DETERMINATION OF INDIUM IN BIOLOGICAL MATERIAL

ON THE DETERMINATION OF INDIUM IN BIOLOGICAL MATERIAL BY NEUTRON ACTIVATION ANALYSIS

By

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SCOPE AND CONTENTS:

Neutron activation has been investigated as a method suitable for the analysis of biological material for indium. Irradiations followed by chemical separation were performed on four materials; calf brain, pine leaves, kale and rabbit liver. The procedure developed was critically evaluated and indium concentrations in the pine leaves, kale and rabbit liver are reported for the first time. The values found were 2.20 ± 0.42 ppb, 0.69 ± 0.10 ppb, and 0.032 ± 0.007 ppb, respectively.

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STATEMENT OF THE PROBLEM

The importance of the role of trace elements in governing life processes has led to a demand for increasingly sensitive analytical techniques for ever more exotic elements. The determination of the rare metal, indium, in biological material has received very little attention to date, but experiments on the effects of trivalent elements on brain tissue have indicated that this is one of the more potentially harmful elements. In order to demonstrate its involvement in causing brain damage, it is necessary to be able to measure concentrations both in healthy tissue and that displaying the characteristic symptoms. The aim of the work presented in this thesis is to develop a method for the determination of indium in biological material.

Indium is scarce, with a geochemical abundance estimated at 0.11 ppm in the earth's crust. Biological materials contain much lower trace element concentrations than do geological matrices and the concentrations sought could be several orders of magnitude less than this. Measurements in the part per billion concentration range would be necessary. A difficulty immediately arises in finding a method which is sensitive enough to make accurate determinations at these concentrations. The establishment of 'normal' levels and distinguishing between these and pathologically significant values demands precision, as well as accuracy, since differences could be small in comparison to the amounts present. The neutron activation method selected was applied to a variety of materials, plant and animal, in order to establish its accuracy and precision and consequently its ability to distinguish differences at these concentrations.

CHAPTER I

INTRODUCTION

1.1 General

Improved techniques for trace analysis have resulted in the demonstration of the presence of nearly every element in plants and animals. Trace elements in mammals and man are usually considered to be those found in concentrations of less than 0.01% of the body's mass and would not include carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, potassium, sodium, chlorine, calcium and magnesium. Trace elements can be divided into two broad categories. The first group, those which have an essential physiological role, includes elements such as cobalt, chromium, copper, fluorine, iron, iodine, manganese, molybdenum, selenium, silicon, tin, vanadium, zinc and possibly nickel.[1,2] Those for which no essential function has yet been found, the nonessential or "accidental" trace elements include, for example, aluminum, barium, beryllium, cadmium, lithium, mercury and lead. Indium would at present also be included in this group. In fact, if sufficiently sensitive methods were available every element, except those mentioned in the first two categories, would be in this group.

The association of certain trace elements with pathological abnormalities and the investigation of correlations with disease states have recently been reviewed by Schroeder and Nason,[1] and Leddicotte,[3] and involvement in cerebral and nerve pathology has been shown.[4] The discovery that aluminum compounds, introduced artificially into rabbit

brains, produced neurofibrillary degeneration similar to that associated with Alzheimer's disease[5,6] indicated that trace elements may play a role in neurological disorders in which unexplained cerebral atrophy occurs. In addition to Alzheimer's disease (presenile dementia, characterized by cortical atrophy in the frontal and temporal lobes of the brain and neurofibrillary degeneration), syringomeyelia (cavitation in the central canal of the spinal cord, frequency extending up into the medulla) and low pressure hydrocephalus (senile dementia in which there is an enlarged ventricular system) are also characterized by neuronal loss and dilation.

The brain is kept in a constant chemical environment provided by the cerebrospinal fluid, insulated from changes occurring in the rest of the body by the blood-brain barrier, a concept describing the hindrance to the exchange of solutes from the blood to the tissue and cerebrospinal fluid. It is possible then, that diseases whose symptoms resemble those caused by the artificial introduction of trace elements to the brain may be due to a breakdown of this barrier.

While aluminum induces only neurofibrillary degeneration and not cell atrophy, it is thought that the latter might be caused by other trivalent elements.[7] Experiments were done by Crapper to study the effects of boron, gallium, indium, lanthanum, scandium, thallium, yttrium, vanadium and chromium (the last two as the trichlorides) on rabbits.[7] Dilute solutions of compounds, which would be soluble at, or near, the physiological pH, were introduced into the cerebral hemisphere near the nucleus caudatus, via a hole drilled through the skull. Neuronal destruction was caused only by indium, scandium and yttrium, the most toxic being indium, which produced widespread cerebral atrophy and marked

dilatation of the lateral and third ventricle, similar to that observed in connection with low pressure hydrocephalus.[7]

Very little is known about the biochemistry of indium. Its toxic properties were originally studied because of concern about the potential hazards to industrial workers in close contact with the metal.[8,9,10] The development of techniques in nuclear medicine which employed indium-113 as a short-lived isotope for internal scanning,[11-13] led to further investigations.[10,14,15] Briefly, these studies revealed that ionic indium and a colloidal suspension of indium hydroxide are cleared rapidly from the blood and are accumulated in the liver, kidney and spleen and to a lesser extent in bone, muscle and lung. Accumulation in brain tissue has not been studied to date.

Indium is one of the most toxic elements when administed parenterally, although only slightly so when injested orally or inhaled. Severe damage occurs to the liver and kidney, similar to that caused by mercury. Lethal doses for parenteral administration are listed below in Table 1.1.

Table 1.1

Lethal Doses of Indium

Animal	Dose (mg/kg body weight)	Chemical Form Administered
mice	.323	ionic chloride[14]
rats, rabbits, dogs	0.33-3.6	chloride chelated as citrate complex[16]
mice	12.5	hydroxide[14]
rabbits	100	trioxide[16]
rats	955	trioxide[16]

In the experiments of Crapper previously mentioned, doses of indium sulfate solution containing 10,1 and 0.1 micromoles of indium (1.15, 0.115 and 0.0115 mg) were administered. The first two quantities produced death in less than 24 hours, while the last dose permitted survival to 84 days, after which the animal was sacrificed. Examination of the brains revealed that cerebral atrophy had occurred in all three animals, although in the case of the 0.0115 mg dose this had not resulted in death.

The rabbit brains weighed approximately 30 grams hence neuronal destruction was caused by less than 0.4 ppm (0.0115 mg indium per 30 g) indium in the tissue. Since some of the injected solution would be lost due to leakage out of the burr hole in the skull and dispersion through blood vessels into the blood stream, amounts as low as 5 ppb to 10 ppb indium in brain tissue were felt to be significant in causing the damage.[7]

1.2 Indium in Biological Material

There are a number of ways in which trace elements are accumulated by animals; through injestion, inhalation and absorption. Concentrations encountered in the environment will give some indication of the levels that can reasonably be expected in biological tissues. Reviews of the relationships between trace elements in the environment and biological systems have been written by Bowen[17] and Underwood.[18]

Indium was placed by Mendeleev in a class of elements which he described as "occurring even more rarely [than other infrequently encountered elements] and without any practical applications".[19] This is an accurate comment on its abundance. Since that time, a number of

important uses have been found; as an antiwear agent in bearings, a coating to prevent corrosion in alloys, an intermetallic in the production of transistors, a surface coating to form mirrors and an ingredient in coloured glass and dental alloys.

It is a rare, widely distributed metal, occurring in concentrations of approximately 0.11 ppm or 10^{-5}% weight of the earth's crust, about the same order of magnitude as silver and antimony and about 200 times higher than gold or platinum.[20,21] No specific indium minerals are known; rather it has a strong tendency to concentrate in zinc blende and is also found in iron, manganese, tin and aluminum ores.[22]

Information on the levels of indium in biological material is scarce. Quite possibly this is a reflection of the lack of commonly available analytical methods and of the low natural abundance, evident in Table 1.2, which shows concentrations in some geochemical matrices.

Table 1.2

Indium in the Environment

Matrix		Concentration (ppm)	Ref.
Rocks			
Standard Rocks G-1		0.025	25
W-1		0.07	25
Skaergaard Intrusion (6 samples)		0.054-0.18	26
6 new USGS Standard Rocks		0.0027-0.109	27
Sea Water			
Atlantic Ocean	Aerosol	$1.30\text{-}8.62 \times 10^{-4}$ ug/m^3	28
	2 ft	0.31×10^{-6}	29
	2000 ft	0.11×10^{-6}	29
Pacific Ocean	0 ft	5×10^{-3}	30
	2588 ft	6×10^{-3}	30
Rain Water		$2.69\text{-}4.68 \times 10^{-6}$	31
Air borne Particulates			
Surrey, England		$0.1\text{-}6.7 \times 10^{-4}$ ug/m^3	32
Indiana		0.1×10^{-3} ug/m^3	33

It has been determined in a wide variety of inorganic materials and in some cases there have been suggestions for modification of techniques so that they might be applied to biological materials.[23,24]

Of the methods commonly applied to the analysis of biological material for inorganic constituents, only neutron activation, mass spectrometry and emission spectrometry have been successful in quantitatively determining indium. In addition, there have been numerous analyses in which indium could not be detected, but from which an upper limit of its concentration could be calculated. For example, an examination of human fluids and tissues by X-ray fluorescence placed the relative detection limit at 0.6 ppm for this technique.[34] Atomic absorption analysis of urine using the standard addition method gave 0.01 ppm as the lowest concentration that could be detected.[35]

While determinations have been made by mass spectrometry and emission spectrometry on some materials, it is not always possible to measure the indium content of every tissue. For example, spectral interferences have prevented the quantitation of indium in whole blood and sugarbeet leaves by mass spectrometry.[36,37] Emission spectrometry has likewise proven unsuccessful in determining indium in blood and citrus leaves.[38,39]

Some materials in which indium has been quantitatively determined are given in Table 1.3, page 7, along with the method used, the detection limit for indium and the concentrations found. All of these experiments were multielement analyses with the exception of the rat brain study, which was concerned with only indium and gallium.[40] In this study

Table 1.3

Analysis of Biological Materials for Indium

Method[1]	Material	Concentration (ppm)	Absolute Detection Limit (ng)	Reference
MS	Human Kidney Tumor	70	0.1	37
	Sheep Bone	0.02		37
	Sheep Lung	0.01		37
		0.23,1.0		41
ES	Sheep Lung	1.3,0.5	10	42
NAA	Rat Brain	0.051	0.005	40
	Meat;	Ash / Bulk		43
	Chicken	0.75 / 0.0047		
	Beef	1.3 / 0.0092		
	Pork	0.47 / 0.0022		
	Ham	0.55 / 0.011		

1. MS, Mass Spectrometry; ES, Emission Spectrometry; NAA, Neutron Activation Analysis.

and in the analyses of the meat samples the determination of indium was one of the objectives of the experiment. The other investigations, by mass spectrometry and emission spectrometry, were concerned with the determination of as many elements as possible and not the optimization of conditions for determining indium.

These analyses show a wide range of concentrations, from 70 ppm for human kidney tumor tissue[37] to 2.2×10^{-3} ppm for pork.[43] To some extent, these variations were due to the methods used to express the final results. Kist and Lobanov,[40] reported concentrations for the rat brain as air-dried tissue, Koch and Roesmer[43] reported values for both the dry ash and the bulk weight of the meat samples and Morrison,[37]

Evans and Morrison[41] and Bedrosian et al.[42] gave concentrations for freeze-dried samples of the kidney tumor, sheep bone and lung. The fact that indium is an "accidental" trace element can lead to sizeable variations as well, from one animal to another in the same tissue.

The concentration of indium determined for the rat brain is of particular interest.[40] This is the only value of indium in brain tissue previously reported and is useful as a guide for levels expected in other mammals and man. It is higher by a factor of ten than the amount suspected of causing damage in rabbit brain[7] and of approximately the same order of magnitude as concentrations reported for a variety of muscle tissues.[43] Since the brain is insulated from the rest of the body by the blood-brain barrier, comparable concentrations in muscle and brain would not be expected. While, as mentioned before, the concentration of indium may vary considerably between different species of mammals, the value reported by Kist and Lobanov for the rat brain will be considered as an upper limit of the amounts expected to be present.

A relative error of ± 15% was quoted for this result. Though the sources of error were not specified, the result was an average of one analysis per brain for six animals. Small samples were used, consisting of 10 mg of dried tissue, and irradiations were only one hour in length so that counting errors might have been large.

The results quoted for the multielement analysis of the meat samples were estimated by the authors to have a maximum error of ± 10%, but errors for individual elements were not given. Only one multielement analysis was performed on each meat, so this error does not include the variations of replicate analyses. Two gram quantities of the dry-

ashed sample were taken so the results are probably quite representative of the materials. No details of the procedures for radiochemical separations were given nor was there any indication of the counting statistics obtained. The latter may be assumed to be the largest source of error and the one on which the quoted value is based. The ± 10% error is probably a good indication of that associated with indium. The authors concluded that their results were indicative of the order of magnitude of the concentrations rather than being typical of those meats.[43]

The mass spectrometric results reported by Morrison[37] and Evans and Morrison[41] were based on a visual comparison of the indium line intensity with that of an internal standard and the assumption that the sensitivity of the recording emulsion was the same for indium and the internal standard in every matrix. This is the reason for the large error associated with their results (a factor of 3) and the values can be considered semi-quantitative at best. The value of 70 ppm for the human kidney tumor seems anomalously high and is most likely due to spectral interferences, which prevented the determination of indium in whole blood and sugarbeet leaves.

By comparison, Samsahl, Brune and Wester in a multielement analysis of cancerous and non-cancerous human liver and kidney tissue by neutron activation with radiochemical separation, reported the presence of faint activity from indium in one of the fractions.[44] This was so small, however, that they made no attempt to measure the indium quantitatively. A concentration of 70 ppm would mean that indium was present in the kidney in larger amounts than copper and manganese which were easily measured by Samsahl *et al.*, and which are much more abundant in nature

than indium. In light of this analysis of kidney tissue, the value of 70 ppm appears erroneous.

