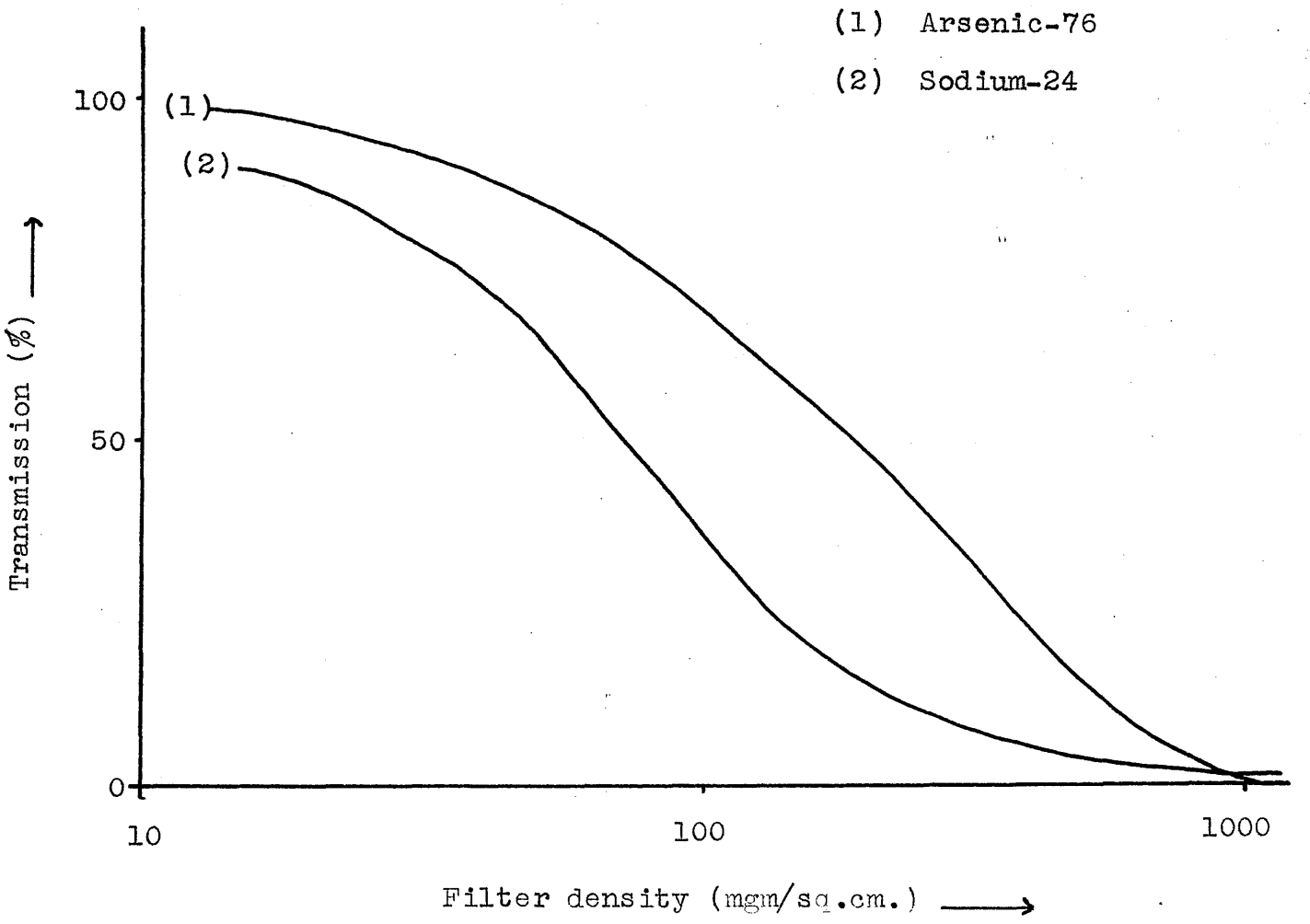
External diameter of absorption tube	12 mm.
External diameter of capillary delivery tube	7 mm.
Internal diameter of capillary delivery tube	1 mm.

The Distribution of Arsenic in Hair

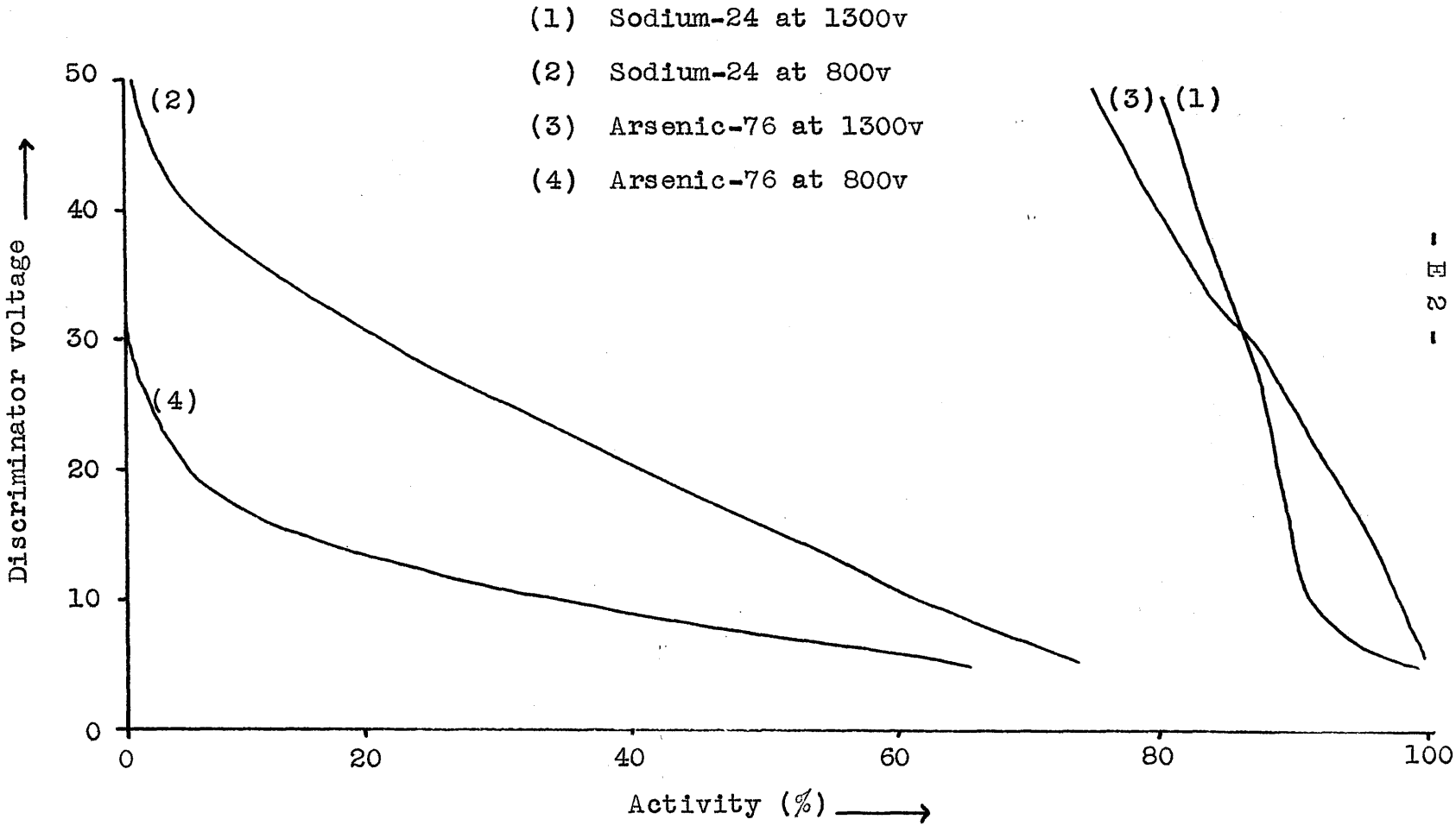



ARSENIC CONTENT OF HAIR
497 SMOKERS
503 NON SMOKERS
604 MEN
396 WOMEN
Per Cent
70
60
50
40
30
20
10
0
0-0.49
0.50-0.99
1.00-1.49
1.50-2.49
Over 2.49
ppm

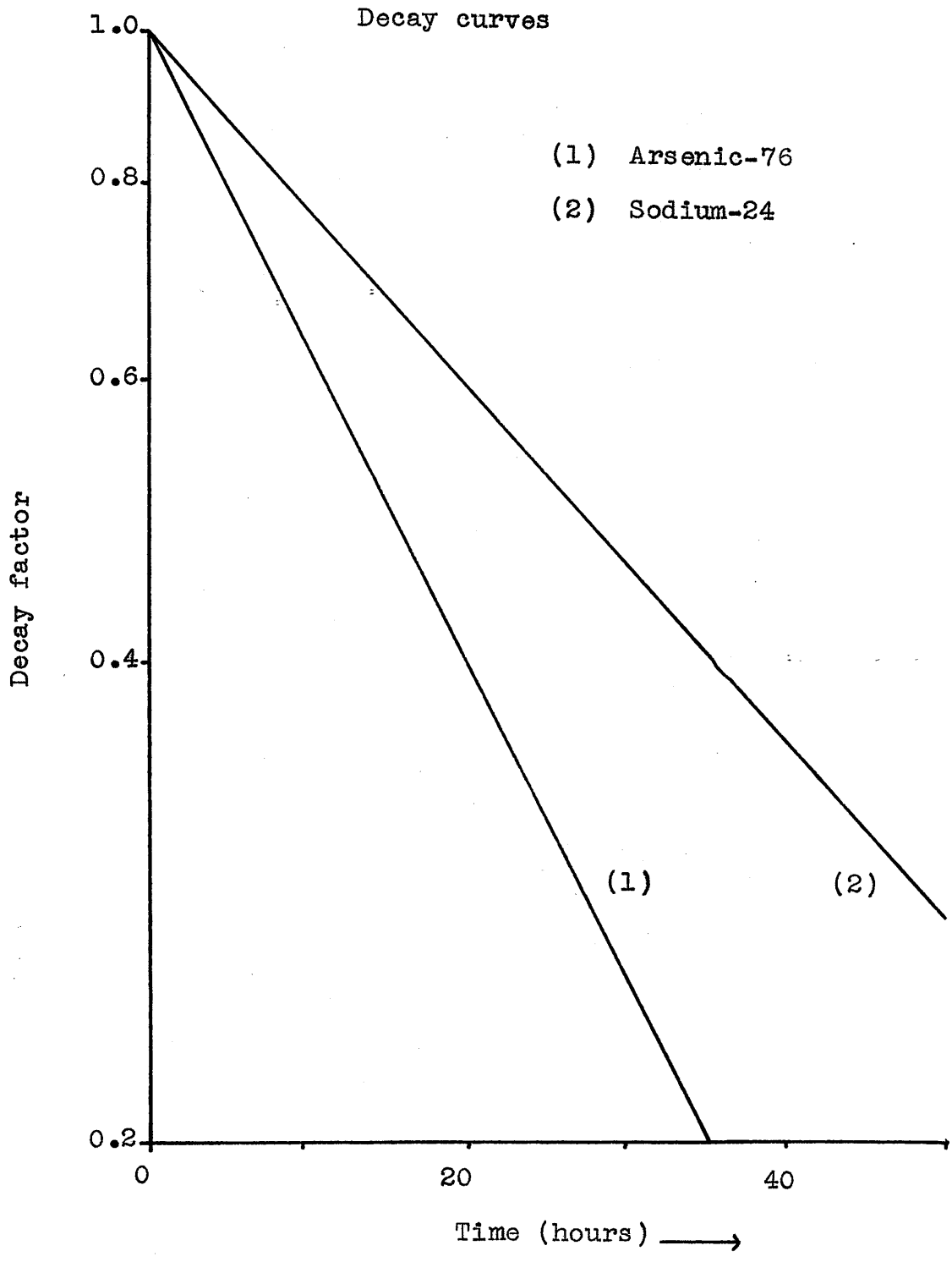
The transmission of radiation through solid filters



