RADIOCHEMICAL AND INSTRUMENTAL NEUTRON ACTIVATION ANALYSES OF NEOPLASTIC TISSUES. (CHEM.)

CHI SANG TSE

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DEDICATED TO MY

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ABSTRACT

Radiochemical and instrumental neutron activation analysis methods coupled with high-resolution Ge(Li) gamma-ray spectrometry have been developed in this work to study the role of trace elements in some models of neoplasia, namely, EL4 lymphoma in C57BL/6J mice and H6 hepatoma in A/J mice.

Because of its simplicity, an instrumental neutron activation analysis (INAA) method has been developed for the simultaneous determination of trace concentrations of up to 23 elements. The method consists of irradiation of the mice tissue samples for 30s, 5min and 8h, and counting for 1min, 5min and 1h after 10s, 3min and a maximum of 14d decay, respectively.

Concentrations of a few elements, namely Fe, Mg, Mn, Se and Zn in mice tissues are high enough so that they can be determined by INAA without much problem. However, the levels of certain elements such as Cu and Mo in tissues are sometimes too low to be determined by INAA in the presence of highly active nuclides of the irradiated matrix. Alternatively, a specially designed rapid radiochemical neutron

activation analysis (RNAA) method has been developed to analyze the tissues after INAA. A few other elements can also be determined by the RNAA method. Among these elements, noble metals are of interest, because of their potential of being used in chemotherapy, as demonstrated by the application of Pt-complexes.

The elements that may be determined by this RNAA method are Ag, As, Au, Cu, Hg, Mo, Pd, Pt, Sb, Se and Zn. Except Ag, Se and Zn which may be better analyzed by INAA, other elements in mice tissues may not be easily determined by INAA even with a high resolution Ge(Li) detector. Instead, the irradiated tissues have been digested and separated into 5 fractions with the removal of major interferences by means of two main stages, namely distillation and iodide column chromatography. Four fractions (containing Mo-Zn; Cu; Cd; Ag-Au-Pd-Pt) have been obtained by the latter method. The chemical yields of all the elements have been observed to be about 100% with the exception of molybdenum which is about 90%. Of particular interest is the determination of copper. Since the separated copper is radiochemically pure, the 511-keV gamma-ray emitted by ^{64}Cu can be used for assaying the copper content, which has an advantage factor of about 200 over the 1346-keV gamma-ray of ^{64}Cu which is normally used in RNAA group separation methods.

Another fraction obtained by distillation of the digested tissues has been found to contain As, Br, Hg, Sb and Se. In

the presence of highly active ^{82}Br, the determination of other elements are seriously hindered. In this work, a simple single-precipitation process has been developed to remove ^{82}Br. The photo-peaks of ^{76}As, ^{197}Hg and ^{122}Sb can then be used for elemental determination.

The results obtained by both INAA and RNAA are reported here. Differences in elemental contents among tumors, tissues of mice with tumors and tissues of normal mice of both strains have been statistically tested using the student's t-test and Wilcoxon two-sample-test. Correlation among pairs of element and multielement regression have also been studied in some cases. The results indicate that copper and selenium levels are lowest in the tumor tissues of H6, and highest in the control among the group of tumor H6, livers of H6-bearing mice, and livers of normal mice. Interestingly, both of the elements have been reported to have some functions in oxidation-reduction processes in biochemistry of cells. Since system of H6-livers is a good model, the results obtained may be indicative of the role of these two elements in the hepatoma H6. The importance of other elements determined are also reported here.

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LIST OF ABBREVIATIONS

n - neutron

b - barn

cm - centimeter

s - second

min - minute

h - hour

d - day

a - annum

g - gram

G - gravitation acceleration constant

mg - milligram

µg - microgram

ppm - parts per million by weight (equivalent to µg/g)

mL - millilitre

M - molar

% - percent

^{o}C - degree Celcius

keV - kiloelectron volt

MeV - megaelectron volt

conc.- concentrated

pptn.- precipitation

1. INTRODUCTION

The activation analysis technique involves irradiation of a sample in an intense flux of bombarding particles (such as neutrons, protons, etc.) or radiations of sufficient energy (such as bremsstrahlung, commonly known as photons) and subsequent detection, identification, and measurement of the intensities of various induced radioactivities. Since the characteristics of the nuclides formed are each unique in their nuclear half-lives, disintegration modes and energies, this technique can be used for both qualitative and quantitative determinations of a number of elements at low concentrations.

Neutron activation analysis (NAA) is the most common type of activation analysis. It was first used by Hevesy and Levi (HEV36) in 1936 for the determination of Dy impurity in Y. Since then NAA has been continously developed as a highly specific and very sensitive analytical technique for measuring multielement content in a number of complex sample matrices.

The activation equation is given by

$$A = \frac{w}{AW} \times N \times \Theta \times \Phi \times \sigma \times (1-e^{-\lambda t_i}) \times (e^{-\lambda t_d}) \quad .... (1.1)$$

where, A = the rate of disintegration,

w = the weight of the element present,

AW = the atomic weight of the element,

N = the Avogadro's mumber,

Θ = the fractional abundance of an isotope of the element,

Φ = the neutron flux,

σ = the neutron cross section,

λ = the radioactive decay constant which is equal to $0.693/t_{\frac{1}{2}}$,

$t_{\frac{1}{2}}$ = the time required for half of the original radioactive isotope to decay,

t_i = the time of irradiation, and

t_d = the time interval between the end of irradiation and the time at which the activity is determined.

In reality, it is the number of counts, c, which is recorded. For nuclides whose half-lives are much longer than the duration of measurement, t_m, the following equation holds.

$$c / t_m = k \times A \quad .... (1.2)$$

where k = a constant for the counting system.

It is possible to use an absolute NAA method for elemental determination by placing the values of the above parameters in equation 1.1. However, these values are not that accurately known for all the elements of interest. Moreover, better precision and accuracy can be obtained by using a ratio technique (otherwise known as a comparator method) in which all the above factors are calibrated simultaneously by a known mass of pure element activated and measured under identical experimental conditions as the unknown sample. By this procedure the activity produced per unit mass should be the same for both the sample and standard, when self-absorption and neutron attenuation are negligible. Mathematically,

$$\left(\frac{A}{W}\right)_{\text{sample}} = A_{sp}, \text{ sample} = A_{sp}, \text{ standard} = \left(\frac{A}{W}\right)_{\text{standard}} \quad (1.3)$$

where the subscript sp stands for specific, and W is the weight of the element. This proportionality allows for more accurate calculations provided that the weight of the element and the physical form of the standard approximate those in the sample, and the isotope abundances are the same in both the sample and standard.

The neutron flux in a reactor may not always be very stable. In that case, a flux monitor (a Cu wire is most commonly used) may be irradiated simultaneously with the sample. A correction term for the flux is made by measuring

the activity of the wire.

In cases where interfering gamma-rays are absent, particularly when high-resolution detectors such as Ge(Li) detectors are used, the gross gamma-ray spectrum can be recorded and the peak intensity can be compared directly with that of a standard. The elemental levels of a sample can then be determined by assaying the gamma-ray intensities of the nuclides produced on neutron irradiation. This technique is known as instrumental neutron activation analysis (INAA). When thermal neutrons are used for activation, the above technique is referred to as thermal INAA (TINAA).

Sometimes one or more nuclides (often called interfering nuclides, e.g., ^{24}Na, ^{38}Cl, ^{82}Br, etc.) in the sample are formed in quantities large enough to mask the gamma-rays emitted by other nuclides of interest. In such situations, determinations of trace elements by TINAA are rather difficult. An alternative to TINAA is to carry out destruction (e.g. wet ashing) of the sample followed by either removal of the interfering nuclides or the extraction of the nuclides of interest from the matrix. Since this process involves some kind of chemical treatment, it is called radiochemical neutron activation analysis (RNAA).

Since NAA is basically a multielement technique, there exists a possibility of determining several elements

simultaneously by RNAA. A multielement separation scheme is usually employed for this purpose, in which traditional analytical methods are generally used for the separation of individual elements. Inactive carriers are added at the beginning of separation and the chemical yields are determined at the end by conventional analytical methods. After the emergence of high-resolution Ge(Li) detectors, a number of elements can be separated into groups and their concentrations can be determined directly from the well-resolved gamma-rays. Even the recovery values (i.e. chemical yields) can be determined by re-irradiating the separated fractions containing the recovered carriers followed by gamma-ray spectrometry.

The extreme case of the former approach will be the analysis of one element at a time. For example, arsenic has been separated by gravimetric precipitation with ammonium hypophosphite after distillation as $AsCl_3$ from the residue of wet digestion of a sample (IRV42). Copper has been quantitatively extracted by diethyldithiocarbamate (DDC) into chloroform phase from an aqueous solution of a sample (LED64a), or more selectively by cadmium carbamate (WYT 75). Acetylacetone has been found to be a selective extraction reagent for molybdenum in ferrous materials (MCK 57). It furnishes a very good method for separating molybdenum from tungsten which has chemical properties so similar to

molybdenum. Vanadium as ^{48}V has been decontaminated from titanium by passing the sample solution through a fluoride loaded anion exchange column (IRV 58). All these methods have been reported to be very specific and to give high chemical yields. Several other such methods have been published (LED 64b).

The most important advantage of the above approach is its freedom from interference. Since the separated element is radiochemically pure, the separated fraction is generally counted in a thallium activated sodium iodide, NaI(Tl), detector which has poorer resolution but higher counting efficiency than a Ge(Li) detector. The sensitivity of determination of the element is thus improved. The most important disadvantage of this approach is that it can not easily be incorporated in a multielement determination scheme. Unfortunately, trace multielement concentrations in a variety of matrices are generally sought.

Several multielement RNAA separation schemes have been published in literature. These schemes can be classified into two categories. In the first category the elements are separated sequentially while in the other the elements are separated into groups. Examples of the first category include separation schemes proposed by Albert and Gaittet (ALB 61), Moiseev et. al. (MOI 65), Aubouin et. al. (AUB 65)

Van den Winkel (WIN 67). The second category includes schemes reported by Ross (ROS 64), Gebauhr (GEB 66), Samsahl et. al. (SAM 65), Girardi et. al. (GIR 65), Jervis et. al. (JER 67), May and Pinte (MAY 69), Morrison et. al. (MOR 69). One of the objectives of the present project is to develop RNAA group separation schemes for trace multi-element determinations in neoplastic tissues.

A few trace elements are well known as potential health hazards. There is an increasing interest in understanding the relationship between several diseases and concentrations of many trace elements. For example, certain trace metals, such as Cr are suspected to be related to cardiovascular diseases (IAE 73). The role of trace elements in cancer has only been studied to a limited extent. Two centuries ago, the induction of tumors in man by chemicals was first discovered (RED70) . Since then, a number of organic chemicals which can induce neoplastic diseases have been found. Several inorganic compounds have also been reported to cause cancer. Compounds of As, Cd, Cr, and Ni have been implicated as human carcinogens. And compounds of Be, Cd, Co, Cr, Fe, Ni, Pb, Ti, and Zn have been reported to induce cancers in experimental animals (SUN 78).

Lympthomas and solid tumors, especially the latter which comprise the majority of human cancer, continue to

challenge the best efforts of both the experimentalist and the clinician. There are so many questions posed by these tumors that some of them can only be answered by the use of animal tumors as models.

Inorganic elements may act as inducers of cancer. Since cancer tissues may be involved in different metabolism mechanisms, certain elemental contents may be elevated while the others may be depressed. For example, concentrations of essential elements such as Na, K, Ca, Mg, and Zn in the breasts with carcinoma have been reported to be significantly higher than those in healthy breasts (MOH 74). If the mechanism is thoroughly understood, the fatality of neoplastic diseases may be suppressed by changing the concentrations of some metabolites involved in the diseases and the technique of using antimetabolites (BRE 64, STO 66, BER 77, CAP 77) in curing cancer will be further improved.

On the other hand, chelating compounds of some elements are being used in cancer treatment, such as in chemotherapy of certain types of neoplasia. The elements which are being studied extensively in cancer treatment are copper, gallium and platinum (SAR 69, HAR 71, ADA 75, GOT 75). Therefore, it is of interest to investigate the possible interactions of metal ions with certain types of cancer. Correlations among concentrations of elements in neoplastic

cells and tissues may lead to a better understanding of the causes and effects of cancer. Such an attempt has been made in the present study.

The primary objective of this project is to develop NAA methods for the determination of trace elements in normal and neoplastic tissues. Several such methods have been developed and described here under two schemes. The first scheme involves RNAA methods only and consists of distillation, iodide column chromatography, chloride column chromatography, and TOPO solvent extraction steps. This scheme utilizes only long-lived nuclides for determining trace concentrations of Ag, As, Au, Br, Co, Cu, Fe, Hg, I, K, Mg, Mn, Mo, Na, Pd, Pt, Rb, Sb, Se and Zn in tissues. The developments of both schemes are described in detail. The precision and accuracy of the methods have been evaluated by analyzing standard reference materials. The limits of detection have been calculated and reported here.

The other objective of this project is to apply the above INAA and RNAA methods to tissues collected from normal and tumor-bearing experimental mice. Trace essential and toxic elements could play an important role in the etiology of neoplastic diseases. Statistical correlation tests between concentrations of pairs of elements have been carried out to determine the role of trace elements in neoplasia. The results

are treated here within the author's limited knowledge of neoplasia, pathology, toxicology and statistics, and with the assumption that the experts in these fields will be able to make a more rigorous interpretation of these results.

2. PRINCIPLES OF ANALYTICAL TECHNIQUES AND LITERATURE SURVEY

The principles of radiochemical and instrumental neutron activation analyses are described here. Various separation methods, such as distillation, precipitation, ion-exchange chromatography and solvent extraction are discussed and their applications to matrices of interest are summarized. Errors involved in activation analysis are assayed. Background materials on neoplastic cells and tumors are also presented here.

2.1 Radiochemical Separation Methods

The most commonly used radiochemical separation methods are distillation, precipitation, ion exchange, and solvent extraction. The principles, advantages and applications of each of these methods are briefly described below.

2.1.1 Distillation

Many elements can be separated from complex matrices by methods based on their volatility (DES72). The separated elements can be either in the elemental state, e.g., halogens (SAM67); as halides, e.g., $AsCl_3$, $HgCl_2$, $SbBr_3$, $SeCl_4$, and

$SnCl_4$ (IYE76); as oxides, e.g., RuO_4 and OsO_4 (MEY64); or as hydrides, e.g., AsH_3 and SeH_4 (GRE63). Some other examples of distillation are given in Table 2.1.

The vapor generated during the distillation process is exposed to a relatively large surface area of the glassware used, and condensed to a liquid. In order to ensure quantitative recovery of microgram quantities of radioactive species by distillation, the addition of milligram amounts of an inactive carrier is necessary. Since the purity of the separated fraction can be rather high, several radiochemical separation schemes have included a preliminary distillation step (MOR69). This step has the advantage of removing the volatile radioactive species from the sample at the very beginning of radiochemical separations, which may otherwise cause health hazard to the experimenter. On the other hand, distillation may require a little bit more space and is slightly more complicated than other methods mentioned later. Distillation methods have been developed in this project to separate As, Br, Hg, Sb and Se from tissues (Section 4.2). Complete volatilization of bromine activity was achieved through the use of large amount of HBr as described.

2.1.2 Precipitation

Precipitation is one of the commonly used separation methods. When the product of activities of the participating

TABLE 2.1 APPLICATIONS OF DISTILLATION IN RNAA

Element separated	Compound distilled	Matrix	Method	Reference
As	$AsBr_3$	hydrocarbon reforming catalyst	Na_2O_2;500°C;HBr,H_2SO_4	SHI58
Sb,As	SbH_3,AsH_3	fission products	Zn,H_2SO_4;heat decomposition	GRE63
Hg,As	$HgCl_2$,$AsCl_3$	biological material	HNO_3,H_2SO_4;$HClO_4$,glycine	SJÖ64
Se	H_2Se	fission products	Zn,H_2SO_4;$CaSO_4$ trap	TOM68
As,Br	$AsBr_3$,Br_2	rocks	HF;HBr,H_2SO_4	MOR69
Tc	TcO_7	reactor alloy fuel	aqua regia;NaOH,NaOCl; HCl;HBr,H_2SO_4	MEY64
As,Br,Hg, Sb,Se,Sn	$AsBr_3$,Br_2, $HgBr_2$,$SbBr_3$, $SeBr_4$,$SnBr_4$	biomedical material	H_2O_2,H_2SO_4;HBr trap1:H_2O_2,H_2SO_4 - As,Hg,Sb,Se,Sn trap2:NaOH - Br	IYE76

TABLE 2.1 Cont'd

Element separated	Compound distilled	Matrix	Method	Reference
As	$AsCl_3$	rocks	NaCl-condensed phosphoric acid	TER76
Se	$SeBr_4$, $SeCl_4$	human tissues	HNO_3, H_2SO_4; H_2O_2; HCl, HBr SO_2 - Se element	PLA78
As, Sb, Se	$AsBr_3$, $SbBr_3$, $SeBr_4$	biological material	H_2O_2, H_2SO_4; HBr H_2SO_3 - As element H_3PO_2 - Se element S^{2-} - Sb_2S_3	ROS79

cation and the added anion is greater than the solubility product (K_{sp}) of the compound, precipitation occurs. It usually requires the addition of a carrier and sometimes a hold-back carrier (of about 10mg each) in order to minimize losses due to solubility of the precipitate and surface adsorption of the ions on the walls of apparatus.

Metal ions can also be coprecipitated with a scavenger. Certain hydrated oxides and hydroxides, especially ferric hydroxide, are frequently used. Ferric hydroxide has been used for separating trace yttrium from strontium (WEL64), hafnium from tantalum (NOV68), etc. The selectivity of such a coprecipitation is generally low. Organic reagents on the other hand, can be used as selective precipitants (WER67). Cocrystallization with organic reagents has been applied to determine trace metal ions (FIR69). Coprecipitation and adsorption on a preformed precipitate may be employed to eliminate the necessity of adding a large amount of carrier. The latter process may involve mechanisms of ion-exchange, isotope exchange, precipitation, and mixed crystal formation by recrystallization (HER69). A few applications of precipitation reactions in RNAA are given in Table 2.2.

In general, precipitation methods are not used in multi-element separation schemes (WEI73), primarily due to incomplete precipitations and interferences from **adsorption**. Yet, it is frequently used in the last step of radiochemical

TABLE 2.2 APPLICATIONS OF PRECIPITATION METHODS IN RNAA

Element separated	Precipitate	Matrix	Method	Reference
Ba	$BaCl_2$	fission-products	HNO_3;$BaCl_2$;HCl-diethyl ether	SEY53
Ag	AgCl;Ag	meteorites	Na_2O_2;HCl;Zn	SCH60
Rh	K-Rh-nitrite complex;Rh	meteorites	Na_2O_2;$FeCl_3$,NH_4OH; KCl,KNO_2;Zn	SCH60
Ag,Bi,Cu, Mo,Te;W; Al;Co,Cr, Mn,Sc;Cd, Ni,Zn;Ca, Sr;K	AgCl,$BiPO_4$,Cu-salicyald-oxime,Mo-α-benzoin-oxime, Te^o;W coprecipitated on Mo-α-benzoin-oxime;$AlCl_3$; $6H_2O$;$Cr(OH)_3$,Co-α-nitroso-β-naphthol,MnO_2,$Sc(OH)_3$; CdS,NiS,ZnS;$CaCO_3$,$SrCO_3$; $KClO_4$.	high purity aluminium	group separations based on several precipitation processes	ALB61
Hg	Hg^o	biological material	HNO_3,H_2SO_4;NaOH;HNO_3; ascorbic acid	SMI63

separation, where the element of interest has already been isolated from its possible interferences. Precipitation reactions have been used in this project to obtain radiochemically pure fractions in solid form and to determine chemical yields of the separated elements (Chapter 4).

The choice of a precipitant is governed by the criteria that it should not cause the precipitation of interfering elements and the K_{sp} of the compound of interest should be less than 10^{-10}. To minimize the adsorption of other elements, the amount of carrier added should be limited to about 10mg.

2.1.3 Ion Exchange Chromatography

Ion exchange chromatography is widely used in radiochemical separations. A few examples are shown in Table 2.3. Ion exchange chromatography includes simple ion exchange, cation exchange with complexation, anion exchange in the presence of high concentrations of strong ligand acids (e.g., HCl and HF) and the use of mixed solvent systems (SAM63, RIE70). Ion exchange separations can be carried out rapidly, selectively, using simple equipment, and under varied conditions. Since several ion exchange methods have been developed in this study (Chapter 4), principles of the basic technique are dealt with here in detail.

The presence of an ion exchanger creates a resin phase

TABLE 2.3 APPLICATIONS OF ION-EXCHANGE IN RNAA

Element separated	Matrix	Method	Reference
As,Cd,Hg,Zn	vegetation human head hair	HCl,HNO_3;in 6M HCl;Dowex 1* 6M HCl - As 0.05M HCl - Zn 0.001M HCl - Cd 0.01M HCl/0.1M thiourea - Hg	CHA72
Na;Au,Cd,Hg,Ir,Mo,Sb, Sn;Co,Cu,Fe;Zn;Ag,Ba, Ca,Cr,Cs,K,Mn,Rb,Sr, rare earths;P	human tissues	HNO_3,H_2O_2;11M HCl;HAP;Dowex 1; Dowex 50**	PLA73
Au	Cu;Cd	$HCl,HNO_3;H_2O$;Dowex 1;0.2M HNO_3; 0.5M HCl	HIR77
As,Cd,Hg	fish	HNO_3;in 6M HCl;Dowex 1 6M HCl - As H_2O - Cd column - Hg	ANA78
^{210}Bi(RaE)	^{210}Pb(RaD)	$(NH_4)_2MoO_4$;silica gel column***	BHA78

* Dowex brand is equivalent to AG brand in Table 2.4 but of lower quality.

** HAP is an abbreviation of hydrated antimony pentoxide, an inorganic exchanger.

*** Silca gel acts as a cation exchanger.

out of the aqueous phase. For monovalent ions of extremely low concentration in resin phase, ion exchange reactions may be regarded as Donnan equilibria (RIE70a). The relationship between the ion activities in the resin phase (r) and those in the external solution (w) can be described by

$$a_{H_r} \cdot a_{L_r} = a_{H_w} \cdot a_{L_w}$$

$$a_{M_r} \cdot a_{L_r} = a_{M_w} \cdot a_{L_w}$$

$$\left(\frac{a_H}{a_M}\right)_r = \left(\frac{a_H}{a_M}\right)_w$$

where a = activity, H = hydronium ion, L = ligand, and M = metal ion.

That is to say the exchange is proportional to the concentration. In the case of multi-valent ions, they may cause swelling of the resin to different extents. And because of being highly charged, their activities can no longer be represented by their concentrations.

In the thermodynamic approach, for the exchange reaction

$$M + HR \rightleftharpoons MR + H$$

where R = resin matrix,

an equilibrium constant K may be defined by

$$\left[\frac{a_M}{a_H}\right]_r \cdot \left[\frac{a_H}{a_M}\right]_w = K$$

It may be rewritten as

$$\left[\frac{X_M}{X_H}\right]_r \cdot \left[\frac{C_H}{C_M}\right]_w \cdot \left[\frac{\gamma_H}{\gamma_M}\right]_w = K \cdot \left[\frac{\gamma_H}{\gamma_M}\right]_r = K_a$$

where X_H and X_M are the equivalent fractions in the resin phase, C_H and C_M are the ion concentrations in the external solution, and γ s are the activity coefficients. In relatively dilute solution, e.g., $\leq$ 0.01M, Debye-Hückel limiting law can be applied:

$$\log \gamma = -0.5091\ Z^2 \sqrt{\mu}$$

where Z = the charge of the ion,

μ = ionic strength

$= \frac{1}{2} \Sigma m_i Z_i^2$

So the ratio of $\left(\gamma_H / \gamma_M \right)_r$ is a constant. Therefore, an apparent equilibrium constant, K_a, can be assigned. And thus the selectivity can be deduced. However, in practice, the concentration of the ions is about 10M in the resin phase — the Debye-Hückel limiting law does not hold anymore. Empirical equations can be used in such cases (ROT19). One such equation is given as

$$\left[\frac{X_M}{X_H}\right]_r \cdot \left[\frac{C_H}{C_M}\right]_w^p = k$$

where k and p are empirical parameters. Their values are not usually determined. So some empirical guidelines may be useful. They are described in the following paragraphs.

a) Electostatic field effect

For strong acid type cation exchanger and strong base type anion exchanger, the charges in the charge-bearing sites of the resin are relatively localized. There is a strong electostatic attraction for the oppositely charged ions. Therefore, ions of high charge density will be retained strongly on the resin.

b) Non-Coulombic forces

For some ions which have completely filled inner electron orbitals outside of inert gas configuration, polarization of electron clouds enhances the strength of binding to the exchanger. For anion exchanger, the situation is more complicated. Electric double layer effect, polarization, stability of metal-halide complexes, and interaction with the water structure may take place. Due to these effects, large differences in binding strength are exhibited by anion exchange reactions.

In short, the combination effect of electrostatic, polarization, and the spatial arrangement of the exchange

sites determines the order of selectivity while the molecular frame-work, which is important to organic species, is of minor consequence in inorganic ionic species. An ion with high affinity to the exchanger will have a large distribution coefficient. The ratio of the distribution coefficients of two ions gives the separation factor. And the difference in affinity increases with increased ionic strength in the resin phase. The affinity of different ions in commonly used exchangers are listed in Table 2.4 (BI076).

Two types of ion exchange chromatography, viz., frontal and displacement chromatography, do not normally give a quantitative separation of the constituents of a sample while elution chromatography does. In the elution chromatography, the ions to be separated are first adsorbed on the column. An eluent passing through the column elutes the adsorbed ions in succession. The ions of lower distribution coefficient will be eluted first. The peak elution volume, v, can be estimated using the following equation (RIE70b).

$$D = \frac{v - i V}{i V}$$

where V = the total volume,

D = the distribution coefficient, and

i = the void fraction of the column.

The width of the peak is approximated by the theoretical plate number, N (GLU55). The higher the number, the better is the resolution of the corresponding column.

TABLE 2.4 ORDER OF SELECTIVITY OF IONS IN COMMONLY USED EXCHANGERS[a]

Resin type	Active group	Selectivity	Order of selectivity
AG 1 (strongly basic anion exchanger)	ϕ -$CH_2N^+(CH_3)_3$*	Cl^-/OH^-=25	$I^- > HSO_4^- > ClO_3^- > NO_3^- > Br^-$ $CN^- > HSO_3^- > NO_2^- > Cl^- > HCO_3^-$ $IO_3^- > H_2COO^- > Ac^- > OH^- > F^-$
AG 3 (weakly basic anion exchanger)	ϕ -$CH_2N^+HR_2$	-	$CrO_3 > H_2SO_4 > H_3PO_4 > H_3AsO_4$ $HNO_3 > HI > HBr > HCl > HF$ $HCOOH > HAc > H_2CO_3$
Chelex 100 (weakly acidic cation chelating exchanger)	ϕ -$CH_2N(CH_2COO^-)_2$	$Cu^{2+}/Na^+ \gg 100$	$H^+ > Li^+ > Na^+ > K^+$ $Cu^{2+} > Fe^{3+} > Cr^{3+} > Ni^{2+} > Zn^{2+}$ $Ag^+ > Co^{2+} > Cd^{2+} > Fe^{2+} > Mn^{2+}$ $Ba^{2+} > Ca^{2+} > Na^+$
AG 50W (strongly acidic cation exchanger)	ϕ -SO_3^-	$Na^+/H^+ \triangleq 1.2$	$Ag^+ > Rb^+ > Cs^+ > K^+ > NH_4^+$ $Na^+ > H^+ > Li^+$ $Zn^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+}$

* ϕ - polymer lattice

[a] Ref. BI076

$$N = \frac{L}{h} = 8\left(\frac{v}{\beta}\right)^2$$

where h = the height of an equivalent theoretical plate

L = the length of the column, and

β = the width of the peak where the concentration is equal to the maximum peak concentration divided by e, the base of natural logaritum. Experiments were carried out in this study to obtain optimum values for above parameters.

In developing the best condition, there are a few factors which affect the elution curve.

a) The effect of the concentration of the eluent:

In an elution with an eluent E for a bound ion M, an equation can be derived as (RIE70c):

$$zE + M\,zR \leftrightharpoons M + z\,ER$$

where z is the charge of the metal ion, M.

$$K = \frac{(M)\cdot(ER)^z}{(E)^z(MzR)}$$

By definition,

$$D = \frac{W}{iV}\cdot Q\cdot\frac{(MzR)}{M}$$

$$= \frac{W}{iV} \cdot \frac{Q}{K} \cdot \frac{(ER)^z}{E^z}$$

So $$v = iV \left(\frac{W}{iV} \cdot \frac{Q}{K} \cdot \frac{(ER)^z}{E^z} + 1 \right)$$

where K = the equilibrium constant,

W = the dry weight of the resin in the column,

Q = the specific exchange capacity of the resin, and

E = the eluent.

From the above equation, it can be seen that an increase in the eluent concentration will decrease the peak volume, i.e., the ion will be eluted faster. A change in the concentration of the eluent will be effective in improving a poor separation of the ions of different valences. If two peaks overlap with the ion of lower valence emerging first, a decrease in eluent concentration should be applied. These factors were taken into consideration in developing an iodide column (Chapter 4).

b) The effect of pH of eluent

In cation exchange, a variation in pH is merely a change in eluent concentration. In anion exchange, if the ion is a conjugated base of a weak acid, a slight change in pH will cause a drastic change in the affinity of the ion to the exchanger. At low pH, a weak acid is only slightly dissociated. The average charge density per anion will be decreased and so

the anion will be eluted more easily. This effect is less noticeable in anions of strong acids. Thus for separating anions of weak acids from those strong acids, optimization of pH is important.

c) The effect of complexing agents in the eluent

In ion exchange, the charge of the metal ions greatly affectstheir affinity to the resin. Many metal ions form complexes with ligands and most ligands are the anions of weak acids. The concentration of free ligands in a given solution may be decreased by decreasing the pH. Variations in pH may be useful in separating complexed metal ions. In anion exchange, simple anionic ligands are usually employed because of their ability of giving maximum coordination such that the complexed metal ions have high negative charge. Multi-dentate ligands have too high an affinity to be eluted from the exchange column, and thus not generally used.