The concentrations for sheep lung, found using emission spectrometry, are approaching the detection limit of 0.01 μg per electrode, or 0.4 ppm, since 25 mg samples were used. The error in the determination is therefore likely to be close to the maximum of 30% quoted for the method. Agreement between these results and sheep lung concentrations obtained by mass spectrometry seems quite reasonable, considering the limitations of the methods. Lungs from two separate animals were employed, hence the reporting of two results.

The possible involvement of indium in neurological disorders, the limited number of determinations and lack of repetition of analysis of one material, inspired the work presented in this thesis. Neutron activation, which is the only method sensitive enough to determine ultra-trace quantities of indium in the part per billion concentration level, was applied to the analysis of a variety of biological samples including pine leaves, rabbit liver, calf brain and a standard kale. Calculations indicated that this method would have the required sensitivity to distinguish differences in concentrations at the part per billion level. It was hoped that the accuracy and precision would be sufficient to permit its eventual application to the analysis of healthy and diseased brain tissue to determine the role of indium in certain neurological disorders.

1.3 Neutron Activation Analysis

1.3.1 General Considerations

In selecting a method for any determination, sensitivity, selectivity,

precision and accuracy should be considered. Because of the low concentrations expected, sensitivity became the essential criterion for the present work. Since biological material normally has a much lower metal content than geological material,[45] many methods successfully applied to quantitating indium in geological material would not be suitable for the present problem. Of the five techniques which have been applied to biological material, only three (neutron activation, mass spectrometry and emission spectrometry) were successful.

Evans and Morrison found the limit of detection for indium by emission spectrometry to be 10^{-8} g per electrode with 25 mg of freeze-dried biological material.[42] Improved sensitivity with decreasing sample size was observed, but the difficulties in ensuring that a representative sample is taken, make the use of smaller amounts impractical. The relative limit, then, is only 0.4 ppm and the method is obviously unsuitable for the present work, where concentrations in the low part per billion region are expected.

Direct spark source mass spectrometry has a lower absolute limit of detection (10^{-10} g) than emission spectrometry.[37,41] The relative limits of detection are also improved by preconcentration of the sample, through ashing and the larger weight of material analysed (80 mg). The ashing is necessary to eliminate spectral interferences from organic ions, but increases the probability of contaminating the sample. Even after mineralization, the major constituents carbon, oxygen, nitrogen, phosphorus, etc., form singly and multiply charged ions, and combine to produce molecular ions, causing interferences with some trace elements. These interferences vary with the particular matrix and, as mentioned

earlier, have prevented the determination of indium in whole human blood and sugarbeet leaves.

The third method, neutron activation, has the lowest limit of detection for indium, which has been reported as 5×10^{-12} g.[26,46,47] This figure refers to the production and measurement of 40 dps at the end of an irradiation and, hence, is a somewhat theoretical number unless it is a sample of pure indium that is being irradiated. Yule has determined the sensitivity of instrumental neutron activation for 65 elements (including indium) in five fluids; whole blood, milk, urine, tap water and "pure" water, as well as in polyethylene vials.[48] The sensitivity for indium varied from 0.1 ppm in whole blood and urine to 0.00006 ppm in "pure" water. It is not surprising that the application of this method to Bowen's standard kale was unsuccessful in quantitating the indium present and sets the lower limit of detection in this matrix at 0.3 ppm.[49] Clearly, a chemical separation is necessary if the ultimate sensitivity obtainable with neutron activation is to be achieved on complex matrices.

The sensitivity was calculated for the conditions expected in the present experiments from the equation

$$\text{Weight of Element} = \frac{C \times M}{6.023 \times 10^{23} \sigma \phi A (1-e^{-\lambda t_1})(e^{-\lambda t_2}) t_3 I E} \qquad (1.1)$$

where;

C is the number of counts desired (taken as 1000),

M is the atomic weight (115 amu),

σ is the thermal neutron cross-section (154×10^{-24} cm^2),

ϕ is the neutron flux (5×10^{13} $n/cm^2/sec$ at the McMaster reactor),

A is the fractional isotopic abundance (.957),

t_1 is the irradiation time in seconds (7200 sec (2 hr)),

t_2 is the cooling time in seconds (7200 sec),

t_3 is the counting time in seconds (taken as 1000 sec),

I is the relative gamma intensity (33% for the 417 keV gamma of indium-116ml), and

E is the relative detector efficiency (7% at 417 keV).

While the lower limit of detection found, 1×10^{-11} g, is higher than the previous value quoted (5×10^{-12} g) it takes into account the cooling period while radiochemical separations are being performed, the intensity of a particular gamma ray and the efficiency of the Ge(Li) detector, while the former refers to the total number of disintegrations produced at the end of the irradiation. The only factor not taken into consideration in the present calculations was the chemical yield of the separation.

Like the other two methods, the detection limit for neutron activation can be lowered further by preconcentration, although this increases the risk of contamination, which is virtually eliminated by performing chemical separations after the irradiation. It is also true, that in general, the maximum sensitivity is obtained only after radiochemical separations have been employed, either to isolate the element of interest or remove major matrix constituents. As mentioned earlier, the detection limit for instrumental neutron activation is highly dependent on the composition of the matrix, the more complex the material, the higher the detection limit will be, because the desired radiation is masked by that of other components.

In the analysis for indium, ashing and separation could be carried out after the irradiation, reducing the danger of contamination of the sample and eliminating the need for reagent blanks, a distinct advantage over the other methods.

Neutron activation was by far the most suitable method for the determination of indium at low concentrations in biological material. Since the basic principles have been discussed in great detail elsewhere, they will not be dealt with here.[50-53]

1.3.2 Neutron Activation of Indium

There are two stable isotopes of indium, indium-113 and indium-115, both of which have been used for its determination by thermal neutron activation. Irradiation of the first isotope gives rise to a long-lived nuclide, indium-114m, which undergoes an internal transformation to indium-114. After equilibrium has been established between parent and daughter nuclides, quantitative measurements can be made using the 1300 keV gamma ray of indium-114, or the beta radiation from its decay to tin-114. Indium-115 undergoes the reaction $In^{115}(n,\gamma)In^{116}$ when irradiated with thermal neutrons. Of the three isomers of the product, indium-116, indium-116m1, and indium-116m2, only the second has a half-life long enough to permit radiochemistry to be performed before counting, and is the only one that will be referred to in this thesis. The capture cross-section for the production of this isotope is 154 barns, making the reaction with indium-115 the more sensitive. Quantitative determinations may be made using any one or a combination of the three most prominent gamma rays from the decay of indium-116m1 to tin-116. The pertinent

nuclear properties of indium-113 and indium-115 are summarized in Table 1.4, page 15.

Table 1.4

Nuclear Data for Indium

Isotope	Abundance (%)	Cross-Section of (n,γ) Reaction (barns)	Product	Half-Life	Gamma Ray Energies (keV)
In^{113}	4.23	8.00	In^{114m}	50 d	189.9
			I.T. ↓		
			In^{114}	72 s	1300.0
In^{115}	95.77	154	In^{116m1}	54 m	417.0 1097.1 1293.4

The advantage of the reaction $In^{113}(n,\gamma)In^{114m}$ is the 50 day half-life of the product, which allows a leisurely separation of indium activity, a necessity if facilities for rapid radiochemistry are not available. However, this must be balanced against the lower natural abundance and smaller cross-section for neutron absorption relative to the $In^{115}(n,\gamma)In^{116m1}$ reaction, resulting in poorer sensitivity.

The latter reaction, used in the present determinations, not only offers a considerable gain in sensitivity, but the time required for complete analysis of the sample is considerably reduced as 2 hour irradiations are sufficient to obtain 75% saturation. One difficulty is that iron-59, a product of the (n,γ) reaction on iron-58, has two major gamma rays with energies of 1099 keV and 1292 keV.[54] In spite of the low natural abundance of iron-58 (0.31%) and the 46 day half-life of iron-59, this reaction will contribute about 15% of the activity observed in the 1095-1100 keV and 1291-1294 keV regions of the spectrum,

assuming concentrations of the order of 10^{-3} ppm for indium (Table 1.3) and 500 ppm for iron.[17,49] Care must be taken to ensure complete separation prior to counting or serious errors will result from calculations based on the 1097 keV and 1293 keV gamma rays of indium-116m1.

Other possible difficulties with the determination of any element by neutron activation arise from interferences caused by primary, secondary and second order nuclear reactions. Of these, primary reactions are the most important in reactor irradiations and are the only ones which may interfere with the determination of indium.[53] They result in the production of the indicator isotope by (n,p), (n,α) or (n,f) reactions on other matrix constituents. The two reactions which are of interest in the present case are the $Sn^{116}(n,p)In^{116m1}$ reaction and the fission of uranium.

The interference from tin-116 may be calculated from the cross-section of the reaction (0.05 mb),[55] the effective neutron flux for a light-water-moderated reactor of the type at McMaster University (1×10^9 $n/cm^2/sec$) [56] and an upper limit of 2 ppm tin in biological material.[17,99] Assuming an indium concentration of 10^{-3} ppm, the contribution from the $Sn^{116}(n,p)In^{116m1}$ reaction would be less than 10^{-8}% of the total indium activity and can be ignored.

The fission of uranium might be of some consequence in the analysis of certain geological samples, but presents no difficulty with biological materials. Smales et al.[23] have experimentally determined an upper limit of the apparent concentration of indium, produced by this reaction, as 1.2 µg per gram U_3O_8. From the decay curves of the separated indium, the majority of the activity seemed to be from indium-117,

a daughter of cadmium-117, which is formed by fission. Only a very small amount of the indium produced was indium-116m1. The concentration of uranium is approximately 0.05 ppm in biological material,[17,49] hence in a one gram sample, the apparent indium concentration would be less than 10^{-14} gm, making the interference negligible.

1.3.3 Radiochemical Separations

Dams et al. found the sensitivity, using indium-116m1 as the indicator isotope, sufficient to perform instrumental analyses on airborne particulates.[33] Interference from iron-59 was avoided by using 5-minute irradiations. Reduction of spectral data by computer gave a calculated limit of detection of 2×10^{-10} g. Hasan and Spyro used the same technique to analyze particulates in Surrey, England.[32]

In the case of biological materials, where the majority of the activity is from five nuclides; 37-minute chlorine-38, 2.6-hour manganese-56, 12-hour potassium-42, 15-hour sodium-24, and 14-day phosphorus-32, isolation of indium is imperative.[51] The separation has to be fairly rapid, because of the 54-minute half-life of indium-116m1.

The general principles and techniques of radiochemical separations have been well reviewed.[50-53,57] The procedure is usually dissolution or mineralization of the sample, removal of the matrix and trace-element activities, preparation of a form suitable for counting and determination of the chemical yield.

The first step, mineralization of the sample was accomplished by wet digestion in a Kjeldahl flask. Gorsuch found no difficulties pertaining to loss of indium with any of the traditionally employed

acid mixtures.[58] For the purposes of this work, ignition in an oxygen flask was also investigated but there was incomplete combustion of samples larger than 100 mg in a 1 liter flask. Since samples as large as 1 g were desired for analysis, it was felt that a commensurate increase in the size of the flask would make it too unwieldly and increase the danger of loss of indium. It was therefore rejected in favour of the slightly slower, but more convenient wet ashing.

Indium is best isolated from other elements in two ways; by ion exchange chromatography or liquid-liquid extraction. These have been used separately and in combination, depending on the elemental composition of the matrix and degree of purity of the final product required.[59-64] A third method, precipitation, either as the sulfide, hydroxide or oxinate, has also been suggested as a separation technique.[65,66] Contamination due to occlusion, absorption or co-precipitation reduces the selectivity, but used in conjunction with the first two methods it is advantageous in removing alkali metals and in preparing the sample for counting.

Separations of indium by cation exchange procedures usually make use of its low affinity for strongly acid resins, such as Dowex 50, in hydrochloric acid or other chloride solutions,[61] due to formation of anionic chloride complexes. Bhakti and Dingle combined this with solvent extraction to separate the indium from the iron carrier in their analysis of rain water.[31] Formation of the chloride complex is used in anion exchange separations also, and isolation from nine elements, cerium, chromium, cesium, iodine, iridium, antimony, selenium, tin and strontium can be effected on Dowex 2 in about 1 hour.[65] This has been

used in pre-irradiation separation from sea water[29] and employed in an automated multielement analysis of biological material, where indium-114m was the isotope determined.[67] The disadvantage of column chromatography relative to solvent extraction is the longer time required to obtain a clean separation.

Solvent extraction of indium has been used in separations from almost every conceivable combination of elements. The most frequently used method relies on the favourable distribution of indium halides between an aqueous and organic phase.[61,65,68-71] Quantitative extraction of the bromide complexes occurs from a 4M to 6M hydrobromic acid solution into diethyl ether,[69] isopropyl ether,[70] and 2-pentanone.[72] The iodide can be extracted from a 0.5M to 2.5M hydriodic acid solution or from a mixture of sulfuric acid and potassium iodide with diethyl ether.[68] The chloride complex is not appreciably extracted from hydrochloric acid by any of the above solvents.[68] The diethyl ether-hydrobromic acid system was selected for the present work. While this is not as selective as extraction from hydriodic acid, hydrobromic acid is more stable than hydriodic acid which decomposes on standing.[71,73] The sulfuric acid-potassium iodide mixture affords less selectivity than the bromide extraction.[68] Isopropyl ether must first be specially purified before use, as the commercially available compound has been found to have a low distribution coefficient for indium bromides.[65] Extraction with 2-pentanone appears to be most suitable in systems where the concentration of indium is greater than 0.2M in the hydrobromic acid layer.[72]

Co-extracted from the bromide solution are iron(III), gallium(III), thallium(III), antimony(V), gold(III), tin(II) and tin(IV), arsenic(III),

molybdenum(IV) and to a smaller degree molybdenum(VI) and selenium(II).[71,73] Indium can be separated from these, with the exception of tin and arsenic, by back-extraction from the ether phase with 6M hydrochloric acid. More than 99% of the iron remains in the organic layer, and as a result, virtually complete separation from indium can be achieved.[71]

Tin and arsenic are of no consequence in these determinations because, although their concentrations in brain are higher than that of indium by a factor of 100 for tin and 10 for arsenic,[17] their nuclear properties are such that only a small amount of activity from each would be produced under the conditions favourable for the activation of indium-116m1.[54]

This combination of liquid phases has been compared to sulfide precipitation and anion exchange separations employed under optimum conditions and was found to give the most complete separation.[65] It has been used in the analysis of rocks[26,27] and biological materials[40] with considerable success.