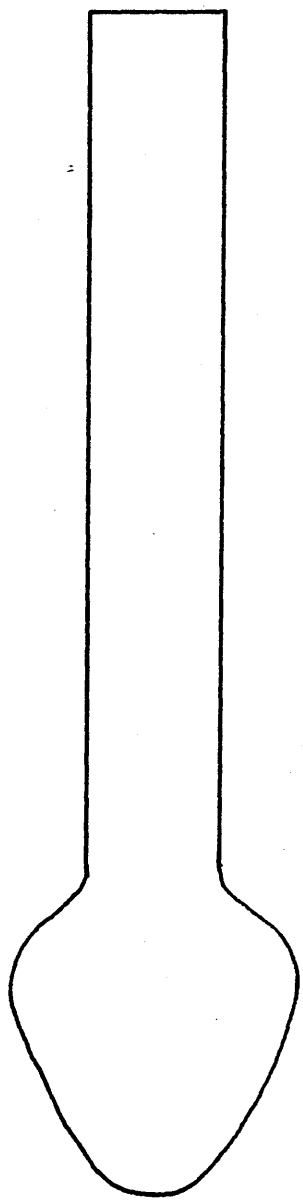
Count variation with varying discriminator voltage and varying E.H.T.



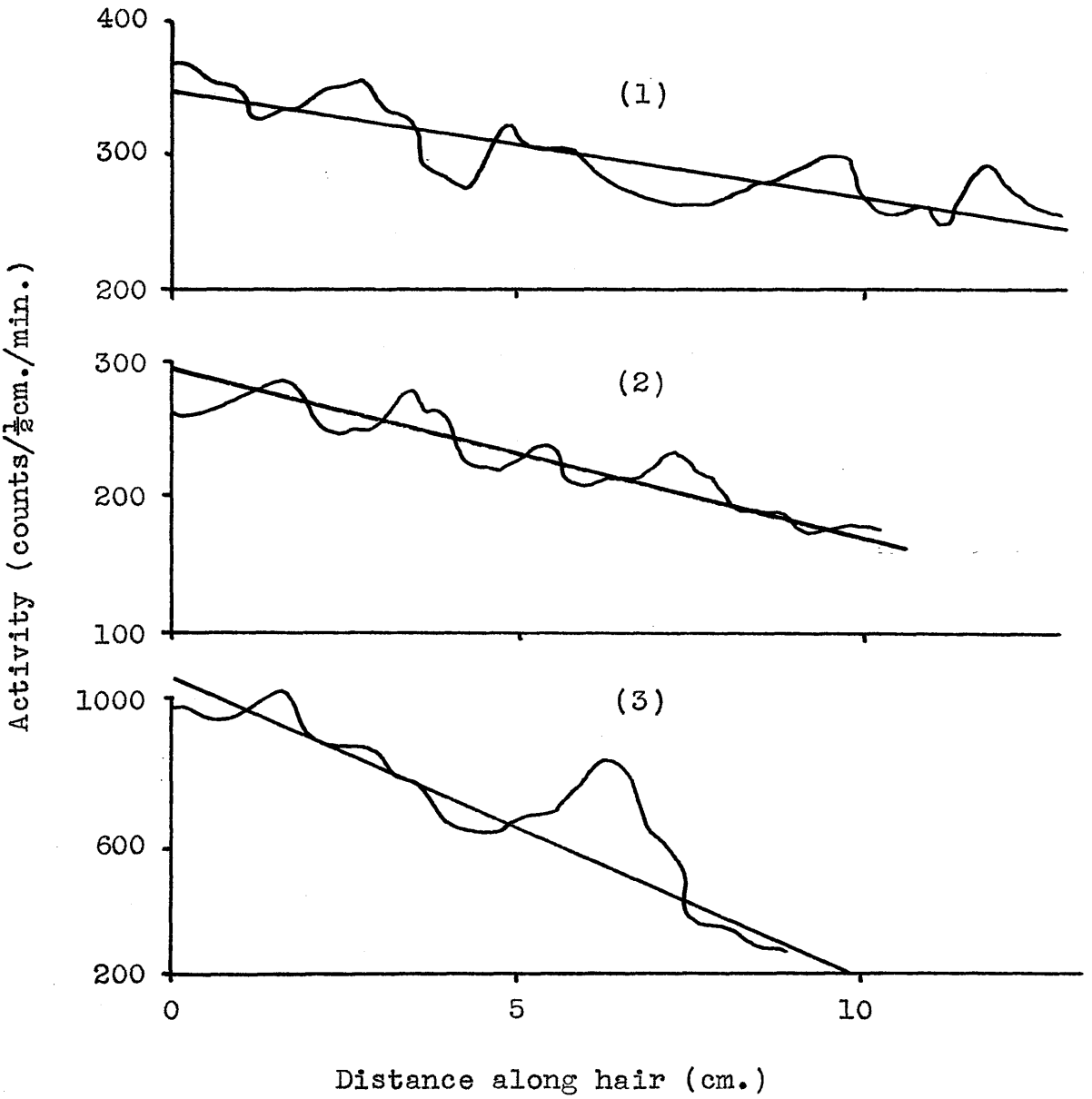
E 2


Decay curves
1.0
0.8
0.6
0.4
0.2
Decay factor
(1) Arsenic-76
(2) Sodium-24
(1)
(2)
0
20
40
Time (hours)

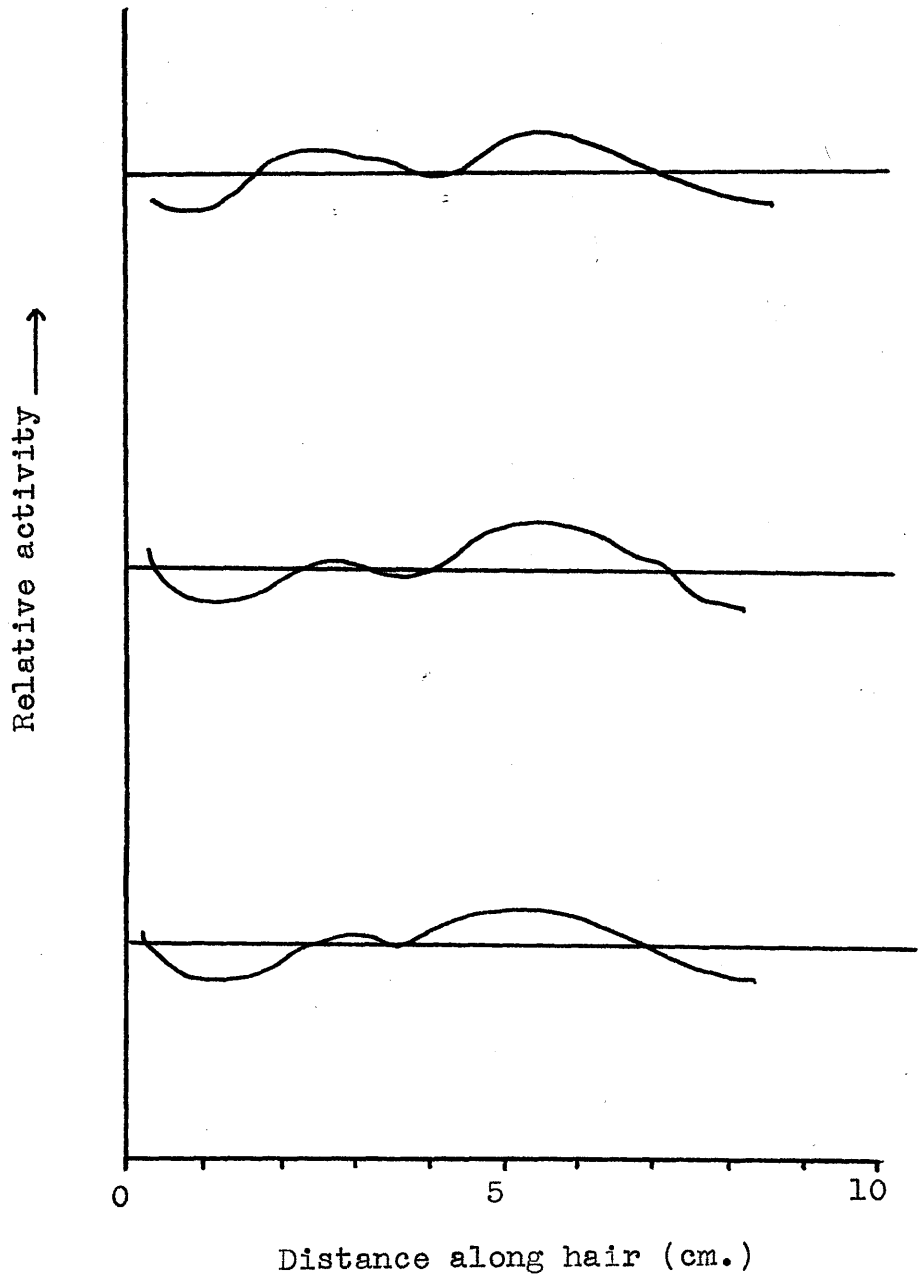
Digestion Flask (full size)