Most metal ions show maxima in the plots of distribution coefficient versus the concentration of anionic ligands, if complexes are formed. An explanation for that is at moderate ligand concentration a slight increase in ligand concentration will bring about more complexes. Thus higher distribution is obtained. As the ligand concentration is increased further, the chance of displacing the complexes by the anion itself will be increased to such an extent that elution is achieved.

These principles have been used in developing the iodide column (Chapter 4).

Neutral complexing agents, such as thiourea, can be used as an eluent. The negative charge densities of the clusters of the simple-anion-complexed metal ions which form complexes with thiourea, are decreased thereby diminishing the tendency of them getting attached to the anion exchanger. So that they can be eluted. The non-complexed metal ions will not be affected, or affected only slightly. Thiourea has been used in the present study for extracting noble metals from the iodide column (Chapter 4).

2.1.4 Solvent Extraction

Liquid-liquid extraction is widely used in radiochemical work (Table 2.5) as it can be carried out rapidly with simple equipment which is capable of being automated. It can be made very selective and can be applied to a wide range of concentrations. Detailed description of solvent extraction is given in the books by Morrison and Freiser (MOR62), and Stary (STA64). The separation depends on the difference in partition coefficients between aqueous phase and an immiscible organic phase. The extracted materials may consist of simple molecules, or as ions made soluble in the organic phase by the formation of chelates, ion pairs or by solvation or ion exchange processes. Solvent extraction methods have been

TABLE 2.5 APPLICATIONS OF SOLVENT EXTRACTION IN RNAA

Element separated	Matrix	Method	Reference
Mo	ferrous material	H_2SO_4;HNO_3;filtration;1:1 chloroform-acetylacetone	MCK57
Co,Mn,Pb,Zn	biological material	HNO_3,H_2O_2,H_2SO_4;1:1 acetylacetone -chloroform (pH2);potassium tartrate;diethyldithiocarbamate (pH6)	ERK61
Cs	reactor fuel	0.01M EDTA/1M TPB;1M HCl-acetone*	CES65
Fe	atmospheric materials	7.5M HNO_3/1.5M HDEHP;4M HCl**	BAG70
Cd,Cu,Mo,Zn	biological materials	6M HCl/TOPO(5% w/w in cyclohexane); TOPO/6M HNO_3***	STE71

* EDTA - ethylenediaminetetraacetic acid;TPB - tri-n-butyl phosphate

** HDEHP - di (2-ethylhexyl) orthophosphate

*** TOPO - tri-octyl phosphine oxide

TABLE 2.5 Cont'd

Element separated	Matrix	Method	Reference
Cr,Mo;Cd,Cu;Zn	biological material	HNO_3,H_2SO_4;K_2CO_3,KNO_3;in 0.5M H_2SO_4; 5% TNOA in CCl_4;0.1% DDDC in CCl_4; 1% DDDC in CCl_4,pH4 *	TRO73
V	biological material	H_2SO_4;HNO_3,H_2O_2;in 5M HCl/BPHA**	BYR78
V	rat liver and blood	dry-ashing;3M HCl;cupferron/$CHCl_3$	COR79

* TNOA - tri-n-octylamine
DDDC - diethylammonium diethyldithiocarbamate

** BPHA - N-benzoyl-N-phenyl-hydroxylamine

developed in this project to separate Cd, Cu and Sc (Chapter 4).

Chelate extraction is the most commonly encountered method.

$$(M^{n+})_w + n\,(HL)_o \leftrightharpoons (ML_n)_o + n\,(H^+)_w$$

$$K_{ex} = \frac{(ML_n)_o}{(M^{n+})_w} \times \frac{(H^+)^n_w}{(HL)^n_o}$$

$$D = \frac{(ML_n)_o}{(M^{n+})_w} = K_{ex} \cdot \frac{(HI)^n_o}{(H^+)^n_w}$$

where the suffixes w and o denote water and organic phases respectively, K_{ex} is the extraction constant, and D is the distribution constant. Assuming that other metal ions do not participate in the reaction or to a lesser extent, then the higher the distribution coefficient, the better is the separation. This technique usually gives high recovery values, and only requires minimum amount of carriers, if at all.

Besides extraction by simple chelate formation, extraction may be enhanced by the formation of a ternary or adduct-complex consisting of the cation, a suitable chelating agent and a neutral donor-molecule (MIT74).

A few things have to be considered in practice. The solubility of the extracted compound should be high in the

extractant. The separation factors should be as large as possible. They may be brought about by the choice of extractant and solvent, the use of masking agents, and pH control. Also the solvents used should be mutually immiscible. The difference in density between them should be large in order to achieve rapid separation of the phases and minimum formation of emulsion. These requirements have been considered in developing solvent extraction methods in the present project.

2.1.5 Possible Errors in RNAA

Besides the indeterminate error associated with the counting statistics, a few possible determinate errors may occur in RNAA. Personal errors are possible but their effect on the result can be minimized by strict displine, such as rechecking data, using clean glassware and developing good work habits. These errors, of course, apply to any analytical measurement.

Chemical yields of separated elements should be determined whenever possible. Tracer studies are often carried out to ensure reproducibility of chemical yields. The accumulative error derived from the determination of the radioactivity of the nuclides and the recovery is generally not high as long as reasonable count rate and recovery of more than 95% are achieved.

Error may arise from using the comparator technique for determining elemental content. The amount of an element may be calculated by using the activation equation where the radioactivities of the sample and standard are corrected to a same reference condition, e.g., saturation in irradiation and zero decay time. In doing so, the inaccuracy in the half-life values used will impart errors in the correction terms. Thus the samples and the standards should be irradiated and counted for approximately the same time periods.

Variation in the geometry of the final precipitates can cause error. It can be eliminated by using identical geometry of samples and standards-such as the one developed in this study and described in chapter 3. Variations in isotopic abundance of up to several percent have been reported in geological samples. It is not likely to occur in tissues of similar sample source used in this work.

Perhaps the most important error can come from the variation in the neutron flux. A flux monitor may be irradiated with the sample and appropriate correction factors may be applied. However, such a correction was not needed as the flux in the irradiation sites of the SLOWPOKE-2 reactor (used in this work) has been found to be steady over a long period of time (RYA78).

Another error may arise in the estimate of the elemental

content due to the possible existence of interfering nuclear reactions. The common interfering reactions are (n,p) and (n,α) type, which are generally caused by epithermal and fast neutrons. Since the cross-sections and the flux of epithermal and fast neutrons are much lower than those of thermal neutrons, the interference is usually not significant in the analyses of tissue samples except for a few elements. The degree of interference can be assessed using the following equation

$$A_{int}/A = 0.22 \times \frac{\theta_{int}\,\sigma_{int}\,W_{int}\,M}{\theta\,\sigma W\,M_{int}}$$

where the subscript 'int' refers to the interfering reaction

A_{int}, A = activities of the nuclide due to the interfering reaction and the reaction of interest, respectively

W_{int}, W = weights of the target elements present

M_{int}, M = atomic weights of the target isotopes

0.22 = the ratio of fast neutrons to thermal neutron flux in DUSR.

The possible interfering reactions and their contributions are discussed in Chapter 4.

2.2 Instrumental Neutron Activation Analysis

Perhaps the most important disadvantage of RNAA is the long time normally required to analyze even one sample. It

is not, most of the time, possible to do multielement analysis of a large number of samples by RNAA. On the other hand, INAA has the advantages of being simple, rapid, non-tedious, sensitive for elements with high cross section and short-lived nuclides, and non-destructive. It can be easily used in routine analyses, especially after the advent of high-resolution Ge(Li) and LEPD detectors and powerful minicomputers.

2.2.1 Thermal and Epithermal INAA

Neutrons produced in a nuclear reactor can be classified into three categories depending on their energy: fast neutrons with energy >1 MeV, resonance neutrons with energy between 0.4eV and 1 MeV, and thermal neutrons with energy of 0.025eV. Resonance neutrons are sometimes called epithermal neutrons. The general features of a reactor neutron spectrum (HUG53, GLA58, BON57) is shown in Fig.2.1.

Intense activities due to thermal neutron activation products of Al, Cl, Na, Sc, V, etc. often seriously interfere in the determinations of other short-to medium- lived nuclides of interest. Resonance activation integrals (I_o) of isotopes of these elements are smaller than the corresponding thermal neutron cross sections (σ_{th}). Also several elements, e.g. As, Au, Cd, I, Mo, etc. have strong resonance peaks at neutron energies between 5 and 500eV. Therefore, if a sample is irradiated with epithermal neutrons,

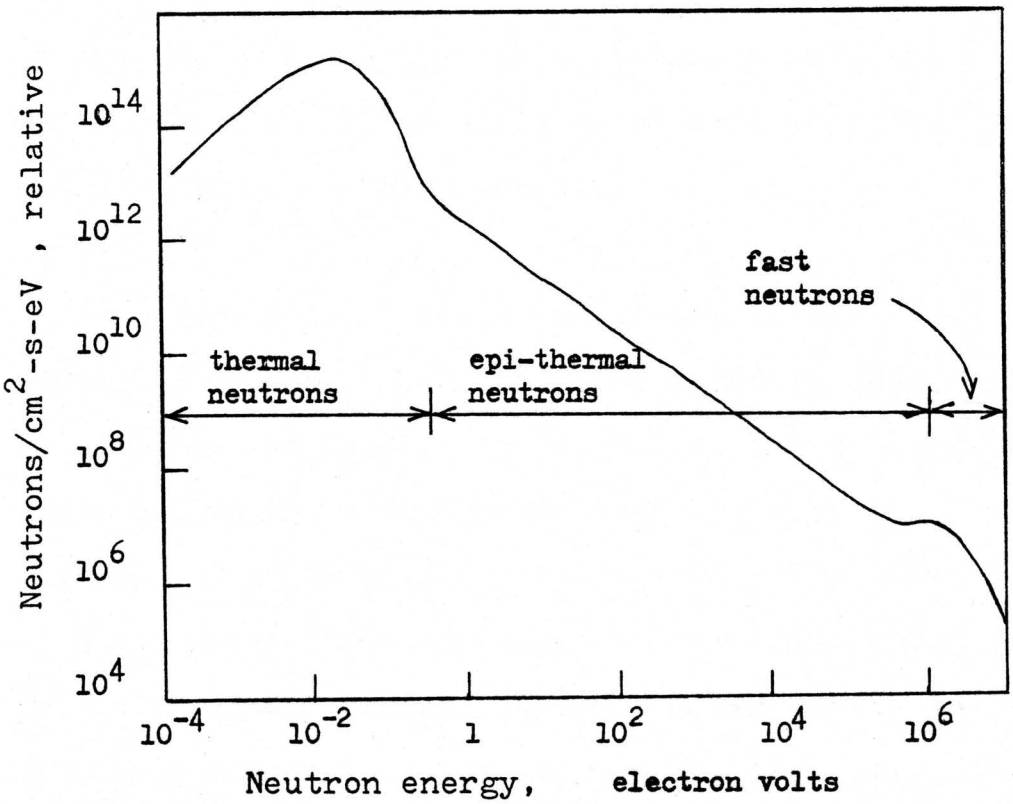


Fig.2.1 General features of a reactor neutron spectrum (HUG53,GLA58,BON57).

activities of the nuclides of interest are expected to be enhanced against a relatively low background of interfering elements. This enhancement can be advantageously used in developing epithermal instrumental neutron activation analysis (EINAA) methods.

Cadmium and boron are commonly used as thermal neutron absorbers. Details of these shields have been discussed by Rossitto et al (ROS72). This technique has been used in biological material (HAN76), coal and fly ash (STE77) and geological materials (MEY71)(UNN77). Attempts have been made

in this work to develop an EINAA method for tissue samples (Chapter 4). Another type of neutrons, namely the fast neutrons, is not used in analyses with reactor neutrons, because of their low flux density.

Using neutrons of different energy is one of the ways of reducing interference. The most commonly used procedure in activation analysis is to coordinate timing parameters so that different nuclides with very different half-lives can be detected. Optimization of the irradiation time, decay time and counting time can suppress the interfering gamma peaks to a certain extent. The optimized conditions vary from sample to sample depending upon matrix composition. Some typical examples are shown in Table 2.6. Biological materials have been analyzed by irradiating the samples for 1-2min then counting for 1-30min at decay times varying between 1min and 12h. The samples have been further irradiated for 80h and counted for 1h after decay at intervals of 3-5 days, 2 weeks, and 1 month (NAD73). The best conditions may not always be practical and a compromise in timing parameters is generally made. A very useful description of experimental conditions for the determination of 20 trace elements in biological samples by INAA has been published (KAS74).

Thermal neutron activation products can be conveniently classified according to their half-lives under five categories: (i) prompt gamma emitters; (ii) those with half-lives less than 10s; (iii) nuclides of half-lives in the range of tens

TABLE 2.6 APPLICATIONS OF INAA

Element of interest	Matrix	Method	Reference
Ag,Al,As,Ba,Br, Ca,Cl,Co,Cr,Cs, Cu,Eu,Fe,Hg,K, La,Lu,Mg,Mn,Mo, Na,Ni,Rb,Sb,Sc, Se,Sr,V,Yb,Zn	biological materials	t_i=1-2min,t_d≙1min,t_c=1-30min t_i=80h,t_d≙1d,t_c=1-5h	NAD73
Hg	brain tissues	t_i=1h,t_d=3d,t_c≙1h	FRI74
Ag,Al,As,Au,Ba, Br,Ca,Cd,Ce,Cl, Co,Cr,Cs,Cu,Eu, Fe,Ga,Gd,Hf,Hg, I,In,K,La,Mg,Mn, Na,Ni,Pt,Rb,Sb, Sc,Se,Si,Sm,Sn, Sr,Tb,Y,Yb,Zn, Zr	kidney tissues	t_i=3min,t_d=30s,t_c≙3min t_d≙1h, t_c≙10min t_i=30min,t_d≙20min,t_c≙30min t_i=2h,t_d=3h,t_c≙30min t_d=2d,t_c≙1h t_i=13h,t_d≙2d and 2w,t_c≙1h	VEL74
Sb	biological and environmental materials	t_i≙5min,t_d≙5min,t_c≙5min	VAL78

TABLE 2.6 Cont'd

Element of interest	Matrix	Method	Reference
Al,Ba,Ca,Ce,Co, Cr,Dy,Eu,Fe,Hf, K,La,Lu,Mg,Mn, Na,Sc,Sm,Sr,Th, Ti,V	geological materials	t_i=90s,t_d=10min,t_c≐3min t_d=30min,t_c≐5min t_i=3h,t_d=10d,t_c≐1h	CHA78
Co,Fe,Rb,Se,Zn	human pineal bodies	t_i=2d,t_d≐2w,t_c≐1h	DEM79
Al,Br,Ca,Cl,Co, Cu,Fe,K,Mg,Mn, Na,Rb,Se,Zn	breast tissues	cyclic-t_i=20s,t_w=0.5s,t_c=20s,N_c≐10 t_i=10min,t_d=90s,t_c=10min t_i=10d,t_d=5d,t_c=1h	OTH79
Se	rat kidneys	epithermal INAA-t_i=20h,t_d=2w,t_c=1h	PAV79
Pt	tissues from Pt administered rat	t_i=4d,t_d=9d,t_c=30min using 158keV gamma of ^{199}Au daughter of ^{199}Pt	ZEI79

t_i= duration of irradiation; t_d= decay time; t_c= duration of counting
t_w= waiting time; N_c= number of cycles;

of seconds to minutes; (iv) nuclides of half-lives in the range of minutes to hours; and (v) long-lived nuclides of half-lives more than hours.

Very short-lived nuclides are presently being utilized for elemental determinations by pseudo-cyclic (CHA79, CHA80) and cyclic (GRA75, KER78, OZE78) INAA methods. In the present project, thermal neutron activation products of the last three categories have been utilized.

2.2.2 Possible Errors in INAA

The errors involved in RNAA and mentioned in the Section 2.1.5 also take place in INAA. The personal error arising from the difference in manual sample transfer time, which is negligible for counting long-lived nuclides, may considerably influence the results of short-lived nuclides. High dead time of active samples can introduce errors in elemental measurements through short-lived nuclides.

In an analyzer system, a signal generated by the interaction of the detector semiconductor material with a gamma-ray is detected, processed through a pre-amplifier and a linear amplifier, converted from analog signal to digital signal, and then further processed by a mini-computer. A certain time is required for the system to register a signal. And during that time period, the system is not able to detect

another gamma-ray. This phenomenon is referred to as the dead time. Correction for dead-time can be automatically done by counting the sample for an additional period of time which is equal to the dead time. This method is adequate for counting long-lived nuclides with low radioactivity. But for highly active short-lived nuclides, the number of counts registered in the additional counting period may not be sufficient for accurate dead time correction. A significant error may also arise if there are large differences in dead times between a sample and a standard. Dead-time corrections have been applied in this study and discussed in Chapter 4.

Another possible source of error in INAA is that caused by gamma-rays of different nuclides overlapping in energy. Interferences of this type can be generally reduced by selecting optimal irradiation, decay and counting times. Use of a high-resolution Ge(Li) detector can also reduce interference in some cases. The extent of interference largely depends on the composition of the sample under investigation. The most common cases of interference due to overlapping gamma-rays are: ^{27}Mg (844keV) and ^{56}Mn (847keV), and ^{65}Zn (1115keV) and ^{46}Sc (1120keV). Details of these interferences on the gamma-rays used in this work are described in Chapter 4.

2.3 Neoplastic Tumors and Cells

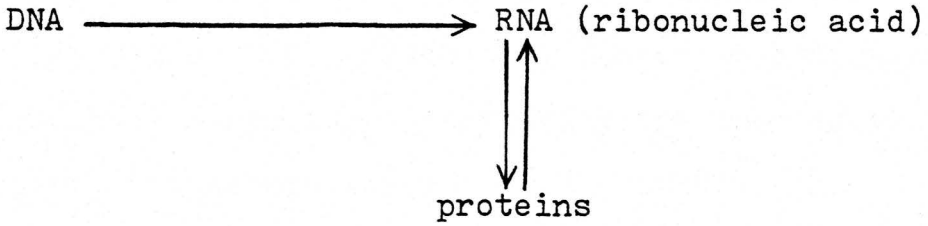
Neoplastic lesions, or tumors, consist of various types of cell which differ in staining properties (BUS74a). These cells have darker staining nuclei, more chromatin, and more mitoses than cells of the tissue of origin. The cancer cells have a number of properties which are biologically different from most cells of the host. The neoplastic cells may undergo multiple division with the appearance of multiple mitotic spindles in some of the cells. They tend to survive under adverse biological conditions. They rely on glycolysis for energy of reactions to certain extent (GRA65, WOO73, FER77). The neoplastic cells have less adhesiveness than the normal cells as determined by the force needed to separate the cells of the same type with a pair of micro-needles (COM44). There are many hypotheses about the cause of cancer (BUS74b). Today they are mostly related to Virchow's principles of cellular pathology instead of the previously postulated humoral pathology (EDM58).

It has been shown that cells in organs and tissues make permeable junctions which provide paths for direct flow of molecules between cells (LOE66). This cell-to-cell membrane channel has been said to control the cell growth (LOE68a, LOE68b). The pore size of the channel in the membrane has been found to be related to the calcium concentration in the cytoplasm (ROS77). By elevating the Ca^{2+} concentration at

the junction from its normal level of $\doteq 10^{-7}$M to 10^{-5}M, the molecular size limit for junctional permeation could be diminished gradually. According to one hypothesis (EWI28), the cell growth is controlled by some growth factors present in the cytoplasm. When the cell growth factor exceeds a certain amount, cell division is stimulated. It has also been suggested that cell-to-cell membrane channels are narrowed in cancer cells. After one stimulation of the generation of growth factors by some means, the growth factors then become difficult to be dispersed to other cells through the cell junctions. So the concentration of growth factors will be high enough to stimulate almost unlimited growth, which is one of the characteristics of cancer (SLA79).

That cancer cells can be transferred from one site to another and then proliferate to form a tumor at the new site was documented by Cornil who in 1891 described the sucessful experimental autotransplantation of human cancer from one breast to the other (COR91). It was suggested that a mutation in the genes of deoxyribonucleic acid (DNA) had occurred. Since the cell properties are associated with the DNA composition, the mutation of DNA will transform one type of a cell to another. The difference between the two types of cell will be propagated with cell divisions ultimately leading to the formation of cancer tissues. This suggestion was supported by the fact that new tumors could be induced by injection of nucleic acid fractions extracted from old tumors (LAM66).

The analysis of DNA is, therefore, important. Although thousands of studies on mutation of genes in DNA are being carried out, no universally acceptable mechanism has yet been put forward. On the other hand, the role of the nucleus in the synthesis of ribonucleoproteins is now well established. The relationship is as follows:



These specially synthesized proteins may impart some sort of feedback mechanism. For example, increased enzymatic activity of soluble ribonucleic acid (sRNA) methylase has been described for neoplastic tissues by Mittelman et al (MIT65), Tsutsui et al (TSU66) and Hancock (HAN67). Anomalous alkylation may change some functional properties of transfer RNA (sRNA) which in turn may result in translational alterations and abnormal proteins. These abnormal proteins (enzymes, histone, etc.) may be essential to the carcinogenic transformation of a cell.

Biochemical reactions in cells can occur with great rapidity through the participation of natural catalysts called enzymes. Enzymes are universally present in living organisms, and the occurrence of metabolic reactions common to all cells reflects the specificity of the responsible enzymes. Since all enzymes are proteins, from the previously mentioned re-

lationship between DNA and protein, it is very much possible that cancer cells may have different enzyme systems. Mitochondria, which contain several important metabolic enzymes, have been under extensive study (WHI76). Mitochondria in tumor cells have been found to have a very high uptake ability of plasm proteins (BUS56). Differences in tumor mitochrondria range from a decrease in the number of mitochrondria per tumor cell (NOV57, HOW55, SV064) to changes in mitochrondrial ultra-structural morphology typified by the loss of cristae and change in the matrix (WHI73, WHI74, SOR71).

Approximately one-quarter of the enzymes known to-day requires metallic cations to achieve their full catalytic activity (SCR77). Metal cofactors are analogous to organic coenzymes in that some are mobile cofactors and others are anchored prosthetic groups as shown in Table 2.7. In the present work, being limited by the capabilities of neutron activation analysis, the metallic parts of the neoplastic cells and tumors have been investigated. In the event there are significant differences between metal concentrations of cancerous and non-cancerous tissues, future studies could be carried out to relate these metals to specific enzymes.

Depending on their biological effects, elements can be classified into three groups: essential, poisonous and undefined as shown in Table 2.8 (TSA78). The term toxic metal is justified only from the practical point of view. For

TABLE 2.7 EXAMPLES OF METAL-CONTAINING ENZYMES

		Enzyme	Metal	Reaction catalysed
metal-activated enzymes		pyrophosphatase	Mg	hydrolysis of inorganic pyrophosphate
		leucine amino-peptidase	Mg or Mn	hydrolysis of peptide bonds
		enolase	Fe,Mg,Mn or Zn	dehydration of D-2-phosphoglyceric acid to phosphoenol pyruvic acid
metallo-enzymes	dissociable	nitrate reductase	Mo	reduction of nitrate to nitrite
		peroxidase	Fe	reduction of hydrogen peroxide
		α-amylase	Ca	hydrolysis of glycosidic linkages
	undissociable	alcohol dehydrogenase*	Zn	oxidation of alcohols to aldehydes
		DPNH-cytochrome -c reductase*	Fe	oxidation of DPNH to DPN and concomitant reduction of cytochrome -c
		succinic dehydrogenase**	Fe	oxidation of succinic to fumaric acid
		xanthine oxidase**	Fe and Mo	oxidation of aldehydes, hypoxanthine, xanthine and other purines

* inhibited by reagents specific for metals

**not inhibited by reagents specific for metals

TABLE 2.8 CLASSIFICATION OF ELEMENTS [a]

Essential elements	Poisonous elements	Undefined elements
Ca, Co, Cr, Cu, Fe, K , Mg, Mn, Mo, Na, Ni, Se, Zn,(Sn, Sr, Ti, V)*	As, Be, Cd, Ga, Hg, Pb, Tl	Ag, Al, Au, Ba, Bi, Cs, Hf, Ir, Li, Nb, Os, Pd, Pt, Rb, Re, Rh, Ru, Sb, Sc, Ta, Te, W , Zr, rare earth

* not yet decided

[a] TSA78

instance, in acute oral toxicity test, copper (an essential element) is just as toxic as cadmium (a poisonous element), and the essential element selenium in the form of selenite is even more toxic than the poisonous element mercury (SCH77). Some values of lethal dose to kill half the number of tested animals can be found in the literature (TSA78).

That some inorganic compounds can cause cancer is well established. Compounds of As, Cd, Cr and Ni have been implicated as human carcinogens. And compounds of Be, Cd, Co, Cr, Fe, Ni, Pb, Ti, and Zn have been reported to induce cancers in experimental animals (SUN78). On the other hand, compounds of some other elements can be used in cancer treatment such as in chemotherapy of some types of cancer. The elements which have been studied extensively in cancer treatment are platinum and gallium (HAR71, ADA75, GOT75). Platinum(II) is believed to be bound to the amino groups of adenosines and cytidines in DNA with the formation of a bidentated chelate ring by the cis-platinum complexes (MAN73). Gallium is said to be highly specific in its affinity to malignant neoplasms (EDW70, VAI70). Some copper complexes have been reported to have similar effects (TAK60, B0066). The copper(II) complex of 2-keto-3-ethoxy butyraldehyde bis(thiosemicarbazone) transports copper into neoplastic cells where it is deposited and inhibits a number of enzymes responsible for the synthesis of DNA (SAR69). Compounds of selenium has been studied in the prevention of cancer (SCH76, YOU79). The medical significance of some elements of biological importance has recently

been reviewed (DAV77). Correlations among concentrations of elements in neoplastic cells and tissues may lead to better understanding of the causes and effects of cancer. Such an attempt has been made in the present study.

Since the mechanism of biological reactions are very complicated, the study of biopsy samples alone is normally not sufficient to draw meaningful conclusions. In general, experiments are carried out with animals under controlled environment where the effects of many other variables can be minimized. In some cases, either human cancer can be transplanted to animals (PEY77, LUM54) or some neoplasms that occur in laboratory animals may have their counterparts in human beings. It, therefore, appears quite reasonable to study the animals first, and then extend the results to human beings (DET78). Animal tumor model is regarded as one of the best models for human cancer (SKI71). Several animal studies have been reported in the literature. Examples include the application of neutron activation analysis to mice for studies on selective elements in metallobiochemistry (GIR77), the analyses of whole blood by neutron activation to search for a biochemical indicator of neoplasia (ZDA76), the presence of metals in reiterative DNA sequences from eukaryotic cells (GUI77), and the study of cellular major and minor elements in normal and abnormal tissues (BER57). The studies involving human biopsy samples include the trace-metal analyses of cancerous and non-cancerous tissues (MUL71), and the use of INAA in

studying trace element change induced by cancer therapy (ALL 78). Since the functions of a few trace metals are related to one another (MAT73, MIL73, PET73), the application of a multielemental analysis technique such as neutron activation analysis would be beneficial in these studies.

A few types of mice tumors were available in Pathology Department of the Dalhousie University. Two of them were used in this work, namely EL4 lymphoma that grew in C57BL/6J mice, and H6 hepatoma that grew in A/J mice. These tumors are of two different kinds and they are even different in physical form. The former is a cell tumor present in a dispersed state and the latter is a solid tumor.

The lymphomas comprise about 6% of all cancer deaths and about 0.8% of deaths from all causes (GUN68). Among them Hodgkin's disease is well known. Because of the difficulties in cytologic identification and the similarity in proliferative capacity of reactive lymphoid tissues to that of malignant lymphoma, there exists confusion in terminology. However, it is generally agreed that malignant lymphoma is a malignant neoplasm of lymphoid tissue. It may be further classified according to Lukes's prescription (LUK67):

a) Lymphocytic, well differentiated
b) Lymphocytic, poorly differentiated
c) Stem cell
d) Histocytic
e) Hodgkin's disease

Each of the above types can be subclassified to two kinds, diffused and nodular.

The EL4 cells originated in a subline of C57BL mice maintained by Gorer, induced by 9,10-dimethyl-1,2-benzanthracene (GOR50). For classification purposes, the EL4 is considered a type of diffuse lymphocytic lymphoma from non-thymic origin, well differentiated. This type of lymphoma consists essentially of a proliferation of small-to-medium size lymphocytes with scanty or indiscernible cytoplasm that are characteristically of uniform size and configuration, but are indistinguishable individually from normal lymphocytes (LUK67). It was first considered as lymphatic leukaemia (which some workers, e.g. JOH75, still use), and later it was transformed to lymphosarcoma, which was a diffuse infiltration of the peritoneal tissues and a terminal ascites, and subsequently lead to the death of all C57BL/6J mice. The relationship between number of EL4 cells inoculated and the survival time had been studied by Ghose (GHO74). The survival time was approximately inversely proportional to the logarithm of the number of cells injected. When a number of $5x10^7$ EL4 cells was injected, the mouse would die in about eleven days.

Since generalized diseases of the lymphoid tissues, such as the various types of malignant lymphoma including Hodgkin's disease, may involve the thymus gland, the study of thymus is of much interest. This endocrine gland can be found near the

neck of the mice. It has at least two functions. Shortly after birth, the thymus gland produces lymphoid cells, which are translocated to the peripheral tissues, notably lymph nodes and spleen, to provide a population of cells that function in immunological responsiveness. The thymus gland also synthesizes and secretes hormones that influence the rate of development and maturity of lymphoid cells. One characteristic of the gland is its shrinkage with age. Examples can be found in Castleman's work (CAS55).

Livers, as a consequence of its size and good blood supply, are the favorable sites of neoplastic lesions. The lesions which appear in livers are greater in number and diversity than those seen in any other organs. Because of its easy growth, hepatomas are most commonly used (WEI72, MOR75). The hepatomas may be classified into two groups, namely the minimal deviation hepatomas and the ordinary transplantable ones. And they can be further subdivided into slow, medium, and fast growing hepatomas (L0073). Detailed information on several transplantable hepatomas of mice are available (POT60, MOR63, HAN69, TH071). Their structures have also been discussed (HRU65).