Any method suitable for determining milligram amounts of indium may be used to determine the chemical yield of the indium carrier at the end of the separation. Precipitation of the oxinate has been well studied and applied to radiochemical procedures.[26m74,75] Another method of interest is re-irradiation of the sample.[27] In this work, these, as well as spectrophotometric measurements of the oxinate complex in chloroform[74,76] and titration with ethylenediamine tetraacetic acid[77] were employed and the results compared.

CHAPTER II

EXPERIMENTAL PROCEDURES

2.1 Materials and Reagents

Four biological materials were analyzed in the course of this work; pine leaves, kale, rabbit liver, and calf brain. All of the experiments were conducted on the dried powder and the results given in the next section refer to the dry weight.

The pine leaves were one of three types of foliage samples; trembling aspen, forage and pine, received by Dr. Fritze for heavy metal analysis from the Air Management Branch of the Ontario Ministry of the Environment. These samples were taken from the Sudbury area and previous determinations of arsenic and selenium had indicated a high heavy metal content. The leaf surfaces were washed with water and the leaves freeze-dried, ground and stored in glass, screw-cap bottles by the Air Management Branch prior to being submitted for analysis. The powder was a medium greenish brown, and consisted of rectangular particles not more than 1 mm in length. The 28 g taken for these experiments were stored in a polyethylene bottle.

The rabbit liver was obtained as freeze-dried chunks, from Dr. Sass-Kortsack of the Hospital for Sick Children, Toronto. It was ground using an agate mortar and pestle used only for the preparation of soft biological tissues and sieved through a 200-mesh silk screen. The fraction which did not pass the mesh was discarded and the remaining powder stored in a polyethylene bottle. Approximately 12 g of a light

brown powder were prepared in this fashion.

The brain powder had been prepared in quantity by another member of the research group, as a material suitable for use in the development of methods to determine various trace metals. Two points of calf brain, purchased from a commercial source, were freeze-dried, ground in a blender and stored in a polyethylene bottle. The mixture was used without sieving and contained powder as well as larger pieces of connective tissue. At a later date, after a number of analyses, it was ground finer, passed through a 200-mesh silk screen and only the resulting powder used.

The preparation and properties of the standard kale dispensed by Bowen have been described in detail elsewhere.[45,78] As suggested it was dried in an oven at 90°C for 20 hours prior to analysis.[79]

Unless otherwise specified all reagents were analytical grade. Aqueous solutions were diluted to volume with distilled, deionized water and stored in polyethylene bottles.

Optronic-grade indium trioxide was obtained from Alpha Inorganics, Ventron Corporation, Chemicals Division (Catalogue number 40133). All indium solutions were prepared by dissolving this in warm aqua regia, care being taken to avoid splattering due to excessive effervescence. A carrier solution with a concentration of 15.2 ± 0.1 mg indium per ml, and a standard solution of 0.0920 mg indium per ml were used during the course of this work. The concentration of the standard was checked by titration with ethylene diamine tetraacetic acid (EDTA), the procedure for which is given on pages 32, 33.

The 8-hydroxyquinoline used in the standardization of the carrier and in chemical yield determinations was recrystallized from ethanol.

Precipitation of the indium oxinate complex employed a 5% solution in 95% ethanol. For spectrophotometric determinations, indium was extracted from the aqueous phase with 0.01M oxine in chloroform.

Acetate buffer of pH 5 used in the above precipitation was prepared from 136 g trihydrated sodium acetate and 30 ml glacial acetic acid diluted to 500 ml.

EDTA, as the disodium salt, (Eastman Chemical Co., lot number 41075) was dried 3 days at 80°C before being used. A 0.09917M stock solution was prepared with deionized water and diluted one hundred fold for the indium titrations. The latter was standardized against an appropriate dilution of a 854 ppm zinc solution, prepared from a metal pellet, 69 grade from Cominco, lot number HPM5205. The indicator used was Eriochrome Black-T (EBT), prepared by dissolving 0.2 g of the powder in 15 ml triethanolamine and 5 ml 95% ethanol.

A lanthanum-aluminum alloy wire (0.47% lanthanum, Reactor Experiments Inc., San Carlos, California) was used to measure flux differences between samples and standards in the reactor.

2.2 Instrumentation

Neutron irradiations were done in the McMaster Reactor which has a thermal neutron flux of 5×10^{13} n $cm^{-2}sec^{-1}$ (increased from 2×10^{13} n cm^{-2} sec part way through the course of this work) and a cadmium ratio of about 20. A pneumatic-rabbit system was used for irradiations of less than 10 minutes. The flux obtained was approximately half that for the in-core positions.

Measurements of the resulting indium-116m1 activity were made with a solid-state, coaxial Ge(Li) detector, which had an active volume of 40 cm^3, in conjunction with an Ortec 118A preamplifier, a Hewlett-

Packard 5582A linear amplifier, two Canberra 1501 stabilization pulsers and 1510 digital stabilizers and a Nuclear Data 2200 series 1024 channel analyser (later increased to 2048 channels). Gamma spectra were displayed on a Fairchild 701 oscilloscope and digital data was printed out by an IBM typewriter.

A Bausch and Lomb Spectronic 20 Spectrophotometer was used for all spectrophotometric determinations.

All weighings of samples and standards were done on a Mettler 'Gram-atic' Balance, model number B5 (E. Mettler, Zurich, Switzerland) which was accurate to ± 0.05 mg.

Volumes of solutions 1 ml or less were pipetted using Eppendorf pipets. These were found to be accurate to within 1% of the stated volume and were precise to ± 1%.

2.3 Pretreatment and Irradiation

A concerted effort was made in the handling of the powdered tissues to avoid contamination. The polyethylene bottles containing the different materials were stored in dessicators, and aliquots transferred to irradiation containers in a "clean" room. These containers were quartz capsules which had initially been boiled in aqua regia and then rinsed thoroughly with deionized water. After each use they were heated in concentrated sulfuric acid, a few drops of nitric acid were added to completely destroy any traces of organic residue, then they were boiled in aqua regia and rinsed with deionized water as above.

The samples were weighed into tared capsules of various sizes, depending on the amount of tissue to be irradiated. Quantities up to 0.5 g could be placed in a capsule measuring 1.5 cm x 1 cm inside dia-

meter, with a slightly larger one (1.7 x 1.3 cm) inverted over it to prevent contamination and spilling. The latter held as much as 1 g of the brain powder and the kale and about 0.7 g leaves and liver. Snuggly fitting covers were made from small polyethylene capsules. The polyethylene charred slightly and became somewhat brittle during the course of the irradiation but did not crack or shatter permitting contamination or leakage of the sample. To analyze larger amounts of the pine leaves and the rabbit liver the powder was pressed into compact pellets using specially hardened steel molds and a hydraulic press (Carver Laboratory Press, model C). Pine leaf discs weighed about 200 mg and were packaged in commercial aluminum foil. The mold used to press the rabbit liver was smaller, the pellets weighing about 100 mg. These were packed in a large quartz capsule and were capped before irradiation as described above. The samples in the capsules were then dried at 90°C for 24 hours and reweighed.

Two comparison methods were used for the quantitative analyses, external standards and standard addition. External standards were irradiated with every sample, while only one standard-addition series was done, on the liver powder.

The preparation of standards at first consisted of heat sealing aliquots of solution in polyethylene bottles. Sealing was done by melting the opening of the container in a hot air jet and pressing the sides together with tweezers. The plastic degraded during the irradiation and leakage occurred with almost half of the standards prepared in this way. Later, 20 µl volumes of the appropriate standard were evaporated in small quartz capsules and covered with a large one. This proved

more satisfactory.

The standard additions were made by pipetting aliquots of a dilute indium solution having a concentration of 2 ng indium per ml onto the weighed powder before drying. This solution was prepared from the 0.0920 mg/ml indium standard solution used to make the comparators. A series of dilutions for one set of standard additions consisted of diluting 20 µl to 100 ml and diluting 10 ml of this solution further to 100 ml giving a final concentration of 1.84 ng/ml. Aliquots ranging from 25 µl to 100 µl were used to spike the samples. The accuracy of the procedure was affected mainly by the eppendorf pipets used and at these low concentrations and with the number of steps involved was probably 3% to 5%.

The final package sealed in aluminum cans and placed in the core of the reactor consisted of a sample container on top of a standard, wrapped in aluminum foil.

To obtain the maximum sensitivity for the indium-116m isotope, each irradiation lasted 2 hours. Three positions in the reactor were used and the position of the aluminum can in each sample tube was specified so that the flux would be as constant as possible along the vertical axis of the can. The difference between sample and standard was measured for each position using 0.25 cm pieces of lanthanum-aluminum wire. One instance of a 5% difference was observed but generally the variation was only 1% to 2%.

2.4 Chemical Separation

Samples were left in the reactor building for approximately

30 minutes to allow the 2.5 minute aluminum-28 activity of the can to decay before removing the sample package. Approximately 45 minutes elapsed from the end of irradiation to the beginning of the radiochemical work.

The sample and standard were unwrapped and the powdered tissue, which normally had darkened and in some cases had shrunk away from the sides of the capsule, was tapped into a 100 ml Kjeldahl flask containing 15.2 mg of indium carrier, and the appropriate mixture of acids.

Animal tissues were digested using 3 ml fuming sulfuric acid ("free" sulfur trioxide 30-33%) in which 0.2 g sodium chloride, as sodium carrier, had been dissolved and 1 ml fuming nitric acid (90%) added after the sample. The flask was heated gently until all the powder had been dissolved, then more strongly until charring occurred. Another milliliter of fuming nitric acid was added dropwise and the flask was again heated strongly. This was usually sufficient to completely dissolve the sample and leave a clear yellow solution. If not, the addition of nitric acid was continued until this was achieved. The last traces of colour were removed by adding 1 ml of 70-72% perchloric acid and heating to fumes.

This procedure was modified slightly for the plant materials as these contain about 100 times the amount of calcium and 12 times the manganese found in soft animal tissues.[17,49] The ashing mixture used consisted of 15 mg indium carrier, 100 mg manganese carrier and 3 ml fuming nitric acid. This was taken almost to dryness, a further 2 ml fuming nitric added, and taken to virtual dryness again. One ml

each of nitric and perchloric acids were added, the solution boiled to fumes of perchloric acid and cooled. Manganese was precipitated as the dioxide from this solution by adding 15 ml of concentrated nitric acid, bringing it just to the boil and adding sodium chlorate crystals. The addition was made in three stages, first a few crystals then two 1 g quantities with a pause each time to let the effervescence subside. The precipitation took about 20 minutes and the sodium chlorate at the same time acted as sodium carrier. The solution containing the manganese dioxide was centrifuged and the supernatent decanted through a filter into another 40 ml centrifuge tube.

The remainder of the separation was identical for all samples. The centrifuge tube containing either the supernatent or ash solution was placed in an ice bath and just enough concentrated ammonium hydroxide added to neutralize the acid. After centrifuging the precipitated hydroxides, the supernatent was discarded and the precipitate dissolved in 9 ml concentrated hydrobromic acid. This solution was transferred, along with 11 ml of water, to a 125 ml separatory funnel and shaken for 1 minute with 25 ml of diethyl ether. The acidic, aqueous phase was discarded and 10 ml of 6M hydrochloric acid containing 10 mg of iron carrier were used to back-extract the indium from the ether. The organic phase was discarded and the aqueous phase washed twice with 10 ml volumes of ether, at which point both layers were completely colourless. The aqueous phase was drained off into a centrifuge tube and indium hydroxide precipitated by the addition of 8M ammonium hydroxide. The heat of neutralization was effective in removing at

least part of the dissolved ether from the solution. The solution containing the precipitated indium hydroxide was centrifuged, the precipitate washed once with water and finally dissolved in 4 drops of concentrated hydrochloric acid. The solution was transferred to a 25 ml safety-cap vial and diluted to 5 ml.

Gamma activity was measured with the sample placed directly on the face of the detector. Reproducibility of the geometry was ensured by using a lucite holder which fitted snuggly over the detector. Data was collected for 400 to 1000 seconds live time, depending on the amount of indium present. Dead time of the samples varied from 1% to 6%.

The evaporated indium standards were leached from the quartz capsules with hot, concentrated nitric acid. Two washes with hot acid and three with distilled water were diluted to 100 ml in a volumetric flask. Microliter aliquots, taken with the Eppendorf pipets, were diluted to 5 ml in the safety-cap vials, like the sample, and counted in the same geometry and for the same length of time. It had been shown[80] that the counts obtained in a particular gamma peak do not vary linearly with the amount of sample over wide ranges of dead-time, so an effort was made to match those of the standard and sample. The dead time of the standards was usually only slightly higher (less than 1% more) than that of the samples but, because there were small amounts of other activities in the samples, as well as that from indium-116m1, the indium activity of the standards was usually higher than that in the samples.

Reproducibility of the transfer was measured by a series of

acid evaporations, rinsing the capsules and diluting the solutions as described above and irradiating 2 ml of the solutions sealed in polyethylene vials for 5 minutes in the rabbit. These were counted in the 25 ml vials.

2.5 Determination of Chemical Yields

As stated in the INTRODUCTION, the four methods employed at various times to determine the recovery of indium from the separation procedure were precipitation of indium oxinate, spectrophotometric determination of the oxine complex in chloroform, re-irradiation and titration with ethylenediamine tetraacetic acid (EDTA).