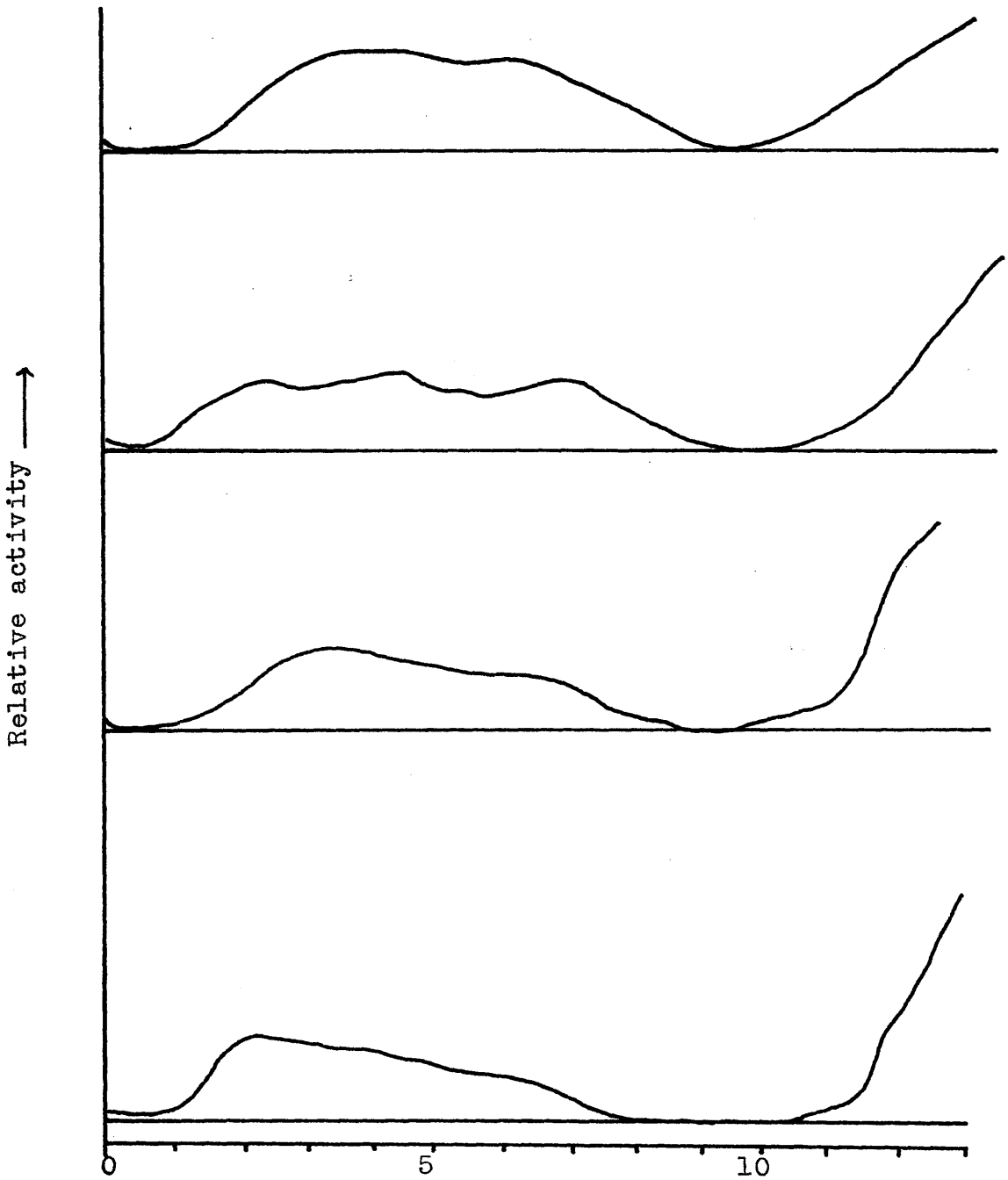
Activity from irradiated hair



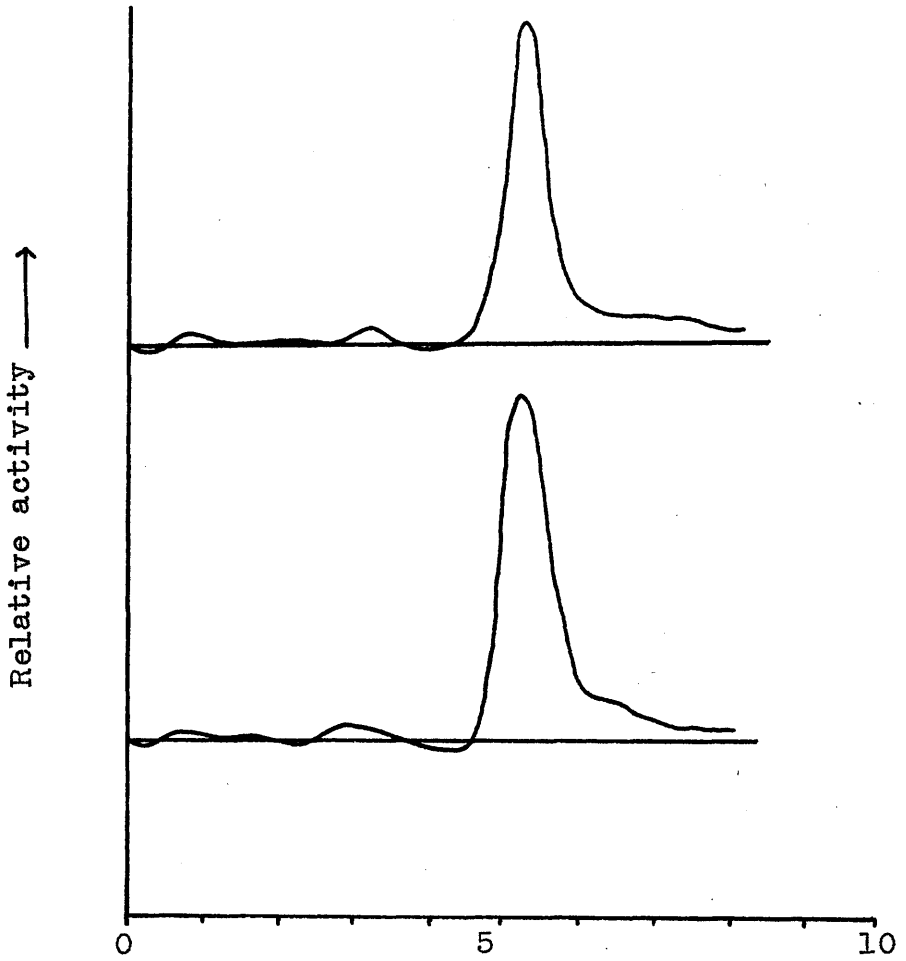
Activity from a normal autoradiograph



Activity from autoradiograph

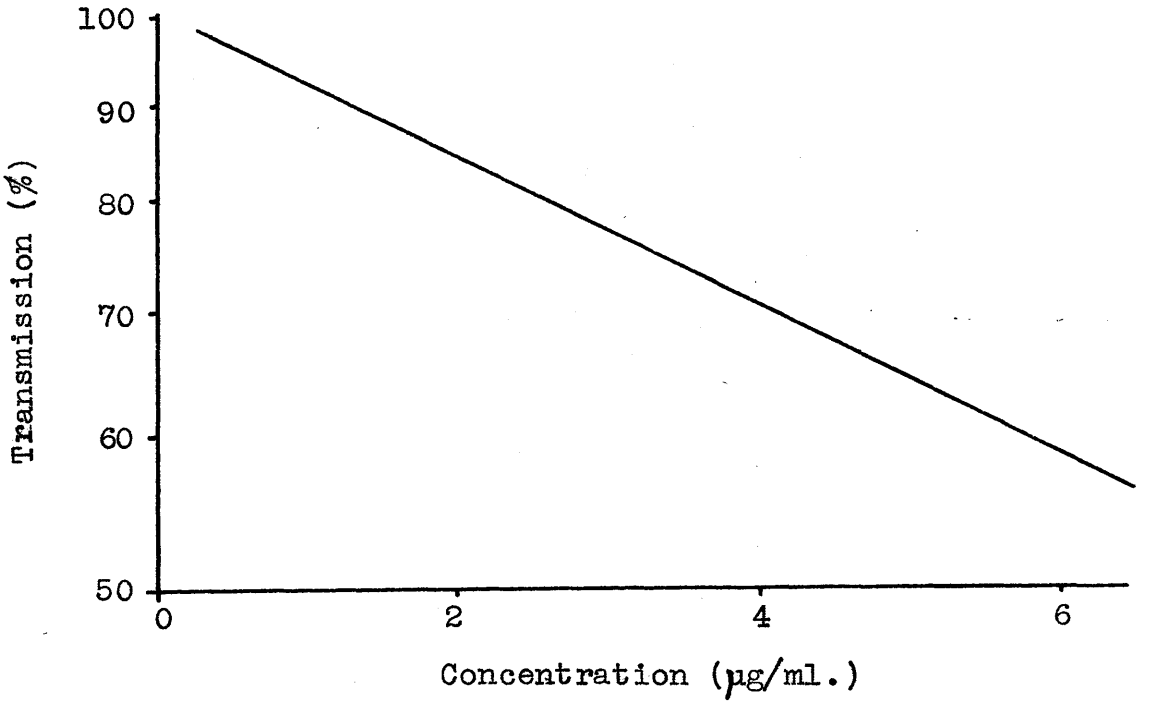


Activity from autoradiograph