The H6 hepatoma, used in this work, originated from an adult A/J male mouse and was transplanted by subcutaneous inoculation with the use of a trocar or by injection of the separated cells. It is a fast-growing tumor among other

spontaneous hepatomas. It can be transplanted every 2-4 weeks on female mice for the sake of easy handling. The H6 tumors are typically well-differentiated hepatomas. They frequently contain small fatty globules, and they are generally organized into compressed cords. They may be further differentiated as H6a, H6b, and H6c under extreme classification scheme (HAN69). And as a reference, the biological and biochemical properties of adult, embryonic, and regenerating livers are available in literature (HRU65).

2.4 Ultrafiltration

In living matter, most trace elements-at both normal and toxic concentrations-are bound to proteins, on which their biological effects and metabolic behavior are dependent to some extent. For example, Hg binds many plasma proteins and dimerizes albumin (HUG64), and much of Cd and Hg absorbed can be sequestered by a soluble kidney protein called metallothionein(PUL66). The effect of a given amount of Co in a tissue for an organism is known to vary considerably. Its bio-availability depends on its presence as an inorganic ion or in the form of Vitamin B_{12}. In order to study these effects one could develop methods which can be applied to the determination of protein-bound trace element fractions by separation and characterization of the proteins in a sample followed by a measurement of trace element levels in them.

A few protein separation techniques with varying degrees of resolution have been reported in the literature. Gel filtration chromatography (FRI68, NOR75, SYV75, OVE77, BEH78, DEC78, ELG78), gel electrophoresis (MAU71), isoelectric focusing (RAN73, SCH75, VAA77), combination of sonification and centrifugation (SAB76), and ultrafiltration (WAN69, CHA71, MAR71, AMI77) techniques have been used for protein fractionation.

Among the preparative protein separation techniques available, ultrafiltration is particularly suitable for several reasons such as low cost, ease of operation, efficiency of separation, convenient automation, and yields of separated fractions in sufficient amounts that can be used for trace element analysis. A simple as well as efficient ultrafiltration system has been set up in the present project and described in Chapter 3. The selection of a buffer solution in ultrafiltration is of utmost importance. Such a buffer solution has been prepared in ultrapure form and reported in Chapter 3.

Some tissues analyzed in this work have been found to contain large amounts of Br, Cl and Na, the neutron activation products of which have been observed to interfere in the determination of other elements. Preliminary experiments have been carried out to separate Br, Cl and Na from the rest of the protein-bound trace elements using the ultrafiltration technique. These experiments are discussed in Chapter 4.

3. EXPERIMENTAL

3.1 Chemicals and Elemental Standards

"Atomic Absorption Spectrometry (AAS) Standard Solutions", obtained from the Alfa Ventron Corporation and Fisher Scientific Company, were used as chemical comparator standards for most elements. Each AAS Standard Solution contained 1000μg of a given element per mL of the solution. These solutions were diluted with deionized water to desired concentrations. The diluted solutions were also used as carriers in the RNAA schemes.

A few of the AAS Standard Solutions had high concentrations of the chloride ion. In INAA, high concentrations of Cl produce highly active ^{38}Cl (half-life = 37.3 min) which interferes in the determination of other short-lived nuclides such as ^{51}Ti (5.8 min). In such cases, other compounds of the element were used. For example, microgram quantities of titanium dioxide powder were used as a comparator standard for Ti.

Chemical comparator standards were generally prepared by

evaporating known quantities of the diluted AAS Standard Solutions on Nuclepore membranes under an infrared lamp. Nuclepore membranes were selected as support because of their very low content of elements of interest (NUC77, ELL79).

The water used in this work for dilution, etc., was high-quality deionized water obtained by passing tap water through a mixed bed column (Ultrapure D0809, Barnstead Sybron Corporation). The typical resistance of such deionized water was 18 megaohms.

Most chemical reagents used in this work were of analytical (certified ACS) grade. Ultrapure ("ULTREX") reagents supplied by the J.T. Baker Chemical Company were used when needed. However, the purity of available acetic acid and ammonia did not meet the standards required for this project. Consequently, they were further purified.

The polyethylene vials used were cleaned by soaking in 2M HNO_3 solution prepared by diluting A.R. grade concentrated HNO_3 with ultrapure deionized water for 24h or in a ultrasonic bath for 30min. They were then thoroughly rinsed with deionized water and vacuum dried.

3.2 Preparation of Ultrapure Ammonium Acetate Solution

Cells and tumors collected from experimental mice needed to be washed in a buffer solution prior to analyses. A buffer

solution was also necessary for the use in ultrafiltration. Ammonium acetate buffer was selected for reasons described in Chapter 4. Available ammonium acetate, acetic acid, and ammonia were found to contain many trace elements in considerable amounts. It was decided to purify acetic acid and ammonia first, and then mix them in appropriate proportions to obtain ultrapure ammonium acetate buffer solution. Chemicals with relatively high vapor pressure can be purified by distillation and sub-boiling distillation. The latter technique has been used to obtain many "ULTREX" reagents (ZIE76), and has been applied in the present work to purify acetic acid and ammonia.

A sub-boiling distillation apparatus was set up as shown in Fig. 3.1. The apparatus consisted of two 500mL Teflon bottles (supplied by the Nalge Corporation) which were connected to each other by a high-density Teflon block with a central tube. One of the bottles containing the impure reagent was heated under an infrared lamp while the collector bottle was cooled by spraying cold water on it.

Analytical grade acetic acid and ammonia were further purified using the above set-up. The purified solution were standardized, and appropriate volumes were mixed to obtain ammonium acetate solution of desired concentration. The purity of this ammonium acetate solution was evaluated by analyzing both the solution and the residue after lyophilization using INAA. Only Cl was detected as a contaminant. The

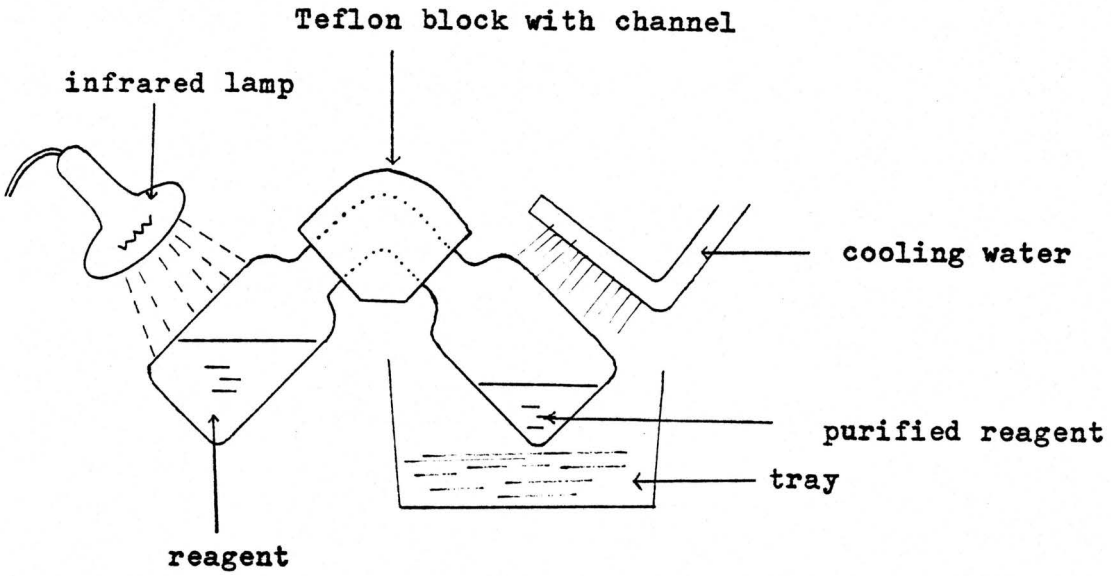


Fig. 3.1 Sub-boiling distillation apparatus

levels of Cl, approximately 0.7μg/mL of the buffer solution, found in the ultrapure ammonium acetate are negligible compared to the Cl content of tissues, viz. 10^3μg/g on dry-weight basis.

3.3 Standard Reference Materials

Standard Reference Materials (SRM) supplied by various agencies were first analyzed to evaluate the precision and accuracy of the methods developed, and then used as multi-element comparator standards in routine analyses for those certified elements. The U.S. National Bureau of Standards (NBS) supplied the Orchard Leaves (NBS SRM-1571), Tomato

Leaves (NBS SRM-1573), Pine Needles (NBS SRM-1575), and Bovine Liver (NBS SRM-1577). The U.S. Geological Survey (USGS) provided the Peridotite (USGS PCC-1) rock standard. Two synthetic Gelatin standards (TEG-50-A and TEG-50-B) were obtained from the U.S. Eastman Kodak company. The SRM were dried prior to use according to the methods prescribed by the supplying agencies.

3.4 Radioactive Precipitate Filtration System

In RNAA, it is essential that the precipitates from both the sample and the standard have identical geometry when they are counted. It is also necessary to have a filtration system which can be easily decontaminated. In order to satisfy these requirements a filtration system was designed as shown in Fig. 3.2. The system was made of polished Teflon which was easily decontaminated by soaking in hot concentrated nitric acid. Due to smoothness of the surfaces, only a negligible amount of particles of a precipitate adhered to the wall of the container.

The filtration system consisted of several removable Teflon parts (Fig. 3.2): a top cylinder, a central perforated holder, a bottom funnel, and a threaded central piece which was used to hold all parts together. This system was attached to a suction funnel for filtering radioactive precipitates. The precipitates obtained using this system were uniform in

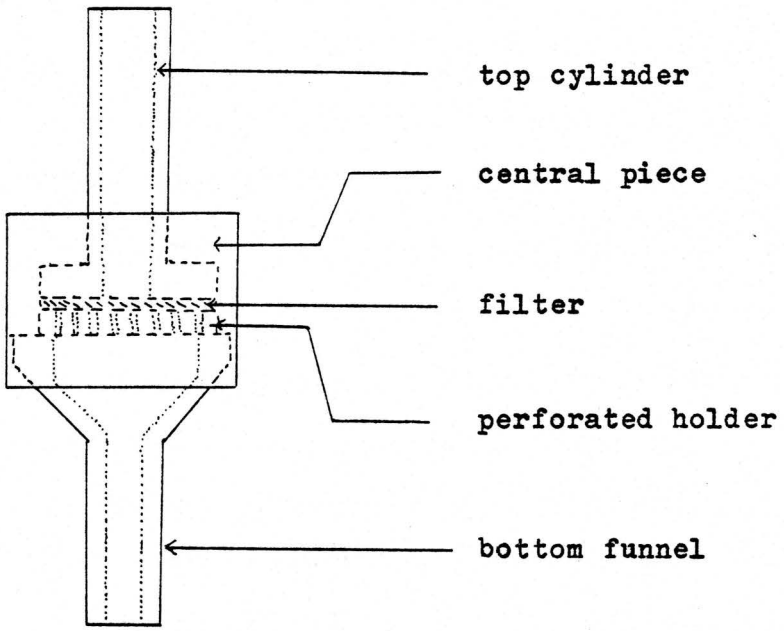


Fig. 3.2 Radioactive precipitate filtration system.

thickness and had identical geometry.

3.5 Preparation of Samples

3.5.1 The EL4 Cells

The EL4 lymphoma originated in 1945 when Gorer(GOR47) treated a C57BL/GO mouse with 9,10-dimethyl 1,2-benz-anthracene. Since 1946 the lymphoma was maintained at the Chester Beatty Research Institute, London(GOR50) from where Ghose, Department of Pathology, Dalhousie University, obtained the tumor

in 1969 and maintained it by serial intra-peritoneal passage in C57BL/6J mice (GH074). We obtained the EL4 cells from Prof. Ghose's laboratory and maintained it by introducing in female mice of strain C57BL/6J purchased from the Jackson Memorial Laboratories, Bar Harbor, Maine. Approximately 10^7 EL4 cells of greater than 90% vitality were intra-peritoneally injected to host mice. The tumor cells were allowed to multiply for about 7d in the mice. The skins of the abdomen of the treated mice were cleaned with 70-95% ethanol. A syringe needle (size 18G-1½) was punched through the skin into the intra-peritoneal. The cells were then collected through the duct of the needle.

The EL4 cells used in this project were collected by a number of procedures depending on the availability of particular samples and method of collection. Although ideally all samples should be collected using the same procedure, in practice it is not always feasible especially when the samples are obtained from another source. Every effort was made to collect the samples using methods which are as similar as possible. However, in the strictest sense, the EL4 cells collected could be classified to four categories (designated as samples A, B, C and D) depending on the method of collection. The methods are described below.

At the beginning of this project, the EL4 cells (designated as EL4 Cells A) were directly obtained from the Pathology

Department and were the left-overs of the cells used for maintaining the EL4 line. Since the number of cells had to be counted before injection, and since the cells had to be kept vital, the cells were first collected into an artificial biological fluid, a phosphate buffered saline (PBS) which was composed of 0.004M Na_2HPO_4, 0.0022M NaH_2PO_4 and 0.15M NaCl. All these chemicals were of analytical grade. The buffer solution was placed in a 50ml polyethylene centrifuge tube. After the collection of the cells, the volume was adjusted to 50ml with the buffer solution. The mixture was homogenized. Then 5µl of the mixture were withdrawn from the bulk with a white-blood cell dilution pipet and diluted to 100µl. Drops of the solution were placed onto the surface of a cytometer. The number of cells in the counting chamber of the cytometer was counted with the aid of a microscope. The total number of cells in the original 50mL solution was calculated to be 10^7, in general. The vitality of the cells in the suspensions was determined by trypan blue exclusion of live cells. Only dead cells would show blue color and the percentage of live cells could be calculated via counting in a cytometer. After the number of cells was counted, the solution was centrifuged for 10 to 15 min at about 400G. The supernatant was discarded. 50mL of 0.15M ultrapure ammonium acetate solution were added and the solution was mixed thoroughly. It was centrifuged again as described above. It was washed once again with another portion of ammonium acetate. The cells were then transferred into a precleaned preweighed

polyethylene vials.

Some cell samples (designated as EL4 Cells B) were obtained by collecting the cells along with the body fluids directly into precleaned preweighed polyethylene vials. This procedure eliminated elemental contaminants from the use of the PBS buffer. On the other hand, the measured elemental contents of the cells, based on the above samples, might be misleading due to the possible presence of elements in the body fluids.

In order to remove body fluids from cell suspensions, some of the samples (designated as EL4 Cells C) were collected by the following procedure. The cells were collected into a centrifuge tube which contained 7 mL of ultrapure 0.15M ammonium acetate solution, centrifuged, and the residue containing only cells was obtained.

The cells (designated as EL4 Cells D) were collected into 25mL of 0.15M ultrapure ammonium acetate solution, centrifuged, and the residue was preserved.

All types of cell sample were placed in precleaned preweighed polyethylene irradiation vials of medium-size (diameter =1.4cm, height=5.2cm). The samples were weighed. The dry weights of the samples were obtained after freeze-drying them for two days. The freeze-dried cells were then pressed into a small volume at the end of the container by introducing a trimmed cap of another vial into the irradiation vial as shown in

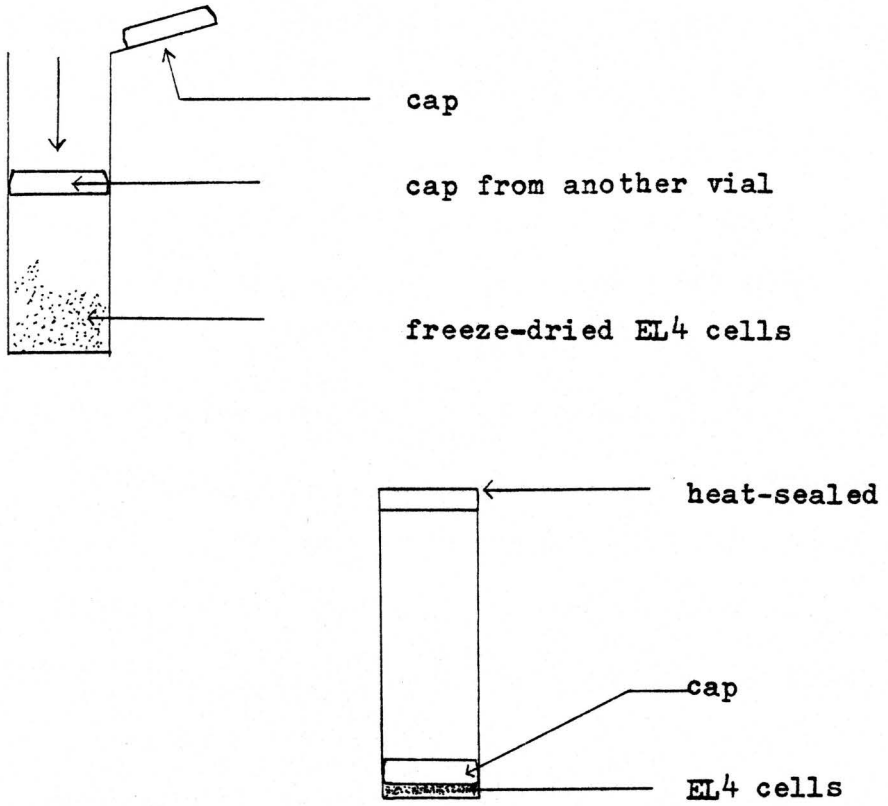


Fig. 3.3 Positioning of cells in an irradiation vial.

Fig. 3.3.

By developing the above procedure, the cells were always placed in a fixed location of the vial and so the irradiation and counting geometry of the samples was identical. Since the cells were packed together at the end of the vial, the counting statistics were significantly improved.

3.5.2 The H6 Tumors

The H6 is a spontaneous solid tumor developed in the livers of mice of strain A/J. The cell line was maintained by passage of 10^6 vital H6 cells into new hosts of A/J mice by subcutaneous

injection at the thighs. The tumor lumps grew beneath the skin and above the muscle at the site of injection. The tumors were allowed to grow for about 10 to 14d depending on the condition of the mice. Then the mice were sacrificed by cervical dislocation. The solid tumors were collected by cutting with a pair of stainless steel scissors. Any detectable necrotic lesions in the tumor were removed. The samples were placed in preweighed precleaned polyethylene vials, and weighed. They were reweighed after freeze-drying for 2d. The dried tumor lumps were pressed at the bottom of the irradiation vials by the procedure described previously.

The experimental procedure followed to maintain the H6 tumor line is described below. Since the tumor was a solid tissue, the tumor lumps were chopped with stainless steel scissors into small pieces inside a petri dish half-filled with PBS. The mixture was withdrawn from the Petri dish by a syringe, and collected. These steps were repeated once more. The remaining solid tissues were pressed against a stainless steel screen with a rubber policeman. The mixture of the PBS and cells passing through the screen were collected. The collected fractions were combined and filtered through a glass wool net to remove some associated fibre tissues. The filtrate was centrifuged for 10min at about 400G. The supernatant was removed by decantation. The number of cells in centrifuged residue and the percentage of vital cells were obtained as mentioned in the section of EL4.

There was a difference in the vitality between the cells of EL4 and H6; in general, that of H6 was lower by about 50%. The reason for this low vitality was that the H6 cells were collected under very severe conditions.

3.5.3 The Other Tissues

The tissues, namely hearts, kidneys, lungs and spleens, of the mice used in this project were collected by cutting them from the trunk of the mice with a pair of stainless steel scissors. Because the vascular vessels were cut, the tissues were soaked in blood during the collection process. To remove the blood, the tissue samples were rinsed with 2mL of deionized water in an ultrasonic bath and later were transferred into medium-size irradiation vials. The wet weights were measured and the dry weights were taken after freeze-drying for 2d. Known quantities of the dried tissue samples were then transferred to small irradiation vials.

3.6 Set-up of the Ultrafiltration System

An ultrafiltration system was set up in an attempt to separate the protein-bound trace elements. This system was also used to study the possible elimination of Br, Cl and Na from homogenized tissues. A schematic diagram of the system is given in Fig. 3.4.

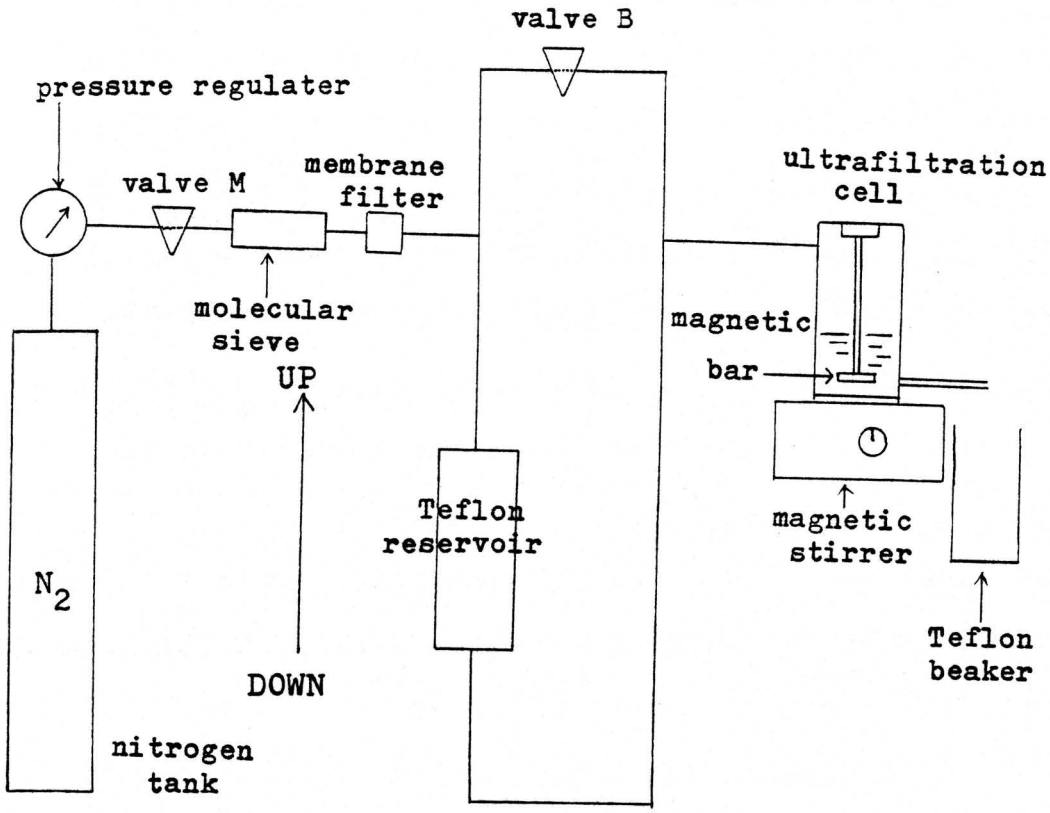


Fig. 3.4 Schematic of the ultrafiltration system

A pressure gradient was produced from a high pressure hyperpure nitrogen tank which was connected to a pressure regulator and then to a molecular seive (Guild Corporation). The nitrogen gas was then passed through an air filter consisting of a Nuclepore membrane (0.4μm pore size, 47mm diameter) placed in a Teflon container. Any particulate contaminant present in the nitrogen gas was removed by these procedures (ZIE76). The outlet from the membrane filter was branched into two lines (Fig. 3.4) where one of the lines went into a Teflon reservoir which was used to contain either 0.05M EDTA solution, deionized water, or 0.15M ammonium acetate

solution. The two branching lines were united together before inserting to the inlet of the ultrafiltration cell. The filtrate coming out of the cell was collected in a Teflon beaker. Contamination from the polyethylene tubing lines, reservoir, and the cell was possible. However, no detectable contamination was observed after treating the entire unit with 0.05M EDTA solution. All the surfaces that came into contact with the homogenized tissue were treated with the EDTA solution at pH7, and were rinsed thoroughly with deionized water.

The various molecular weight cut-off membranes (supplied by the Amicon Corporation) were removed from the protective envelopes with a tweezer. To remove traces of UV-absorbing materials (AMI) , the membranes were soaked in 5% NaCl solution for 30min. They were then rinsed 5 times with deionized water for a total soaking time of about one and half hours. The membranes were further treated as follows:

(i) rinsed the membrane and the plastic membrane support three times with 30mL deionized water each time;

(ii) rinsed them with 70% ethanol/water solution twice, 30mL each time;

(iii) rinsed them twice with 30mL deionized water;

(iv) soaked them in 30mL of 0.05M EDTA solution at pH7 for 30min at room temperature; and finally

(v) rinsed them three times with deionized water.

The samples were composed of about 1g wet tissue and 5mL

of 0.15M ultrapure ammonium acetate solution at a concentration of about 20% w/v. This mixture was minced and sonicated with a Vertis ultrasonic homogenizer for 6min at a scale setting of 5 in a 0-10 scale system. Before the sample was homogenized, the probe of the homogenizer was immersed in one liter of 0.05M EDTA solution (pH7), with the power set at half, for 15min. It was then washed with deionized water 6 times for 2min each time. Finally, it was immersed in one liter deionized water at half power for 18min. This one-liter water was saved, and later freeze-dried and analysed by INAA. It was used to check the presence of any contaminants coming from the contact with the probe.

The tissue mixture was then homogenized with the probe. The homogenate obtained was placed in a precleaned polyethylene bottle, and it was kept in the refrigerater at $0^{o}C$. The homogenate was used within a few days. Prior to use, it was first defrosted and then shaken well. A certain amount of it was withdrawn with a syringe for analysis. Another equal amount was also withdrawn, which was freeze-dried to obtain the dry weight. Since ammonium acetate was removed during the freeze-drying process, the dry weight obtained was that of the organic tissue.

After the insertion of a selected membrane, the ultrafiltration cell was tightened. The sample was then injected into the cell via the inlet. The valves M and B (Fig. 3.4) were opened, followed by the opening of the nitrogen tank re-

gulators. The pressure was adjusted to about 70psi with the aid of the regulators. The magnetic stirrer was kept rotating during this process. After the homogenate ran dry, the valve B was closed. The nitrogen gas now could only pass through the reservoir containing 0.05M ammonium acetate solution which was previously by-passed. The residue on the membrane was thus washed with ammonium acetate solution. The residue was kept wet to avoid problems in filtering until the entire process was completed. The filtrate effluent and the washings were collected in a Teflon beaker. The residue, membrane along with the plastic support were transferred to a Teflon beaker containing 30mL of 0.15M ammonium acetate solution. The mixture was stirred in an ultrasonic bath. This step was repeated twice. The residue and the washings were combined and subjected to freeze-drying. The dried material was transferred to a medium-size polyethylene irradiation vial and analysed by INAA. Finally, the membrane and the support were removed and soaked in 1M NaCl solution over-night. Then they were rinsed three times with deionized water, once with 0.15M ammonium acetate solution followed twice by deionized water. And they were soaked in 10% ethanol/water and stored for the next run.

3.7 Irradiations

At the beginning of this work, samples were irradiated at the McMaster University Nuclear Reactor at a flux of 1×10^{13}n cm^{-2} s^{-1} for various lengths of time. After a reactor was installed here, all samples, standards, and tracers were irradiated at

the Dalhousie University SLOWPOKE Reactor (DUSR). SLOWPOKE (an acronym for Safe Low Power Kritical Experiment) is a small swimming-pool-type research reactor developed by the Atomic Energy of Canada Limited. It uses enriched 235-uranium fuel with a light water moderator and beryllium reflector. The neutron flux generally used was 5×10^{11} n $cm^{-2} s^{-1}$. The water moderator reached a maximum temperature of approximately 50°C at this flux. Because of this low temperature, loss of volatile elements of interest from freeze-dried samples used in this work was not observed. The stability of the neutron flux at a given operating power level of the DUSR has been measured to be within ±3% for a given pneumatic site over long periods. The variation in both vertical and radial homogeneity for the inner pneumatic sites has been observed to be less than ±1% (RYA78).

The presence of bromine, chlorine and sodium in the sample matrix is known to hinder the detection and measurement of other trace elements. Thus not too many elements in tissues could be determined by instrumental neutron activation analysis (INAA). For the elements detected by INAA, irradiation, decay and counting periods were optimized in a trial and error process by maximizing peak to background ratios for the low-activity nuclides without making the experimental time too long. Different irradiation, decay and counting periods finally selected for multielement analysis of various types of tissue samples by INAA are described in Chapter 4.

Since some of the nuclides of interest either were not detected at all or gave very poor counting statistics by INAA, radiochemical neutron activation analysis (RNAA) methods were developed for certain elements. Ideally, the samples should be irradiated for days at high flux for sensitive RNAA measurements. However, due to the limitation in the length of irradiation at the DUSR facility at high flux and lack of hot cell facilities, the samples were only irradiated for seven to sixteen hours at a flux of 5×10^{11} n $cm^{-2} s^{-1}$.

3.8 Gamma-ray Spectrometry Systems

Three independent gamma-ray spectrometry systems were available at the DUSR facility. One system consisted of a Harshaw 7.5-cm-diameter x 7.5-cm-thick thallium activated sodium iodide, NaI(Tl), crystal optically coupled to a 7.5cm photomultiplier tube hermetically sealed in an aluminum housing with a well of dimensions of 2.5cm in diameter and 5.0cm in depth. The resolution of this detector at 662 keV of ^{137}Cs photopeak was 7.4%. The NaI(Tl) detector was coupled to a Canberra model 8100/e 4096-channel pulse height analyzer. This system was primarily used for counting radiochemically separated nuclides and for tracer studies.

The other two gamma-ray spectrometry systems consisted of lithium drifted germanium, Ge(Li), detectors. A Princeton Gamma-Tech coaxial Ge(Li) detector, of 38mm diameter, 34mm length, and an active volume of $40 cm^3$, was coupled to a Tracor

Northern TN-1700 model 4096-channel analyzer. The resolution of this detector was 2.02 keV (FWHM) at 1333keV gamma-ray of ^{60}Co, and the efficiency was 7.1% (relative to a standard NaI (Tl) detector) at a 25cm source-to-detector distance. The other system consisted of a Canberra coaxial Ge(Li) detector of 42.5mm diameter, 42.5mm length, and an active volume of 60 cm^3. The resolution was 1.9keV at the 1333keV photopeak of ^{60}Co, and the efficiency was 9.5%. This detector was connected to a Tracor Northern TN-11 model 4096-channel analyzer assisted by a 16-K memory PDP11/05 mini-computer.