Chemical yields by precipitation of the oxinate were used for all but three of the calf brain samples. The acid solution containing active indium and carrier was left for a day, then was transferred quantitatively to a 150 ml beaker and diluted to 20 ml. The precipitate formed on addition of 5 ml pH 5 acetate buffer followed by 5 ml of 5.0% oxine in ethanol, was digested on a hot plate at 60°C to 70°C with stirring for about 30 minutes. It was cooled to room temperature, filtered into a tared, medium-porosity, 3 ml glass frit and washed with water. After drying at 110°C for a half hour, it was allowed to cool and weighed. The drying was repeated for another half hour and the sample reweighed. This procedure was also used in the standardization of the carrier solution, 1 ml of which was precipitated and carried through the procedure, along with every sample. Some of the oxinates were checked for impurities by igniting them to the oxide at 1000°C and reweighing them.

In the chemical yield determinations by spectrophotometry, the active indium was allowed to 'cool' for one day before the solution was diluted in a 100 ml volumetric flask. Two aliquots of the diluted sample, 10 ml and 20 ml, along with 10 ml pH 3.5 acetate-hydrochloric acid buffer were further diluted to 100 ml. The most concentrated solution, corresponding to 100% yield, would then have a concentration of about 30 ppm. The procedure for colour development was based on that described by Moeller.[81] Twenty-five ml of the buffered solution were shaken with four successive 5 ml portions of the oxine-chloroform reagent. The combined extracts were diluted to 50 ml, filtered into polyethylene bottles to remove any traces of water and refrigerated. All solutions were brought to room temperature before taking measurements with the spectrophotometer.

A series of standard solutions were prepared from the indium carrier and the 0.9 mg per ml indium standard. The oxine-chloroform reagent, after extraction of the appropriately diluted buffer solution, served as a blank. The calibration curves prepared were usually reproducible over a period of about 2 weeks if the solutions were kept in tightly capped bottles and refrigerated. Longer storage resulted in significant evaporation of the chloroform and standards were usually re-prepared for each new series of irradiations. The concentration of indium in the sample solutions was calculated from the standard curve by means of a least squares analysis of the data. Chemical yields were determined by taking the ratio of this concentration to that of the carrier.

Re-irradiation to measure the chemical yield has been used by

Johansen and Steinnes in their analysis of standard rocks.[27] A comparison of this method to spectrophotometry in the analysis of rabbit liver samples was made using aliquots from the initial 100 ml dilutions for the spectrophotometric determinations. Carrier, diluted 200 fold to make the activity similar to that of the samples, was used as a comparator. Volumes of 0.05 ml were sealed in 1 ml snap-cap polyethylene vials by running a hot glass rod around the seam between the lid and the vial.

Samples and standards were irradiated for 1 minute in the rabbit, and left for 1.5 hours to allow the 38-minute chlorine-38 activity to decay. Spectra obtained in preliminary experiments on vials containing deionized water, indicated that no indium could be detected under these experimental conditions, and consequently the samples could be counted in the irradiation containers. The vials were washed with nitric acid and acetone to remove any surface activity before counting for 200 seconds. Sufficient indium activity was obtained to permit placing them 20 cm from the detector crystal in a lucite frame. This position eliminated any effects of the geometry of the liquid on the counting rates.

Samples were counted before the standards and, because of the short half-life of indium, the counts obtained for the standards had to be corrected to the same time interval as the samples. As well, a correction had to be made for the flux gradient along the vertical axis of the rabbit. Irradiations with two standards in the rabbit were made to determine the difference in the effective flux between the top and bottom position.

The determination of micro amounts of indium by titration with EDTA was investigated originally as a check of the standard indium solutions. As a matter of interest, some chemical yields were also determined and the results compared with those found by spectrophotometry and re-irradiation.

The dilute EDTA solution was standardized against 1 ml of the zinc solution described earlier.[114] This was diluted to approximately 25 ml, in an Erlenmeyer flask and 2 ml pH 10.5 buffer and 3 drops EBT indicator were added. Four titrations agreed within 0.5%.

The procedure used for the determination of indium was as follows:[77] 2 ml aliquots of the indium solution were neutralized to Methyl Red with 0.1M sodium hydroxide in a 15 ml centrifuge tube, 2 ml of 5% tartrate solution and 2 ml of pH 10.5 buffer were added and the centrifuge tube placed in a boiling water bath. Two drops of EBT indicator were added prior to the titration with EDTA. At the end of the titration, the tube was dipped in the boiling water for a few seconds to determine whether or not another drop of EDTA was required.

A 5 ml microburet was used in all of the determinations. The centrifuge tubes and stirring rods were washed with a strongly ammoniacal, 2% EDTA solution and rinsed well before use, to avoid errors caused by the leaching of metal ions from the glass into the basic and very dilute indium solution.

CHAPTER III

RESULTS AND DISCUSSION

Results were obtained for the concentration of indium in four biological materials; calf brain, pine leaves, kale and rabbit liver. The materials had been removed from their natural environment, washed, dried and, with the exception of the rabbit liver, ground before being received for analysis. While due care was probably taken during these processes, contamination or loss of indium might have occurred and so the results obtained in these experiments relate to the dry, powdered material as received.

Accuracy in analytical chemistry is a measure of how closely the mean of a series of determinations for a constituent approaches the most probably correct value of its concentration. This is usually determined only after analyses by a number of fundamentally different methods yields results that are in good agreement.[66] In the present situation, only one method, namely neutron activation, was sensitive enough to perform the analyses. A measure of the accuracy can therefore only be obtained by eliminating or accounting for all the systematic errors inherent in the method.

3.1 Investigation of the Method

A compilation of some possible sources of error in activation analysis is given in Table 3.1, page 35. Not all of these apply in the present analyses, however. The thermal flux, cadmium ratios of

Table 3.1

Sources of Error in Neutron Activation Analysis[7,83]

Type of Error	Approximate Magnitude (%)[1]
Systematic Errors	
Chemistry:	
Surface contaminants	
Faulty preparation of standards	
Incomplete exchange with carrier	
Insufficient decontamination from other activities during chemical separation	
Irradiation:	
Flux gradients	± 2
Self-shielding	± 4
Thermal enhancement	± 2
Absolute value of thermal flux	± 5
Value of the cadmium ratio	± 2
Nuclear reactions	
Nuclear Constants:	
Half-life	± 2-10
Decay scheme	± 2-50
Cross-section	± 5-30
Counting:	
Geometry of sample and standard	
Detector calibration	± 3
Dead-time corrections	± 4
Random Errors	
Chemistry:	
Sample homogeneity	
Sample weight	(< 1 mg), ± 0.5 [2]
Standard weight	± 2
Chemical yield	± 2
Irradiation:	
Variations in flux	
Irradiations (< 1 min.)	± 3
Counting:	
Gain and zero drift caused by electronic instability or variations in count rates	± 4
Statistics	
Geometrical factors	± 1

1. All values, except where noted, were taken from Kruger.[4]
2. Taken from De Soete *et al.*[50]

sample and reactor, and cross-section for neutron absorption and thermal activation do not influence the accuracy of the final result directly, although a knowledge of the approximate values is essential for determining optimum experimental conditions. Random variations of the neutron flux may influence the precision.

The length of the irradiation time was not crucial either, as each sample was irradiated together with a standard and direct comparisons of activity from one irradiation to another were not made. This was the case even for short rabbit irradiations with the exception of the experiments to determine the reproducibility of the standards. In these, however, five minute irradiations were used and differences of a few seconds were not critical.

Errors caused by a lack of knowledge of the decay scheme were virtually non-existent in the present case as indium-116m1 has a relatively simple decay pattern which has been well studied.[54] Only the major gamma rays were used in the calculations and these were easily distinguished because of the clean separations achieved. More difficulties in this respect would undoubtedly be encountered in non-destructive, multielement neutron activation analyses, where single and double escape peaks and sharp compton edges may be mistaken for gamma rays. Even in single element analyses, a knowledge of nuclides with gamma rays of similar energies to those being studied is essential so a conscious effort can be made to eliminate or compensate for possible interferences.

Because the spectra were relatively simple, detector calibration was necessary only at first, until the spectra became familiar. Again, in multielement analyses this would be more critical.

The errors which are relevant to the present determinations will be discussed in some detail in the following subsections, with the exception of nuclear reactions which are pertinent but have already been covered in the INTRODUCTION.

The random errors associated with counting statistics will be discussed with the results for the individual materials as they varied from sample to sample depending on the amount analyzed, the indium concentration, chemical yield and time from irradiation to counting. They were calculated using the formula:

$$\sigma_A = (C + B)^{\frac{1}{2}} \qquad 3.1$$

where C is the gross number of counts in a peak and B is the peak background counts.

3.1.1 Irradiation

Indium was determined by a comparison of the induced indium-116m1 activity in the samples to that in a known indium standard using the relationship:

$$\frac{C_u}{C_s} = \frac{W_u}{W_s} \qquad \text{or} \qquad W_u = \frac{C_u}{C_s} W_s \qquad 3.2$$

where C_u and C_s are the counts of corresponding gamma peaks in the sample and standard spectra, respectively, and W_u and W_s are the weight of indium in each. Implicit in this relationship are the assumptions that the sample and standard are exposed to the same neutron flux and are counted with the same efficiency. A bias will be introduced by relative differences in the flux caused by flux gradients in the reactor, self-shielding and enhancement of the thermal flux in one

relative to the other.

To minimize as much as possible the effect of flux gradients along the length of the aluminum can, its position in the irradiation tube was specified so that it would correspond to the maximum neutron flux. The differences in the flux reaching the sample and standard were measured for the in-core positions used during the course of this experimental work, by taping pieces of lanthanum-aluminum wire to the sides of the quartz capsules for typical two-hour irradiations. The flux differences were determined by comparing the counts of the 487 keV and 1597 keV lanthanum-140 gamma peaks after adjusting them for differences in the weight of wire irradiated. The results, shown in Table 3.2, page 40, indicated that there were no systematic differences which would cause a bias in the results. The observed variation in the count rates can be seen to be of the same order of magnitude as the calculated error in the number of counts obtained due to the statistical nature of radioactive decay. Small random flux variations between sample and standard would appear to affect the precision of the results by only ± 2%.

Flux depression in either the samples or the standards due to self-shielding was not a difficulty in the present experiments. The samples were low-density, biological materials, which do not contain large concentrations of strong neutron absorbers.[78] The effect in the standard can be calculated using the equations of Reynolds and Mullins.[84] The attenuation or ratio of the average flux inside the standard to that when the standard is absent for the thermal and resonance components of the neutron spectrum are given by the following equations:

Table 3.2

Variations in the Neutron Flux Between Sample and Standard Positions in the Reactor Core

Position	Counts per minute[1] Sample	Standard	Difference	% Difference[2]
8B	7945 ± 48[3]	7789 ± 48	156	+2
	5091 ± 39[4]	5003 ± 39	88	+2
8B	7320 ± 46	6359 ± 46	62	+0.8
	4574 ± 37	4588 ± 37	14	-0.3
8D	6635 ± 45	6753 ± 45	118	-2
	4217 ± 36	4227 ± 36	10	-0.2
8D	6809 ± 45	6581 ± 44	228	+3
	4414 ± 36	4191 ± 35	283	+6
6F	9600 ± 54	9830 ± 50	229	-2
	5975 ± 42	6280 ± 43	305	-5
6F	9814 ± 54	9637 ± 54	177	+2
	6252 ± 44	6189 ± 43	63	-1

1. The counts for each pair of numbers have been adjusted to the same weight of wire. One standard deviation is shown, calculated from the counts obtained in one 200-second count.

2. The percent difference between standard and sample counts relative to the sample.

3. 487 keV lanthanum-140 gamma ray.

4. 1587 keV lanthanum-140 gamma ray.

$$f_{thermal} = 1 - \frac{\tau}{2}(0.923 - \ln \frac{1}{\tau}) \qquad 3.3$$

$$f_{resonance} = -0.29 \log \frac{tI\ n}{I_{Co} n_{Co}} \qquad 3.4$$

where;

τ is a shape parameter, approximated as a foil by the evaporated standard and given by $n\sigma t$, n is the density in atoms/cm^3, σ is the thermal activation cross-section in cm^2.

t is the thickness in cm.

I is the resonance integral, 3500 barns for indium and 75 barns for cobalt.[94]

Only 1.8 μg of indium was irradiated, and the calculated values of $f_{thermal}$ and $f_{resonance}$ were equal to 1, indicating that the degree of self-shielding was negligible.

The moderation of epithermal and fast neutrons is, in general, only of consequence in matrices composed of hydrogen and other light atoms, for example organic material and water. Enhancement of thermal activation by this moderation has been studied by Reynolds and Mullins[84] and by Johnson.[85] Reynolds and Mullins found a 5% enhancement of thermal activation occurred when metal wires were irradiated in 1.5 ml of water and a 12% enhancement in 30 ml of water relative to irradiations without water. Johnson, on irradiating sodium solutions of different volumes found a 4% enhancement on varying the volume from 5 ml to 25 ml. Since organic material will have approximately the same effect as water in moderating the flux, an error could arise because of an effectively different neutron spectrum in the sample to that in the standard. In the present experiments, sample sizes were varied from 0.1 g to 1 g to

observe the effect of a ten-fold increase in size on the calculated indium concentrations. From a graph in which the sample weight was plotted against the calculated unit concentration of indium, shown in section 3.2, page 63, for the pine leaves, it can be seen that there is no variation in the concentration with sample weight. Indium has a resonance cross-section of 3500 barns, compared to a thermal cross-section of 154 barns and, while thermalization would increase the thermal component of the neutron spectrum by about 1% in a reactor of the type at McMaster, the decrease in the epithermal and fast neutron component would result in fewer resonance reactions. For this reason, the overall activity remained constant for indium instead of increasing as in the experiments mentioned above on elements such as sodium, whose cross-sections vary as the inverse of the neutron energy even in the epithermal region of the neutron spectrum.