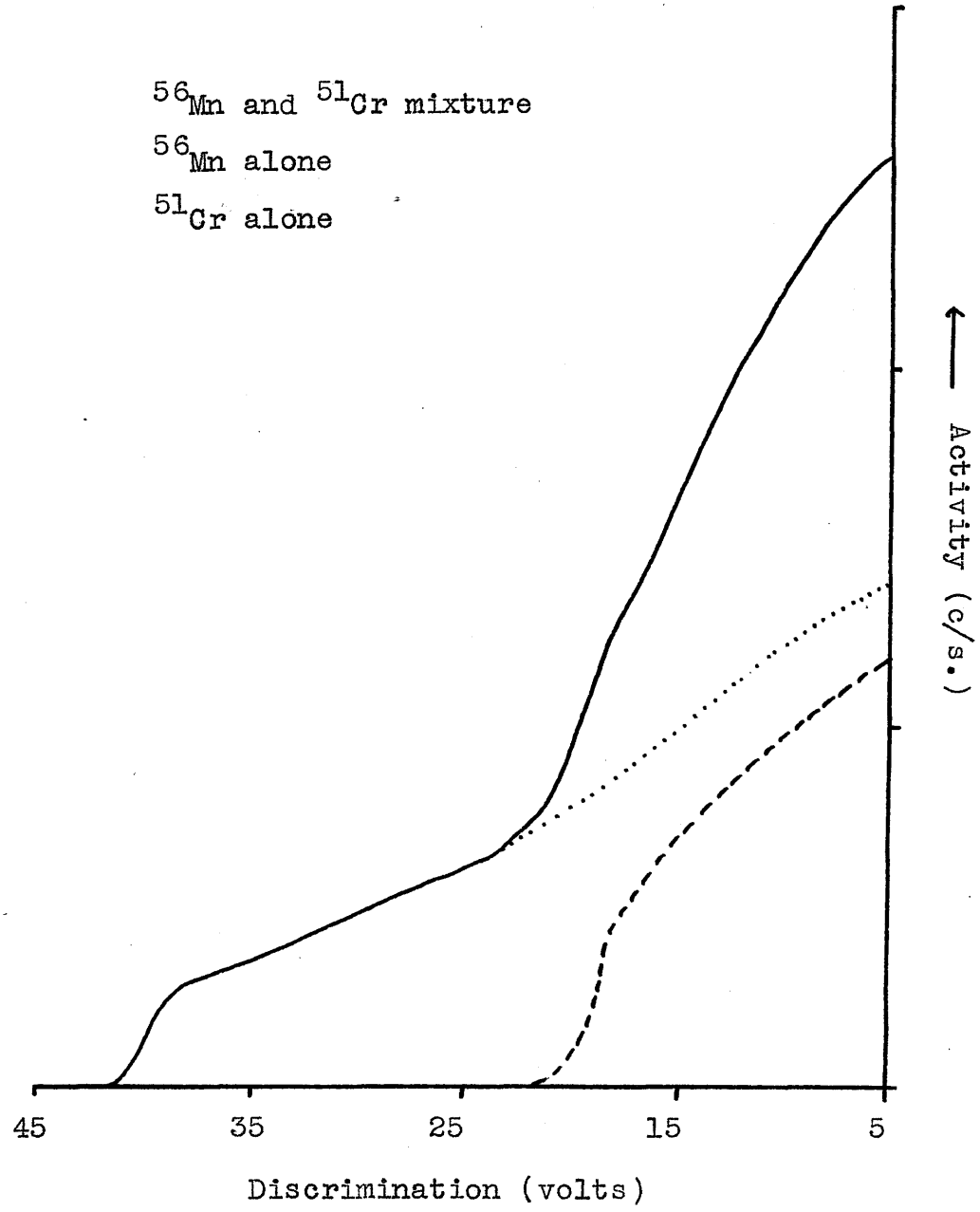


Transmission curve

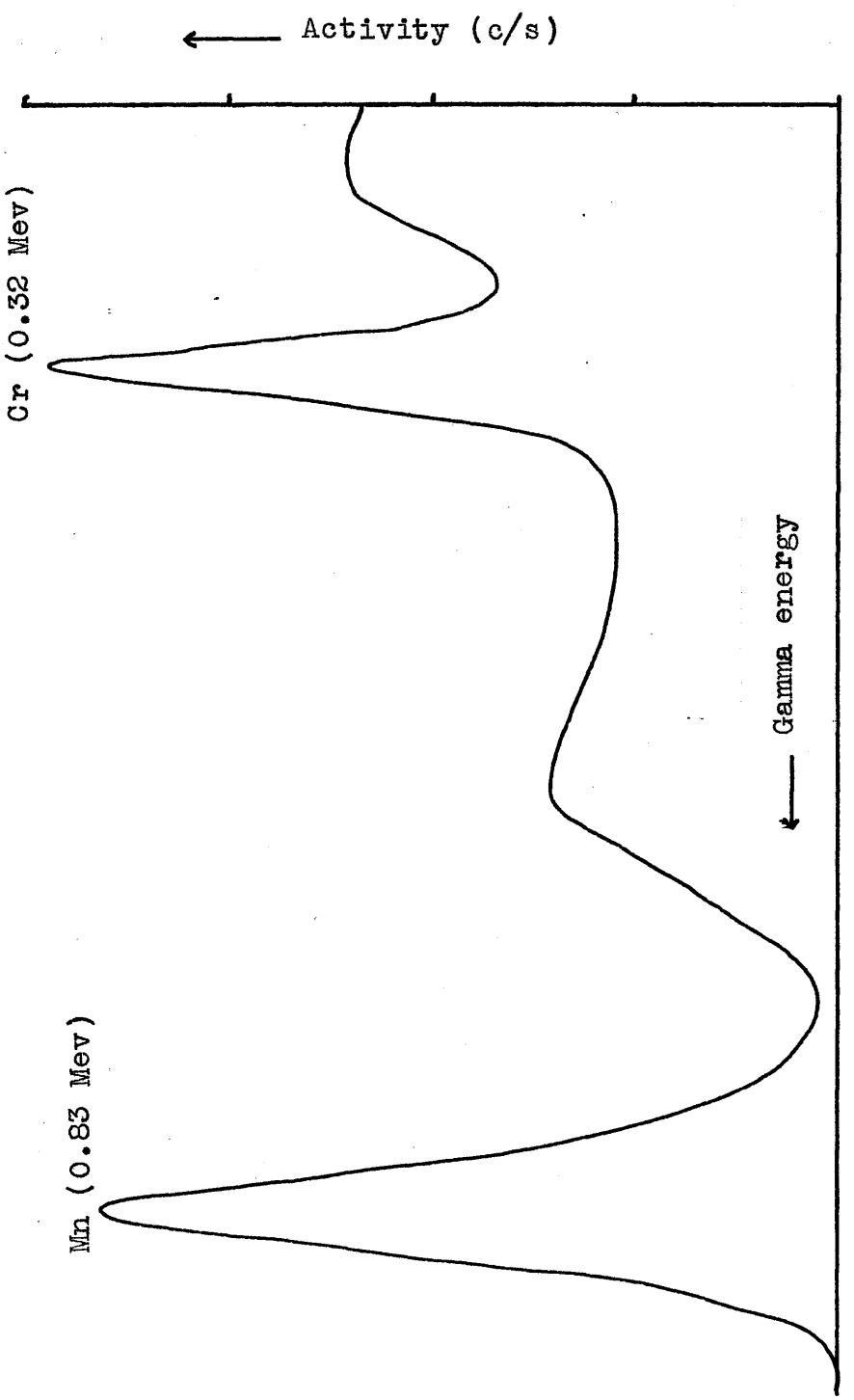
(Permanganate ion in chloroform)



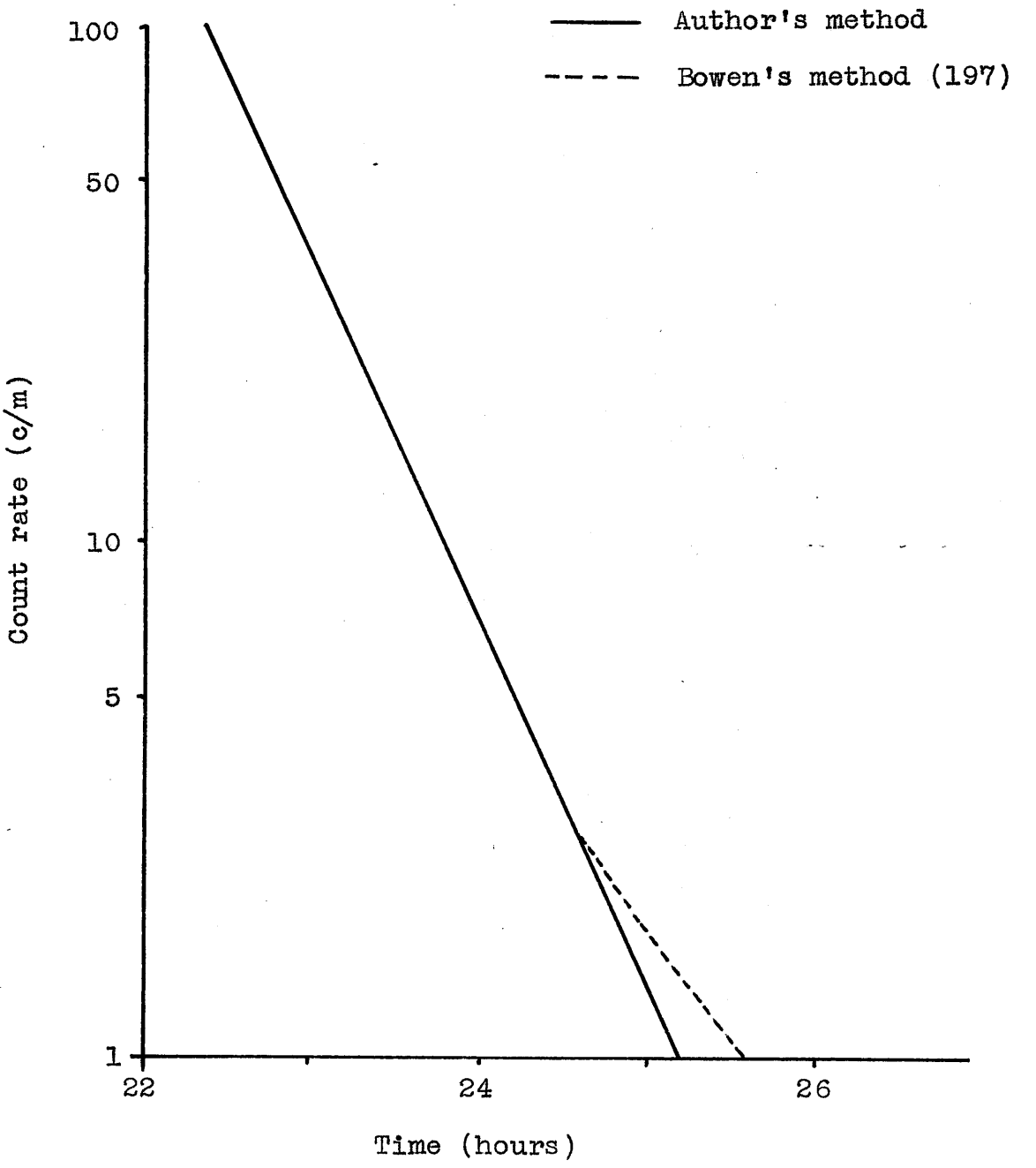
Discrimination separation of ^{56}Mn and ^{51}Cr