4. DEVELOPMENT OF ANALYTICAL METHODS

It was stated in the literature survey that no suitable analytical methods were available at the beginning of this work for the determination of trace concentrations of the elements of interest in tissues. Consequently, several nuclear analytical methods were developed in this study. Initially emphasis was placed in developing radiochemical neutron activation analysis (RNAA) methods primarily because of the lack of irradiation facilities close to our laboratories and for other reasons stated earlier. Subsequent to the installation of the Dalhousie University SLOWPOKE-2 Reactor (DUSR) facility, instrumental neutron activation analysis (INAA) methods were developed in addition to the continual development of RNAA. The various RNAA and INAA methods developed during the course of this study are described in this chapter. Radiochemical purity, interferences, and sensitivity of measurements are discussed. Precision and accuracy of the methods have been evaluated by analyzing standard reference materials (SRM) and are reported here.

Since several neutron activation products and their various gamma-rays were utilized in developing RNAA and INAA

methods, it will be useful to summarize them. The nuclear data (NDS, BRU72, LED67, PAG71) of the products studied are shown in Table 4.1. In cases where a nuclide is known to decay by emitting several intense gamma-rays, the one used in this study is designated by an asterisk. In order to minimize, if not to completely eliminate, interferences from gamma-rays overlapping in energy, nuclides were grouped together and separated by radiochemical methods. Attempts were also made to group nuclides with similar half-lives and elements of similar chemical properties. These RNAA group separation schemes are described below in detail.

4.1 Radiochemical Separation of Ag, Au, Hg, Pd and Pt by Ion Exchange Chromatography

Many metal ions are known to form complex compounds with halides. The instability constants of these compounds depend on the type of both metal and halide ions, among other factors. Depending on the difference in the instability constants and elution behavior, metal-halide complexes can be separated from one another. These observations have been utilized in developing ion exchange chromatographic methods for separating nuclides of Ag, Au, Hg, Pd and Pt. The details of chloride, bromide and iodide exchange methods developed are given below.

4.1.1 Chloride Column

Noble metals are known to form strong chloro-complexes

TABLE 4.1 NUCLEAR DATA OF NEUTRON ACTIVATION PRODUCTS STUDIED[a]

Element	Target isotope	Isotopic abundance,%	Cross section,b	Nuclide	Half-life	Major γ-ray energy,keV	γ-ray intensity,%
Aluminium	^{27}Al	100	0.232	^{28}Al	2.3min	1779	100
Antimony	^{121}Sb	57.2	6.2	^{122}Sb	2.80d	564*,693	66.3
Arsenic	^{75}As	100	4.3	^{76}As	1.104d	559*,657	44.0
Bromine	^{81}Br	49.5	3.26	^{82}Br	1.479d	776*,554	25.7
Cadmium	^{114}Cd	28.86	0.3	^{115}Cd	2.25d	335*,528	52.8
Calcium	^{46}Ca	0.0033	0.25	^{47}Ca	4.53d	1297	81.9
	^{48}Ca	0.185	1.1	^{49}Ca	8.5min	3083	89.0
Chlorine	^{37}Cl	24.5	0.43	^{38}Cl	37.3min	2167*,1642	47.0
Chromium	^{50}Cr	4.31	16	^{51}Cr	27.8d	320	9.0
Cobalt	^{59}Co	100	37	^{60}Co	5.25a	1173,1333*	100
Copper	^{63}Cu	69.1	4.5	^{64}Cu	12.8h	511*,1346	38.6
	^{65}Cu	30.9	2.3	^{66}Cu	5.1min	1039	9.3
Gold	^{197}Au	100	98.5	^{198}Au	2.693d	412	96.0
Iodine	^{127}I	100	6.2	^{128}I	25min	443	14.3

TABLE 4.1 Cont'd

Element	Target isotope	Isotopic abundance,%	Cross section,b	Nuclide	Half-life	Major γ-ray energy,keV	γ-ray intensity,%
Iron	^{55}Fe	0.33	1.23	^{56}Fe	45d	1099*,1292	56.0
Magnesium	^{26}Mg	11.17	0.003	^{27}Mg	9.5min	844*1014	70.0
Manganese	^{55}Mn	100	13.3	^{56}Mn	2.58h	847*,1811	99.0
Mercury	^{196}Hg	0.146	3110	^{197}Hg	2.672d	78	19.6
	^{202}Hg	29.8	4.9	^{203}Hg	46.57d	279	82.0
Molybdenum	^{98}Mo	23.78	0.51	^{99}Mo	2.779d	140*,739	88.7
Osmium	^{192}Os	41	106	^{193}Os	1.292d	139,460*	3.9
Palladium	^{108}Pd	26.71	12.26	^{109}Pd	13.6h	88	8.9
Platinum	^{198}Pt	7.21	4.0	^{199}Pt	31min	543	17.0
				^{199}Au	3.15d	158*,208	37.0
Potassium	^{41}K	6.88	1.30	^{42}K	12.5h	1525	18.0
Rubidium	^{85}Rb	72.15	0.91	^{86}Rb	18.7d	1077	8.8
			0.1	^{86m}Rb	1.04min	556	100
Scandium	^{45}Sc	100	23.0	^{46}Sc	84.0d	1121*,889	100

TABLE 4.1 Cont'd

Element	Target isotope	Isotopic abundance,%	Cross section,b	Nuclide	Half-life	Major γ-ray energy,keV	γ-ray intensity,%
Selenium	^{74}Se	0.87	30.0	^{75}Se	120.4d	136,264*,279	63.0
	^{76}Se	9.02	21.0	^{77m}Se	17.5s	162	50.6
Silver	^{109}Ag	48.65	89.0	^{110}Ag	24.0s	658	12.0
			3.2	^{110m}Ag	253d	658	96.0
Sodium	^{23}Na	100	0.53	^{24}Na	15.0h	1368*,2754	100
Tin	^{112}Sn	0.95	1.20	^{113}Sn	115d	392	60.0
Vanadium	^{51}V	99.7	4.8	^{52}V	3.8min	1434	99.0
Zinc	^{64}Zn	48.9	0.82	^{65}Zn	243d	1116	50.6
	^{68}Zn	18.56	0.097	^{69m}Zn	13.9h	439	95.0

a
Ref.: NDS, BRU72, LED67, PAG71.

which can be retained on anion exchange resins. The distribution constants of Ag and Hg in Dowex 1x10 at different concentrations of HCl (MAR69) are shown in Table 4.2. The analytical grade anion exchange resin AG 1x8, supplied by the Bio-Rad Laboratories, is similar to the Dowex resin in retention property and was used in this work.

The chloride exchange column was prepared by the following procedure. Approximately 50g of the resin (AG 1x8, 200-400 mesh) was transferred to a clean beaker to which 100mL of water were added. The resin slurry was stirred with a clean glass rod and the very fine particles in the supernatant were decanted off. The process was repeated twice. The final resin slurry was transferred to a burette. The burette was fitted with a quartz wool plug at the bottom in addition to the stop-cock. The strongly basic anion exchange resin was converted to the chloride form by treating it three times with bed volume of 6M HCl.

Tracer solutions were prepared by irradiating 100µg of $AgNO_3$ for 30min and 100µg of $Hg(NO_3)_2$ for 15min at a flux of $5x10^{11}$n $cm^{-2}s^{-1}$. The radioactivities of the tracer solutions were sufficient to give statistically significant counts. Ten milligram quantities of each of the carrier solutions (i.e. inactive Ag and Hg) were added to the combined radioactive tracer solutions, and the volume of the mixture was adjusted to 25mL with 6M HCl.

TABLE 4.2 THE DISTRIBUTION CONSTANTS OF Ag AND Hg IN DOWEX 1x10 IN DIFFERENT CONCENTRATIONS OF HYDROCHLORIC ACID[a]

	Distribution coefficient	
HCl (M)	Ag(I)	Hg(II)
1	$7x10^2$	$5x10^4$
4	$1.1x10^1$	$6x10^3$
7	3	$7x10^2$
10	—	$9x10^1$

[a]Ref. MAR69.

The mixture was added to the prepared column and was allowed to pass through. The column was washed with 5mL water. The nuclides of Ag and Hg were eluted with successive additions of 10mL of 1.5M ethylenediammine (en), 15mL of 3M en, 5mL of 7.5M en, and 30mL of 15M NH_4OH. The above eluents were used because they are known to form strong complexes with Ag(I) and Hg(II) ions and these complexes are positively charged which are not retained on the anion exchange column. The metal ions came out from the column with the eluents were precipitated by sulfide after neutralization with acetic acid.

Several tracer experiments were carried out to optimize for maximum yields. A flow rate of 2mL min^{-1} was finally selected for a column with an internal diameter of 1.1cm. The average yields of triplicate analyses at two different column lengths under optimum flow rate and elution conditions are shown in Table 4.3. The short column gave poor recoveries and was, consequently, discarded. The 7-cm-long column produced yields around 90% which were not entirely satisfactory as yields around 100% were desired . Consequently, the reasons for low yields were investigated. First the resin was counted and no nuclides of Ag and Hg were detected in the resin. It was then concluded that loss could not have been occurred due to inefficient elution of the elements. And later about 80-90% of the added Ag(I) and about 30% of that of Hg(II) were found in the effluent from the short column. Subsequently it was also found that a few percent of both elements were not precipitated.

TABLE 4.3 RECOVERIES OF Ag AND Hg FROM THE CHLORIDE COLUMN

Element	Column length	
	1.3-1.5cm	7cm
Ag(I)	10±5%[a]	92±4%[a]
Hg(II)	60±10%[a]	87±5%[a]

[a] average of three determinations.

Several attempts to increase the efficiency of precipitation met with little success. Since the retention of silver(I) ion in an chloride exchange column is seriously influenced by the concentration of the hydrochloric acid used in dissolving the sample, development of a bromide column was attempted as a substitute.

4.1.2 Bromide Column

It is known that polarizing ions such as Ag(I) and Hg(II) can bind strongly to a polarizable anion such as bromide (VEL74). This observation was used in developing a bromide column for separating Ag(I) and Hg(II).

The bromide column was freshly prepared before use according to the following procedure. After the removal of very fine particles of the resin (AG 1x8, 200-400mesh) by decantation, the resin-water slurry was poured into a column (1.1cm in diameter) to a height of 7cm. A tribromide solution was prepared separately in a beaker by thoroughly mixing 8mL liquid bromine with 30mL of 5M KBr solution, and by allowing the excess bromine, which did not form soluble tribromide complex, to evaporate in a fume hood. The tribromide solution was introduced in the resin column which turned orange in colour.

Tracer experiments were carried out to evaluate various

column-separation parameters. The results obtained with tracer solutions at 9M, 6M and 3M HCl concentrations are shown in Table 4.4. The nuclides of Ag and Hg were retained on the columns and the chemical yields of the elements were determined.

It was obvious that the chemical yield depended on the concentration of HCl used. At both very high and very low HCl concentrations, yields of metal ions were poor. Surface adsorption and/or precipitation of metal halides could account for the low yields in low HCl concentration. The best results were obtained at 6M HCl as shown in Table 4.4.

A comparison between the chloride and bromide columns (Tables 4.3 and 4.4) showed that there were no significant differences between the two columns (7-cm-long, 6M HCl) for retaining Ag(I) and Hg(II). However, the effect of different HCl concentration on the retention of Ag and Hg was less pronounced in the bromide column compared to that in the chloride column. Consequently, the bromide column could be used with sample solutions where acidity and Cl^- ion concentration are slightly varied from optimum conditions.

Although Ag, Au, Hg, Pd and Pt were separated with more than 90% yield using the 7-cm-long tribromide column as presented in Tables 4.4 and 4.5, the column failed to adequately separate them using shorter column length. Also the determination of yields of the separated noble metals by re-irra-

TABLE 4.4 RECOVERIES OF Ag AND Hg FROM THE BROMIDE COLUMN

Element	HCl concentration		
	9M	6M	3M
Ag(I)	68±10%[a]	93±6%[a]	72±15%[a]
Hg(II)	76±7%[a]	90±5%[a]	91±5%[a]

[a] average of three determinations

TABLE 4.5 RECOVERIES OF Au(III), Pd(II) AND Pt(IV) FROM THE BROMIDE COLUMN[a]

Element	HCl concentration		
	9M	6M	3M
Au(III)	97±2%	99±3%	99±2%
Pd(II)	71±7	90±5	95±3
Pt(IV)	88±3	93±3	97±2

[a] average of three determinations

diation were interferred with by numerous intense gamma-rays of ^{82}Br. The further use of a bromide column in the present work was therefore discarded. A fresh attempt was made to separate the noble metals using an iodide column.

4.1.3 Iodide Column

It has been stated earlier that the noble metals are known to form complex compounds with halides where instability constants depend on many factors including the type of both metal and halide ions. A comparison of the instability constants of a few selected metal iodide and chloride complexes, presented in Table 4.6, shows that iodide forms stronger complexes than chloride. The retention and subsequent measurement of these complexes on an anion exchange resin could prove to be a simple as well as efficient method for determining the noble metals and possibly other elements of interest in biological materials.

After several preliminary experiments, conditions were standardized for the preparation of an iodide column. Following the general procedure described earlier, the very fine particles of the resin (AG 1x8, 200-400mesh) were removed. Approximately 1.5mL of the resin were placed in a glass column (diameter of 1.1 cm). About 4.5mL of 4M NH_4I solution were introduced into the column. The column was freshly prepared before use.

TABLE 4.6 INSTABILITY CONSTANTS OF SELECTED METAL IODIDE AND CHLORIDE COMPLEXES[a]

Iodide complexes				Chloride complexes			
Complex ion	Temp., °C	Ionic strength	pK	Complex ion	Temp., °C	Ionic strength	pK
AgI_3^{2-}	20	1.6	14.0	$AgCl_3^{2-}$	25	5.0	5.4
AgI_4^{3-}	20	1.6	13.8	$AgCl_4^{3-}$	25	5.0	5.3
				$AuCl_4^-$	18	-	21.3
CdI_4^{2-}	25	3.0	6.5	$CdCl_4^{2-}$	18	1.0-1.6	2.0
CuI_2^-	25	0.02-0.5	8.76				
				$CuCl_3^{2-}$	18	0.67	5.3
HgI_4^{2-}	25	0.5	29.8	$HgCl_4^{2-}$	25	0.5	14.9
				$PdCl_4^{2-}$	25	1.0-4.0	13.2
				$PtCl_6^{2-}$	-	-	16

[a]Ref. RIN63

Preliminary experiments involving the iodide column were carried out using a mixture of radioactive Ag(I), Au(III), Hg(II), Pd(II) and Pt(IV) tracer solutions. Tracers of known activities were added to known quantities of the above metals and then mixed with 6M HCl. The mixture was introduced in the iodide column. The liquid, after passing through the resin, was collected. Both the resin and liquid were counted to evaluate the efficiency of separation. The results are presented in Table 4.7. It can be seen that the noble metals can be quantitatively retained on the iodide column.

The analytical application of the iodide column for separating the noble metals depends on the radiochemical purity of the fraction containing the nuclides of these metals. The desired radiochemical purity can possibly be achieved in two ways: (i) by designing experimental conditions which will allow the retention of only the noble metals on the iodide column, or (ii) by developing a method which is highly selective for eluting the noble metals from the iodide column. Both of these approaches were studied in this project and are described below in detail.

The preliminary experiments (Table 4.7) showed that Ag(I), Au(III), Hg(II), Pd(II) and Pt(IV) were quantitatively retained on the iodide column in absence of other elements. Since biological materials are known to contain several elements in addition to the above metals, the retention behavior of these

TABLE 4.7 RETENTION OF Ag(I), Au(III), Hg(II), Pd(II) AND Pt(IV) ON THE IODIDE COLUMN

Element	Percentage retained on the column[a]
Ag(I)	100±3%
Au(III)	100±2%
Hg(II)	100±2%
Pd(II)	99±3%
Pt(IV?)	100±2%

[a]average of three determinations.

metals must be restudied in presence of commonly found major, minor and trace elements. Consequently, a mixture was prepared containing nuclides of interest: Ag, As, Au, Br, Cd, Co, Cu, Hg, In, K, Mo, Na, Pd, Pt, Sb, Se and Zn. This mixture of radioactive tracers was introduced into the iodide column and various elution experiments were then carried out.

The principal objective at this stage was to develop conditions for retaining only the noble metals on the column while eluting other elements. No special attempts were made to elute 100% of any given element in a single elution step. In other words, the elution of a given element at several elution steps could be tolerated as long as almost 100% of the element was eluted. After several initial experiments, the conditions were finally selected for eluting the undesirable elements while retaining the noble metals.

A flow-sheet for eluting 16 major, minor and trace elements from the iodide column is shown in Fig. 4.1. It can be seen that K passed through the column quantitatively. Similarly most of Na ($\simeq$95%) also passed through the column, the remaining ($\simeq$5%) was removed by washing with deionized water. Much of Br ($\simeq$20%) remained on the column prior to elution. Several elements, namely As, Br, Co, Cu, Mo, Sb, Se and Zn, were eluted in several fractions as shown in Fig. 4.1. Two elements, namely Cd and In, were eluted quantitatively in

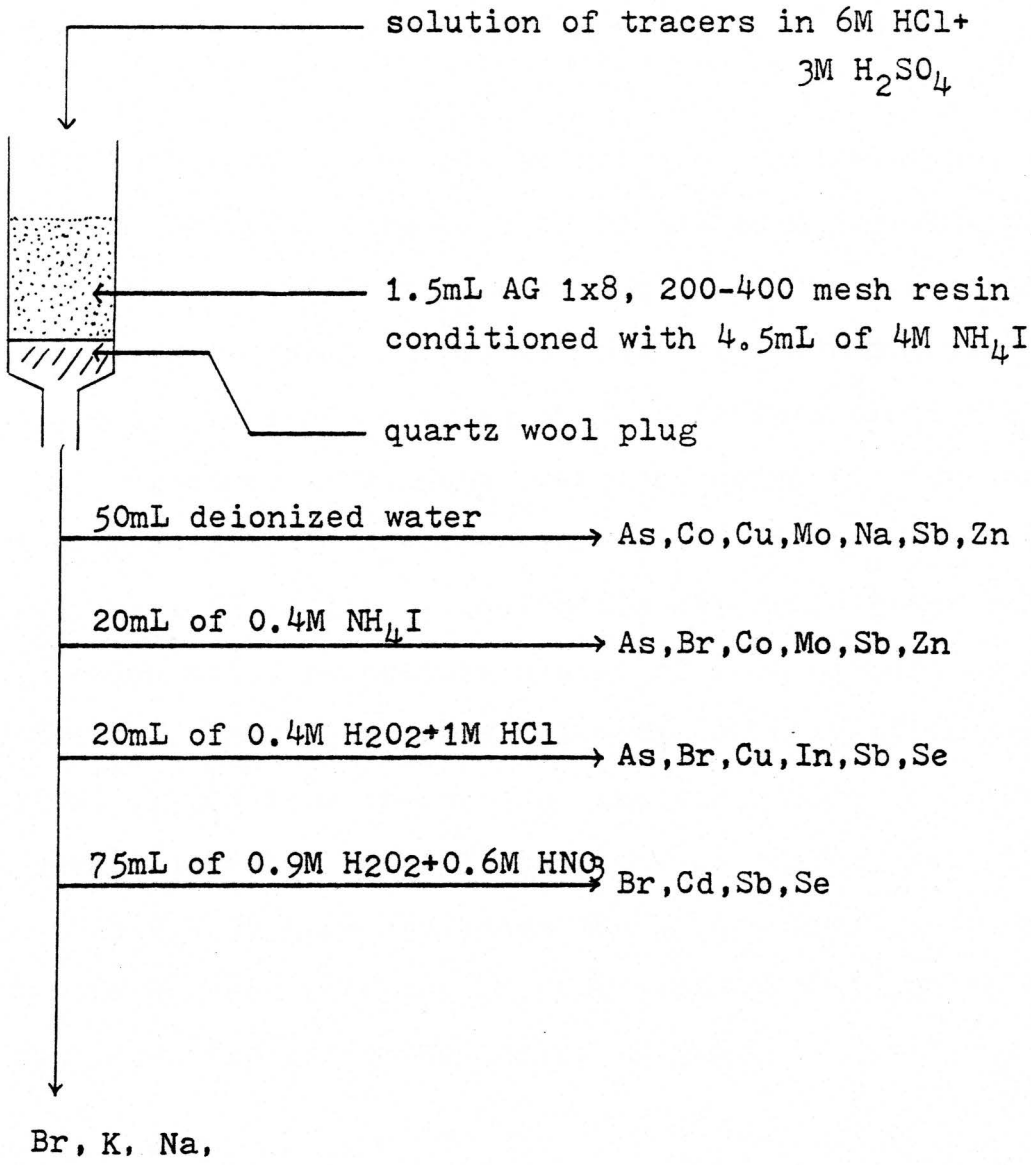


Fig. 4.1 Flowsheet for eluting elements from the iodide column.

single steps, and perhaps could be used for their determinations. The metals of interest, namely Ag, Au, Hg, Pd and Pt, were quantitatively retained on the column as originally desired.

The number of net counts before and after elution, in the integrated photopeak area of each of the nuclides studied are shown in Table 4.8. These counts for the 'retained on the column' category was obtained by counting the resin from the column in the same geometry as that of 'before elution', using a Ge(Li) detector. The counts were not corrected for the decay (t_d). A more practical representation of the retention and elution characteristics of the iodide column is given in Table 4.9 in which total percentage eluted of each element studied is tabulated. All undesirable elements were quantitatively (95-100%) eluted from the column, and the metals of interest were quantitatively (99-100%) retained on the column. This table (Table 4.9) also indicates the large amounts, not necessarily the highest amounts, of each element that can be efficiently separated using the iodide column.

Following the removal of the undesirable elements by the method described in Fig. 4.1, the resin along with the quartz wool from the iodide column were pushed into a polyethylene vial, and counted using a Ge(Li) detector. A typical gamma-ray spectrum of the resin is shown in Fig. 4.2. The gamma-rays emitted by the nuclides of interest, namely ^{110m}Ag, ^{198}Au,

TABLE 4.8 RADIOTRACER STUDY OF THE IODIDE COLUMN

Nuclide	γ-ray energy (keV)	number of net counts in the photopeak area	
		before elution [a]	retained on the column [b]
^{110m}Ag	885	3060	3030
^{76}As	559	70000	ND
^{198}Au	412	96000	74700
^{199}Au(from ^{199}Pt)	158	30490	24720
^{82}Br	776	50400	330
^{115}Cd	529	8500	77
^{60}Co	1333	3600	ND
^{64}Cu	1346	830	ND
^{203}Hg	279	1660*	1650
^{42}K	1525	6190	ND
^{99}Mo	739	1150	24
^{24}Na	1369	18000	ND
^{109}Pd	88	78000	23800
^{122}Sb	564	77500	2650
^{75}Se	264	4860	170
^{69m}Zn	439	1240	ND

ND- not detected; *- corrected for ^{75}Se

a- t_c= 300s; b- t_d= 22.8h, t_c= 300s.

TABLE 4.9 ELUTION OF ELEMENTS FROM THE IODIDE COLUMN

Element	Amount added (μg)	Amount retained (μg)	Amount eluted (μg)	Percent eluted (%)
Ag	100	99	1	1
As	10	0	10	100
Au	0.5	0.495	0.005	1
Br*	50	0.5	49.5	99
Cd	50	0.6	49.4	99
Co	50	0	50	100
Cu	100	0	100	100
Hg	10	10	0	0
K *	10	0	10	100
Mo	50	1.3	48.7	97
Na*	10	0	10	100
Pd	100	98	2	2
Pt[a]	100	100 ?	0	0
(by ^{199}Au)	100	100	0	0
Sb	10	0.4	9.6	96
Se	100	4	96	96
Zn	50	0	50	100

*- these tracers were prepared under different conditions.

a- determined by the absence of 191-keV gamma from the precipitate formed by the effluent with sulfide.

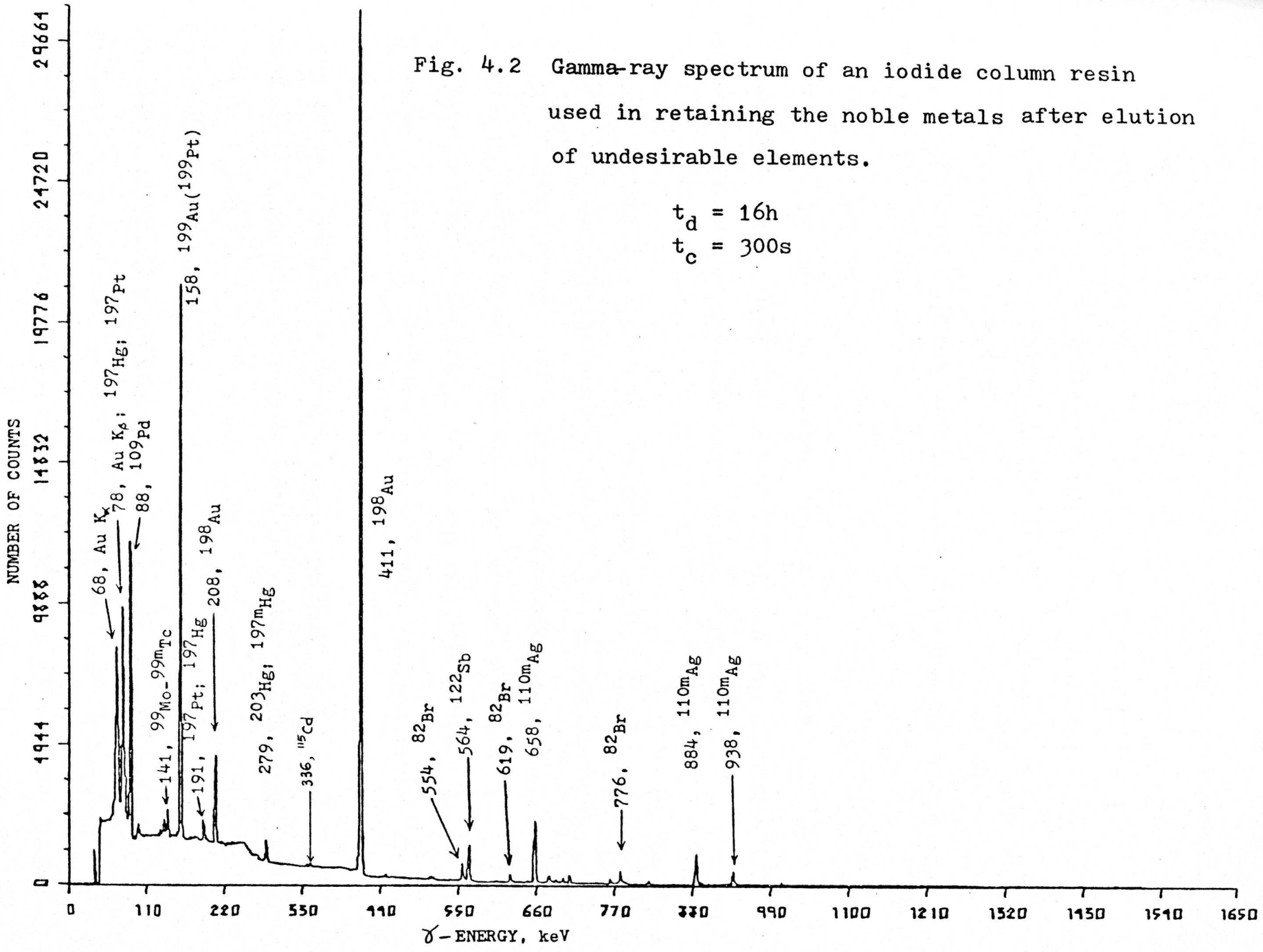


Fig. 4.2 Gamma-ray spectrum of an iodide column resin used in retaining the noble metals after elution of undesirable elements.

t_d = 16h
t_c = 300s

^{203}Hg, ^{109}Pd and ^{197}Pt, were all easily detected. In addition the gamma-rays emitted by the ^{99}Mo-^{99m}Tc and ^{122}Sb nuclides were detected in the spectrum as expected (Table 4.9 shows that 3% of Mo and 4% of Sb added were retained on the resin). Although tracer studies showed that 4% of Se added were retained on the column (Table 4.9), the most intense gamma-ray, 265-keV, of ^{75}Se was barely detected in the spectrum (Fig. 4.2). Consequently, any interfering contribution by the 279-keV gamma-ray of ^{75}Se to the 279-keV gamma-ray of ^{203}Hg was considered negligible. On the other hand, the gamma-rays emitted from ^{82}Br of the 1% of the total bromine remaining on the column were observable, as shown.

Following the success of the tracer study, it was decided to evaluate the precision and accuracy of measurement by the iodide column method. Two standard reference materials (SRM), namely the Bovine Liver (NBS SRM-1577) supplied by the U.S. National Bureau of Standards (NBS) and the Gelatin (TEG 50-B) provided by the U.S. Eastman Kodak Company, were analyzed. About 1g of the Bovine Liver and 100mg of the Gelatin SRM were dried according to the procedures prescribed by the respective agencies. The SRM were then irradiated for 16h at a flux of 5×10^{11} n $cm^{-2} s^{-1}$ in the DUSR, "cooled" for 24h to reduce radiation hazard from ^{24}Na, wet ashed by a method described later, and subjected to the radiochemical separation by the iodide column method. The average of three determinations are shown in Table 4.10. The agreement between the values

TABLE 4.10 Ag, Au AND Hg CONTENT OF STANDARD REFERENCE MATERIALS USING THE IODIDE COLUMN.