Relative differences between the sample and standard can be eliminated by irradiating liquid standards. However, the heat and pressure produced during two-hour irradiations made the volumes of water comparable to that of the samples somewhat hazardous. The best compromise seemed to be to use evaporated standards and, if variations with sample size were observed, to calculate the indium concentration by extrapolating the concentration to zero sample weight. According to Johnson, this effect is quite small relative to other errors that may occur in the analysis.[85]

3.1.2 Radiochemical Procedures

3.1.2.1 Preparation of the Samples: To ensure that the portions of the powders taken for analysis were representative, they

were handled as little as possible before the irradiations and under conditions that would reduce the opportunities for introduction of foreign material to a minimum. Sample sizes varied from 0.1 g to 1 g. This was an interval recommended by Bowen for the analysis of his standard kale, which had been demonstrated to be homogeneous.[78] The pine leaf powder was coarser than the kale and the particles not as uniform in size or shape. However, no fractionation of smaller and larger particles was observed. Homogeneity had been demonstrated by the precise results obtained for concentrations of selenium and arsenic by another member of the research group, for samples weighing 0.1 g indicating that portions weighing between 0.1 g and 1 g were sufficient for representative sampling. The rabbit liver and calf brain powders were not tested for homogeneity. The rabbit liver had been ground in a mortar and sieved through 200 mesh silk. This resulted in a fine powder and provided thorough mixing. The brain was ground in a blender which mixed the sample thoroughly, but resulted in a wide range of particle sizes. The range for sample weights was deemed acceptable for both materials.

Bowen has also noted that relative errors of up to ± 1% have been introduced into results obtained for his standard kale through variations in the drying procedures used. As a result, he recommended that the kale be dried at 90°C for 20 hours prior to analysis.[45] This procedure was followed for all the tissues analyzed in the present experiments. Average weight losses of 6% were observed for the rabbit liver, 5% for the calf brain, and 5% for the kale and pine leaves. The relative moisture content of the kale concurs with Bowen's estimate

of 4.9 - 5.1%. No comparative figures are available for the other tissues. Under these conditions however, surface moisture should be removed without significant volatilization of organic components.

The weight of the samples was determined to an accuracy of ± 0.5% mg which meant that the error for a 0.1 g sample of powdered tissue was approximately ± 0.1%. The powder was tapped out of the container in which it had been irradiated. The small amount of residue which invariably remained in the container was determined by reweighing the capsule. This was never more than 0.5% of the original sample weight and averaged about 0.2%. The final weight on which calculations were based was the weight of the sample plus container before irradiation less the container weight after removal. The random error using this procedure was not more than ± 0.2%.

3.1.2.2 Standards: The standards were prepared from a stock solution of indium containing 0.09 mg indium per ml, which was stored at a low pH in a polyethylene bottle, conditions which were shown by Robertson to result in no observable adsorption of indium on the walls of the container.[87] The concentration was checked by microtitration with EDTA and found to be 0.0915 ± 0.002 mg per ml which was in good agreement with the value of 0.092 mg per ml calculated from the weight of indium trioxide dissolved.

Removal of the standards from the quartz capsules was checked on two occasions by comparing the activities of a total of eight standards, two series of four each. From the results shown in Table 3.3, page 45, it can be seen that the total random error in the standards prepared in this manner was approximately 2% to 3%. Having washed and

Table 3.3

Reproducibility of Standards

	Counts per minute[1]		
	417 keV	1097 keV	1293 keV
Series 1	1712 ± 11	1243 ± 10	2217 ± 13
	1809 ± 12	1318 ± 10	2178 ± 13
	1729 ± 11	1226 ± 10	2044 ± 12
	1754 ± 11	1246 ± 10	2157 ± 13
Mean ± σ_{mean} (%)	1751 ± 21(1)	1258 ± 20(2)	2149 ± 23(1)
Series 2	1440 ± 10	1049 ± 9	1709 ± 11
	1396 ± 10	1032 ± 9	1658 ± 11
	1337 ± 10	977 ± 9	1740 ± 11
	1470 ± 10	1039 ± 9	1724 ± 11
Mean ± σ_{mean}(%)	1411 ± 20(2)	1024 ± 16(2)	1692 ± 17(1)

1. One standard deviation is shown, based on the recorded activity for a 1000 second live-time count.

rinsed the capsules, they were heated with another ml of acid and this, together with the washings, was made up directly to 2 ml, irradiated and counted as described. The activity obtained by this procedure corresponded to 0.1% of the indium standard. Further washings with acid showed no indium activity after being irradiated. Thereafter, the capsules were washed twice with hot acid to ensure that all the standard was removed for counting.

3.1.2.3 Chemical Separations: Dissolution of the sample after irradiation is a critical step, as it is important that complete exchange occur between the indium in the sample and the carrier. The chemical yield determined for the carrier must be an accurate measure of the fraction of the original activity counted. As there is only one oxidation state of indium that is stable in aqueous solution, indium(III),[88] incomplete exchange because the indium in the sample and the carrier were in different oxidation states did not present a problem. The strong oxidizing conditions under which the sample was ashed insured that all the organic material had been destroyed and only inorganic indium was in the digested solution.

The method proposed by Smales, Smit and Irving for the radiochemical separation of indium from biological materials included specific steps to remove iron, copper, barium and nickel.[22] The only elements that were found to cause difficulties with present determinations, however, were iron and, in the case of the plant materials only, manganese. A triple extraction of the iron from a 6M hydrochloric acid solution containing the dissolved hydroxides, into ether as described in Section 2.4 was found to give a satisfactory separation from indium. To ensure that there was no contribution to the 1097 keV and 1293 keV indium-116m1 gamma peaks from the 1099 keV and 1292 keV iron-59 gamma rays, the samples were counted again six hours after the original indium spectra were taken. In this manner a correction could be made by subtracting the iron activity from the original peak area to determine the activity due to indium only. It was found, though, that this correction was unnecessary as the number of counts in the two regions were never above

background in the second spectrum.

In the calf brain and rabbit liver samples, the activity from manganese-56 was very minor. The 847 keV gamma had approximately the same intensity as the indium-116m1 819 keV gamma resulting in a doublet being observed in this energy region. Since the 819 keV peak was not used in the calculations and the manganese contributed very little to the overall background or dead-time of the sample, no special steps were taken to separate it along with the other matrix activities.

Kale, on the other hand, contains 15 ppm manganese, or about 10 times the amount found in the animal tissues analyzed[17] and the pine leaves appeared to have an even higher concentration. The activity from manganese-56 distorted the sample spectrum to the extent that comparison with the standard spectra was not feasible. The separation procedure was modified, therefore, to remove manganese and thereby reduce this activity. The procedure adopted was a modification of the Ford-Williams method, in which manganese dioxide was precipitated from a boiling, concentrated nitric acid solution by the gradual addition of sodium chlorate, as described in Section 2.4.[89] While this procedure was somewhat time consuming, requiring approximately 20 minutes to precipitate the dioxide, it was found to be effective in reducing the manganese activity and was convenient as the precipitation could be carried out directly on the acid digest without having to adjust the pH. The rest of the separation could be continued as with the animal tissues.

The only other activities observed in the spectra were a small

sodium-24, 1390 keV gamma and potassium-40, 1440 keV gamma from the room background. It appeared then, that no systematic errors were introduced because of insufficient decontamination of the indium in the radiochemical separation procedures used.

3.1.3 Chemical Yield Procedures

The chemical yield of the separation was determined by three different methods; precipitation of indium oxinate, spectrophotometric measurement of the oxinate in chloroform and titration with EDTA. The procedures were straight forward and have been described in Section 2.5. In addition, re-irradiation, which had been used by Johansen and Steinnes in analyses of geological samples,[27] was investigated as a fast, simple method for determining yields and was compared to the spectrophotometric method and EDTA titration. It was essential in all of the determinations that the iron and manganese carriers were either quantitatively removed during the separation or would not cause interferences and introduce a systematic error to the results.

3.1.3.1 Precipitation of Indium Oxinate: The oxinate precipitation procedure was used for all but three of the brain samples (spectrophotometry was used for these). Iron oxinate is completely precipitated in the same pH range (pH 2.8 - 11.2)[74] as indium oxinate (pH 2.6 - 7.5)[75] and could cause a positive bias in the chemical yields determined by this method. The fact that no iron activity was observed in the spectra of the separated indium, made this possibility most unlikely, but as a qualitative check, some oxinates were dissolved in a small volume of 2N hydrochloric acid (1 to 2 ml) and a few drops of

3M potassium thiocyanate were added. As the typical red colour of the iron complex was either absent or only very faint, it was concluded that virtually all had been removed in the separation and that it was not a source of error. To further test the purity of the precipitations, the oxinates from five samples were heated to 1000°C so they could be determined as indium trioxide. The results in Table 3.4, page 49, indicate that there was no systematic error introduced by the chemical yields determined in this manner. The precision of the method, determined from repeated precipitation of the carrier was ± 1%.

Table 3.4

Conversion of Indium Oxinate Precipitates to Indium Trioxide

Indium Oxinate (mg)	In_2O_3 (mg)		Percent
	Experimental	Theoretical	Difference
24.7	6.2	6.3	-1
47.2	11.5	12.0	-4
67.9	16.9	17.2	-2
50.2	13.0	12.8	+2
54.8	14.3	13.9	+3

3.1.3.2 Spectrophotometry: Spectrophotometry was used to determine chemical yields for all the pine leaf and kale samples and some rabbit liver and calf brain samples. This method was used for the plant samples as the manganese carrier had not been quantitatively precipitated as the dioxide. The remaining carrier would interfere with the oxinate method, as manganese(II) oxinate starts precipitating

at pH 4.5 [74] and indium oxinate was precipitated in the pH range 4.5 - 5.0 in the present experiments. The two elements are extracted into a 1% oxine in chloroform solution at different pH's however, indium being completely extracted from pH 3.5 - 4.5, while manganese is not extracted below pH 5.8.[64] Iron extracts in the same pH range as indium, but is removed quantitatively in the chemical separation.

Since the presence of manganese carrier would be indicated by the manganese activity in the sample, a comparison was made of the chemical yields of the seven samples with the highest manganese-56 activity with the seven with the lowest activity. The mean yield of the first set was 50% while that of the latter was 51%, indicating that the manganese carrier was not influencing the chemical yields determined by this method.

The samples were diluted to 1 litre and 10 and 20 ml aliquots were extracted. The chemical yields of the first series of pine leaf samples were determined a total of four times, using two 10 ml and two 20 ml volumes, to estimate the precision of the method. From Table 3.5, page 51, it can be seen that the standard deviation was approximately ± 2%. In addition, the percent yields obtained for the two volumes were the same, within the experimental error, indicating that no constant errors were occurring in the determinations.

One drawback of the method however, was the instability of the standards. The volatility of the chloroform and relative instability of the complex caused the slope of the standard curve to vary by about 10% over a period of two weeks, even though the solutions were tightly sealed in screw-cap bottles covered with parafilm and refrigerated.

Table 3.5

Reproducibility of Spectrophotometric Determinations

Sample	Volume taken for analysis (ml)	Indium Carrier Found (mg)			Average[1]
				Mean	
1	10	9.00	9.14	9.07	9.03 ± .14
	20	9.00	9.03	9.02	
2	10	4.22	4.31	4.26	4.23 ± .12
	20	4.19	4.20	4.19	
3	10	6.72	6.76	6.74	6.69 ± .15
	20	6.59	6.71	6.65	
4	10	8.08	8.15	8.12	8.14 ± .10
	20	8.14	8.18	8.16	
5	10	6.72	6.88	6.80	6.83 ± .17
	20	6.85	6.89	6.87	
6	10	5.92	6.07	6.00	6.04 ± .21
	20	6.01	6.13	6.07	
7	10	5.16	5.26	5.21	5.13 ± .28
	20	4.98	5.12	5.05	
8	10	8.68	8.65	8.66	8.63 ± .15
	20	6.84	8.53	8.59	

1. Average of four determinations, with one standard deviation shown.

This meant determinations were rather lengthy, since new standards had to be prepared with each series of samples.

3.1.3.3 EDTA Titration: Titration with EDTA, which had been used to check the concentration of the standards, was also used as an alternative method for determining chemical yields of the rabbit liver samples. As in the oxinate precipitation, manganese interferes and must be absent from the solutions being titrated,[77] hence the method could not

be used with the plant samples. Iron can be masked with cyanide and presented no problem. The main advantage of this method was the greater speed relative to the spectrophotometric and gravimetric methods.

The spectrophotometric procedure and titration with EDTA were compared by performing replicate analyses on a series of eight rabbit liver samples. The microtitrations were performed on 2 ml aliquots of the diluted sample solutions used for the spectrophotometric method. Results are shown as mg of indium carrier in the original sample solution, but, in fact, amounts of the order of 10 μg were titrated. Hence, the values in Table 3.6, page 52, appear to be much less precise than those obtained for the indium standards, in which 100 μg of indium were titrated.

From the results shown in Table 3.6, the agreement between the methods can be seen to be very good. The mean values obtained by each method differed by only 1% to 2% with the exception of sample 4 in which there is a 4% variation. The precision of the titration method for three determinations is slightly worse than the variation of the two spectrophotometric determinations, but is the same as that obtained for four results on the pine leaves in Table 3.5. The titration method was used for the remainder of the chemical yield measurements.