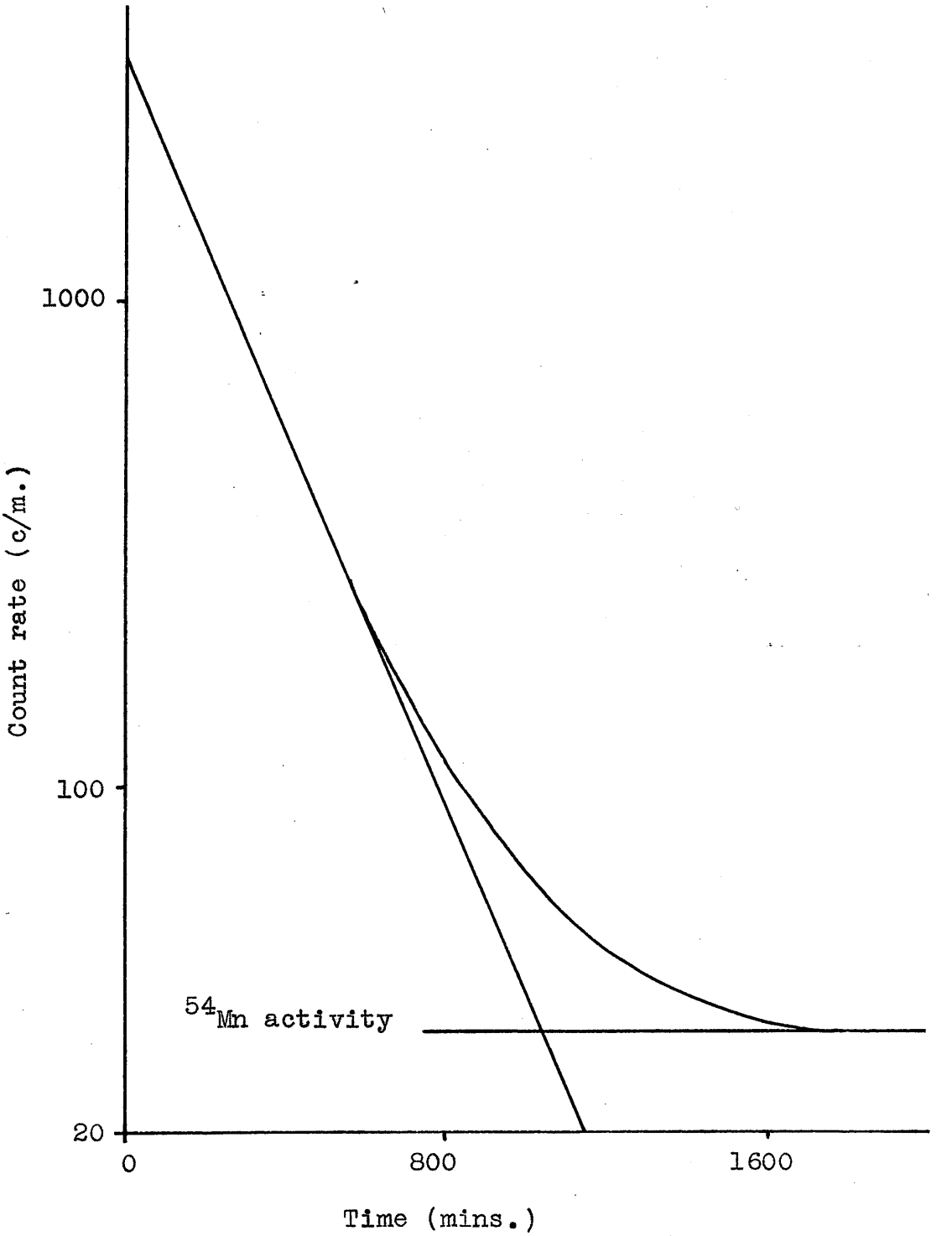
Gamma spectrum of ^{54}Mn and ^{51}Cr mixture