Element	Bovine Liver (NBS-SRM-1577)		Gelatin (TEG-50-B)	
	This work[a]	NBS[b]	This work[a]	Kodak[d]
Ag	58±11ng/g	(60ng/g)	104±15µg/g	100µg/g
Au	40±10ng/g	30ng/g[c]	-	-
Hg	-	16±2ng/g	54.4±6µg/g	55µg/g

[a] average of three determinations

[b] NBS certified values, otherwise specified; value in parentheses is provided for information only;

[c] NAD78;

d AND77.

obtained in this work and those reported by the NBS and Kodak is generally very good, wherever comparison can be made, indicating the reliability of the method developed. Levels of Pd and Pt could not be measured in either of the SRM-none was available from the suppliers. Obviously, these levels were below the detection limits of the method developed here.

Both qualitative detection limits and quantitative determination limits of measurements were calculated according to methods prescribed by Currie (CUR68). These limits for Ag, Au, Hg, Pd and Pt in the Bovine Liver are shown in Table 4.11. The detection limits ranged between 6 nanogram (ng) for Au to 1 microgram (μg) for Pd. These limits could be substantially lowered if the samples were irradiated in a considerably higher flux of neutrons (e.g. $5x10^{13}$n $cm^{-2}s^{-1}$) than the one used in this work (viz. $5x10^{11}$n $cm^{-2}s^{-1}$).

Having achieved the first objective, i.e. the development of experimental conditions which allowed the quantitative retention of Ag, Au, Hg, Pd,and Pt on the iodide column, attention was focussed on the second objective, i.e. the development of a method which would be highly selective for eluting the above metals from the iodide column. There were several reasons for pursuing the second objective. It was earlier stated that the resin containing the metal ions was later transferred to a polyethylene vial for re-irradiation and subsequent determination of chemical yields; this transfer

TABLE 4.11 THE DETECTION AND DETERMINATION LIMITS OF Ag, Au, Hg, Pd AND Pt USING THE IODIDE COLUMN

Element		Detection limit[a,b]	Determination limit[a,b]
Ag		10ng	38ng
Au		6ng	21ng
Hg	^{203}Hg	0.1µg	0.4µg
	^{197}Hg	25ng	90ng
Pd		1µg	4µg
Pt		0.1µg	0.4µg

[a] as defined by Currie, CUR68.

[b] t_i=16h at a flux of 5×10^{11}n $cm^{-2}s^{-1}$; t_d=1.5d; t_c=1800s.

process might result in a loss of a few mg of the resin, which could be avoided if the metals were eluted from the resin first, precipitated and then irradiated. Another reason for eluting the metals prior to re-irradiation would be the elimination of high background activity from the irradiated resin itself. A selective elution method could also eliminate Br and Se in eluate, which were partially ($\leqslant 5\%$) retained on the iodide column as explained earlier (Table 4.9). The detection limits for Ag, Au, Hg, Pd and Pt could be lowered slightly if the elution method was quantitative.

After several preliminary experiments, Ag, Au, Hg, Pd and Pt were eluted from the iodide column by a solution containing 4.4g thiourea dissolved in 100mL of 0.05M HCl. A solution of about 15mL containing 0.1g thioacetamide in deionized water was added to the eluate. The combined solutions were warmed for 20min at about 60°C. A black precipitate was obtained, which was collected using the radioactive precipitate filtration system.

A few factors were found to affect the recovery of elements from the iodide column. These are described below. (i) A certain amount of iodide ions were eluted by 6M HCl. The extent of elution was reduced by using low concentration of HCl, such as 0.05M HCl, and an optimum total volume (sample solution and wash) of 25mL. (ii) The amount of thiourea used also affected recoveries. If 11g of thiourea were used, about 50% of Ag(I) and Au(III) were not precipitated from the solution. (iii)

Other factors were the temperature and the duration of heating of the combined solutions of the eluate and thioacetamide.

The first two factors are related to the problem of precipitation in the presence of complexing ions. The other one is dependent on the different paths thiourea can be hydrolyzed.

In an experiment, the combined solution was boiled for more than 20min. The precipitate, which formed at first, redissolved upon heating. It did not reprecipitate even after cooling for two hours. Similar tests on long time heating at lower temperatures indicated that even heating for 45min at about 70°C did not alter the result very much. These observations could be explained in terms of the different hydrolysis mechanisms of thiourea:

$$3H_2O + H_2N\text{-}\overset{\overset{S}{\|}}{C}\text{-}NH_2 \xrightarrow{\text{warm}} CO_3^{2-} + 2\,NH_4^+ + S^{2-} + 2H^+$$

$$H_2N\text{-}\overset{\overset{S}{\|}}{C}\text{-}NH_2 \xrightarrow{\text{boiling}} SCN^- + NH_4^+$$

The second process is favoured at higher temperatures. The hydrolyzed product, thiocyanate, is a strong complexing agent. It will complex with the platinum metal ions and, in doing so, will dissolve the precipitate. The first reaction might be also faster at elevated temperatures. Thus it could produce a large amount of ammonium sulfide which would dissolve

the platinum metal sulfides. Therefore, the degree of heating must be controlled carefully. Warming the solution would hydrolyze the thiourea to sulfide and coagulate the precipitate, while strong heating would make precipitation almost impossible.

Since the above hydrolysis and precipitation processes might not be reproduced easily, if the reaction was not controlled properly, attempts were made to destroy thiourea. At first, methyl iodide was used; it was not successful. The possibility of using sodium nitrite was studied later.

$$H_2N\text{-}\overset{\overset{S}{\|}}{C}\text{-}NH_2 + 2\,NO_2^- + 2\,H^+ \longrightarrow 2\,N_2 + O{=}C{=}S + 3\,H_2O$$

A reaction as shown above takes place when thiourea is added to a nitrite solution.

An apparatus (shown in Fig. 4.3) was designed for carrying out the above process. Microgram amounts Ag, Au, Hg, Pd and Pt (in the form of solution) were spiked onto a Whatman 1 filter paper and irradiated for 16h at a flux 5×10^{11}n $cm^{-2}s^{-1}$. 10mg of each element (inactive) were added during the digestion of the irradiated sample. The solution was adjusted to 6M in HCl. It was loaded on to the iodide column, and eluted with 4.4g thiourea dissolved in 100mL of 0.05M HCl. Ten g of sodium nitrite were added to 80mL 2M HCl in the 250mL round-bottom flask used before elution. The flask was chilled in an ice-

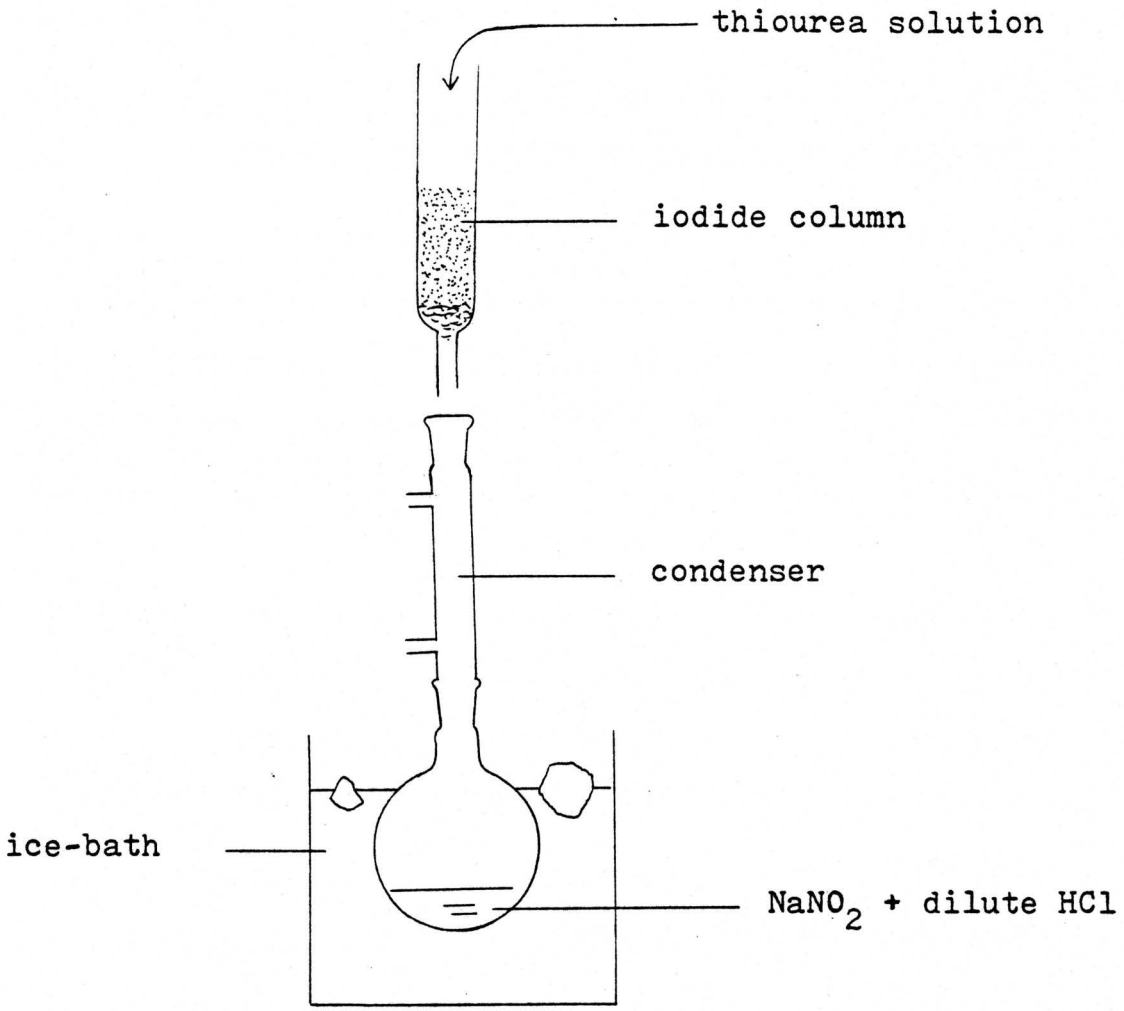


Fig. 4.3 The apparatus for the destruction of thiourea.

bath to avoid side reaction which was favored at elevated temperature. After the elution, the whole set-up was put aside for about 4h at room temperature. About 15mL of thioacetamide (0.1g) in water were added. The mixture was heated for 20min at about 60°C. It was cooled. The precipitate was collected on a filter and counted. The recoveries of triplicate analyses are given in Table 4.12, which shows quantitative elution and precipitation of the metal ions.

TABLE 4.12 THE RECOVERIES OF Ag, Au, Hg, Pd AND Pt FROM THE IODIDE COLUMN BY THE ELUTION METHOD[a]

Element	Amount added, μg	Percentage recovery
Ag	500	91 ± 5
Au	0.1	95 ± 2
Hg	5	98 ± 3
Pd	9	97 ± 2
Pt	10	100 ?
	10(by ^{199}Au)	95 ± 2

a- average of triplicate analyses.

It was stated earlier that the levels of Pd and Pt are very low in biological SRM. One geological SRM (USGS PCC-1) is known to contain these metals at ppb levels. It was decided to analyze this SRM. The concentrations of Br, Mn, Na, etc. in the PCC-1 are reported to be very high. Consequently, radiation hazards to the experimenter. Alternatively, the platinum metals could be preconcentrated using the separation method developed earlier, while the major interfering elements could be eliminated. This approach was followed up.

Approximately 2g of the PCC-1 were transferred to a Teflon beaker with 50μg of Pd and Pt, to which 80mL of ULTREX conc. HF were added in four equal portions. The mixture was heated at 110-120°C for a prolonged period. Ten mL of ULTREX conc. HNO_3 and 20mL of ULTREX conc. HCl were then added. The solution was evaporated to dryness. Fifty mL of ULTREX conc. HCl were added and boiled until the volume was reduced to 10mL. The process was repeated once. The final solution was adjusted to 6M in HCl. It was passed through the iodide column. The platinum metals were eluted and precipitated afterwards by the method described above. The dried product was irradiated for 16h at a flux of $5x10^{11}$ n $cm^{-2}s^{-1}$ and later counted with a Ge(Li) detector. The concentrations of Au, Pd and Pt obtained using this preconcentration method are shown in Table 4.13. The values reported in the literature have been generally found to agree well with the values obtained in this work. The limits

TABLE 4.13 CONCENTRATIONS OF Au, Pd, AND Pt IN THE USGS PCC-1 ROCK STANDARD USING PRECONCENTRATION AND IODIDE COLUMN ELUTION METHODS

Element	Concentration, ppb	
	This work	Literature values
Au	0.78±0.03	0.74±0.04[a], 3.4±1.4[b]
Pd	6.5±1.5	6.6±1[a], 3.0±0.1[b]
Pt	11±1.2	11.6±2.3[a], 5.1±1.5[b]

[a] Ref. : GAL78

[b] Ref. : NAD74

of detection and determination were calculated and are presented in Table 4.14. The limits obtained using the preconcentration and elution methods are generally good, particularly when the elements could not be determined by either INAA or RNAA. Although Ag and Hg were detected in the PCC-1, their concentrations could not be accurately measured due to large reagent blanks in chemicals added during the process.

In summary, several ion exchange chromatographic methods were developed in this study for separating Ag, Au, Hg, Pd and Pt, and are described above. It would be useful to compare all three halide columns under conditions suited best to each one. Such a study was carried out, and the results are shown in Table 4.15. The bromide column was perhaps the worst because the elements could only be partially retained under the condition used. The chloride column gave satisfactory results for some elements. However, the iodide column produced the best results-all the elements were quantitatively and reproducibly retained.

Since the iodide column can efficiently separate Ag, Au, Hg, Pd and Pt from a matrix, it would be useful to compare the advantages and disadvantages of the iodide column. These are listed in Table 4.16. The advantages are numerous. However, the column has two main weaknesses: it is not stable towards oxidizing agents and it is slightly contaminated with Br and Sb. The later weakness can cause problems when the resin

TABLE 4.14 THE DETECTION AND DETERMINATION LIMITS OF Ag, Au, Hg, Pd, AND Pt IN THE USGS PCC-1 USING PRECONCENTRATION AND IODIDE COLUMN ELUTION METHODS

Element	Detection limit,[a] ng	Determination limit,[a] ng
Ag	4	14
Au	1	4
Hg	5	18
Pd	19	63
Pt	11	37

[a] as defined by Currie, CUR68.

TABLE 4.15 THE COMPARISON OF CHLORIDE, BROMIDE AND IODIDE COLUMNS FOR SEPARATING Ag, Au, Hg, Pd AND Pt[a]

Element	Percentage retained		
	Cl^- column	Br_3^- column	I^- column
Ag	0	45	100
Au	100	96	100
Hg	100	40	100
Pd	94	10	99
Pt	90	0	100

a **Washing** used was 10 ml 6M HCl.

The sample matrix was composed of 10 ml solution in 6M HCl and 3M H_2SO_4.

The columns used were composed of 1.5 ml AG 1 x 8, 200 - 400 mesh resin.

TABLE 4.16 ADVANTAGES AND DISADVANTAGES OF THE IODIDE COLUMN

Advantages	Disadvantages
1. Quantitative retention of Ag, Au, Hg, Pd and Pt	1. Oxidizing agents must be absent
2. Quantitative elution of the above metals	2. Up to 5 % Sb and 1 % Br can be retained.
3. Sample solution in up to 6M HCl can be used	
4. Chemical yields can be determined by re-irradiation of the resin	
5. Excellent reproducibility	
6. High specificity	
7. Good sensitivity	
8. Good precision and accuracy	
9. Not too time consuming	

itself is re-irradiated for chemical yield determinations. Since Br is volatile, and since Sb can form volatile compounds, it will be advantageous to include a distillation step prior to separation by the iodide column.

4.2 Radiochemical Separation of As, Br, Hg, Ru, Sb, Se and Sn by Distillation

Since the iodide column developed cannot be exposed to oxidizing agents such as nitric acid, used in the wet digestion of the tissues, these chemicals have to be removed by some procedure prior to the introduction of the solution into the iodide column. Moreover, the eluate from the iodide column has to be evaporated to reduce the volume and to remove the iodide ion and iodine present in the solution. During this evaporation, certain elements such as As, Sb and Se can be lost as volatile halides. Both the elimination of nitric acid and the determination of volatile elements can be accomplished by incorporating a distillation step before separation by the iodide column. The first step in a radiochemical separation scheme is generally the dissolution of the sample by digestion with acids. The apparatus set-up for digestion is similar to that required for distillation. Only a small effort is needed to switch the set-ups. Considering all these aspects, it was decided to develop a distillation method for separating As, Br, Hg, Ru, Sb, Se and Sn. The method is described below in detail.

A mixture containing microgram amounts of As, Br, Hg, Ru, Sb, Se and Sn deposited on Whatman No.1 filter was irradiated for 16h at a flux of $5x10^{11}$n $cm^{-2}s^{-1}$. The distillation method was studied using tracers of the above elements. The first distillation method developed (called the old version) consisted of the following step:

(i) The irradiated filter containing tracers was transferred to a 100mL round-bottom flask to which 100µg carriers of each element were added.

(ii) A condenser was placed on top of the round-bottom flask and the quick-fit joint was wetted with concentrated sulfuric acid.

(iii) Four mL concentrated sulfuric acid were added to the flask, and the mixture was heated for 15min.

(iv) After the black slurry was cooled to about 50°C, 4mL 30% H_2O_2 solution were added. The solution was heated slowly to boil until all the excess hydrogen peroxides was decomposed.

(v) The digested solution changed colour to pale yellow. It was ice-cooled. Then 2mL of concentrated perchloric acid were added to it.

(vi) Ten mL of concentrated hydrochloric acid were added through the condenser.

(vii) The digestion set-up was then changed to a distillation set-up.

(viii) Two mL of concentrated hydrobromic acid were added. The quick-fit joints were checked for leaks. Distillation was

then carried out.

(ix) The flask was heated until fumes of SO_3 were observed.

(x) The flask was cooled. Two mL ice-cooled concentrated HBr were added. Distillation was resumed as in step (ix).

(xi) Step (x) was repeated once.

The distillate was collected in a 50mL graduated Erlenmeyer flask, stoppered, and counted using a Ge(Li) detector. Recoveries of all the nuclides were calculated. The average of 6 determinations for each element studied is given in Table 4.17.

It is clear from Table 4.17 that the distillation method gave satisfactory yields for As, Br, Hg, Sb, Se and Sn. Tracer of Au was also added to check its behavior during distillation. Results showed that 10% of Au added was distilled along with other elements. Gold halides might be partially volatile at a temperature of about 360°C at which fumes of SO_3 would also appear from sulfuric acid. This was also observed by other researchers (DES72) who found that only 1% gold would be lost if the temperature was controlled below 220°C. It was thought that the distillation efficiency would be improved if an adding device, which was made by heat-sealing the bottom end of a Quick-Fit adaptor to a small hole, was used. The flow rate of liquids passing the hole was regulated by a small glass rod on the hole. Concentrated nitric acid was used in addition to concentrated sulfuric acid to destroy the organic matter present in materials such as liver tissues.

TABLE 4.17 RECOVERIES OF SOME ELEMENTS BY THE DISTILLATION METHOD

Element	As	Au	Br	Hg	Ru	Sb	Se	Sn
Old version (N = 6)	95±6	10±3	100±2	90±5	n.d.	96±5	94±7	100±2
New version (N = 6)	100±2	2±1	100±2	98±3	100±2	98±3	100±2	100±2

n.d. = not determined.

The modified distillation method (called the new version) consisted of the following steps:

(i) The irradiated filter containing tracers was transferred to a 100mL round-bottom flask with 100μg carriers of each of the volatile element.

(ii) A condenser was placed on top of the round-bottom flask as before.

(iii) Two mL deionized water were added followed by 2mL concentrated sulfuric acid and then 1mL concentrated nitric acid.

(iv) The solution was warmed to about 40-50°C. Heating was continued until fuming of nitric oxide ceased. Six mL of 30% hydrogen peroxide solution were added through the condenser. Then the temperature was slowly increased to about 80°C. Oxidative digestion was almost complete by this time. Finally, the solution was boiled for additional 5 min to remove any hydrogen peroxide that might have remained undecomposed.

(v) The flask was then cooled and changed into distillation arrangement. Two pieces of glass boiling chips were added to the flask. About 4mL concentrated hydrobromic acid were added to the solution. Distillation was proceeded. After most of the solution had been distilled, about 8mL concentrated hydrobromic acid were added dropwise to the flask through the adding device, while the flask was maintained at about 220°C via the reading of a pyrometer and the regulation of a power regulator. In the experiments with Ru, 2mL of concentrated perchloric acid were added followed by 4mL of 30% H_2O_2, before the addition of concentrated hydrobromic acid.

After the distillation was completed, the set-up was allowed to cool to room temperature. The remaining solution (residue) in the round-bottom flask was saved for processing in the iodide column. The distillate, which was collected in a 50mL Erlenmeyer flask, was counted using a Ge(Li) detector. The average recovery values of the elements studied are listed in Table 4.17. It can be seen that the recoveries in the new version of the distillation method are superior to those of old version. These enhancements can be explained by the increase in the distillation efficiency and minimizing losses during the addition of acids.

4.3 Radiochemical Separation of Co, Fe, Mo, and Zn by Ion Exchange Chromatography

Although several elements could be determined using a combination of distillation and iodide column chromatographic methods, a few elements such as Co, Cu, Fe, Mo, Sc, Zn, etc. could not still be measured in tissue samples. The nuclides of these elements were not detected by counting the eluate from the iodide column, because of the presence of large amounts of highly radioactive ^{24}Na. A chloride conditioned anion exchange chromatographic method was developed for the separation of some of the above elements from the matrix.

A solution containing radioactive tracers (50µg each) of Co, Fe, Zn, and Mo was adjusted to 6M in HCl. A chloride

loaded anion exchange column was set up (8-9mL AG 1x10, 100-200 mesh resin). The resin was packed in a buret as described before. It was then conditioned with three times of the resin bed volume of concentrated hydrochloric acid. The sample solution was passed through the column at room temperature. Cobalt, iron, molybdenum and zinc were retained on the column. The column was washed with 3mL of 6M HCl. Cobalt was eluted first with 15mL of 4M HCl. Another 15mL of 1M HCl were used to elute iron and molybdenum. Finally, zinc was eluted with 30mL deionized water and 20mL 1M NaOH. The yields of Co, Fe, Mo and Zn obtained using tracers are shown in Table 4.18. The results show that all four elements were quantitatively recovered.

4.4 Radiochemical Separation of Cu and Sc by Solvent Extraction

An RNAA method was developed for determining Cu and Sc using the information published (DAT63) on trioctylphosphine oxide (TOPO) extraction.

A solution of radioactive Cu and Sc tracers in 15mL of 6M HCl was prepared and placed in separating funnel. An equal volume of 5% TOPO in toluene was added to it. The solution was shaken well and allowed to separate. The supernatant (organic phase) was removed using a micropipet. The aqueous phase was extracted three more times under the same condition.

TABLE 4.18 RECOVERIES OF Co, Fe, Mo, AND Zn BY THE CHLORIDE EXCHANGE CHROMATOGRAPHIC METHOD

Element	Percentage recovered[a]
Co	98±2
Fe	98±2
Mo	85±6
Zn	96±5

[a]average of 3 determinations

The results of the triplicate analyses of the tracers are shown in Table 4.19. The recovery of Cu was improved using a solution of 6M in HCl, whereas that of Sc did not change much. Hence, it is recommended that 6M HCl be used in the TOPO extraction of Cu and Sc.

4.5 A complete RNAA Scheme for Analyzing Neoplastic Tissues

A careful examination of the typical values of elemental contents of tissues, as listed in Table 4.20, and the nuclear characteristics (Table 4.1) of the nuclides produced from these elements on neutron activation revealed that some, but not all, of the elements of interest perhaps could be determined easier by INAA than by RNAA. For example, ^{60}Co, ^{59}Fe, ^{86}Rb and ^{46}Sc with half-lives of 5.25a, 45d, 18.7d and 84d, respectively, could be measured by INAA. Although there would be a slight advantage in analyzing the above nuclides with greater sensitivity and shorter decay period by RNAA, the time and labour required in carrying out radiochemical separations would not make them worthwhile. Moreover, an irradiation facility (DUSR) was available on-site in the meantime. Consequently, a modified RNAA scheme based on the above methods (Sections 4.1 through 4.4) was designed to measure the elements (viz. As, Au, Cd, Cu, Hg, Mo, Pd, Pt, Sb and Zn) which could not or might not be determined by INAA. The entire RNAA scheme, as used for analyzing neoplastic tissues, is described below in detail.

TABLE 4.19 RECOVERIES OF Cu AND Sc BY THE TOPO EXTRACTION METHOD

	Percentage extracted [a,b]	
	aqueous phase in 4M HCl	aqueous phase in 6M HCl
Cu(II)	70±3%	85±4%
Sc(III)	94±4%	95±5%

a- extracted 4 times with equal volume 5% TOPO in toluene;
b- average of triplicate determinations.

TABLE 4.20 RANGES OF ELEMENTAL CONTENT OF DRIED TISSUES OF NORMAL RATS[a,b] (IN PPM)

Element	Heart	Kidney	Liver	Lung	Spleen
Ag	-	0.01-0.02	0.003-0.03	0.02-0.03	0.02-0.05
As	-	0.05-0.4	0.09-0.26	0.09-0.69	0.26-1.7
Au	-	$(1.5\text{-}8.2)10^{-5}$	$(0.2\text{-}9.5)10^{-5}$	$(7.5\text{-}20)10^{-5}$	$(2\text{-}160)10^{-5}$
Br	7-12	15-26	11-15	17-40	-
Co	4.5-8.8	0.018-0.06	$(6\text{-}9)10^{-3}$	$(5\text{-}15)10^{-3}$	0.9-1.3
Cr	1-5.7	1-4.6	0.03-41	0.1	17-42
Cu	-	4.4-8.1	1.0-7.6	0.84-1.5	0.4-8.1
Fe	280-680	35-520	29-390	44-78	120-2300
Hg	0.04-0.2	0.02-1.2	0.03-1.2	$(1.7\text{-}20)10^{-3}$	0.003-1.7
Rb	-	4.7-5.9	2.1-2.5	5.3-5.8	8.2-15.4
Sb	1.2-2.5	0.005-2.4	0.02-5.8	0.02-0.03	0.02-7.6
Se	-	0.044-0.34	0.08	0.03-0.07	0.04-0.14
Zn	80-110	23-38	15-110	23-44	72-110

a Ref. : GIR77,KOS77 ; b Age : = seven months.

4.5.1 Digestion

The tissues obtained from the experimental mice were rinsed with deionized water in an ultrasonic bath for 3 min. The samples were weighed, then freeze-dried, and reweighed. The samples were first subjected to INAA as described in the Section 4.6. After a long decay, the samples were irradiated again for sixteen hours in a neutron flux of $5x10^{11}$n $cm^{-2}s^{-1}$. These samples were processed for RNAA within two days.

After washing the outside of the vials with 2M HNO_3 solution, they were centrifuged to settle the irradiated solid lumps down at the bottom of the vials. The top of the vials were carefully removed by a scalpel. The tissues were transferred to a 100mL round bottom flask. Inactive carriers of Ag, As, Au, Cd, Cu, Hg, Mo, Pd, Pt, Sb, Se and Zn were added to the flask. Two mL of deionized water followed by 2mL concentrated sulfuric acid and 1mL of concentrated nitric acid were added. A condenser was fitted on the top of the flask. The mixture of tissue, carriers, and acids were warmed to 40-50°C. Six mL of 30% hydrogen peroxide were added through the condenser and heated to about 80°C for several hours. The tissues were completely dissolved by this procedure.

4.5.2 Distillation

The modified distillation procedure (the new version)

described in the Section 4.2 were essentially followed with minor changes. Briefly, the distillation was carried out at about 220°C with the addition of 12mL concentrated hydrobromic acid without the addition of perchloric acid. The residue (solution in the distillation flask) was kept aside for further processing by the iodide column. The distillate was collected and treated to recover As, Br, Hg, Sb and Se.

However, a simple distillation process like this cannot separate the trace elements of interest from the highly active ^{82}Br which is present in large amounts in the distillate. This problem can be solved by removing from the distillate either Br as described by Samsahl (SAM67) or the elements of interest as reported by Rosenberg (ROS79). The first method requires quite an extensive set-up of glass apparatus and a large number of separation steps, and cannot be easily carried out in a small laboratory. Similarly, the second method is fairly cumbersome. Consequently, a method involving sulfide precipitation was developed to remove Br from the distillate as described below.

The distillate was collected in a round-bottom flask immersed in an ice-bath. After the completion of distillation, the condenser was rinsed with 1M HCl to wash down any deposited volatile condensate. The combined solution in the flask was removed from the distillation apparatus and was placed in a fume-hood. Some bromine vapor emitted from the

distillate was removed by sucking with a water suction device. The other volatile elements were not lost during this process in any detectable amount as revealed by tracer experiments. Not all the Br was removed by the above process. The flask containing the solution was then fitted back with the condenser. About 10g of hydrazine sulfate were added through the condenser until the solution changed color from orange to colorless. By then bromine was reduced to bromide. A solution prepared by dissolving 0.2g thioacetamide in 20mL deionized water was introduced into the flask. The solution was adjusted to about 1M in HCl and then heated to about 100°C. It was then set aside for several hours to age the precipitate at about 50°C. The precipitate was collected on a Whatman No. 1 filter with the radioactive precipitate filtration system, and washed with 10mL of 1M HCl and then rinsed with deionized water. The precipitate with the filter paper was then transferred to an irradiation vial at a fixed geometry. The radioactive precipitate was counted using a Ge(Li) detector. The precipitate was later reirradiated for 15min and counted for determining the recovery values of these elements after a day's decay. The average recovery values of three experiments were 98±2, 95±3, 97±3, 99±2 and 0.01% for As, Sb, Hg, Se and Br, respectively.

The gamma-rays emitted by ^{76}As and ^{122}Sb could be mutually interfering. However, since the detector used in this work was a high-resolution Ge(Li) detector, the 559- and 564-keV

photopeaks of ^{76}As and ^{122}Sb, respectively, were well resolved. Their determinations through the above peaks were feasible as long as the ratio of As to Sb levels was not too different from 1. Thus further separation of these elements into single element fractions was not required.