3.1.3.4 Re-irradiation: As mentioned in the INTRODUCTION, re-irradiation has been used in the analysis of geological samples to determine the chemical yield of indium.[27] The method appeared attractively simple; the sample was diluted to one litre, and an aliquot was irradiated in the rabbit along with a standard, equivalent to 100%

Table 3.6

Comparison of the Spectrophotometric Method and EDTA Titration for the Determination of Chemical Yields on Rabbit Liver Samples

Sample	Calculated Indium Carrier Present After Chemical Separation (mg)						
	Spectrophotometry		Mean[1]	Titration			Mean[1]
1	11.2	11.1	11.1 ± 0.1	11.0	11.0	11.1	11.0 ± 0.1
2	8.1	8.0	8.0 ± 0.1	8.3	8.3	7.9	8.2 ± 0.4
3	9.4	9.3	9.4 ± 0.1	9.5	9.4	9.4	9.5 ± 0.1
4	10.5	10.4	10.4 ± 0.1	10.1	9.9	10.1	10.0 ± 0.2
5	7.1	7.0	7.0 ± 0.1	7.0	6.8	6.8	6.9 ± 0.2
6	10.9	10.9	10.9 ± 0.03	10.9	10.9	10.8	10.9 ± 0.1
7	8.4	8.4	8.4 ± 0.05	8.5	8.3	8.4	8.4 ± 0.2
8	10.4	10.2	10.3 ± 0.2	10.0	10.1	10.2	10.1 ± 0.2

1. One standard deviation shown.

yield. After adjusting the counts to the same time interval, the ratio of the indium activity of the sample to that of the standard would give the chemical yield.

It was found in the present experiments that there were some difficulties and the procedure was not as promising as it looked at first. The position of the rabbit in the core of the reactor was such that a large flux difference existed along its length. The activities of the sample and standard had to be adjusted for this gradient in order to make a comparison valid. With each set of irradiations, two measurements of the flux differences were made with

indium standards occupying the two positions in the rabbit, one at the beginning and one at the end of each series of samples. This was not an ideal way to measure the gradient as the magnitude could change from one irradiation to the next. However, with the samples run in as short a space of time as possible, variations in the gradient should be minimal.

Chemical yields were determined by this method on the same set of rabbit liver samples as had been determined by EDTA titration and spectrophotometry, discussed in the previous subsection. To make comparison with the other methods possible, the results for re-irradiation, shown in Table 3.7, page 54, have been calculated as mg of indium present in the undiluted solution. Included in the Table is the ratio of the effective flux at the top position of the rabbit (occupied by the standard) to that at the lower position (occupied by the sample).

Because the counting was carried out with the solutions in the irradiation vials, there was some interference from 110-minute argon-41, which has a major gamma ray at 1293.8 keV. This was evident notably in the first set of irradiations in which the irradiation times were longer than the other two sets and the cooling period shorter. The result was a higher calculated indium content based on the 1293.4 keV gamma. The 1293 keV gamma from indium in the first series was not used in the calculation of the mean.

It was also apparent from the results, that some of the samples had leaked slightly during the first irradiation, although there were no visible signs at the time they were removed from the rabbit. This occurred with samples 1, 6, 7 and 8 and again these were not used to compute the mean.

Table 3.7

Chemical Yields by Re-irradiation

Sample[1]	Gamma Energy (keV)	Calculated Indium Carrier Present After Chemical Separation (mg) Irradiation 1 (.94)[2]	Mean	Irradiation 2 (.94)[2]	Mean	Irradiation 3 (.90)[2]	Mean	Mean[5]
1	417	6.9		10.0		10.8		
	1097	7.2	7.1[4]	10.6	10.3	10.2	10.5	10.4 ± 0.3
	1293	7.7[3]		10.2		10.5		
2	417	7.8		7.8				
	1097	8.3	8.1	7.6	7.7			7.9 ± 0.3
	1293	9.4		7.7				
3	417	8.7		9.2				
	1097	8.7	8.7	9.0	9.2			9.0 ± 0.3
	1293	10.4[3]		9.4				
4	417	11.0		10.8				
	1097	10.6	10.8	10.3	10.6			10.7 ± 0.3
	1293	11.8[3]		10.6				
5	417	6.6		6.5				
	1097	6.4	6.4	6.4	6.5			6.5 ± 0.1
	1293	7.9[3]		6.6				
6	417	9.6		11.1		10.8		
	1097	10.2	9.9[4]	10.9	11.1	11.5	11.1	11.1 ± 0.3
	1293	11.4[3]		11.2		10.9		
7	417	7.4		8.4		8.4		
	1097	7.4	7.4[4]	8.2	8.2	8.2	8.4	8.3 ± 0.1
	1293	8.7[3]		8.2		8.5		
8	417	9.1		10.5		10.8		
	1097	9.3	9.2[4]	10.5	10.3	10.4	10.5	10.4 ± 0.3
	1293	9.6[3]		10.0		10.3		

1. Sample numbers correspond to those in Table 3.6, page 53.
2. Ratio of the flux on the top half of the rabbit to that in the lower half.
3. This value was not used to calculate the mean for the irradiation.
4. Not used to calculate the mean of all results.
5. One standard deviation shown.

Ignoring these abberant results, the precision of the mean values of two irradiations is about ± 2%, the same as the precision obtained for the EDTA titrations. The random error in the mean value for each irradiation is ± 5% however, due to variations in the values calculated from the individual gamma rays. The agreement between the means was quite acceptable, although the variation in results calculated from each gamma ray seemed excessive.

The accuracy of the results, judged by comparison with those obtained by spectrophotometry and titration was questionable. For samples 2, 6, 7 and 8 the three methods agreed within 2%, sample 4 by re-irradiation was 5% high and samples 1, 3 and 5 were 5% low. By contrast, all but one of the results obtained by the other two methods agreed within ± 2%.

It was felt that the flux gradient was responsible for the poor agreement between re-irradiation and the other methods. A gradient had been found to exist around the circumference of the rabbit as well as along its length, and variations of the magnitude from one irradiation to the next were also possible. The correction made was only an average over the time of eight irradiations.

Re-irradiation was abandoned in favour of titration because of the poor agreement with the other methods. Better results would no doubt be obtained in the absence of flux gradients or if a method of averaging them could be found. In the present experiments, however, a lack of time and the availability of alternate and more suitable methods made it impractical to pursue the matter further.

3.1.4 Counting and Calculations

As mentioned earlier, not only must the sample and standard be irradiated under the same conditions for a comparison of their activities to be valid, but they must also be counted with the same efficiency.

Care was taken to reduce the possibility of systematic errors caused by non-uniformity of counting conditions to a minimum. The experimental conditions employed to ensure reproducibility of the geometry on the detector have been described in Section 2.4, as has the preparation of the standards, which were diluted to match as closely as possible the dead-time of the samples, minimizing electronic drift due to variations in the count rate. The dead-time of the samples was approximately 1% for the rabbit liver, 2% to 3% for the pine leaves and kale and 2% for the calf brain. The difference in dead-time between sample and standard was never more than 0.5%. To reduce drift caused by electronic instability, zero and gain stabilizers were incorporated in the analyzer system.

The sample was counted first, followed by the two standards. Indium-116m1 has a half-life of 54 minutes and the standards decayed considerably during the 1000 seconds (approximately 17 minutes) of the sample count and had to be adjusted to the same time interval as the sample. This was done using the equation:

$$A_{(t_2)} = A_{(t_1)} e^{\lambda(t_2 - t_1)} \qquad (3.5)$$

where; A is the number of counts,

t_1 is the starting time of the count,

t_2 is the time to which the count is being adjusted,

λ is the decay constant of the nuclide under consideration.

The errors introduced by using an incorrect half-life or by faulty measurement of the interval "$t_2 - t_1$" have been calculated and are shown in Table 3.8, page 58. The total error in the calculated

Table 3.8

Errors Incurred by Adjusting Counts from One Time Interval to Another

	Calculated Error in the Corrected Counts (A_t)[1]		
$t = t_2 - t_1$	$\frac{1}{2}t_{\frac{1}{2}}$	$t_{\frac{1}{2}}$	$2t_{\frac{1}{2}}$
$A_t = 1000\ e^{\lambda t}$	1026	2000	4000
Error in t			
1 second	< 1	< 1	1
0.5 minutes	9	13	26
1 minute	18	26	52
2 minutes	36	52	103
Error in $t_{\frac{1}{2}}$			
0.3 minutes	2	8	31
0.5 minutes	3	13	52
1 minute	7	26	103

1. $E_{(t)} = \frac{\partial}{\partial t} Ae^{\lambda t} \cdot dt$

$$E_{(t_{\frac{1}{2}})} = \frac{\partial}{\partial t_{\frac{1}{2}}} Ae^{\frac{\ln 2}{t_{\frac{1}{2}}} t} \cdot dt_{\frac{1}{2}}$$

count rate is the sum of these errors. It can be seen that as the length of the interval "$t_2 - t_1$" increases, the errors increase rapidly. In the present experiments, this interval was always less than 2 half-

lives and care was taken to ensure that the interval was timed to at least the nearest minute. Hence, the maximum error incurred from this source was about 1%.

The half-life in all calculations was taken as 53.7 minutes.[54] An older value of 54 minutes was quoted by some sources,[26,62] but the one used was assumed to be a refinement of this figure. The discrepancy of 0.3 minutes would result in a maximum error of less than 1%.

The total error that could result from these two areas, then, is 2% and is less than the 3% random error that would be associated with 1000 counts.

3.2 Analyses of Some Biological Materials for Indium

Following preliminary investigations of the method, neutron activation accompanied by radiochemical separation was applied to the analysis of pine leaves, rabbit liver, kale and calf brain for indium. Indium concentrations of 2.20 ± 0.42 ppb, 0.032 ± 0.007 ppb and 0.69 ± 0.10 ppb, respectively, were obtained for the first three materials. The precision of the calf brain results was considerably worse, the average concentration being 0.55 ± 0.54 ppb.

3.2.2 Pine Leaves

The known high concentrations of some heavy metals (arsenic and selenium) in the pine leaves, indicated that indium might also be present at somewhat elevated concentrations. Analysis of this material was done as part of an initial testing of the method on biological materials. Once it was shown that indium could be determined in this material, the procedure could be applied to other materials which had

a lower metal content.

A wide range of sample weights was used in the pine leaf experiments (0.1 g to 1.0 g) with a fairly even distribution between these extremes, to determine whether or not the measured indium concentrations were dependent on the size of sample analyzed.

The levels of indium found in the pine leaves were the highest encountered in all the materials studied. The results of eleven analyses are given in Table 3.9, page 61, which shows the sample weight, the indium concentration determined for each sample, calculated using the three major gamma rays and two aliquots of standard, the standard deviation of each result and the error due to the counting statistics. Samples which were similar in weight were considered to be duplicates and have been grouped together.

The standard deviation of individual results was, for most samples, less than the error due to the counting statistics. The two cases for which this was not true exhibited fairly large manganese-56 peaks at 847 keV. The manganese activity caused some distortion in the sample spectrum resulting in larger variations in the indium concentrations calculated from the gamma rays than could be accounted for by the counting statistics.

The measured concentrations varied from 1.90 ppb to 3.31 ppb. The latter value seemed anomalously high compared to the rest of the results and the "t"-test was applied to determine whether or not it should be retained.[90,91] While some caution must be exercised in comparing a single value to an average rather than comparing two averages, the test would provide a guide as to whether the result should be

Table 3.9

Determination of Indium in Pine Leaves

Sample Number	Sample Weight (g)	Mean Indium Concentration (ppb)[1]	Counting Error (ppb)
1	0.1007	2.52 ± 0.05	0.07
2	0.1039	2.66 ± 0.05	0.08
3	0.2737	1.98 ± 0.07	0.07
4	0.2757	1.84 ± 0.14	0.06
5	0.5079	2.20 ± 0.05	0.09
6	0.6664	2.19 ± 0.02	0.04
7	0.7048	2.16 ± 0.04	0.06
8	0.9445	3.31 ± 0.06	0.06
9	1.0470	2.39 ± 0.20	0.04
10	1.0471	2.20 ± 0.03	0.08
11	1.1480	2.08 ± 0.03	0.04

Average Concentration of Indium in Pine Leaves:	2.32 ppb
One Standard Deviation:	0.37 ppb
Standard Deviation of the Mean:	0.08 ppb

1. Calculated based on values for two standards and three gamma rays for each standard. One standard deviation is shown.

rejected or retained. The value of "t" in this instance was 4.38, which is somewhat higher than the value corresponding to agreement at the one percent level of significance, 3.25, for nine degrees of freedom. But the test did not seem conclusive enough for rejection of the result

on this basis alone. A second test, the Q-test was applied, in which the ratio of the difference between the outlying result and its nearest neighbour to the difference between the largest and smallest values is compared to a "critical" value of Q.[92] Q was calculated to be 0.44 for this set of data. The critical value of Q for rejection at the ten percent level of significance for eleven measurements is 0.39 and again it was doubtful that rejection should be employed. As this sample had been treated in a manner identical to samples seven through eleven, all being pressed into pellets before irradiation, there seemed to be no justification for rejection because of increased risk of contamination due to different experimental conditions.

Since the inclusion of the outlying result in the set was dubious, the median of the eleven results, 2.20 ppb ± 0.42 ppb was taken as the best estimate of the concentration of indium in pine leaves, rather than the mean, 2.32 ppb ± 0.37 ppb which would be influenced to a larger extent by the high result.[92]

The standard deviation of the eleven results about the median was ± 0.42 ppb or 19%. This is larger than the random error of the method for this material, which was estimated to be ± 6%, assuming that the various factors contributing to the random error were independent. The discrepancy is due, at least in part, to the fact that the standard deviation was calculated from the distribution about a median value rather than the mean. If sample 8 (which is questionable) is ignored the standard deviation about the mean for the remaining 10 samples is ± 11%, which is approaching the precision of the method.

To determine whether or not there was a significant change in

the indium concentration with sample size, the calculated concentration was plotted as a function of sample weight. Samples having similar weights were considered duplicates and were averaged to give one result. The influence of sample 8 on the slope of the line and the intercept was again found to be inordinately large and this sample was omitted from the calculations. The slope of the line (-0.065 ± 0.22 ppb/g) determined from the remaining six points was found to be essentially equivalent to a slope of zero at the fifty percent level of significance, using the "t"-test.[90] Similarly, the intercept, 2.23 ± 0.16 ppb was in good agreement with the median value for the eleven samples of 2.20 ppb. It was therefore concluded that there was no dependence of the indium concentration on the sample size.