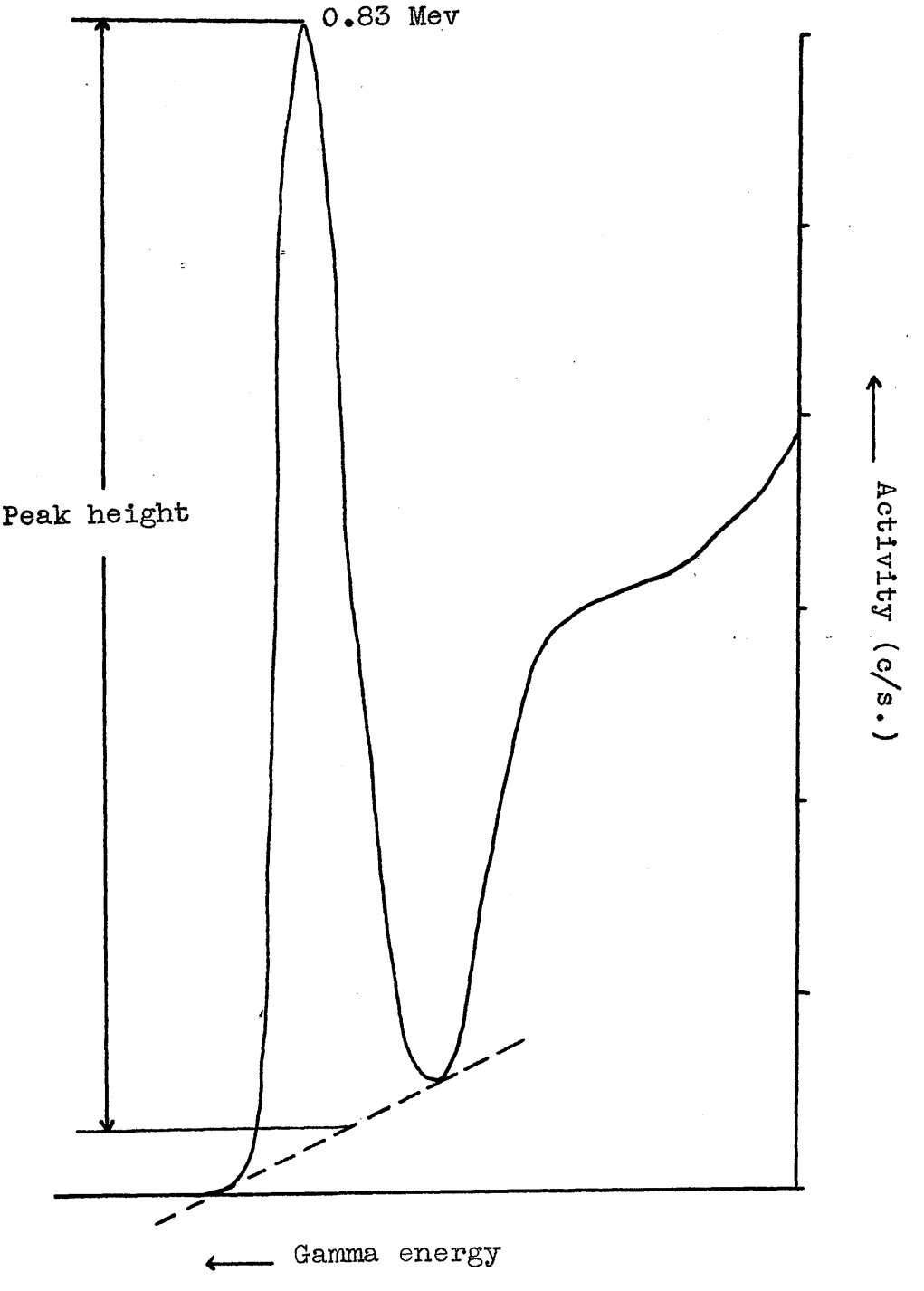
Decay curves of purified ^{56}Mn



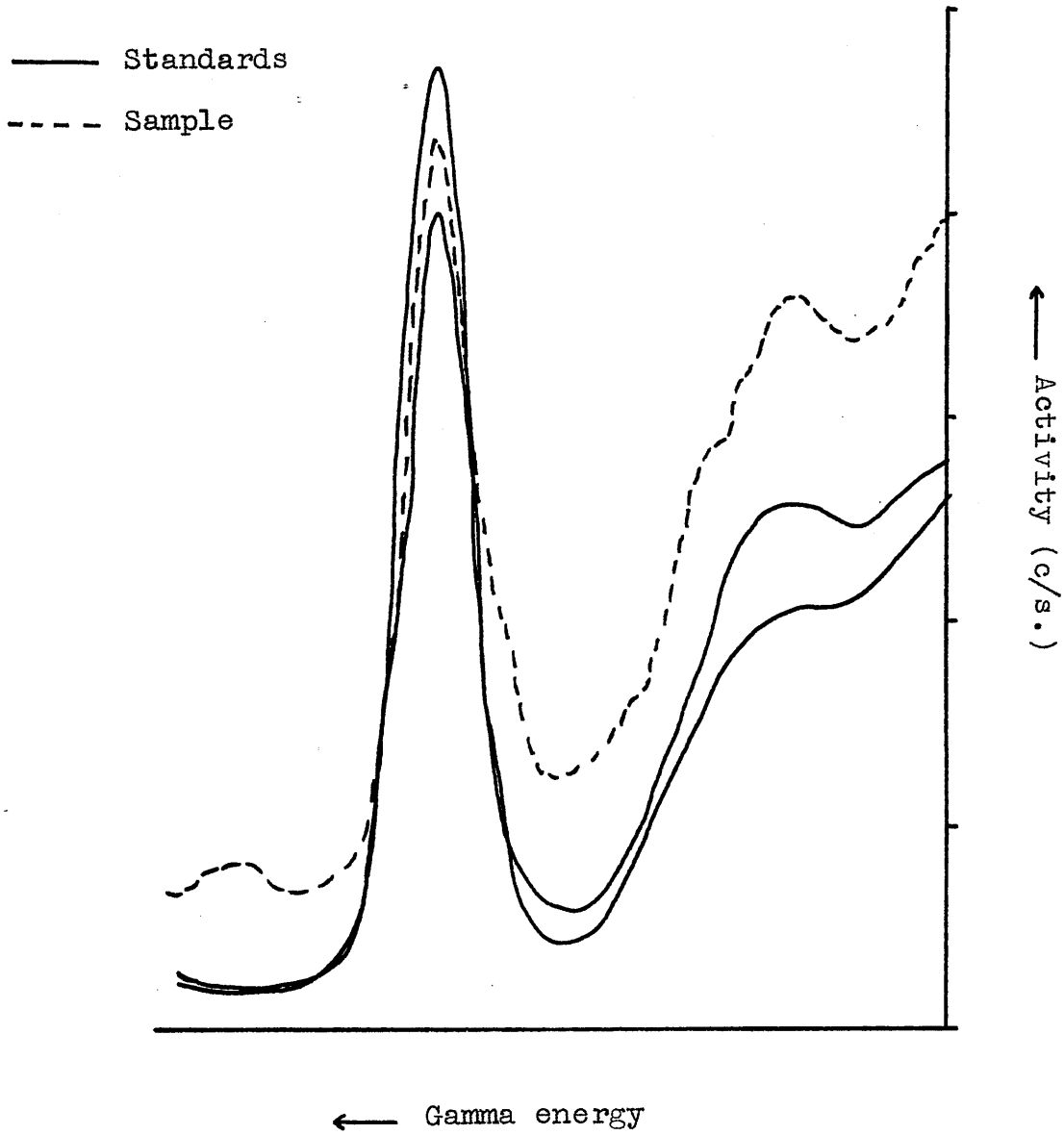
The decay curve of an ^{56}Mn and ^{54}Mn mixture



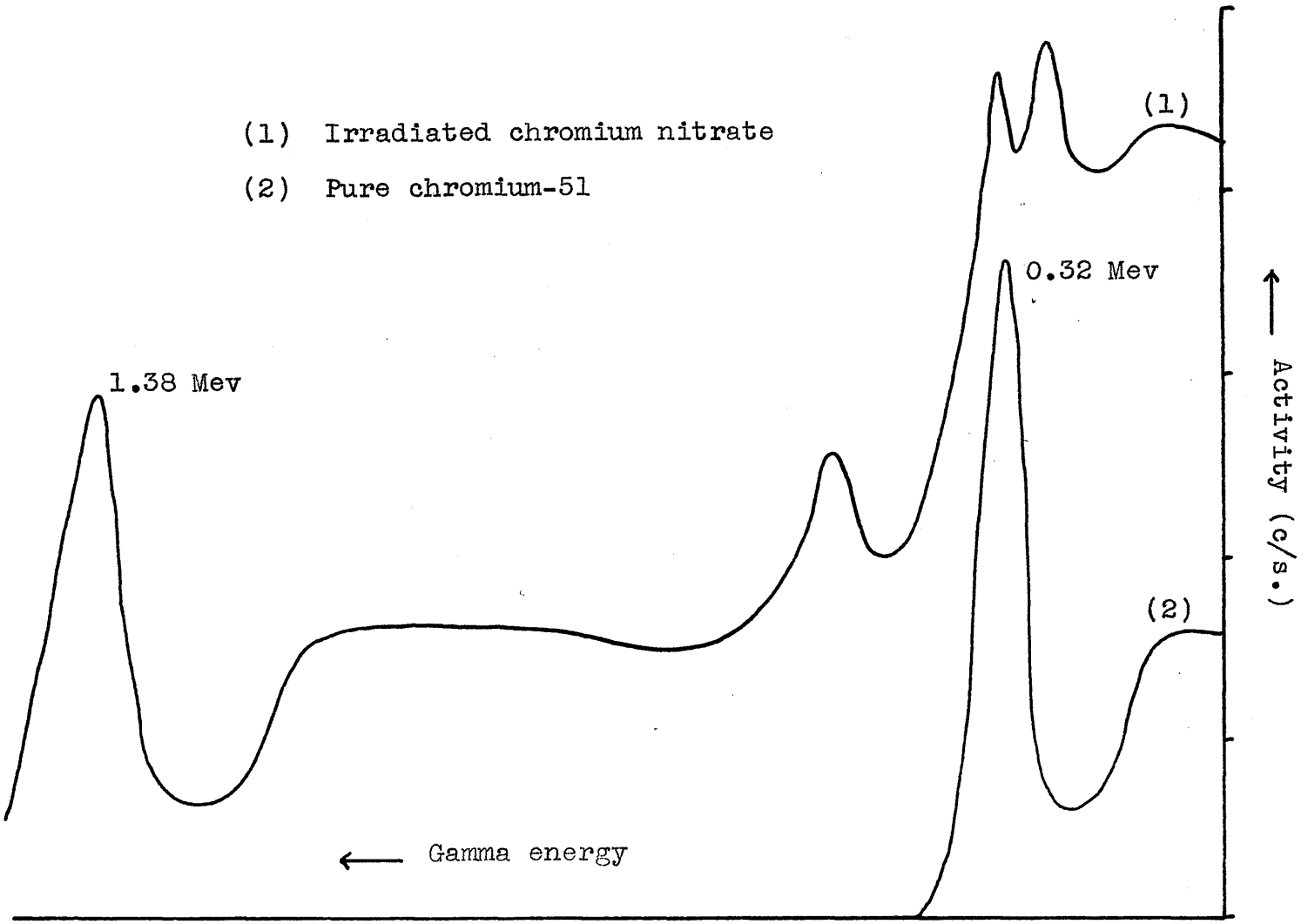
Gamma energy trace of ^{54}Mn



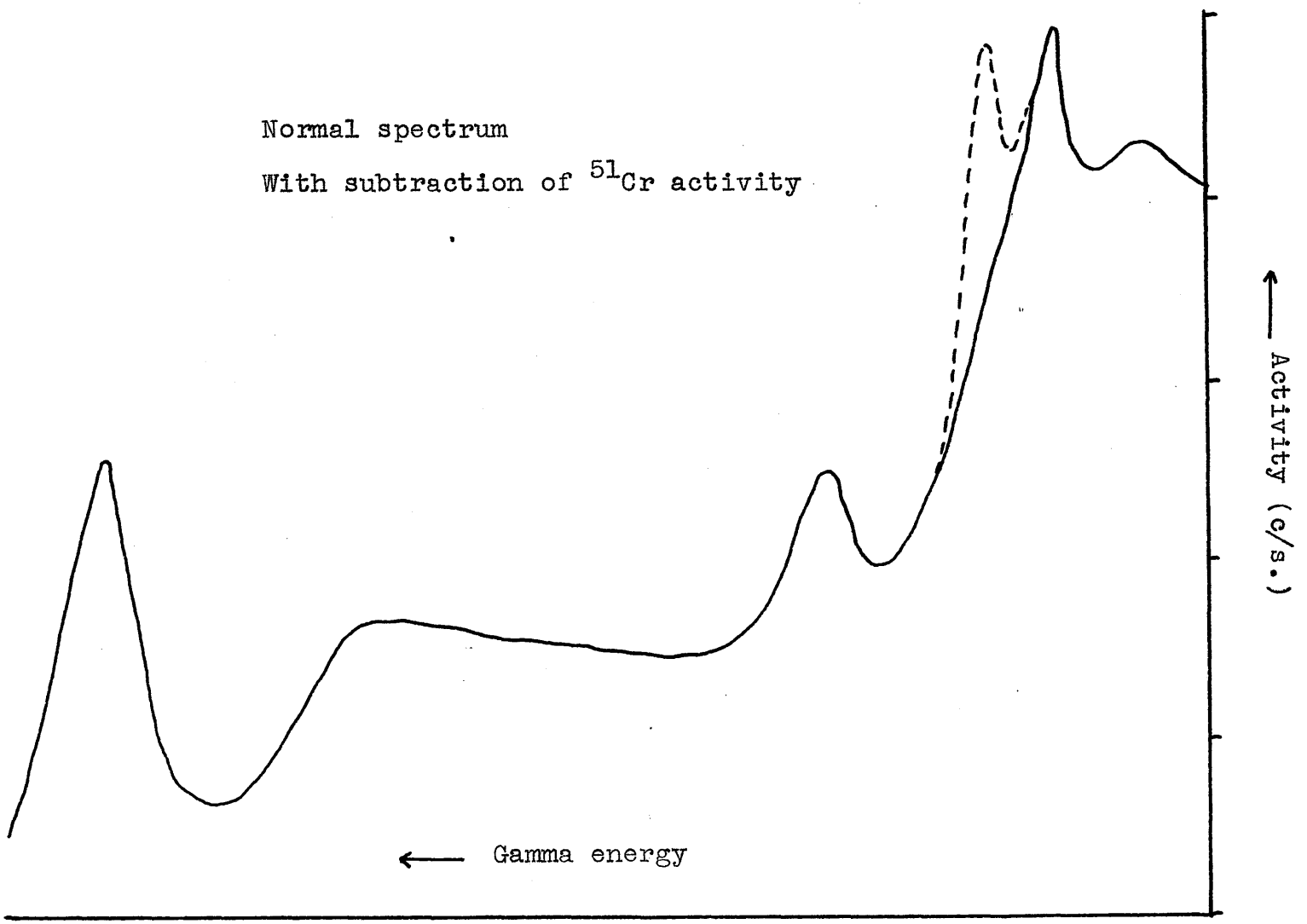
Peak height analysis (^{56}Mn)



Gamma energy spectra



Gamma energy spectra



BIBLIOGRAPHY

1. I. Curie & F. Joliot : Nature 133, 201, (1934).

2. J.D. Cockcroft, C.W. Gilbert & E.T.S. Walton, Nature 133, 328, (1934).

3. E.O. Lāwrence & D. Cooksey, Phys. Rev. 50, 1131, (1936).

4. H.R. Crane & C.C. Lauritsen, Phys. Rev. 45, 430, (1934).

5. E. Amaldi, O. D'Agostino, E. Fermi, B. Pontecorvo, F. Rasetti and E. Segre, Proc. Roy. Soc. (London) A.149, 522, (1935).