4.5.3 Ion Exchange Chromatography

The iodide column method developed earlier (Section 4.1.3) was extended to include the determination of Cd, Cu, Mo and Zn in neoplastic tissues. The modified methodologies are described here. As discussed in the Section 4.1.3, iodide column has the ability to reduce certain metal ions. Although a trace amount of copper was retained on the iodide column, the possibility of retaining copper(II) was obviously questioned in view of the above statement. A tracer solution of copper was loaded on an iodide column. It was found that the eluent contained no detectable amount of radioactive ^{64}Cu. A mathmetical calculation has been done and is shown in the appendix. The result supported that copper(II) was reduced to copper(I). It also predicted that for half-reactions with reduction potentials greater than 0.416 V, the reduction reaction would take place. The observation that copper ion was eluted from the column in earlier experiments suggested that under certain conditions it might be separated by the iodide column. Under extensive tracer study, it was found that copper ions would be completely retained on an iodide column as long as the

matrix was in 0.4M I^-. It might be eluted with 1M HCl. The problem of tailing existed in such an elution chromatography. To avoid elution, copper fraction was first oxidized using mild oxidizing agents. Oxidation of copper(I) was expected to be faster than iodide ion because there was no phase change after the reaction. This deduction was supported by the fact that under stronger oxidative condition, copper ion would adhere to the column strongly instead of being eluted. After such a treatment, the elution of copper was unsuccessful by all means. Using these results, the following procedures were established.

The residue remaining after the distillation step was adjusted to 20mL with 6M HCl. The iodide column was prepared as described in the Section 4.1.3. After the passage of the sample solution, the iodide column was washed twice with 6mL solution composed of 5mL of 4M HCl and 1mL of 4M NH_4I. The effluent and the washings were discarded. The column was eluted to recover the elements as follows.

(i) Fifty mL of 0.4M NH_4I were added to the column and the flow rate was adjusted to 1 drop/10 s. Zinc and molybdenum were recovered in this fraction with yields of about 97 ± 2 and $90\pm4\%$, respectively, in triplicate tracer experiments.

(ii) Ten mL solution composed of 0.5mL 30% H_2O_2 in 1M HCl were added to the column. In this fraction copper was

recovered with a yield of 100±1%.

(iii) Then a 75mL solution consisting of 6mL of 30% H_2O_2, 1mL of concentrated HNO_3 and deionized water was added to the column. Cadmium was recovered in this fraction with a yield of 97±2%.

(iv) The column was counted after these treatments. Gold, silver, palladium and platinum were determined with yields of 98±2, 100±2, 100±5 and 98±2%, respectively, obtained in triplicate tracer experiments. However, later on-during the analyses of the tissues, it was found that noble metals could not be measured. Their concentrations in the real samples were lower than the detection limits which were limited by the low flux of neutrons used in this work.

(v) In view of the observations in step (iv), it was decided not to elute Cd from the column by the method in step (iii). Instead, the column, after step (ii) elution, was directly counted with a Ge(Li) detector. The recovery of Cd was 100±2% as determined by numerous experiments.

The recoveries of all elements separated by the above methods were between 97-100% with the exception of Mo. Extensive studies were carried out to determine the yields of Mo and Zn in fraction collected from the step (i). Five mL glacial acetic acid and 4mL 30% hydrogen peroxide were added

to this fraction. The solution was warmed to 40°C for 30min. It was then evaporated to almost dryness at about 120°C. The salt formed was volatile to certain extent and thus was partially removed. The solution was then transferred to an irradiation vial with rinsates, and dried under an I.R. lamp. The vial was heat-sealed and then subjected to INAA by an irradiation of 10min. It was concluded from these experiments that the loss of Mo due to the above yield determination process was less than 2% and some Mo remained on the iodide column (as shown in Fig. 4.8).

4.5.4 Summary

The complete RNAA scheme for analyzing neoplastic tissue samples is presented in Fig. 4.4. Following the wet digestion of the irradiated sample and exchange of radioactive isotopes and inactive carriers, distillation was carried out. The elements of interest, namely As, Hg, Sb and Se, were quantitatively recovered from the distillate after sulfide precipitation; the interfering element Br was removed at this step. The residue from distillation was introduced on the iodide column. The interfering elements, such as Cl, K, Na, etc., passed through the column. The elements of interest, namely Au, Cd, Cu, Mo, Pd, Pt and Zn, were either eluted or measured by counting the iodide column itself as shown in Fig. 4.4.

A multielement tracer experiment was carried out using

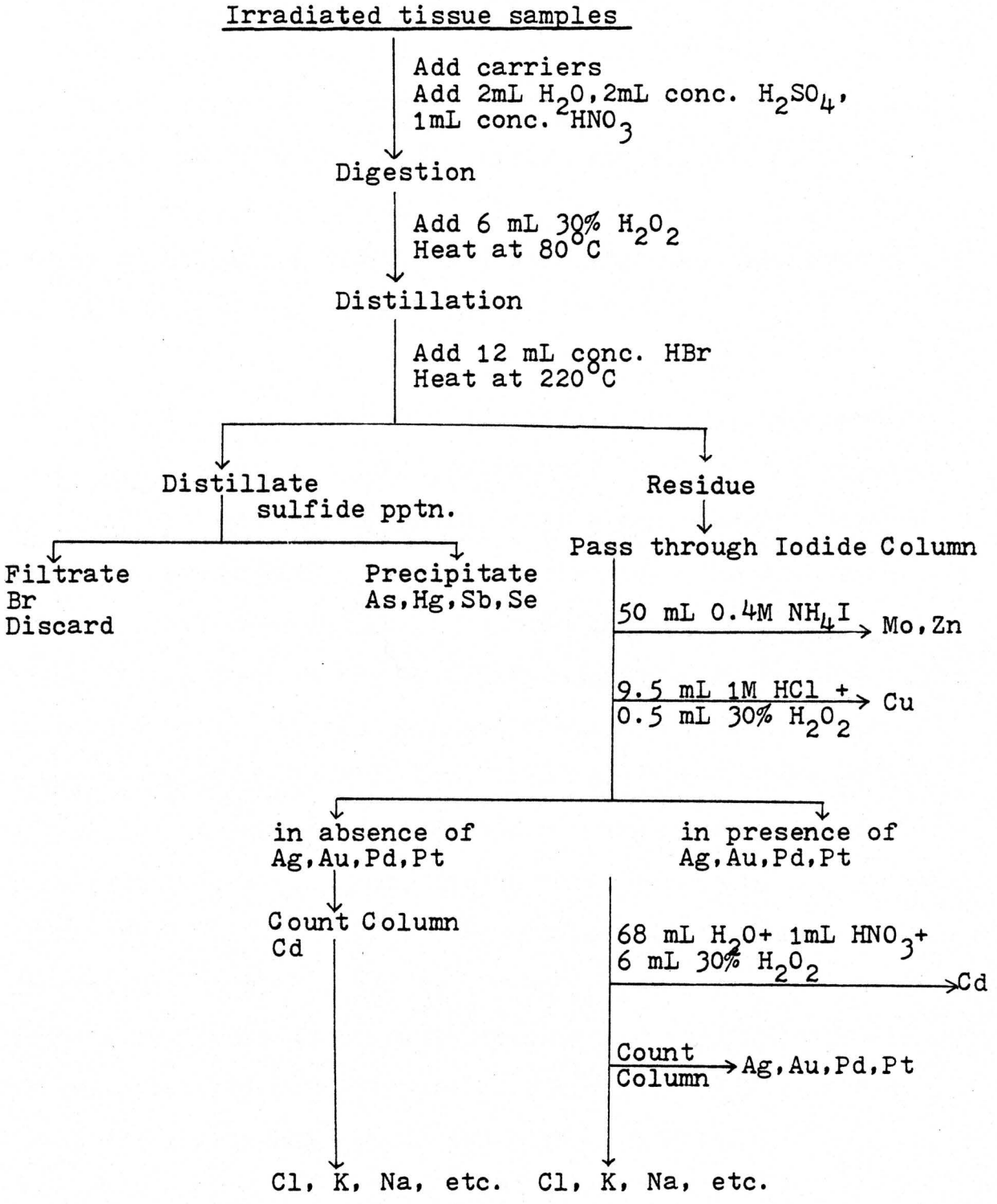


Fig.4.4 Summary of the RNAA scheme used for analyzing neoplastic tissues.

the above scheme. Various radioactive tracers were first produced and then added to 0.5g of freeze-dried pork liver (to simulate mice liver which was at scarce supply). The mixture was digested as usual and the tracers were separated and counted using a Ge(Li) detector. The results of this study are presented in Table 4.21. It can be seen that the tracers were quantitatively separated and recovered by the RNAA scheme developed in this work.

The RNAA scheme was then applied to various neoplastic tissues for determining their elemental levels. The gamma-ray spectra of separated fractions of a real sample (mice liver) are presented in the next few pages. The spectrum of the distillate fraction containing the nuclides of As, Hg, Sb and Se is shown in Fig. 4.5. The spectrum of the fraction eluted from the iodide column and containing the nuclides of Mo and Zn is given in Fig. 4.6. The spectrum of the eluted Cu fraction is presented in Fig. 4.7. The gamma-rays of radio-cadmium can be identified in the spectrum of the iodide column itself shown in Fig. 4.8. It has already been pointed out that the levels of Ag, Au, Pd and Pt were too low in normal mice livers to be detected; none is seen in Fig.4.8. The gamma-rays of ^{99}Mo-^{99m}Tc were identified in Fig. 4.8 as Mo was not completely eluted from the iodide column.

The precision and accuracy of the RNAA method developed were evaluated by analyzing various standard reference

TABLE 4.21 MULTIELEMENT TRACER STUDY WITH THE ADDITION OF 0.5GRAM FREEZE-DRIED PORK LIVER

Element	Tracer added (µg)	Carrier added (mg)	Tracer recovered									
			distillation precipitate		iodide column							
					Mo-Zn		Cu		Cd		column	
			µg	%	µg	%	µg	%	µg	%	µg	%
As	10	3	9.8	98	-	0	-	0	-	0	-	0
Br	10	-	-	0	-	0	-	0	-	0	-	0
Hg	5	3	5	100	-	0	-	0	-	0	-	0
Sb	5	5	4.7	94	-	0	-	0	-	0	0.1	2
Se	20	3	20	100	-	0	-	0	-	0	-	0
Mo	5	2	-	0	4.7	94	-	0	-	0	0.1	2
Zn	10	4	-	0	9.6	96	-	0	-	0	-	0
Cu	50	1	-	0	-	0	49	98	-	0	-	0
Cd	10	1	-	0	-	0	-	0	9.9	99	-	0
Ag	10	1	-	0	-	0	-	0	-	0	9.5	95
Au	0.05	-	0.001	2	-	0	-	0	-	0	0.048	96
Pd	5	-	-	0	-	0	-	0	-	0	4.7	94
Pt	50	-	-	0	-	0	-	0	-	0	48	96

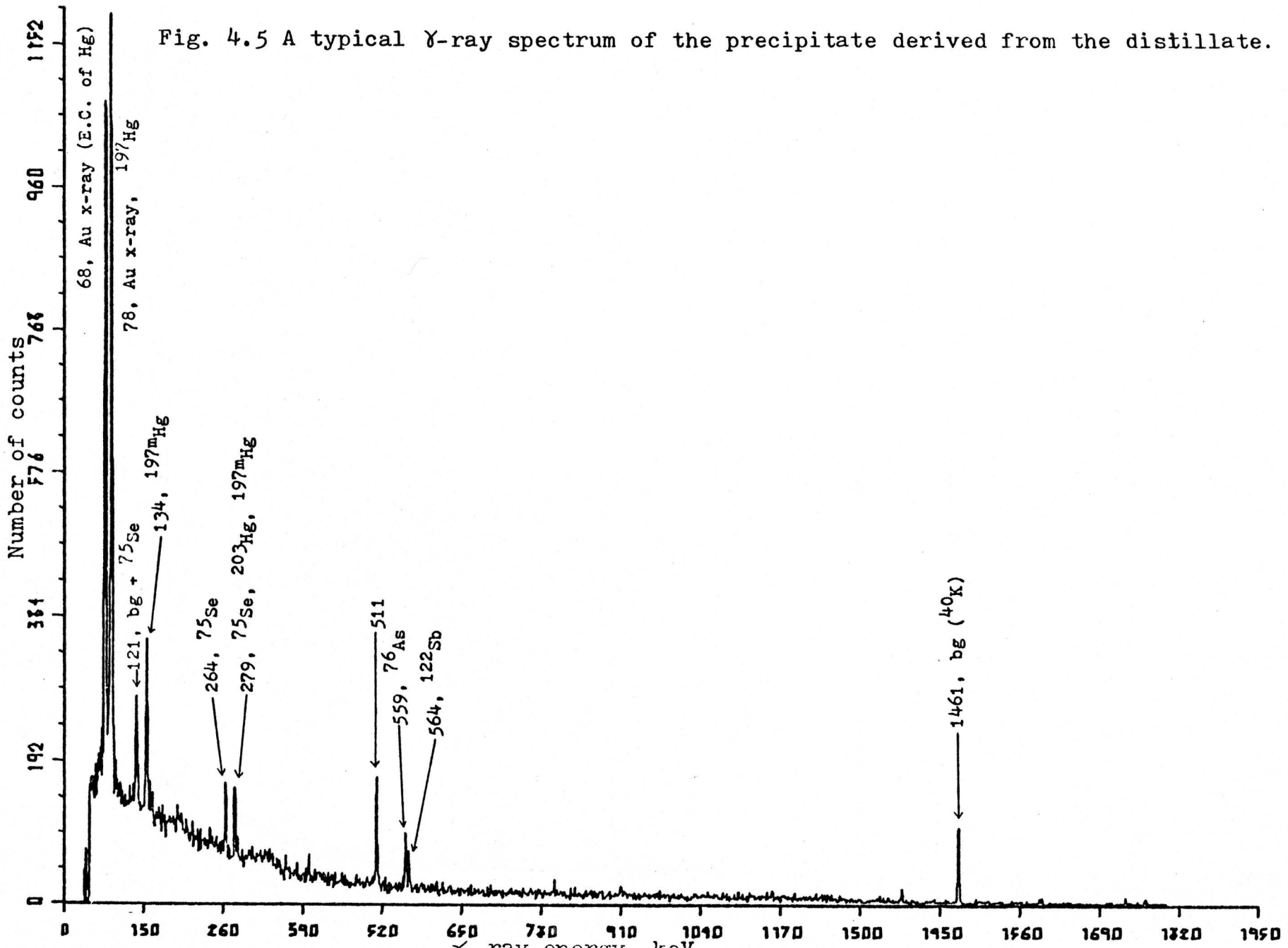


Fig. 4.5 A typical γ-ray spectrum of the precipitate derived from the distillate.

Fig. 4.6 A typical γ-ray spectrum of Mo-Zn fraction.

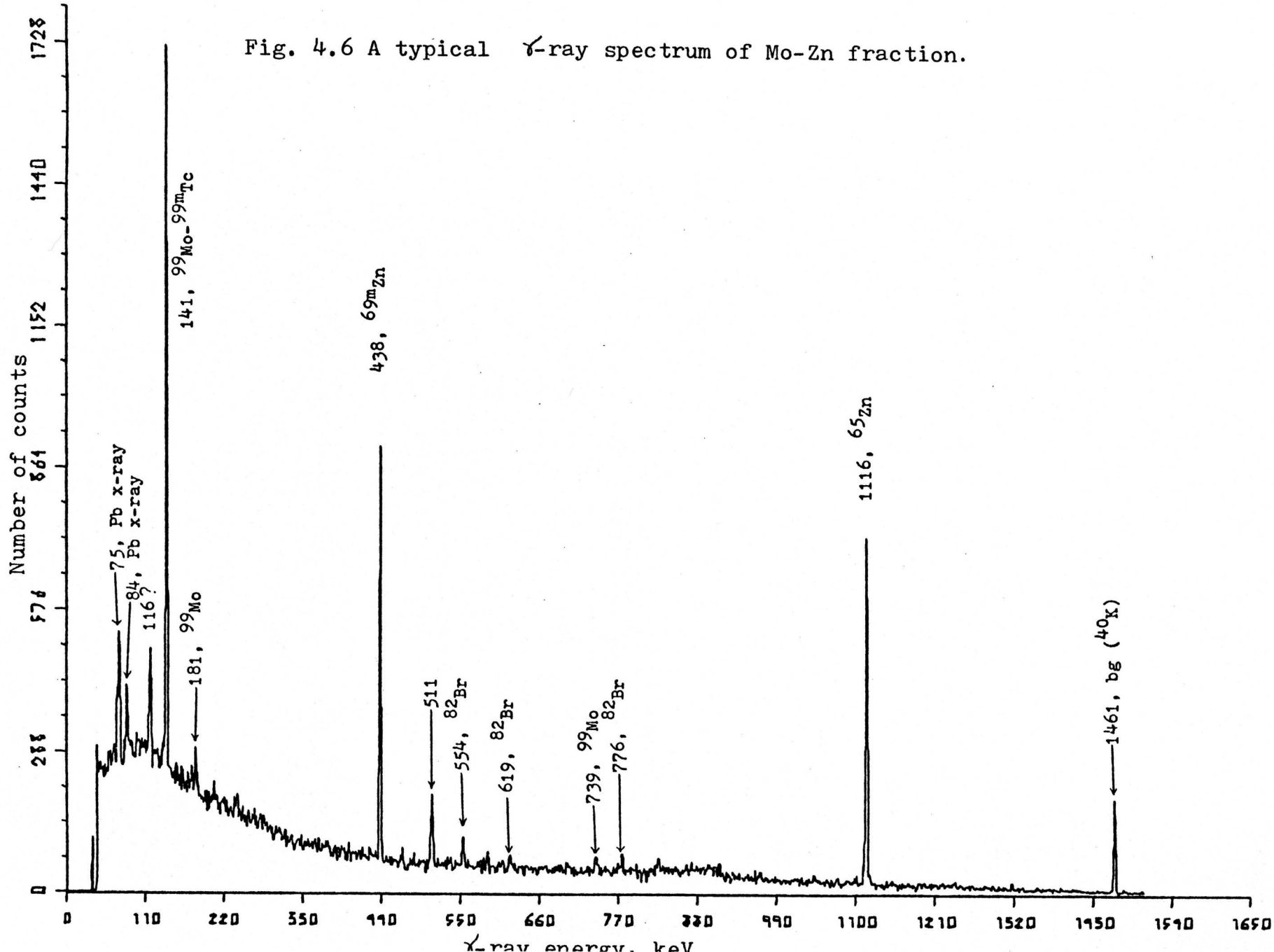


Fig. 4.7
A typical γ-ray spectrum of the Cu fraction.

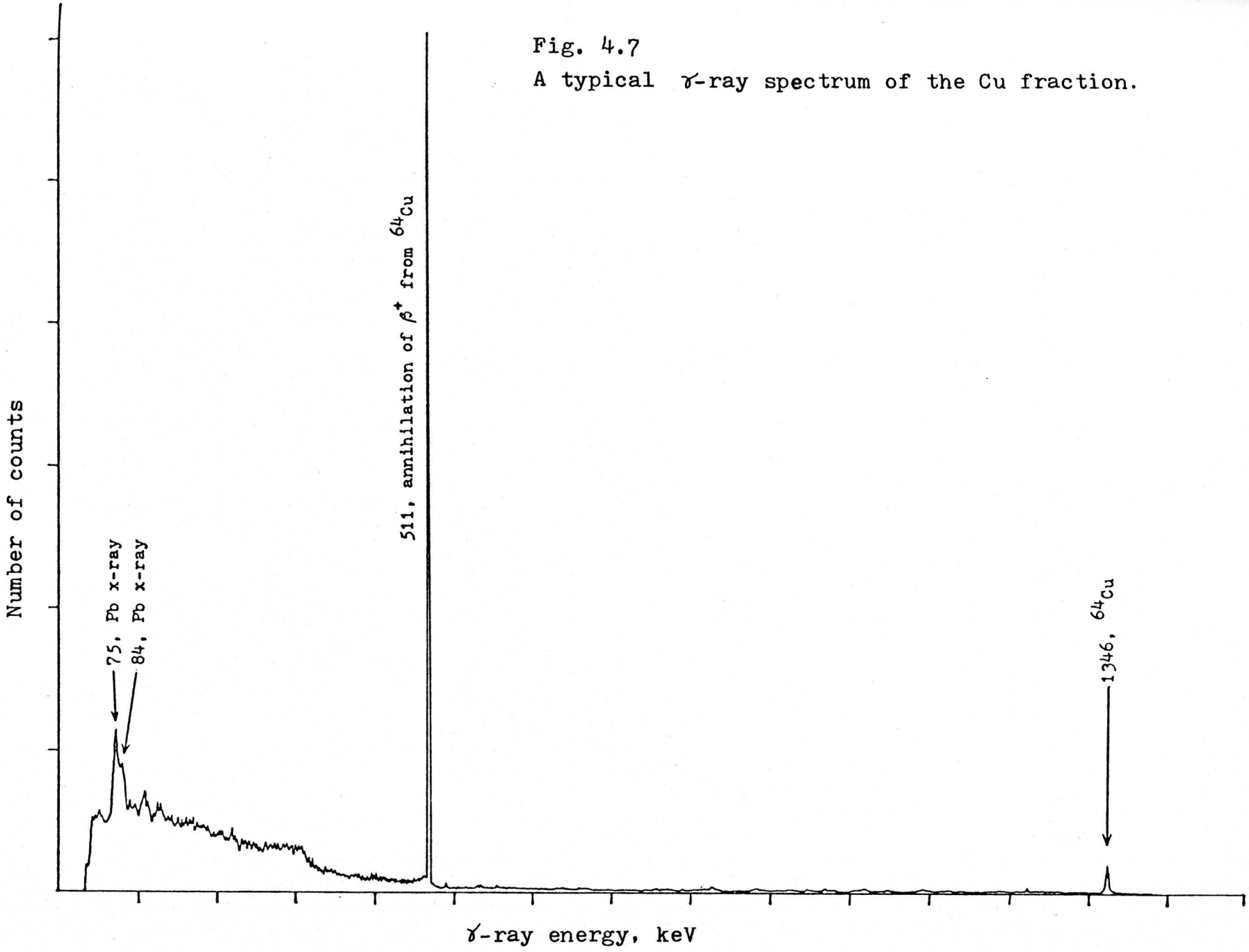
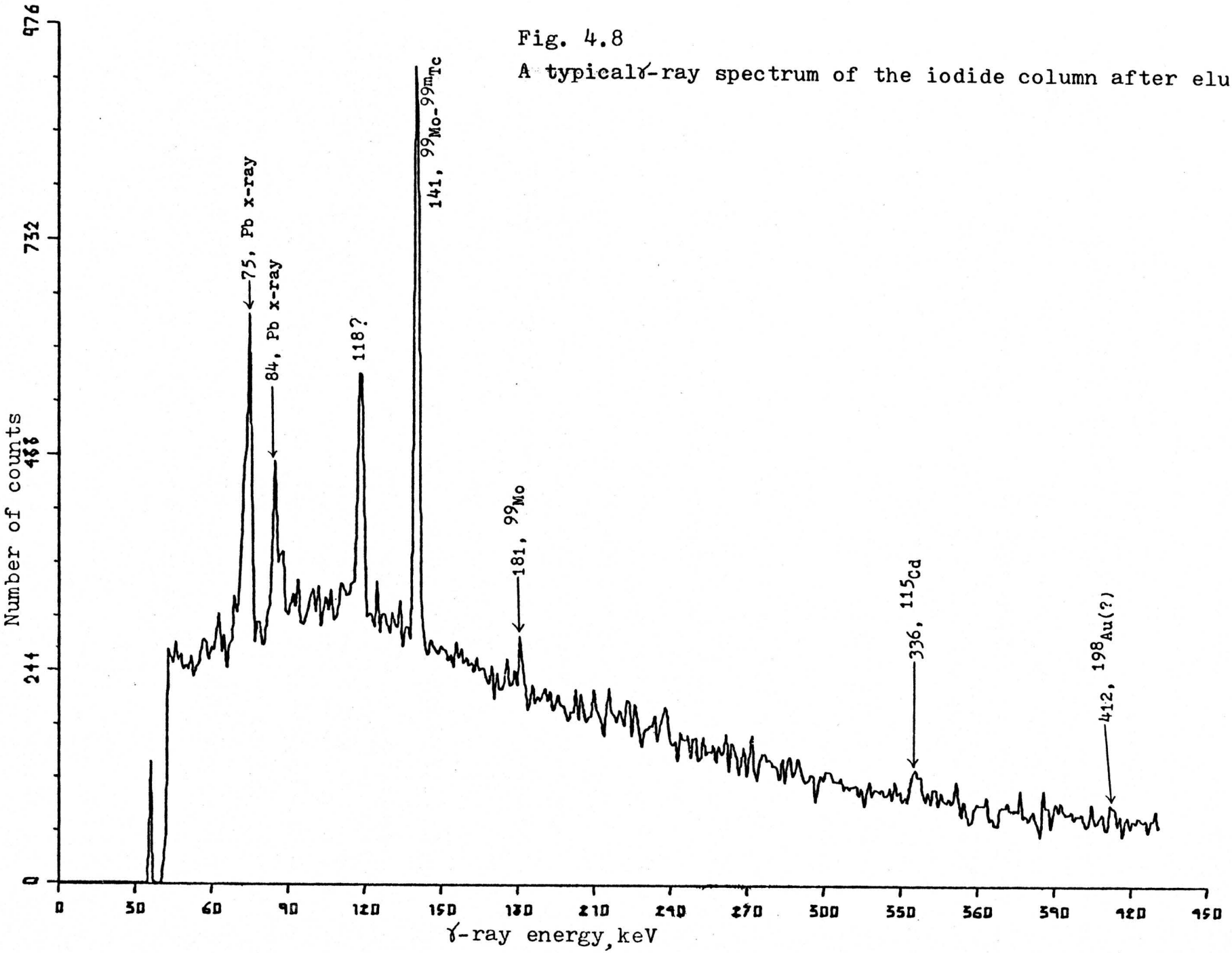


Fig. 4.8
A typical γ-ray spectrum of the iodide column after elution.



materials (SRM). The concentrations of several elements in different types of SRM analyzed by RNAA are shown in Table 4.22. The agreement between the values obtained in this work and those reported in the literature (some are certified by issuing agencies) is generally very good, and is within ±10% in most cases. And since part of this work was developed for the analysis of copper, several types of SRM analyzed by RNAA are shown in Table 4.23. The accuracy and precision can be observed from this table. The detection and determination limits of analyses of tissues by the above RNAA scheme are shown in Table 4.24.

4.6 An INAA Scheme for Analyzing Neoplastic Tissues

With the availability of the on-site reactor facility (DUSR), determination of elements, which produce short-lived neutron activation products, by INAA became a realistic proposal. It has been mentioned in the Section 2.2.1 that INAA has a few advantages over its sister technique RNAA. These advantages were used in developing an INAA methods for determining several elements of interest in neoplastic tissues. Both thermal and epithermal (epi-cadmium in this project) neutrons were used to evaluate the best possible experimental conditions for simultaneous multielement determinations. Various aspects of the INAA scheme are described below.

TABLE 4.22 PRECISION AND ACCURACY OF MEASUREMENT USING THE RNAA METHOD DEVELOPED FOR ANALYZING TISSUES

Element	Bovine Liver (NBS SRM-1577)		Gelatin(Kodak TEG-50-B)[c]	
	This work[a]	NBS[b]	This work	Literature[c]
Ag	0.057±0.017	(0.06)	1 04±5	100
As	0.054±0.002	0.055±0.005	74.5±8	86
Au	0.039±0.08	-	-	-
Cd	0.28±0.03	0.27±0.04	42±3	45
Hg	-	0.016±0.002	48.2±4	50-55
Mo	3.5±0.4	(3.4)	-	-
Pd	-	-	-	-
Pt	-	-	-	-
Sb	-	(0.005)	37±3	37-51
Se	0.96±0.06	1.1±0.1	28±4	26-43
Zn	123±25	130±13	52±5	59

[a] triplicate analyses;
[b] Ref. : NBS certified values; [c] Ref. ; AND77;
[d] all values are in ppm , dry weight basis.

TABLE 4.23 ANALYSIS OF Cu BY MEANS OF IODIDE COLUMN CHROMATOGRAPHY

Matrix	Cu content, ppm	
	This work	NBS[a]
Bovine Liver (NBS SRM-1577)	203±8[b]	193±10
Orchard Leaves (NBS SRM-1571)	11.8	12±1
Pine Needles (NBS SRM-1575)	3.5	3.0±0.3
Spinach (NBS SRM-1570)	11.9	12±2
Tomato Leaves (NBS SRM-1573)	12.3	11±1

[a]Ref : NBS certified values;

[b] triplicate analyses.

TABLE 4.24 THE DETECTION AND DETERMINATION LIMITS OF THE RNAA METHOD DEVELOPED FOR ANALYZING TISSUES

Element	LD[a]	LQ[a]
As	4	14
Hg	6	22
Sb	3	11
Se	25	90
Mo	20	74
Zn	24	88
Cu	1.6	5.7
Ag	4	14
Au	0.8	3.0
Pd	24	89
Pt	14	50
Cd	88	350

a - Ref. : CUR68
LD - detection limit, in ppb;
LQ - determination limit, in ppb.

4.6.1 Thermal INAA

Among the large number of nuclides and photopeaks detected in gamma-ray spectra, a few could be used for interference-free and quantitative measurements. Following the identification of nuclides and assignment of half-lives to them, the nuclides of interest were grouped in three categories: (i) those with half-lives between 17s and 65s; (ii) those between 1 min and 3h; and (iii) the rest with half-lives ranging up to several years. The irradiation, decay and counting periods for each of the above groups of nuclides were optimized by maximizing peak-to-background ratios for the low-activity nuclides in a trial and error process. The contributions from interfering reactions and gamma-rays overlapping in energy were also considered in the optimization process. Finally, three separate irradiation and four different decay and counting periods were selected for analyzing neoplastic tissues. These experimental conditions are shown in Table 4.25.

Up to 23 nuclides could be detected in tissues by thermal INAA. The nuclear data of these nuclides have already been presented in Table 4.1. The first set of experimental conditions consisting of t_i=30s, t_d=10s, and t_c=60s were designed to measure concentrations of the short-lived nuclides, namely ^{110}Ag, ^{86m}Rb and ^{77m}Se. In addition, photopeaks due to ^{28}Al, ^{27}Mg and ^{52}V were detected in the same gamma-ray spectrum. However, counting statistics of these nuclides were very poor. The dead time at the beginning of counting was about 10%.