The best estimate of the indium concentration in pine leaves was therefore taken to be 2.20 ± 0.42 ppb.

3.2.2 Rabbit Liver

The powdered rabbit liver was the most extensively studied material in these experiments. Preliminary work had shown that indium was present in concentrations that were lower than one part per billion and analysis of this material would prove or disprove the worth of the method for measuring ultra-trace quantities of indium. Determinations were made by both comparison of the indium radioactivity in the sample to that of a standard and the method of standard addition, in which a series of samples were spiked with a standard solution.

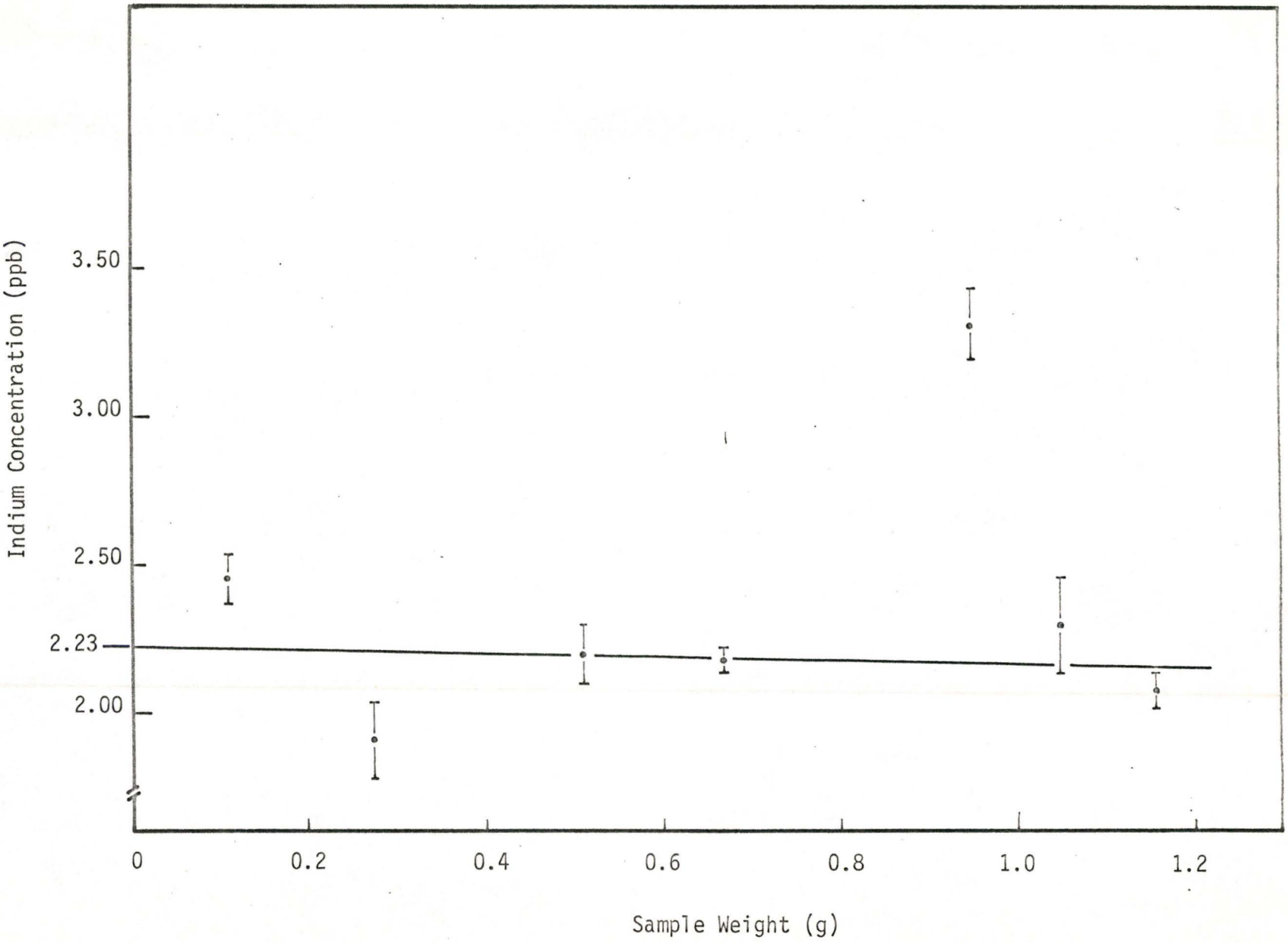
3.2.2.1 Determination of Indium by the Method of Comparison:

The Method of Comparison was used for all the materials studied and

Figure 3.1. Indium Concentration as a Function of Sample Weight for the Powdered Pine Leaves.

The error bars bracketting each point indicate two standard deviations.

The equation of the line: $y = -0.065x + 2.23$


Indium Concentration (ppb)
3.50
3.00
2.50
2.23
2.00
0
0.2
0.4
0.6
0.8
1.0
1.2
Sample Weight (g)

basically involved irradiating a sample and standard simultaneously and comparing the indium activity of each. The powdered rabbit liver samples varied in weight from 0.3 g to 1 g and were irradiated and analyzed using the same procedure as for the pine leaves with omission of the step to remove manganese as the dioxide.

The results are given in Table 3.10, page 65. Inspection of the results for the nine samples reveals a range of concentrations from 0.022 ppb to 0.11 ppb. Since the value of 0.11 ppb for sample 9 is more than twice the value of the next highest result (0.043 ppb, sample 1) it was rejected. This could be justified for experimental reasons, as it was the only rabbit liver sample which had been pressed into a pellet before irradiation. It is possible that contamination during this process was responsible for the aberrant result, as the difference in concentration between this and the other results was less than a tenth of a part per billion or 10^{-10} g of indium.

Taking a statistical approach to the problem, the value 0.11 ppb was compared to the mean of the remaining eight samples, again using the "t"-test.[90,91] In this case, "t" was found to be 10.51, indicating that 0.11 ppb did not agree with the value 0.032 ppb at the one percent level of significance for which "t" is 3.50 for seven degrees of freedom. The result, therefore, was rejected both on the basis of the experimental conditions which had increased the opportunity for contamination of this sample and on the basis of the "t"-test.

The indium concentration of the powdered rabbit liver was

Table 3.10

Analysis of Rabbit Liver for Indium Using the Method of Comparison

Sample Number	Sample Weight (g)	Mean Indium Concentration (ppb)[1]	Counting Error (ppb)
1	0.2800	0.0431 + 0.0017	0.004
2	0.5740	0.0258 + 0.0028	0.002
3	0.5745	0.0293 + 0.0042	0.002
4	0.6066	0.0390 + 0.0015	0.002
5	0.6294	0.0227 + 0.0007	0.001
6	0.7040	0.0335 + 0.0019	0.002
7	0.7110	0.0376 + 0.0026	0.002
8	1.0087	0.0261 + 0.0023	0.004
9[2]	1.0473	0.109 + 0.0034	0.003
Average Concentration of Indium in Rabbit Liver:			0.032 ppb
One Standard Deviation:			0.007 ppb
Standard Deviation of the Mean:			0.002 ppb

1. Calculated based on values for two standards and three gamma rays for each standard. One standard deviation is shown.
2. This result was not used to calculate the mean.

taken as the mean of the remaining eight values, 0.032 ppb + 0.007 ppb (one standard deviation).

The relative precision of the method for this material was estimated in the same manner as for the pine leaves and was found to be ± 6%. The standard deviation of the results was higher than this (22%) but was still deemed acceptable because of the low concentrations and the problems of accurate, representative samples.

3.2.1.2 Analysis of the Rabbit Liver by the Method of Standard Addition: It was hoped that at least two pieces of information could be gleaned from the standard addition experiments on the rabbit liver. One result would be a determination of indium with both sample and standard having an identical matrix, providing a check on the method of comparison in which a dissimilar standard was used. The other was to determine the recovery of the method. This would show whether or not there was complete exchange between the active indium in the sample and the indium carrier.

A series of five samples were prepared, four spiked with various amounts of indium, one not. Each sample was irradiated together with a standard and the separation conducted as in the previous section.

For the determination of indium by standard addition, it was necessary that the activities from the five samples be directly comparable. Sample weights, therefore, were made as similar as possible, the maximum difference being 2 mg in 630 mg or 0.3%. The activity

from each was adjusted for the chemical yield obtained, for variations in the length of time between the end of irradiation and the start of counting and for variations in the neutron flux. The time between irradiation and counting was measured to the nearest minute. The median time for the five samples was chosen as the reference point to which all samples would be adjusted to make the correction factors as small as possible.

The flux variations between irradiations were measured by comparing the activities of a constant aliquot of the standard irradiated with each sample. The first sample was arbitrarily taken as the reference point and a correction factor was found for each standard, to adjust their activities to this value. These factors were then applied to samples 2 through 5.

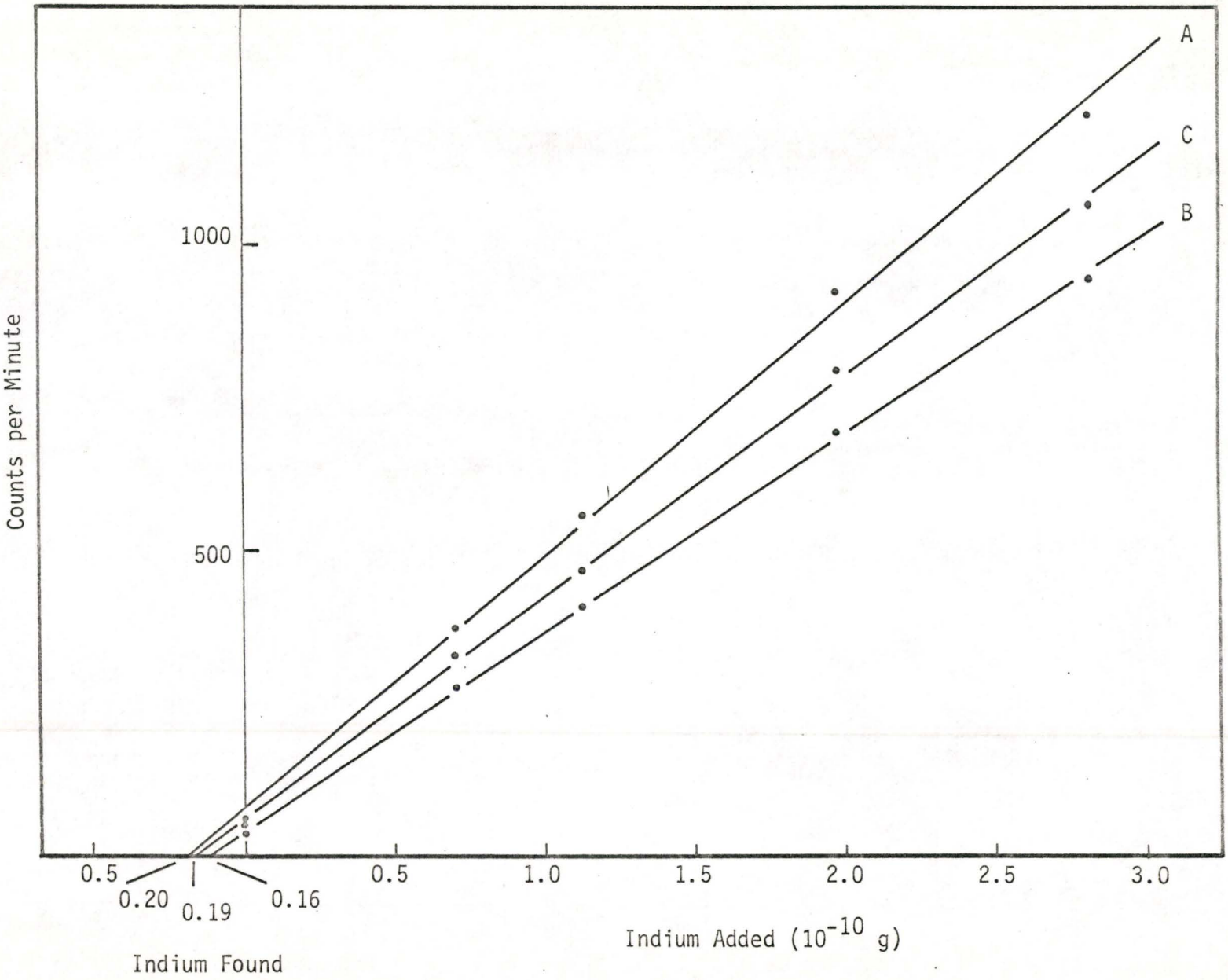
The final activity of the samples was plotted as a function of the added spike and a straight line drawn, based on a least-squares fit of the data, as illustrated in Figure 3.2, page 68. The equations of the lines calculated from the data for the 417 keV, 1097 keV and 1293 keV gamma rays and the x-intercept, which is a measure of the amount of indium present in the unspiked samples are shown. These results were converted to parts per billion by dividing by the average weight of the five samples (628.5 mg). Standard deviations of the x-intercepts were calculated from the standard error of regression, using the method of Larsen, Hartmann and Wagner.[93] The concentrations calculated from this set of experiments were 0.031 ppb ± 0.014 ppb, 0.026 ppb ± 0.012 ppb and 0.030 ppb ± 0.009 ppb, respectively, with a

Figure 3.2. Standard Addition Curves Obtained by Spiking a Series of Rabbit Liver Samples with Indium

Line A (417 keV gamma): $y = 412x + 81$
x-intercept = 0.197×10^{-10} g In

Line B (1097 keV gamma): $y = 320 x + 53$
x-intercept = 0.165×10^{-10} g In

Line C (1293 keV gamma): $y = 361x + 67$
x-intercept = 0.186×10^{-10} g In


A
C
B
1000
500
Counts per Minute
0.5
0.20
0.19
0.16
0.5
1.0
1.5
2.0
2.5
3.0
Indium Found
Indium Added (10^{-10} g)

mean of 0.029 ppb ± 0.012 ppb. This agrees with the previous result, 0.032 ppb, at the five percent level of significance. It can be seen, however, that the error associated with these values is much larger than in the case of the direct determinations, the relative standard deviation being 50% while the standard deviation of eight results obtained by the Method of Comparison is only 22%.