6. L.B. Ridenour & D.M. Yost, Chem. Rev. 18, 457, (1936).

7. J.J. Livingood & G.T. Seaborg, Rev. Modern Phys. 12, 30, (1940).

8. L.W. Alvarez & R. Cornog, Phys. Rev. 56, 613, (1939).

9. S. Ruben and M.D. Kamen, Phys. Rev. 57, 549, (1940).

10. G.B. Cook & J.F. Duncan, Modern Radiochemical Practice (1952).

11. Nuclear Data, U.S. Dept. of Commerce. Nat. Bureau of Standards.

12. G. Hevesy & H.B. Levi, Kgl. Danske, Videnskab, Selskab, Math-fys, Medd. 15, No.11, (1938).

13. G.T. Seaborg, Chem. Revs. 27, 199, (1940).

14. G. Hevesy, Ann. Rev. Biochem. 9, 641, (1940).

15. C.C. Cassil, J. Ass. Off. Agri. Chem., 20, 171, (1937).

16. E.G. Young & F.A.H. Rice, J. Lab. Clin. Med., 29, 439, (1944).

17. J.S. Satterlee & G. Blodgett. J. Ind. Eng. Chem. Anal. Ed., 16, 400, (1944).

18. Texas Reports Biol. Med., 9, 27, (1951).

19. A.A. Smales & B.D. Pate. The Analyst, 77, 196, (1952).

20. J. Bourret, A. Badinand & F. Serusclat. Ann. Med. Legale, 33, 144, (1953).

21. K.N. Bagchi, Indian Med. Gaz., 72, 477, (1937).

22. G. Middleton & R.E. Stuckey, The Analyst, 78, 532, (1953).

23. Kjeldahl, Z. Anal. Chem., 22, 336, (1883).

24. A.K. Klein & H.J. Wichmann, J. Ass. Off. Agri. Chem., 28, 257, (1945).

25. D.M. Hubbard, J. Ind. Eng. Chem. Anal. Ed., 13, 915 (1941).

26. N. Strafford, P.F. Wyatt & F.G. Kershaw, The Analyst, 70, 232, (1945).

27. E. Kahane & M. Pourtoy, J. Pharm., 23, 5, (1936).

28. W.E. Lawson & W.O. Scott, J. Biol, Chem., 64, 23, (1925).

29. F.V. Zaikovskii, Aptechnoe Delo, 4, 37, (1952).

30. N.K. Zagumennikova, Gigiena i Sanit, I, 41, (1953).

31. G. Middleton & R.E. Stuckey, The Analyst, 79, 138 (1954).

32. J.L. Bartlet, M. Wood & R.A. Chapman, Anal. Chem., 24, 1821, (1952).

33. G.R. Kingsley & R.R. Schaffert, Anal. Chem., 23, 914,(1951).

34. W.M. Allcroft & H.H. Green, Biochem. J., 29, 824, (1935).

35. W.J. Stainsby & A.M. Taylor, The Analyst, 66, 233, (1941).

36. R. Milton, Thesis for Ph.D. London External (1952).

37. R.J. Evans & S.L. Bandemer, Anal. Chem., 26, 595, (1954).

38. A. Fleuret, Bull. Soc. Chim., 12, 133, (1945).

39. F.P. Carey, G. Blodgett & H. Satterlee, J. Ind. Eng. Chem. Anal. Ed., 6, 327 (1934).

40. B. D. H. B.P. (1953), (appendix VI).

41. Aptechnoe Delo (3) 6, 12, (1954).

42. P.A. Mills, J. Ass. Off. Agri. Chem., 18, 506, (1935).

43. N.I. Goldstone, J. Ind. Eng. Chem. Anal. Ed., 18, 797, (1946).

44. A.K. Klein, J. Ass. Off. Agri. Chem., 3, 51, (1920).

45. C.E. Lachele, J. Ind. Eng. Chem. Anal. Ed., 6, 256, (1934).

46. M.D. Thomas & T.R. Collier, J. Ind. Hyg. Tox., 27, 201, (1945).

47. T.J. Ward, The Analyst, 51, 457, (1926).

48. A.E. Osterberg & W.S. Green, J. Biol. Chem., 155, 513(1944).

49. D. Rogers & A.E. Heron, The Analyst, 71, 414, (1946).

50. G. Taylor & J.H. Hammence, The Analyst, 67, 12, (1942).

51. T. Crawford, Biochem. J., 41, 129, (1947).

52. Ind. Chim. Belge, 17, 119, (1952).

53. Bull. Soc. Pharm. Bordeaux, 91, 245, (1953).

54. Science Reports, Tohoku Univ. Ser. A., 7, 140, (1955).

55. Chemical Abstracts, 42-2, 4487a, (1948).

56. M.B. Jacobs, J. Ind. Eng. Chem. Anal. Ed., 14, 442, (1942).

57. D.F. Boltz & M.G. Mellon, J. Ind. Eng. Chem. Anal. Ed., 19, 873, (1947).

58. E.W. McChesney, Anal. Chem., 21, 880, (1949).

59. E. Cahill & L. Walters, J. Ind. Eng. Chem. Anal. Ed., 14, 90, (1942).

60. A.E. How, J. Ind., Eng. Chem. Anal, Ed., 10, 226, (1938).

61. H. Almond, Anal. Chem., 25, 1767, (1953).

62. W.I. Stringer, J. Inst., Brewing, 60(3), 249, (1954).

63. J. Soleil, Ann. Pharm. Franc., 12, 562, (1954).

64. G.A. Levy, Biochem. J. 37, 598, (1943).

65. R. Milton & W.D. Duffield, The Analyst, 67, 798, (1942).

66. H.W. Berkhout & G.H. Jongen, Chemist Analyst, 43(3), 60, (1954).

67. Anales Asoc. quim Argentina, 37, 274, (1949).

68. W.A. Dewar & J.M.A. Lenihan, Scot. Med. J., 1, 236, (1956).

69. E.G. Young & R.P. Smith, Brit. Med. J., --, 251, (1942).

70. A. Lucas, Forensic Chem. & Sci. Criminal Investigation 2nd, Ed. p. 268.

71. D.M. Hubbard, J. Ind. Eng. Chem. Anal. Ed., 13, 915, (1941).

72. E. Schaaf & J. Maurer, Z. Anal. Chem., 126, 298, (1943).

73. W.D. Treadwell, Helv. Chim. Acta, 5, 818, (1922).

74. Ann. Chim. Applicata, 37, 384, (1947).

75. J.A. Sultzaberget, J. Ind. Eng. Chem. Anal. Ed., 15, 408, (1943).

76. Klug & Lampson, Proc. South Dakota Acad. Sci., 27, 50,(1948).

77. A.A. Smales & B.D. Pate, Anal. Chem., 24, 717, (1952).

78. A.A. Smales & B.D. Pate, The Analyst, 77, 188, (1952).

79. G.H. Morrison & J.F. Cosgrove, Anal. Chem., 28, 320, (1956).

80. H.J. Magnuson & E.B. Watson, J. Ind. Eng. Chem. Anal. Ed., 16, 339, (1944).

81. A.L.,Chaney & H.J. Magnuson, J. Ind. Eng. Chem. Anal. Ed., 12, 691, (1940).

82. T.H. Maren, J. Ind.,Eng. Chem. Anal. Ed., 18, 521, (1946).

83. J.C. Bartlet, H. Wood & R.A. Chapman, Anal. Chem., 24, 1821, (1952).

84. The Analyst, 45, 8, (1920).

85. Ann. Chim. Anal., 28, 24, (1946).

86. S. Kaye, Am. J. Clin. Path. Tech. Sect., 8, 36, (1944).

87. J.G.A. Griffiths, The Analyst, 66, 491, (1941).

88. A.O. Gettler & S. Kaye, J. Lab. Clin. Med., 35, 146, (1950).

89. J. Chem. Soc. Japan, Pure Chem. Sect., 74, 79, (1953).

90. A.I. Vogel, A Text Book of Macro & Semimicro Qualitative Inorganic Analysis., 4th Ed. p.245.

91. W.B. King & F.E. Brown, J. Ind. Eng. Chem. Anal. Ed., 5, 168, (1933).

92. J. Chem. Soc. Japan, Pure Chem. Sect., 73, 501, (1952).

93. B.S. Evans, The Analyst, 54, 523, (1929).

94. J.A. James & D.H. Richards, Nature, 175, 769, (1955).

95. Yukito Yosida, J. Chem. Soc. Japan, 61, 1239, (1940).

96. L. Couchak, Ann. Chim. Anal. Chim. Appl.(2), 4, 138, (1922).

97. H. Kleinmann & F. Pangritz, Biochem. J., 14, 185, (1927).

98. C.B. Goyanes, E.S. Serrano & C. Gomes, Bol. Radioactiv., 26, 37, (1954) and Z. Anal. Chem., 120, 217, (1940).

99. R. Pieruccini, Spectrochimica Acta., 4, 189,(1950).

100. W.C. Davies & C. Rey, The Analyst, 72, 17, (1947).

101. C.L. Luke, J. Ind. Eng. Chem. Anal. Ed., 15, 626, (1943).

102. F.H. Burstall, G.R. Davies & R.P. Wells, Nature, 163, 64, (1949).

103. F.H. Burstall, G.R. Davies, Linstead & R.P. Wells, J. Chem. Soc., 516, (1950).

104. Jentzsch & Frotscher, Analytical Abstracts, --, 1133,(1955).

105. Arch. Ital. Sci. Farmacol.(3), 226, 255, (1955).

106. I.I.M. Elbeih, J.F.W. McOmie & F.H. Pollard, Disc. Farad. Soc., 7, 183, (1949).

107. Anales Asoc. quim Argentina, 37, 274, (1949).

108. C.C. Cassil, J. Ass. Off. Agri. Chem., 24, 196, (1941).

109. C.C. Cassil & H.J. Wichmann, J. Ass. Off. Agri. Chem., 22, 436, (1939).

110. H.L. Burrill, J. Ass. Off. Agri. Chem., 26, 489, (1943).

111. A.O.A.C. Methods of Analysis, 1935. p.391.

112. A.K. Klein & F.A. Vorhes, J. Ass. Off. Agri. Chem., 22, 121, (1939).

113. Naunyn Schmiedebergs, Arch. Exptl. Pathol. Pharmakol., 226, 255, (1955).

114. D.K. Gullstrom & M.G. Mellon, Anal. Chem., 25, 1809,(1953).

115. J. Thuret, J. Pharm. Chim., 26, 18, (1937).