TABLE 4.25 THERMAL INAA SCHEME FOR ANALYZING NEOPLASTIC TISSUES

Duration of irradiation(t_i)	Decay time (t_d)	Duration of counting (t_c)	Nuclides detected
30s	10s	60s	^{110}Ag, ^{28}Al, ^{27}Mg, ^{86m}Rb, ^{77m}Se, ^{52}V
5min	3min	5min	^{28}Al, ^{49}Ca, ^{38}Cl, ^{66}Cu, ^{128}I, ^{42}K, ^{27}Mg, ^{56}Mn, ^{24}Na, ^{52}V
8h	2d	1h	^{76}As, ^{82}Br, ^{42}K, ^{99}Mo, ^{122}Sb
	14d	2h	^{60}Co, ^{51}Cr, ^{59}Fe, ^{203}Hg, ^{86}Rb, ^{46}Sc, ^{75}Se, ^{65}Zn

The same samples were re-irradiated after 2d for 5 min, and were allowed to decay for 3 min followed by a counting for 5 min. Up to 10 nuclides (shown in Table 4.25) were detected and used for elemental measurements. The 844- and 847-keV gamma-rays of ^{27}Mg and ^{56}Mn, respectively, could be mutually interfering. The results shown in the Chapter 5 indicate that the ratio of these two elements in mice tissues analyzed is greater than 100 to 1. On the other hand, the sensitivity ratio of thses two nuclides is approximately 1 to 100. However, the high-resolution Ge(Li) detector used in this work was able to separate the 844- and 847-keV gamma-rays ; consequently, no correction factor was either needed or applied. The interference-free 1014- and 1811- keV gamma-rays of ^{27}Mg and ^{56}Mn, respectively, could not be used in this study because of very poor counting statistics.

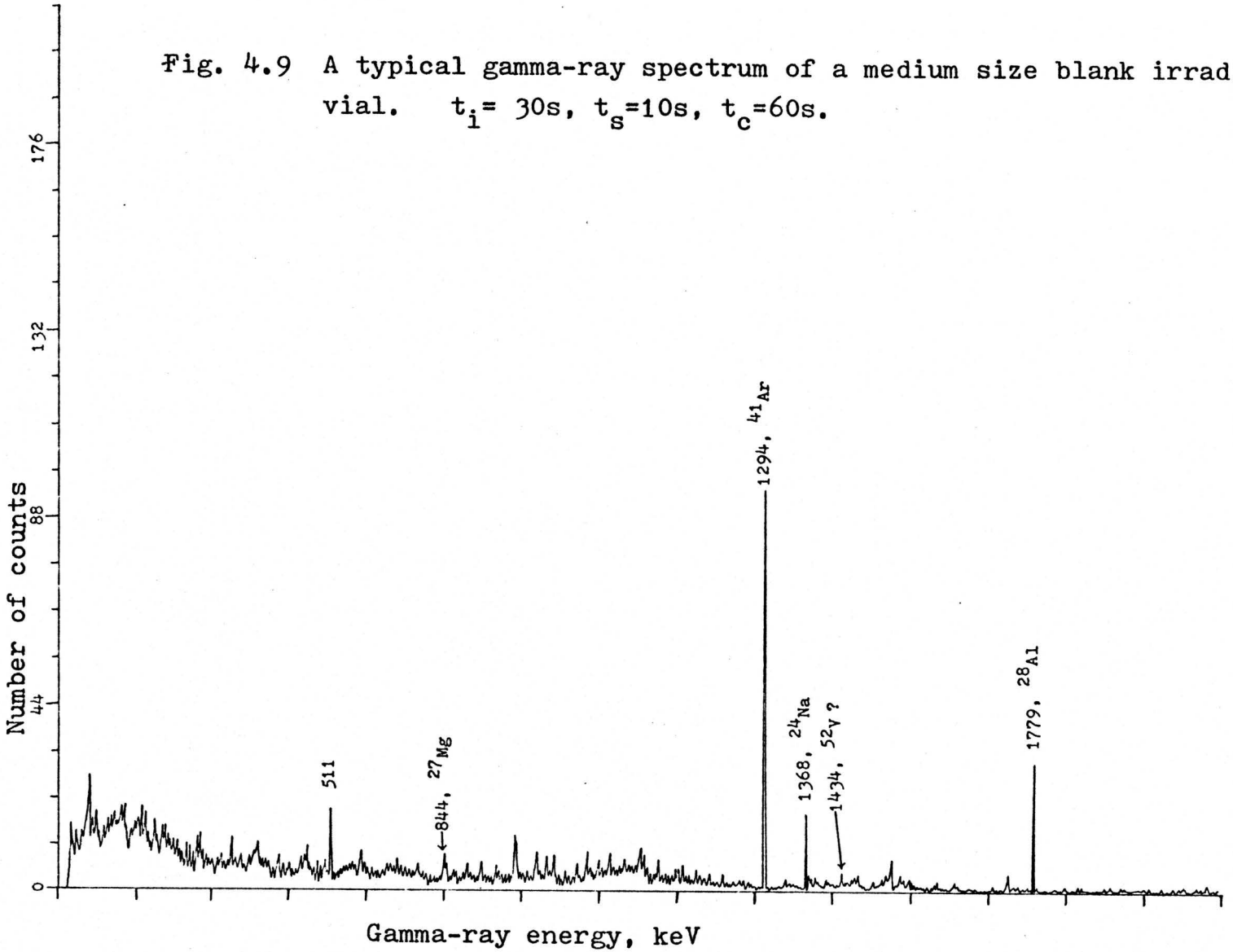
Variable dead times were recorded at the beginning of counting under the second set of experimental conditions (viz., t_i=5 min, t_d=3 min, t_c=5 min) due to the variable amounts of Al, Cl, Na and V in the samples analyzed. A significant error may occur due to this variability and due to the difference in dead times between a sample and standard. A number of elemental comparator standards with different dead time values were prepared and analyzed. The dead times of the samples and standards were matched within $\pm$2% to minimize, if not to completely eliminate, errors due to dead time variation. This method was successful as revealed by the close agreement in values between

this work and certified ones of the standard reference materials analyzed.

In the case of the 8-h irradiation, samples were counted twice to detect the medium- and long-lived nuclides. Concentrations of As, Br, K, Mo and Sb were determined after a 2-d decay and 1-h counting. A 14-d decay and 2-h counting was used to measure levels of Co, Cr, Fe, Hg, Rb, Sc, Se and Zn in tissues. The 1120-keV photopeak of ^{46}Sc could be interfered with by the 1115-keV photopeak of ^{65}Zn. Concentrations of Sc determined in the tissues were very low compared to those of Zn, although the former is more sensitive. Levels of Sc were also measured using the interference-free 889-keV gamma-ray of ^{46}Sc. The two values were found to agree very well with each other and also with that obtained using the short-lived ^{46m}Sc nuclide. Consequently, any interference from the 1115-keV peak of ^{65}Zn to 1120-keV peak of ^{46}Sc was discarded.

Since the samples were irradiated and counted in the same vial in the INAA methods developed here, elements present in the irradiation vials could be a potential source of error. Consequently. several vials from the same shipment were analyzed by INAA under the same experimental conditions as those of samples.Three typical gamma-ray spectra of a vial under 3 different experimental conditions are shown in Figs. 4.9, 4.10 and 4.11.

Fig. 4.9 A typical gamma-ray spectrum of a medium size blank irradiation vial. t_i= 30s, t_s=10s, t_c=60s.



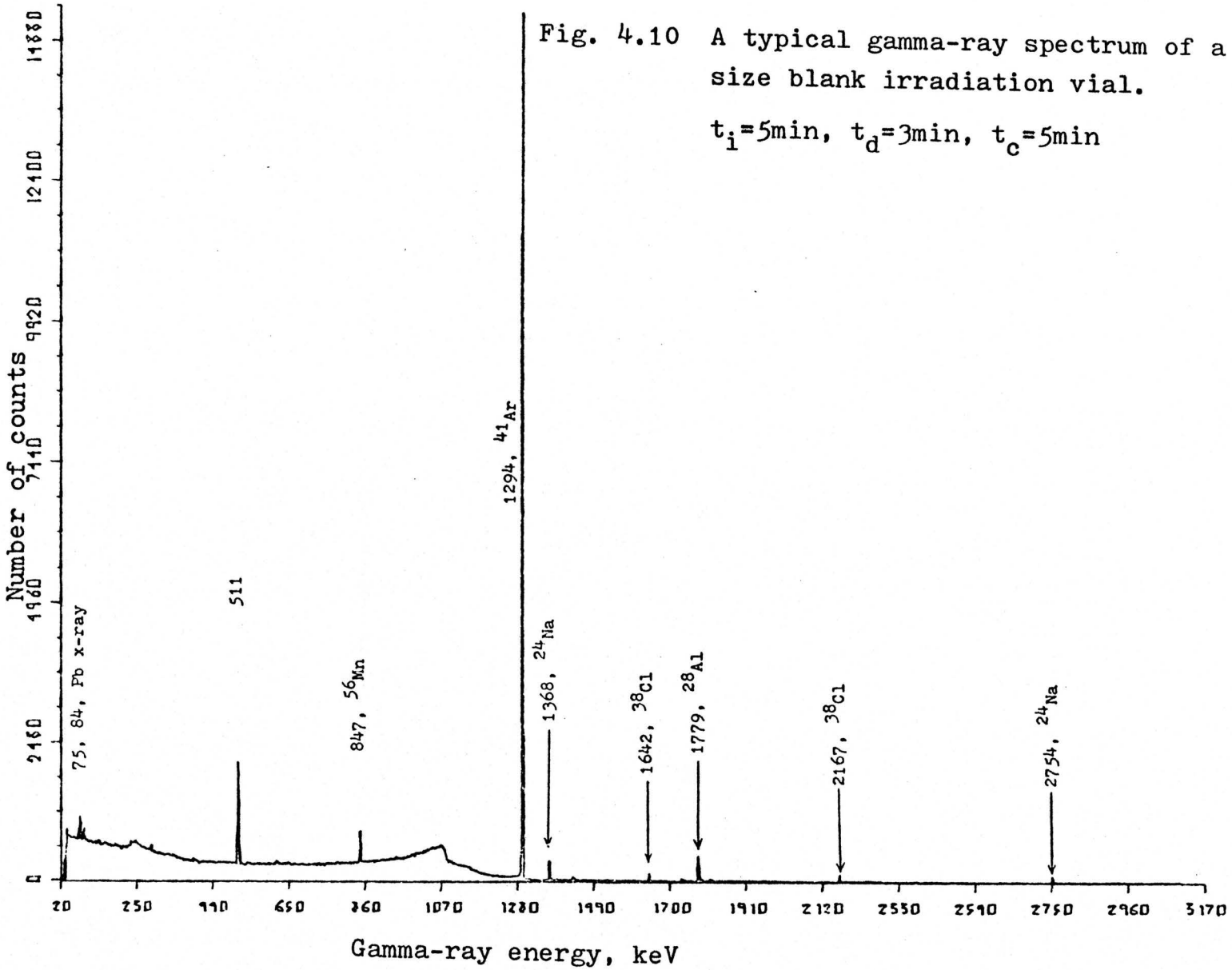
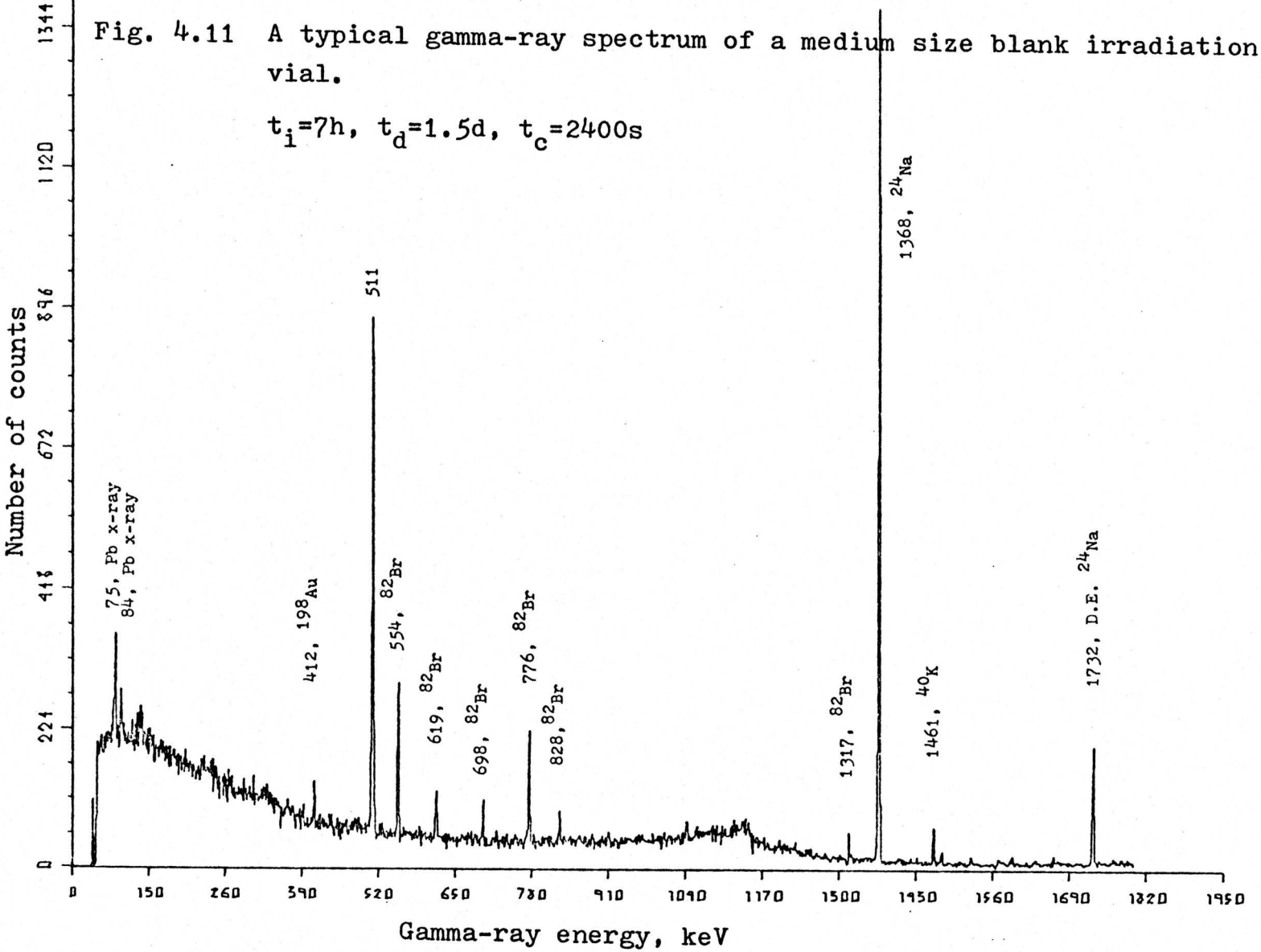


Fig. 4.10 A typical gamma-ray spectrum of a medium-size blank irradiation vial.

t_i=5min, t_d=3min, t_c=5min

Fig. 4.11 A typical gamma-ray spectrum of a medium size blank irradiation vial.

t_i=7h, t_d=1.5d, t_c=2400s



The following nuclides were detected in vials after several experiments under the conditions given in Table 4.25: ^{28}Al, ^{41}Ar (from air in empty vial), ^{198}Au, ^{82}Br, ^{38}Cl, ^{56}Mn and ^{24}Na. The average concentrations of Al, Cl and Na in vials were calculated and appropriately subtracted from the elemental levels of the tissues.

It has been mentioned in the Section 2.15 that the nuclides of interest may also be produced by reactions other than the usual (n,γ) type. The two most common types of interfering reactions are (n,p) and (n,α). The maximum conditions from these two types of interfering reactions to the nuclides of interest were calculated using the equation given in the Section 2.1.5. The results are presented in Table 4.26. Most of the interfering reactions are negligible as revealed by the ratio A_{int}/A except that of ^{28}Al. Up to 50% of ^{28}Al could be produced by the $^{31}P(n,\alpha)\ ^{28}Al$ reaction. Consequently, appropriate correction factors were applied to Al values in the samples analyzed in this work.

The precision and accuracy of the INAA methods developed in this study were evaluated by analyzing the Bovine Liver (NBS SRM-1577). The average of 3 determinations is shown in Table 4.27. The agreement between this work and certified values is generally within $\pm$10%.

The limits of detection and determination for elements analyzed by INAA in neoplastic tissues were calculated

TABLE 4.26 CALCULATED MAXIMUM CONTRIBUTIONS FROM INTERFERING NUCLEAR REACTIONS

Element	Data for Reaction of Interest				Data for Interfering Reaction				Aint/A
	Activation reaction	θ (%)	σ (b)	W (ppm)	Interfering reaction	θ_{int} (%)	σ_{int} (b)	W int (ppm)	
Al	$^{27}Al(n,\gamma)^{28}Al$	100	0.232	100	$^{31}P(n,\alpha)^{28}Al$	100	1.9×10^{-3}	100,000	1.6
Cl	$^{37}Cl(n,\gamma)^{38}Cl$	24.5	0.43	2000	$^{41}K(n,\alpha)^{38}Cl$	6.7	7.6×10^{-4}	8000	3.8×10^{-4}
Mg	$^{26}Mg(n,\gamma)^{27}Mg$	11	0.03	700	$^{27}Al(n,p)^{27}Mg$	100	4×10^{-3}	100	3.4×10^{-2}
Mn	$^{55}Mn(n,\gamma)^{56}Mn$	100	13.3	2	$^{59}Co(n,\alpha)^{56}Mn$	100	1.56×10^{-4}	0.2	2.4×10^{-7}
					$^{56}Fe(n,p)^{56}Mn$	92	1.07×10^{-3}	500	4.0×10^{-3}
Na	$^{23}Na(n,\gamma)^{24}Na$	100	0.53	2000	$^{24}Mg(n,p)^{24}Na$	79	1.53×10^{-3}	700	1.7×10^{-4}
					$^{27}Al(n,\alpha)^{24}Na$	100	7.25×10^{-4}	100	1.3×10^{-5}

TABLE 4.27 ELEMENTAL CONTENT OF THE NBS BOVINE LIVER BY INAA

Element	Content in ppm	
	This work[a]	NBS[b]
Ag	0.058±0.005	(0.06)
Al	---	---
As	---	0.055±0.005
Br	6.7±0.5	---
Ca	120±20	124±6
Cl	2640±200	(2700)
Co	0.19±0.03	(0.18)
Cr	---	0.088±0.012
Cu	201±12	193±10
Fe	275±19	268±8
Hg	---	0.016±0.002
I	---	(0.18)
K	9500±700	9700±600
Mg	609±25	640±9
Mn	9.8±1.0	10.3±1.0
Mo	3.2±0.4	(3.4)
Na	2360±160	2430±130
Rb(^{86m}Rb)	17.9±2.0	18.3±1.0
(^{86}Rb)	18.4±1.2	18.3±1.0
Sb	---	(0.005)
Se(^{77m}Se)	1.06±0.2	1.1±0.1
(^{75}Se)	1.08±0.1	1.1±0.1
V	---	---
Zn	117±12	130±13

a - average of triplicate analyses
b - Ref. : NBS certified values, values in bracket are given for reference only.

according to the methods prescribed by Currie (CUR68). These limits are shown in Table 4.28. Several elements could be determined in most of the tissues analyzed in this work. Some of the elements, which exhibited high detection limits by INAA and a few others which gave poor precision, were determined by RNAA as described in the Section 4.5.

4.6.2 Epi-cadmium INAA

It has been stated in the Section 2.2.1 that elements (such as Al, Cl, Na,V, etc.) which cause spectral interference in thermal INAA generally have small resonance activation integrals. The use of epithermal neutrons can not only reduce this interference but also might assist in determining several elements which possess high resonance activation integrals (such as As, Au, Cd, I, Mo, etc.). Consequently, attempts were made in this study to develop epithermal INAA methods for determining elemental concentrations in neoplastic tissues.

The samples were wrapped in 0.5-mm-thick cadmium foil, and were irradiated for 5 min , allowed to decay for 5 min and counted for 10 min. Two typical gamma-ray spectra of the livers of tumor-bearing mice (C57BL/6J) irradiated without and with cadmium shielding are shown in Fig. 4.12 and Fig. 4.13, respectively. A comparison of the two spectra revealed no new photopeaks. Moreover, the net counts under each photopeak (Fig. 4.13) were significantly decreased due to the lower flux of epi-cadmium

TABLE 4.28 DETECTION AND DETERMINATION LIMITS OF ELEMENTS IN TISSUES BY INAA

Element	Detection limit[a] (ppb)	Determination limit[a] (ppb)
Ag	16	90
Al	340	1900
As	30	110
Br	70	240
Ca	18000	188000
Cl	8600	34000
Co	30	120
Cr	200	740
Cu	2200	8000
Fe	15000	65000
Hg	60	200
I	150	540
K	132000	460000
Mg	15000	56000
Mn	80	320
Mo	160	500
Na	8800	30000
Rb(^{86m}Rb)	720	3500
(^{86}Rb)	840	3200
Sb	6800	23000
Se(^{77m}Se)	120	490
(^{75}Se)	160	540
V	30	110
Zn	740	3400

a - Ref. : CUR68

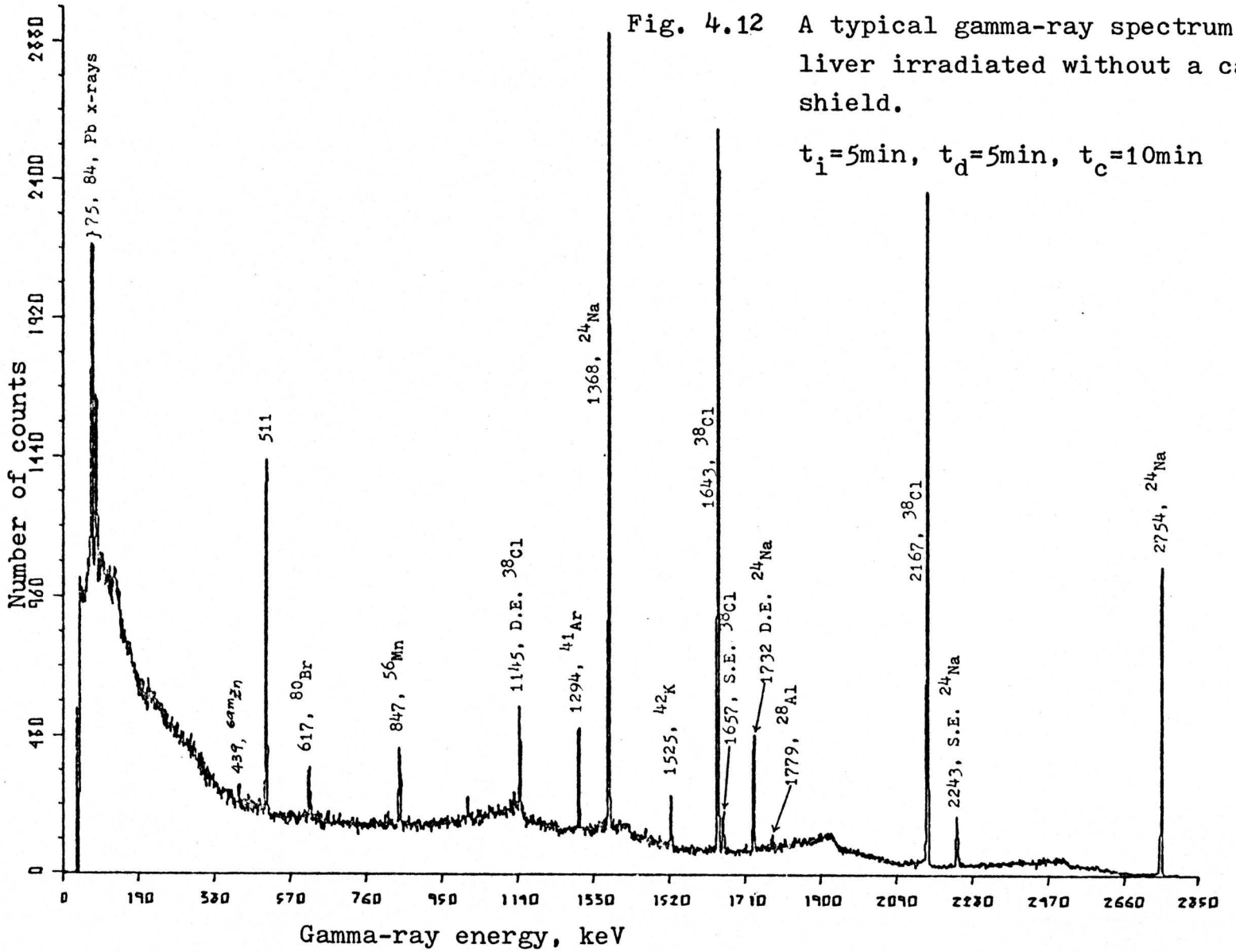


Fig. 4.12 A typical gamma-ray spectrum of a mouse liver irradiated without a cadmium shield.

t_i=5min, t_d=5min, t_c=10min

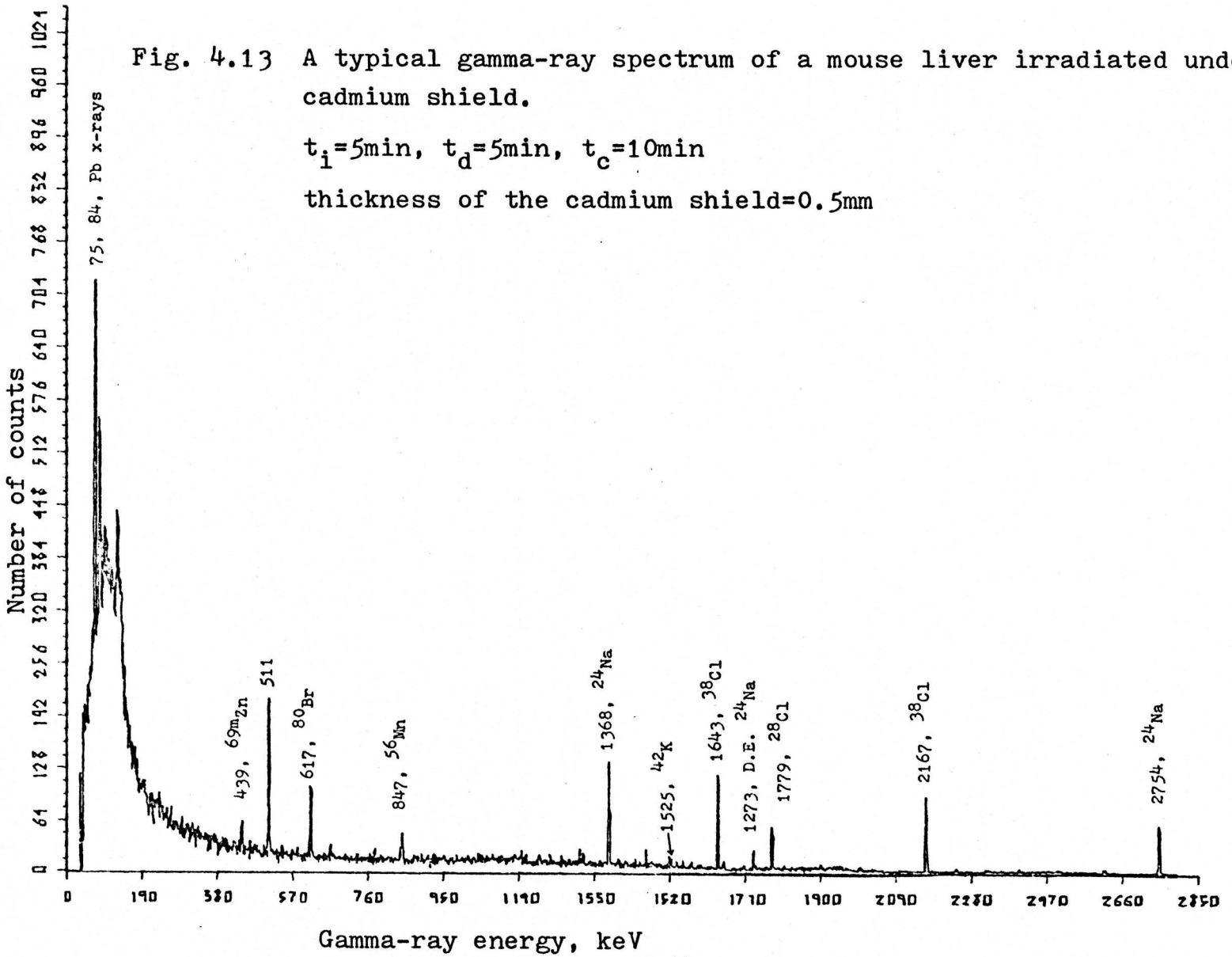


Fig. 4.13 A typical gamma-ray spectrum of a mouse liver irradiated under a cadmium shield.

t_i=5min, t_d=5min, t_c=10min

thickness of the cadmium shield=0.5mm

neutrons compared to thermal neutrons. It was then concluded that epi-cadmium INAA did not provide any advantage over thermal INAA for detecting short-lived nuclides in neoplastic tissues.

The production of medium- and long- lived resonance neutron activation products were then considered. Samples of mice liver (C57BL/6J) were irradiated for 1h (maximum length of irradiation that was available for a cadmium-shielded sample in the DUSR facility without affecting other users and changing neutron flux), allowed to decay for 2d and counted for 2400s. The typical gamma-ray spectra of samples irradiated without and with cadmium shielding are shown in Fig. 4.14 and Fig. 4.15, respectively. Numerous gamma-rays of ^{82}Br were detected in Fig. 4.15 because of the advantage factor of 15 for ^{82}Br under epi-cadmium conditions. ^{65}Zn was the only nuclide of interest which was detected in Fig. 4.15 and not in Fig. 4.14. However, ^{65}Zn was already detected easily by thermal INAA (Table 4.24). Consequently, epi-cadmium INAA was not used for routine determinations of elements in neoplastic tissues.