The analysis of the standard addition samples to determine the total indium present was carried out using the same procedure as was employed for the Method of Comparison. The difference between the measured total indium in the sample and the indium added in the spike should be comparable to the indium content of the rabbit liver as determined by the previous two methods. The results of the recovery determinations are given in Table 3.11, page 69, which includes the sample weight, the weight of indium in the spike and the recovered indium.

Table 3.11

Recovery of Indium from Spiked Rabbit Liver Samples

Sample Number	Sample Weight (g)	Indium Added (10^{-10} g)	Total Indium in Sample[1] (10^{-10} g)	Difference (10^{-10} g)	Difference (ppb)
1	0.6304	0.690	0.878 + 0.027	0.188	0.0298
2	0.6285	1.103	1.300 + 0.030	0.197	0.0313
3	0.6288	1.932	2.206 + 0.081	0.274	0.0436
4	0.6297	2.760	2.898 + 0.014	0.138	0.0319
Average Concentration of Indium in Rabbit Liver:				0.032 ppb	
One Standard Deviation:				0.007 ppb	
Standard Deviation of the Mean:				0.004 ppb	

1. The average of six values calculated from three gamma rays and two standards. One standard deviation is shown.

In the final columns, the difference between the spike and the recovered indium, and the corresponding concentration of indium in rabbit liver have been given. These values range from 0.0219 ppb for sample 4 to 0.0436 ppb for sample 3, with a mean of 0.0316 ppb ± 0.0072 ppb. This agrees with the mean determined by the Method of Comparison and with the standard addition value at the five percent level of significance.

The standard addition experiments demonstrated two things; that the use of a standard in a matrix unlike that of the sample did not introduce a bias in the results, and that complete exchange occurred between the indium in the sample and the indium carrier. From the point of view of obtaining accurate data quickly however, there are draw-backs to the use of standard addition. The error in the determined indium by the extrapolation of a straight line is much larger than in comparing the activity of the sample directly to that of a sample, and many more irradiations must be performed to obtain one value. For these reasons, further standard addition experiments were not performed.

Results from the three methods of determining indium were pooled to find the best value for the concentration of indium in dried, powdered rabbit liver. The mean of thirteen measurements (eight by the Method of Comparison, one by Standard Addition and four from the recovery experiments) was 0.032 ppb ± 0.007 ppb.

3.2.3 Calf Brain

The calf brain was the first material to be studied in these experiments, because the original purpose of the project was to find a suitable method for analysing brain tissue for indium. The concentrations

encountered in this material might also give an indication of the magnitudes to be found in human brain tissue.

Quantities of the powdered material ranging from 0.1 g to 0.7 g were irradiated and analyzed, following the same procedure described for the rabbit liver.

The results of these analyses are presented in Table 3.12, page 72. The values for the first set of analyses on the coarse tissue ranged from 0.16 ppb for sample 4 to 1.40 ppb for sample 3, with a mean of 0.56 ppb ± 0.46 ppb. When the first few analyses produced discordant results, it was thought to be due to inexperience with the method. However, as more analyses were performed, the precision did not improve, and inhomogeneity of the material was investigated as a possible cause. The coarse material was ground to a powder in an agate mortar and sieved through a 200 mesh silk screen. The sieved fraction of the material, retained for analysis, consisted of particles 74 μ and less in size. Bowen felt that 220 μ particles (which will pass through a 100 mesh screen) were sufficiently fine to ensure the homogeneity of his standard kale and it was felt that the mesh size used in the present experiments was fine enough to remove inhomogeneity caused by particle size.[78]

The precision of the results obtained from analyses of this powder was no better than that obtained for the coarse tissue, with a variation from 0.060 ppb to 1.19 ppb and a mean of 0.527 ppb ± 0.590 ppb. The reduction of particle size and removal of large pieces of tissue appeared to have virtually no effect on the distribution of indium in this material.

Table 3.12

Determination of Indium in Calf Brain

Sample Number	Sample Weight (g)	Mean Indium Concentration (ppb)[1]	Counting Error (ppb)
Coarse Tissue			
1	0.1045	0.211 ± 0.028	0.04
2	0.2112	1.40 ± 0.093	0.05
3	0.2576	0.165 ± 0.008	0.01
4	0.2818	0.401 ± 0.036	0.02
5	0.3035	1.01 ± 0.006	0.03
6	0.3079	1.19 ± 0.016	0.06
7	0.3258	0.408 ± 0.036	0.04
8	0.3429	0.193 ± 0.010	0.01
9	0.3674	0.360 ± 0.022	0.02
10	0.6323	0.262 ± 0.026	0.01
Mean:			0.56 ppb
One Standard Deviation:			0.46 ppb
Standard Deviation of the Mean:			0.14 ppb
Fine Powder			
11	0.2128	0.331 ± 0.044	0.01
12	0.4163	1.19 ± 0.016	0.02
13	0.7380	0.060 ± 0.006	0.01
Mean:			0.53 ppb
One Standard Deviation:			0.59 ppb
Standard Deviation of the Mean:			0.34 ppb
Average Concentration of Indium in Calf Brain:			0.55 ppb
One Standard Deviation:			0.54 ppb
Standard Deviation of the Mean:			0.18 ppb

1. Calculated based on values for two standards and three gamma rays for each standard. One standard deviation is shown.

The scattered results indicated that there had been contamination from an external source. The tissue had been prepared prior to being received for the indium analysis and the blender, used in the initial chopping of the dried sample, could have been one such agent. It has been suggested that even in the preparation of soft tissues, such as the calf brain, plastic utensils be used rather than metal.[17] It is also apparent that contamination occurred prior to its being received by the laboratory.

Random errors in the method of analysis and inexperience with the techniques employed were rejected as causes of the poor precision, as analyses of pine leaves and rabbit liver were performed following those on the coarse brain tissue and before the fine brain tissue powder. The precision obtained for the leaves and liver was very much better than that found for the brain. A standard kale was analyzed during the same period as the fine calf brain powder, and, while the indium concentration in the kale was of the same order of magnitude as that in the brain, the precision for a similar number of analyses was, again, far better.

Since the additional grinding and sieving of the tissue did not appear to affect either the average concentration obtained, or the precision of brain powder results, the two sets of data were combined to give an average for all determinations, of 0.55 ppb ± 0.54 ppb. Unfortunately, there was not time to secure further brain samples for analysis.

The possibility that this sample had been contaminated, meant that the concentration obtained should be regarded, at best, as an upper

limit of the indium concentration in calf brain tissue. By contrast, the only other published result for indium in brain tissue that has come to the author's attention is 51 ppb obtained for rat brain tissue.[40] This is one hundred times higher than the average concentration found in the present experiments, and thirty-five times the highest concentration obtained, 1.40 ppb for sample 2. The rat brain result has been discussed in the INTRODUCTION. It must be conceded that the precision of these results was superior to that for the present determinations. Six analyses on samples from six different animals gave an error of ± 15%. The discrepancy between the values for these two types of brain may, at least in part, be attributed to differences in the physiology of the mammals. Rats may have a facility for concentrating indium relative to cows. The history of the animals prior to analysis may also have influenced the results. Exposure of the rats to higher doses of indium might increase the concentration found, relative to the calf.[1] In the absence of another set of data for either rat brain or calf brain further discussion of the differences in the indium concentrations would be merely speculative.

The average concentration found in the present determinations is also lower than the 5 ppb to 10 ppb indium that is suspected of causing brain damage.[7] Nonetheless, an elevation in concentration of this magnitude would be observable in brain tissue normally having indium concentrations of the same magnitude as the calf brain, even with the scatter of the results obtained for this material. These estimates were only qualitative, however, and cannot be taken as a guide to concentrations that might be found in brain tissue.

3.2.4 Kale

The analysis of the standard kale prepared by Dr. Bowen was undertaken because, as far as was known, no accurate values for the indium concentration had been reported. An instrumental neutron activation analysis of kale had revealed only that indium if present was there in concentrations below 0.3 ppm, the detection limit of the method for this material.[49]

Determinations were made on four samples using the same procedure employed in the pine leaf experiments. Again the sample sizes were chosen to determine whether or not an indium concentration-sample weight correlation existed. In these analyses, however, the range of sample weights was not as wide as in the case of the pine leaves, with the extremes being 0.2 g and 0.9 g.

From the results shown in Table 3.13, it can be seen that the standard deviation of the average concentration for each sample is of the same magnitude as the random error due to counting statistics.

Table 3.13

Analysis of a Standard Kale for Indium

Sample Number	Sample Weight (g)	Mean Indium Concentration (ppb)[1]	Counting Error (ppb)
1	0.1717	0.801 ± 0.024	0.020
2	0.1723	0.577 ± 0.018	0.020
3	0.4375	0.744 ± 0.017	0.030
4	0.8654	0.644 ± 0.020	0.020
Average Concentration of Indium in Kale:			0.69 ppb
One Standard Deviation:			0.10 ppb
Standard Deviation of the Mean:			0.05 ppb

1. Calculated based on two standards and three gamma rays for each standard. One standard deviation is shown.

Values from 0.577 ppb to 0.801 ppb were found, with a mean of 0.69 ppb ± 0.10 ppb. The precision of the method for this material was ± 6%, while the standard deviation of the results corresponded to ± 14%. This relative deviation is similar to that obtained in the analysis of the pine leaves (± 11%), whose concentration was only slightly higher than that of the kale (2.20 ppb). By contrast, the precision of the powdered calf brain results, which were determined at the same time as the kale, was of the order of ± 100% for three analyses.

There appeared to be no correlation between the calculated indium concentration and the weight of sample analyzed. The extremes in the concentrations determined were both for samples of similar weight, 0.17 g. Because there were so few determinations (only four) a graph of the indium concentration as a function of sample weight was not plotted nor was a statistical analysis performed on the data to determine the variation of the slope of the line from zero. The effects of any such correlation, if one existed, were again smaller than the random error of the method. As the kale analyses were not intended for the purposes of developing the method, further experiments to demonstrate this correlation were felt to be unnecessary.

CHAPTER IV

CONCLUSIONS

It has been postulated that only neutron activation analysis, accompanied by radiochemical separation, has the potential sensitivity to measure indium at the part per billion and part per trillion levels, and therefore is the only method which can be used to analyze biological materials for this element. Experiments contained in this thesis on both plant and animal tissues have demonstrated the ability of this method to determine indium at these concentrations.

An attempt was made to account for possible sources of systematic error in the method and to eliminate or compensate for them to ensure the accuracy of these results. The difference in composition and geometry between the sample and standard matrices appeared to have no influence in biasing the indium concentrations determined. This was demonstrated in two separate experiments. The more convincing was the analysis of the rabbit liver by both the comparison of the sample activity to that of a standard which was in a different matrix and the method of standard addition in which aliquots of standard were added to a series of samples. The concentrations obtained by both methods were in agreement at the five percent level of significance, 0.032 ppb by the method of comparison, 0.029 ppb by standard addition. The second experiment employing the pine leaves, in which a series of samples varying in weight from 0.1 g to 1 g were analyzed, showed no significant variation in the indium concentration with sample weight. If the neutron spectrum was modified by the sample, the effect was

smaller than the random error inherent in the method and sampling techniques, and hence was not observed. In the absence of corroborative results by another independent method, these results and the investigation of other possible sources of error were taken as an indication (although not proof) of the accuracy of the method.

To the author's knowledge, this is the first time that indium has been determined in pine leaves and rabbit liver. The concentrations in the particular samples analyzed were 2.20 ± 0.12 ppb and 0.032 ± 0.002 ppb, respectively. (The errors shown for results in this section are the standard deviations of the means.) These concentrations refer to the freeze-dried, powdered material and it should be noted that the pine leaf sample was known to have a higher than usual heavy metal content, so the indium concentration found in the present experiments may be higher than in other samples of pine foliage.

Kale has previously been analyzed for indium by instrumental neutron activation analysis. However, the indium concentration could not be determined quantitatively. In the present experiments, using neutron activation with radiochemical separation, the concentration was found to be 0.69 ± 0.05 ppb.

Results of determinations on the freeze-dried calf brain were not as precise as those obtained for the other three materials. An indium concentration of the order of 1 ppb was found. The scattered results prohibited a more precise estimate. This poor precision was felt to be due to contamination of the sample prior to its introduction to the laboratory for analysis, or to the freeze-drying and grinding processes. Reduction of particle size part way through the series of

analyses had no effect on the precision of the results. Unfortunately, there was insufficient time for the preparation and analysis of a second brain.

As time did not permit a comparative analysis of healthy and diseased brain tissue, there is no experimental evidence that the method developed here could distinguish elevated levels of indium in affected tissue relative to healthy tissue, if such exist. The difference which would cause destruction has been estimated to be of the order of 5 ppb to 10 ppb. If the concentration of the calf brain (1 ppb) is indicative of concentrations in all brain tissue, then values elevated by 5 to 10 ppb would easily be detected and distinguished from normal levels by this method. At the concentration levels found by Kist and Lobanov in their studies on rat brain, 51 ppb, the precision of the present method would be of the same magnitude as the differences sought, and it is possible that the elevation in concentration would not be detected. The precision of the method in the present experiments, for all tissues except the calf brain, was of the order of ± 20%, and the relative difference between the normal and abnormal levels would have to be at least this large to be detected.

In conclusion, neutron activation analysis, accompanied by radiochemical separation has been applied to the determination of indium in pine leaves, kale, rabbit liver and calf brain. This is the first time that quantitative results for the indium concentration in the first three materials have been obtained. The method is considered capable of determining concentrations at the part per billion and part per trillion levels. The analysis of diseased and healthy tissue remains a project for future consideration.

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