116. R.F. Milton & W.A. Waters, Methods of Quantitative Micro Analysis, 395.

117. K. Bambach, J. Ind. Eng. Chem. Anal. Ed., 14, 265, (1942).

118. Zhur. Anal. Khim., 3, 16, (1948).

119. Zhur. Anal. Khim., 4, 89, (1949).

120. Bengt Forss. Acta Acad. Aboensis Math. & Phys., 17 (3), 1-120, (1951).

121. W.J. Ramsey, P.S. Farrington & E.H. Swift, Anal. Chem., 22, 332, (1950).

122. G.W. Everett & C.N. Reilly, Anal. Chem., 26, 1750, (1954).

123. W.J. Ramsey, P.S. Farrington & E.H. Swift, Anal. Chem., 22, 332, (1950).

124. N.H. Furman & A.J. Fenton, Anal. Chem., 28, 515, (1956).

125. M. Milbourn & H.E.R. Hertley, Spectrochim, Acta, 3, 320, (1948).

126. G.H. Morrison & J.F.,Crosgrove, Anal. Chem., 25,1095, (1953).

127. H. Griffon & J. Barbaud, Comptes Rend., 232, 1455, (1951).

128. H. Griffon & J. Barbaud, Annales Pharm. Franc., 9, 545, (1951).

129. A.A. Smales, Internat. Confer. on the Peaceful Uses of Atomic Energy, (1955).

130. E.N. Jenkins & A.A. Smales, Quart. Revs., 10, 83, (1956).

131. R. Belcher, Annual Reports, Chem. Soc., 52, 370, (1955).

132. J.T. Odencrantz & W. Tieman, Anal. Chem., 22, 1066, (1950).

133. W.W. Meinke, Science, 121, 177, (1955).

134. Bertrand, Gauthier & Beltier, Treatise.

135. V. Brustier, P. Bourdon & R. Vignes, Ann. Pharm. Franc., 7, 729, (1949).

136. L. Derobert & R. Le Breton, Ann. Med. Leg., 21, 35,(1951).

137. J.M.A. Lenihan et al., West. Reg. Hospital Board Physics Dept., Glasgow, Personal Communication.

138. J.M.A. Lenihan, H. Smith & J.G. Chalmers, Nature, 181, 1463, (1958).

139. J.M.A. Lenihan, H. Smith, 2nd International Conference on Peaceful Uses of Atomic Energy (1958), Paper 69.

140. E.J. Bailey, E.L. Kennaway & M.E. Urquhart, B.J. Cancer, 11, 49, (1952).

141. R.E. Remington, J.A.C.S., 49, 1910, (1927).

142. O.A. Gross & R.E. Nelson, Amer. J. Public Health, 24, 36, (1934).

143. M.E. Daff & E.L. Kennaway, B.J. Cancer, 4, 174, (1945).

144. Royal Beatson Memorial Hospital, Brit. Emp. Cancer Camp. -, 298, (1954).

145. H. Griffon, Ann. Pharm. Franc. 13, 258, (1955).

146. H. Griffon & R. Le Breton, J. Med. Bordeaux et Sud-Ouest 134, 776, (1957).

147, I.M. Kolthoff & E.B. Sandell, J. Ind. Eng. Chem. Anal. Ed., 1. 181, (1929).

148. F. Nydahl, Anal. Chim. Acta, 3, 144, (1949).

149. H.C. Davis & A. Bacon, Selected Govt. Research Reports, 3, 296, (1951).

150. J. Andrews, G. Harrison & J. Pierce, J. Inst., Brewing, 59, 293, (1953).

151. K. Kimura & Y. Murakami, Mikrochim, Acta, 36, 727, (1951).

152. A.I. Vogel, Quantitative Inorganic Analysis 2nd. Ed., P.648.

153. R. Copeman, J. Forensic Med. 2, 55, (1955).

154. E.M. Gates & G.H. Ellis, J. Biol. Chem, 168, 537, (1947).

155. C.H. Gentry & L.G. Sherrington, The Analyst, 75, 17, (1950).

156. P.F. Wyatt, The Analyst, 78, 656, (1953).

157. J. Hoffmann, Chem. Zentr, 2, 642, (1941).

158. M. Tsutomu, J. Chem. Soc. Japan, Ind. Chem. Sect. 55, 418, (1952).

159. C. Barcia-Goyanes & E. Serrano, Bol. Radiactividad. (Madrid), 24, 34, (1951).

160. L, Barzaghi, Anais. Assoc. quim Brazil, 2, 187, (1943).

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

162. F. Hein & H. Holzapfel, Chem. Zentr. 1, 1114, (1942).

163. S. Hikime, Japan Analyst, 3, 239, (1954).

164. E. Hluchan & J. Mayer, Chem. Listy. 47, 846, (1953).

165. R.N. Golovatzi & V.M. Sologub, Lab. Prakt. (U.S.S.R.), 15, 23, (1940).

166. L. Gordon, H. Teicher, & B.P. Burtt, Anal. Chem., 26, 992, (1954).

167. K. Neelakantam, Proc. Indian Acad. Sci., 27A, 202, (1949).

168. K. Neelakantam, Current Sci., 10, 21, (1941).

169. A. de Sousa, Anal. Chim. Acta. 7, 393, (1953).

170. M. Borrel & R. Paris, Anal. Chim. Acta., 4, 267, (1950).

171. H. Bode, A. Anal. Chem. 144, 165, (1955).

172. A.K. Majumdar & A.K. Dee, J. Indian Chem. Soc, 30,123, (1953).

173. T.S. West, Metallurgia, 47, 97, (1953).

174. P.H. Bell, J. Ind. Eng. Chem. Anal. Ed., 10, 579, (1938).

175. E. Batt, R. Nussbaum, T. Gilmour & S. Didio, Amer. J. Clin. Pathol. 24, 385, (1954).

176. J. Hess, J. Owens, L. Reinhardt, J. Ind. Eng. Chem. Anal. Ed., 11, 646, (1939).

177. E.V. Jaycox, Anal. Chem., 27, 347, (1955).

178. D. Ewing, M. Wilson & R. Hibbard, J. Ind., Eng., Chem., Anal., Ed., 9, 410, (1937).

179. J. Perman, Bull, Sci. Conseil Acad. R.P.F., Yougoslav., 1, 42, (1954).

180. T. Yosimura, J. Faculty Sci. Hokkaido Imp. Univ. 4, 113, (1938).

181. T. Vanstone & R. Philcox, Ann. Repts. East Malling Research Station Kent. --, 105, (1949).

182. J. Dolezal & J. Adam., Chem. Listy. 49, 138, (1955).

183. R. Kalvoda & J. Zyka, Chem. Listy. 45, 462, (1951).

184. G.B. Jones, Anal. Chim. Acta. 11, 88, (1954).

185. V. Vandenbosch, Ind. Chim. Belge. 16, 668, (1951).

186. G. Carleson, Acta. Chem. Scand., 8, 1673, (1954).

187. D.E. Carritt, Anal. Chem., 25, 1927, (1953).

188. H.H. Strain, Anal. Chem., 24, 356, (1952).

189. K. Lakshminarayanan, Proc. Indian Acad. Sci. --, 167, (1954) and Chem. Abs., --, 7635f, (1955).

190. K.A. Kraus & G.E. Moore, J. Am. Chem. Soc., 75, 1460,(1953).

191. J. Charney & W.P. Fisher, Science, 114, 687, (1951).

192. R. Hara & R.W. West, Anal. Chim. Acta. 12, 72, (1955).

193. H. Flaschka, Mikrochim Acta. 38, 38, (1952).

194. G. Duyckaerts, Anal. Chim. Acta. 5,2233, (1951).

195. G. Goffart, G. Michael & T. Pitance, Anal. Chim. Acta, 1, 393, (1947).

196. T. Shiokawa & S. Suzuki, Sci. Repts. Res. Inst. Tohoku Univ. Ser A, 3, 419, (1951).

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

198. C. Fisher & J. Beydon, Bull. Soc, Chim. France, --, c102, (1953).

199. P. Iredale, A.E.R.E. report EL/M 96, (1957).

200. D.H. Peirson, Atomics, 7, 316, (1956).

201. P. Copeman, J. Forensic Med., 2, 118, (1955).

202. G. Bertrand & F. Medigreceanu, Ann. Inst. Pasteur, 26, 1013, (1913).

203. C. Reiman & A. Minot, J. Biol. Chem., 42, 329, (1920).

204. R. Kehoc, J. Cholak, R. Story, J. Nutrition, (1940).

205. G. Bruckmann & S. Zondek, Biochem. J., 33, 1845, (1939).

206. J. Sykora, Pracovni Lekarstvi, 7, 22, (1955).

207. R. McCracken & E. Passamaneck, Arch., Path. Lab. Med., 1, 585, (1926).

208. A. Broek & L. Wolff, Acta Brevia Neerland, Physiol. Pharmacol. Microbiol. 5, 80, (1935).

209. S. Tribalat & J. Beydon, Anal. Chim. Acta, 8, 22, (1953).

210. H. Willard & G. Smith, J. Ind. Eng. Chem. Anal. Ed., 11, 305, (1939).

211. H. Willard & G. Smith, J. Ind. Eng. Chem. Anal. Ed., 11, 269, (1939).

212. H. Willard & G. Smith, J. Ind. Eng. Chem. Anal. Ed., 11, 186, (1949).

213. R. Thomson & H. Askew, New Zealand J. Sci. Technol, 37A, 584, (1956).

214. D.H. Peirson, Nature, 173, 990, (1954).

215. D.H. Peirson, Brit. J. Appl. Phys., 6, 444, (1955).

216. J.L. Putman & W.H. Taylor, Intern. J. App. Radiation and Isotopes, 1, 315, (1957).

217. R.C. Hendrix, Dept. of Pathology, Michigan, Personal communication.

218. P.R. Peacock, Royal Beatson Memorial Hospital, Glasgow, Personal communication.

219. E. Rentoul, Dept. Forensic Med., Glasgow, Personal communication.