4.7 Ultrafiltration

It has been stated in the Section 2.4 that there is increasing interest in determining protein-bound trace elements in tissues. Preliminary studies were carried out

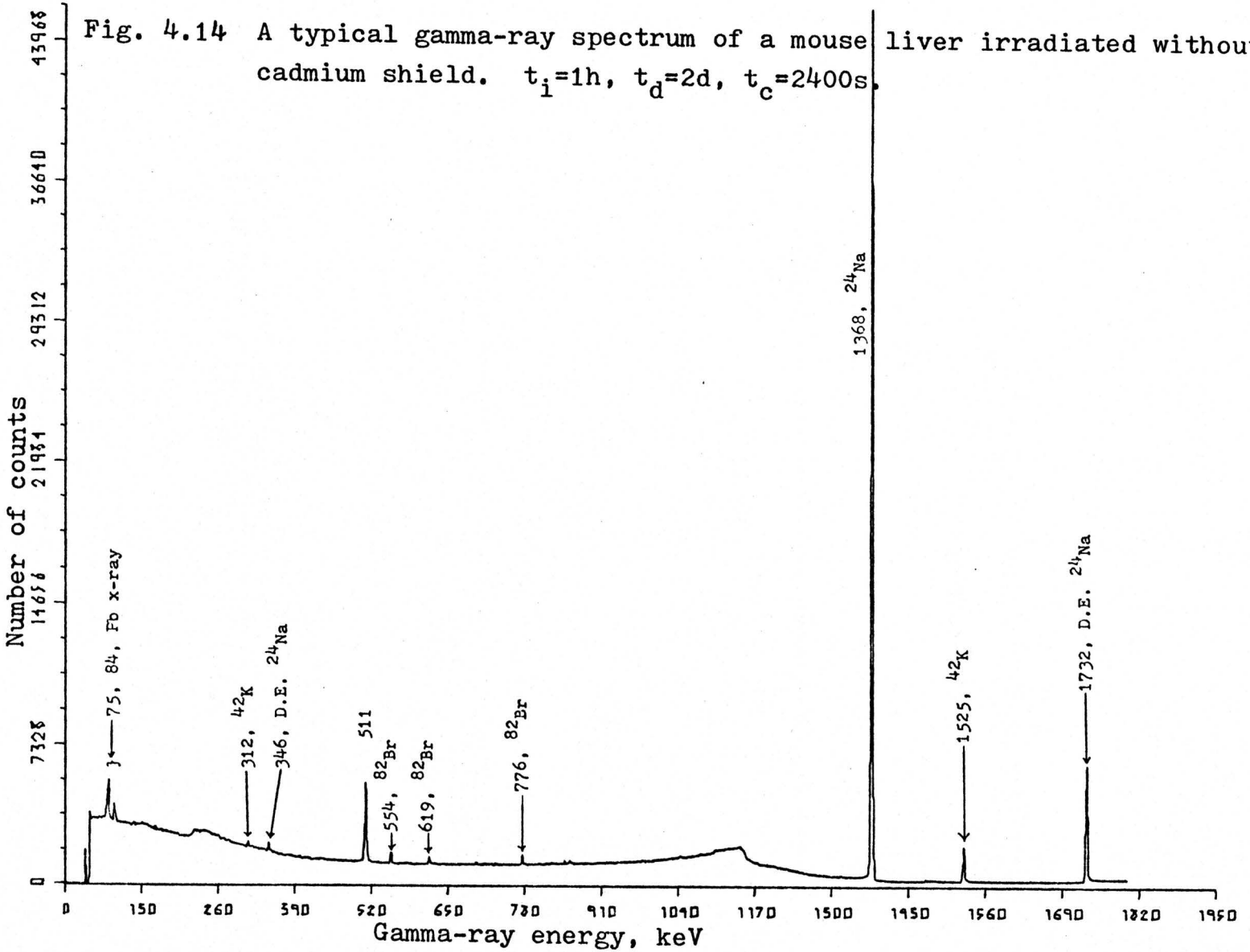
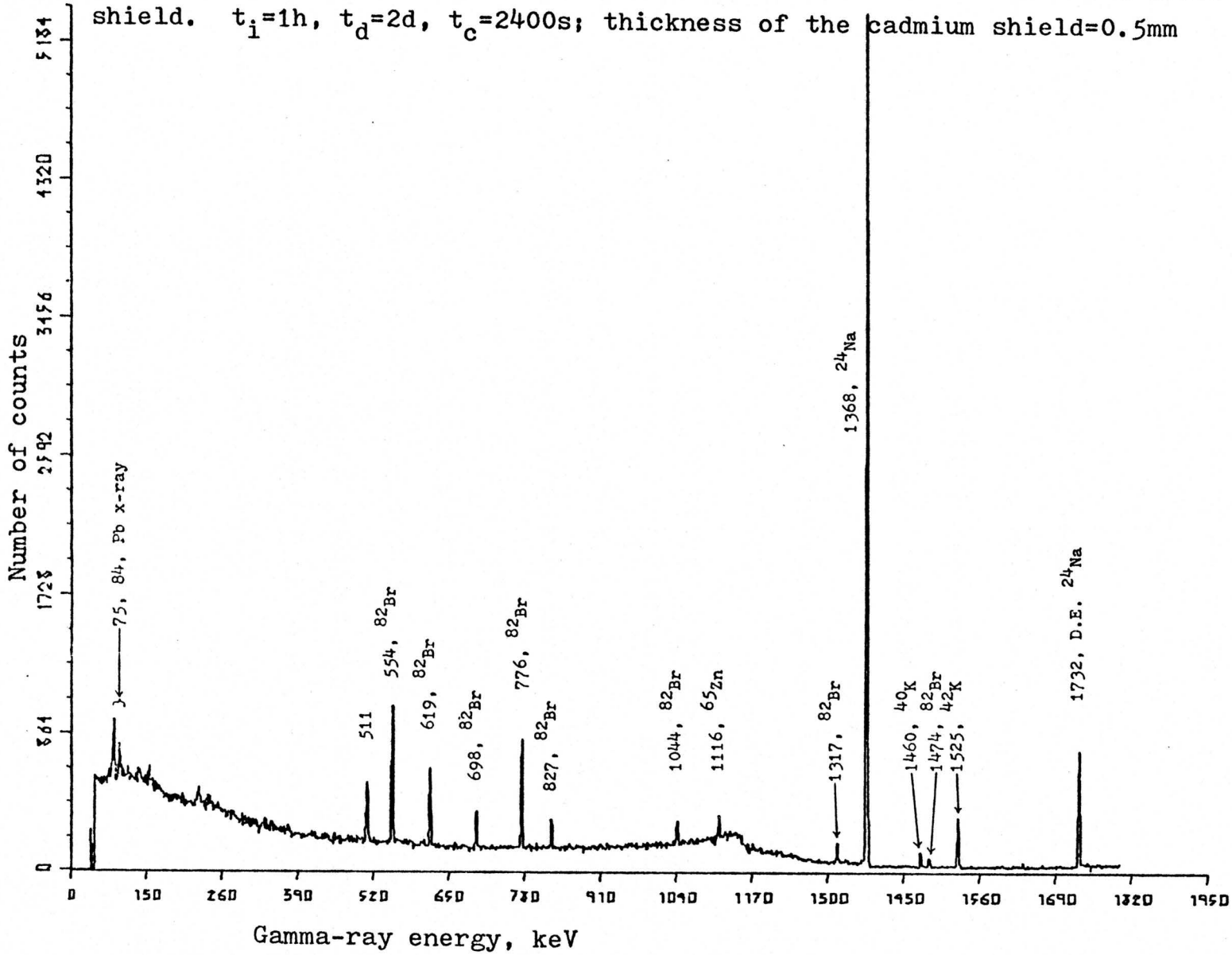


Fig. 4.14 A typical gamma-ray spectrum of a mouse liver irradiated without a cadmium shield. t_i=1h, t_d=2d, t_c=2400s.

Fig. 4.15 A typical gamma-ray spectrum of a mouse liver irradiated under a cadmium shield. t_i=1h, t_d=2d, t_c=2400s; thickness of the cadmium shield=0.5mm



to evaluate the scope of the combination of ultrafiltration and INAA in achieving the above goal. Consequently, an ultrafiltration system was set up as described in the Section 3.6. This system was also used to study the possible elimination of Br, Cl and Na from homogenized tissues.

One of the first things needed to be developed was a suitable buffer solution in which the tissues could be homogenized. A buffer solution should meet several criteria: (i) should have very low levels of contaminating elements; (ii) should be able to effectively elute different protein fractions; (iii) should be removed easily, say on freeze drying; and (iv) should not leave cations and anions (which produce interfering nuclides on neutron activation) as counter ions of the proteins. Acetic acid-ammonium acetate mixture was considered in this study to be an ideal buffer solution for ultrafiltration and INAA studies. (Since the optimum pH value for cells is about pH7, only ammonium acetate is being used.) However, commercially available reagents were found to be highly impure, and needed further purification. A sub-boiling distillation apparatus was set up to purify the reagents as described in the Section 3.2. The products obtained were highly pure.

Due to the non-availibility of a few grams of mice liver, it was decided to carry out preliminary experiments with commercially available edible pork liver. A sample of pork liver was first washed with water in an ultrasonic bath to

remove blood. The sample was then placed in 5mL of 0.15M ammonium acetate solution, and homogenized with a Vertis ultrasonic homogenizer. A portion of the homogenate was passed through a 500 Dalton molecular weight cut-off (MWC_o) membrane using the set-up shown in Fig. 3.4 and described in the Section 3.6. The residue (portion remaining in the ultrafiltration cell) was collected.

Samples of original pork liver (no treatment), homogenized liver, and residue in ultrafiltration cell were freeze-dried and analyzed by INAA using three different experimental conditions. At first, t_i=30s, t_d=10s, and t_c=60s were used to detect the short-lived nuclides. The gamma-ray spectra of the original, homogenized, and residue samples are shown in Fig. 4.16, Fig. 4.17, and Fig. 4.18, respectively. The gamma-rays of ^{38}Cl and ^{24}Na were detected in both Fig.4.16 and Fig. 4.17. The spectrum of the residue (Fig. 4.18) did not contain any peaks of ^{38}Cl and ^{24}Na indicating that these two elements were removed by ultrafiltration. However, a new photopeak, namely 59-keV of ^{60m}Co, was observed in the spectra of both homogenized and residue samples.

Following decay for several days, all the above samples were further irradiated for 5min, "cooled" for 5min, and counted for 5min. The gamma-ray spectra of the original, homogenized, and residue samples are shown in Fig. 4.19, Fig. 4.20, and Fig. 4.21 , respectively. The gamma-rays of

Fig. 4.16 A typical gamma-ray spectrum of a freeze-dried pork liver sample. t_i=30s, t_d=10s, t_c=60s

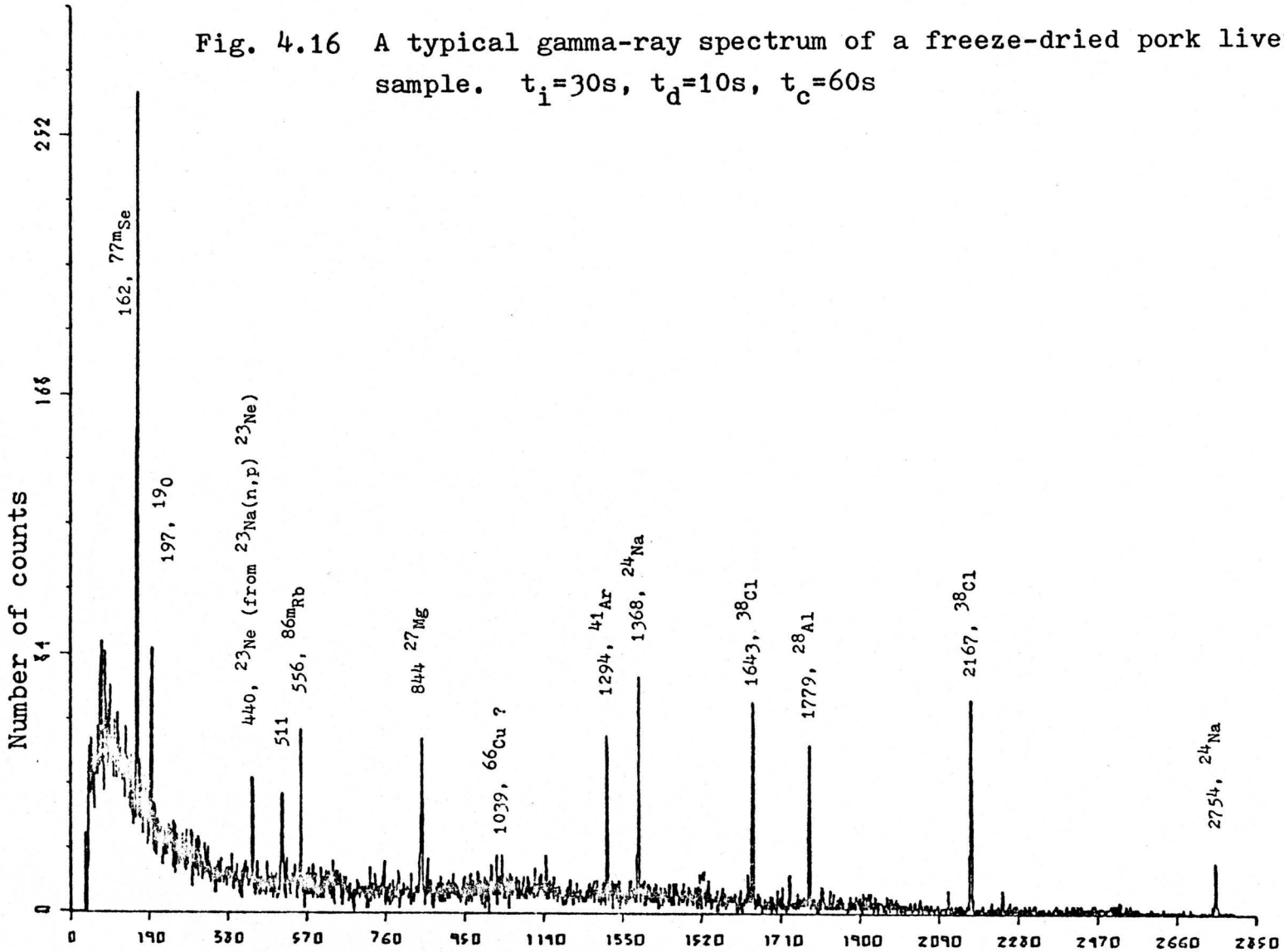
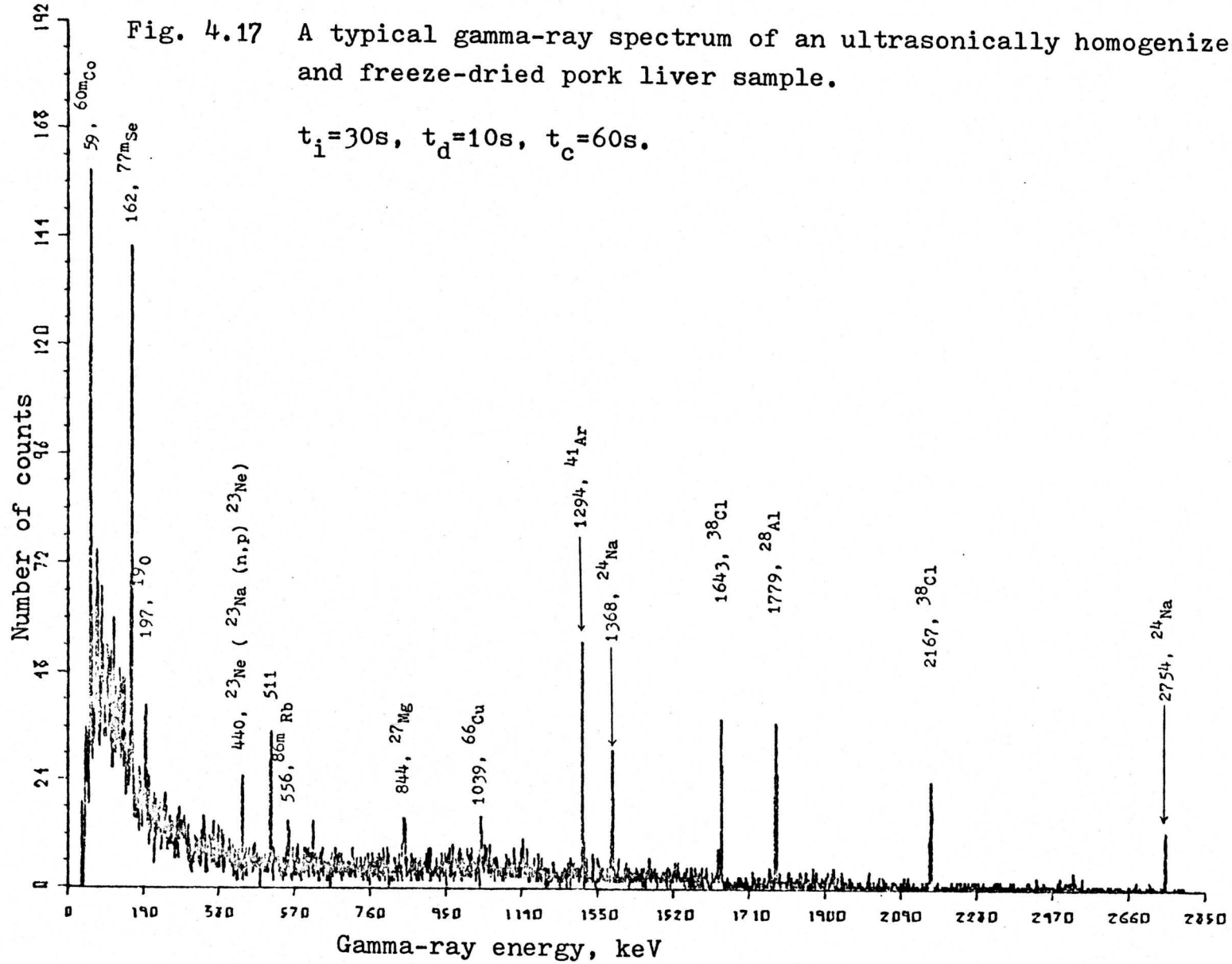


Fig. 4.17 A typical gamma-ray spectrum of an ultrasonically homogenized and freeze-dried pork liver sample.

t_i=30s, t_d=10s, t_c=60s.



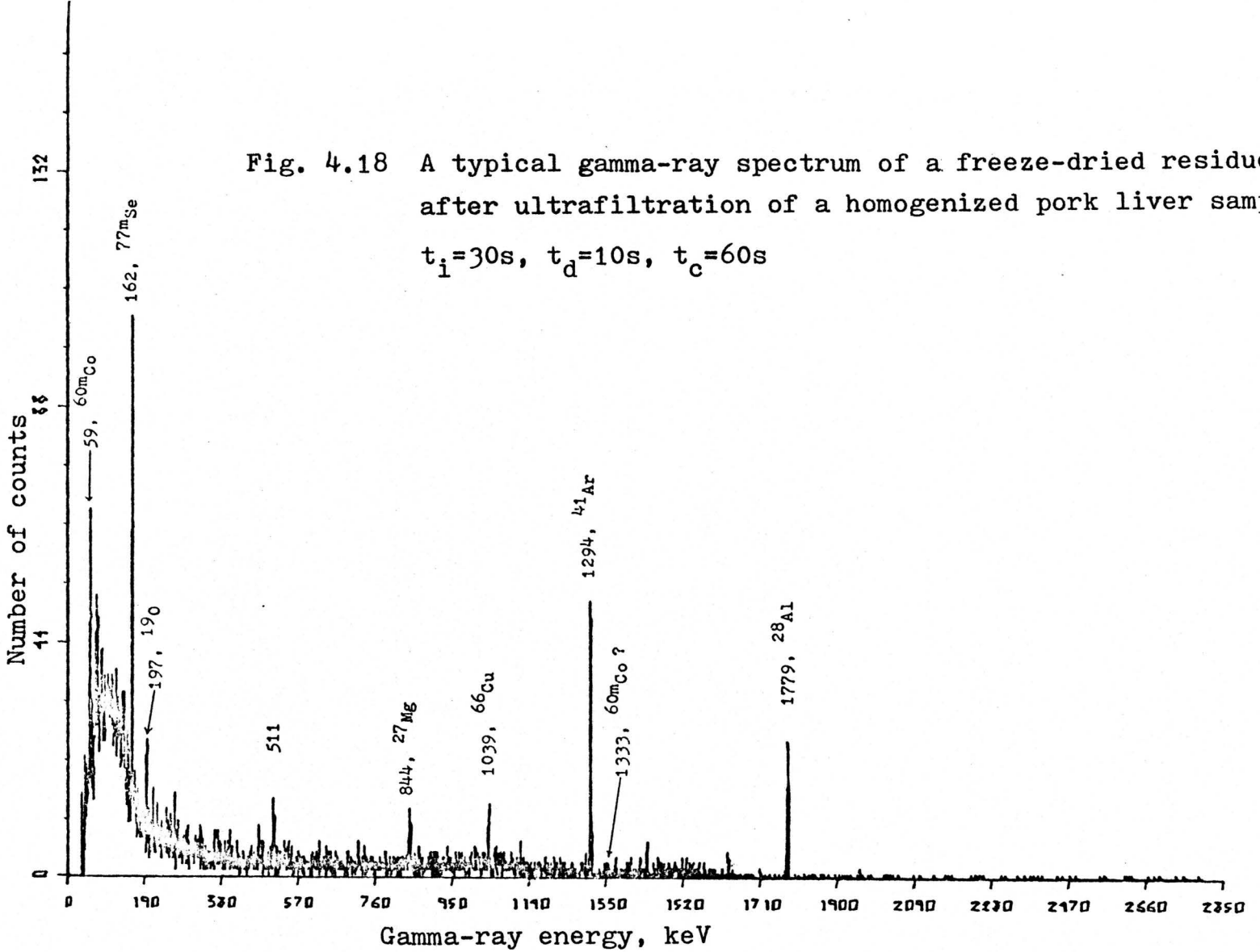


Fig. 4.18 A typical gamma-ray spectrum of a freeze-dried residue after ultrafiltration of a homogenized pork liver sample. t_i=30s, t_d=10s, t_c=60s

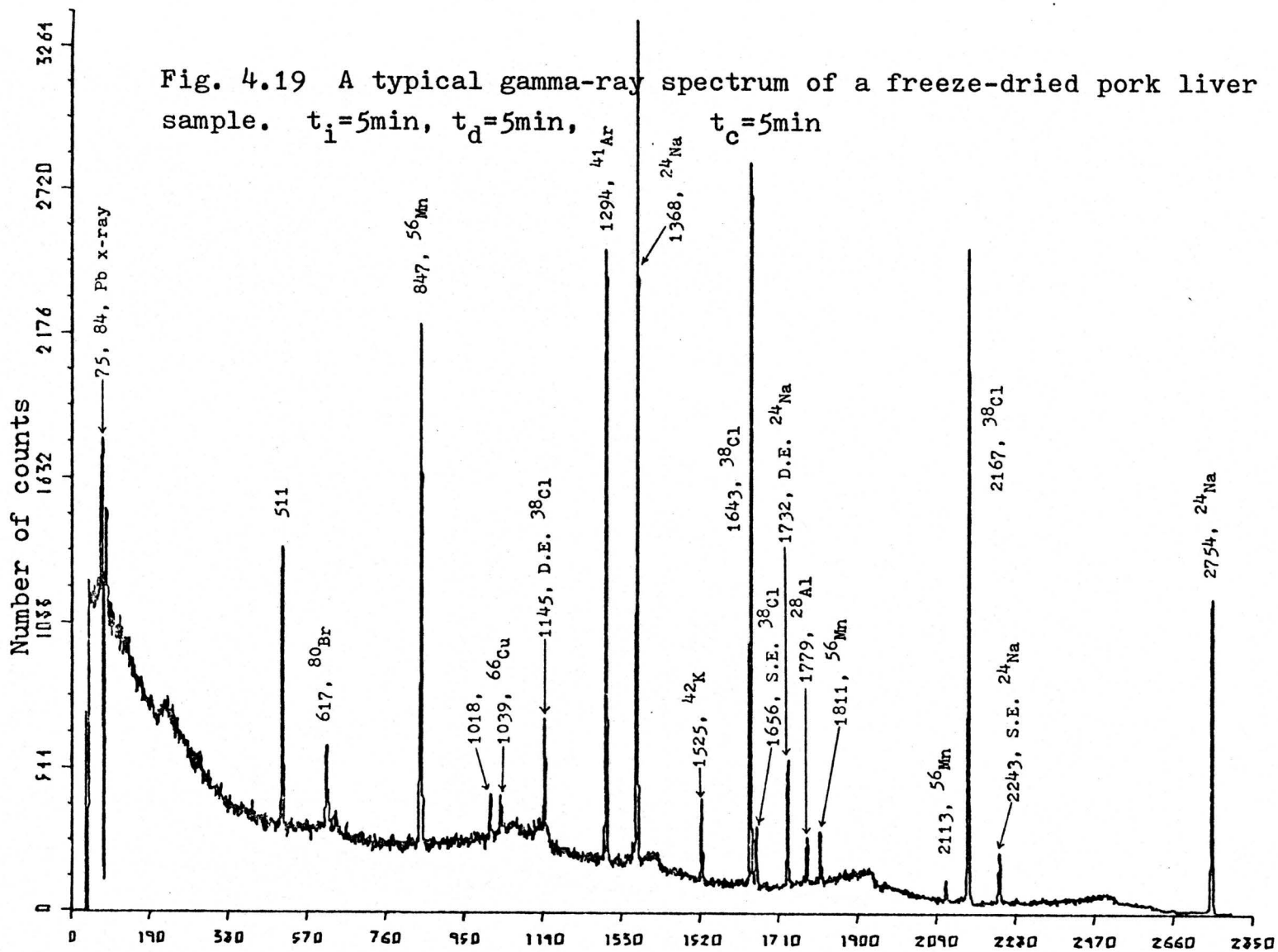


Fig. 4.19 A typical gamma-ray spectrum of a freeze-dried pork liver sample. t_i=5min, t_d=5min, t_c=5min

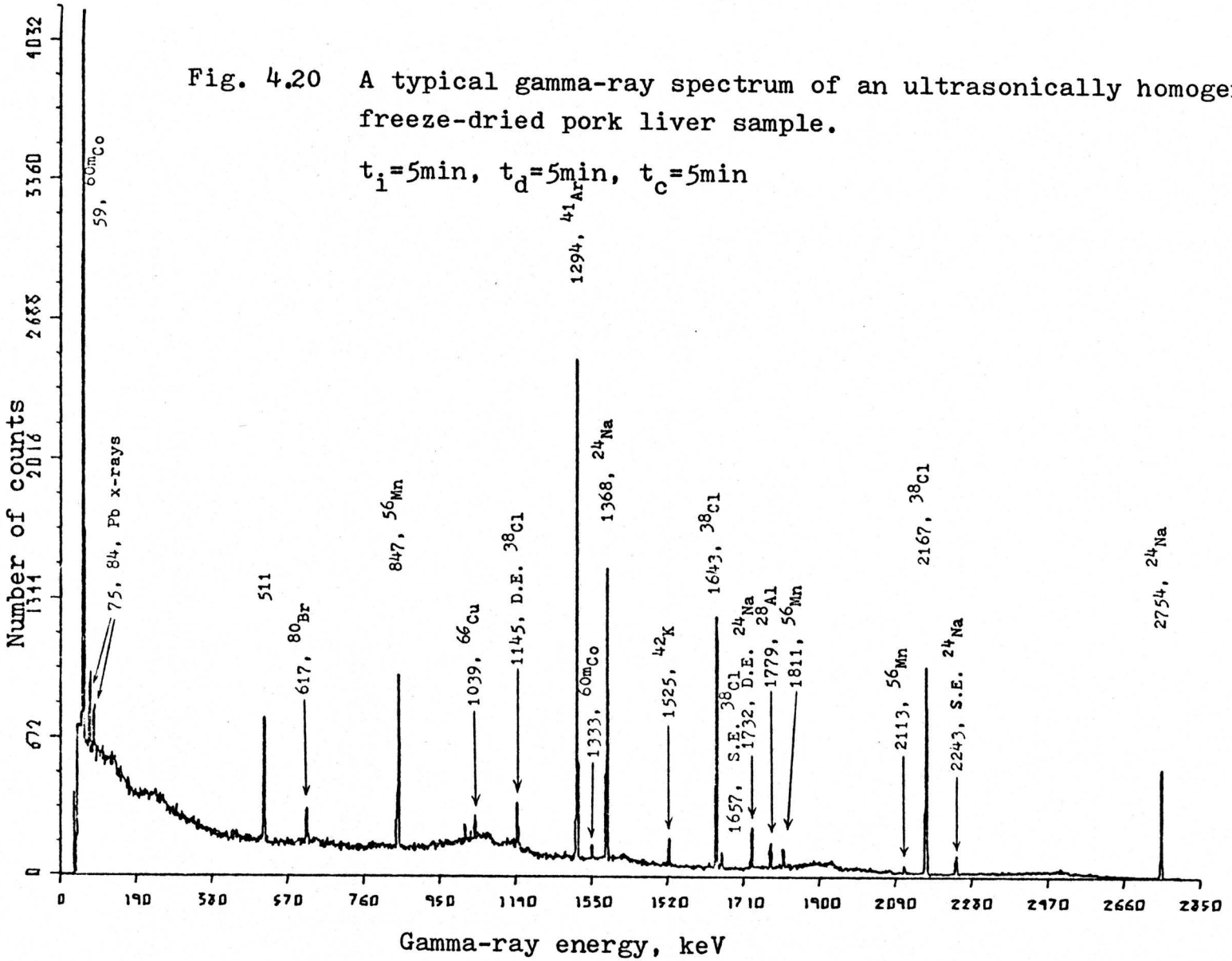


Fig. 4.20 A typical gamma-ray spectrum of an ultrasonically homogenized and freeze-dried pork liver sample.

t_i=5min, t_d=5min, t_c=5min

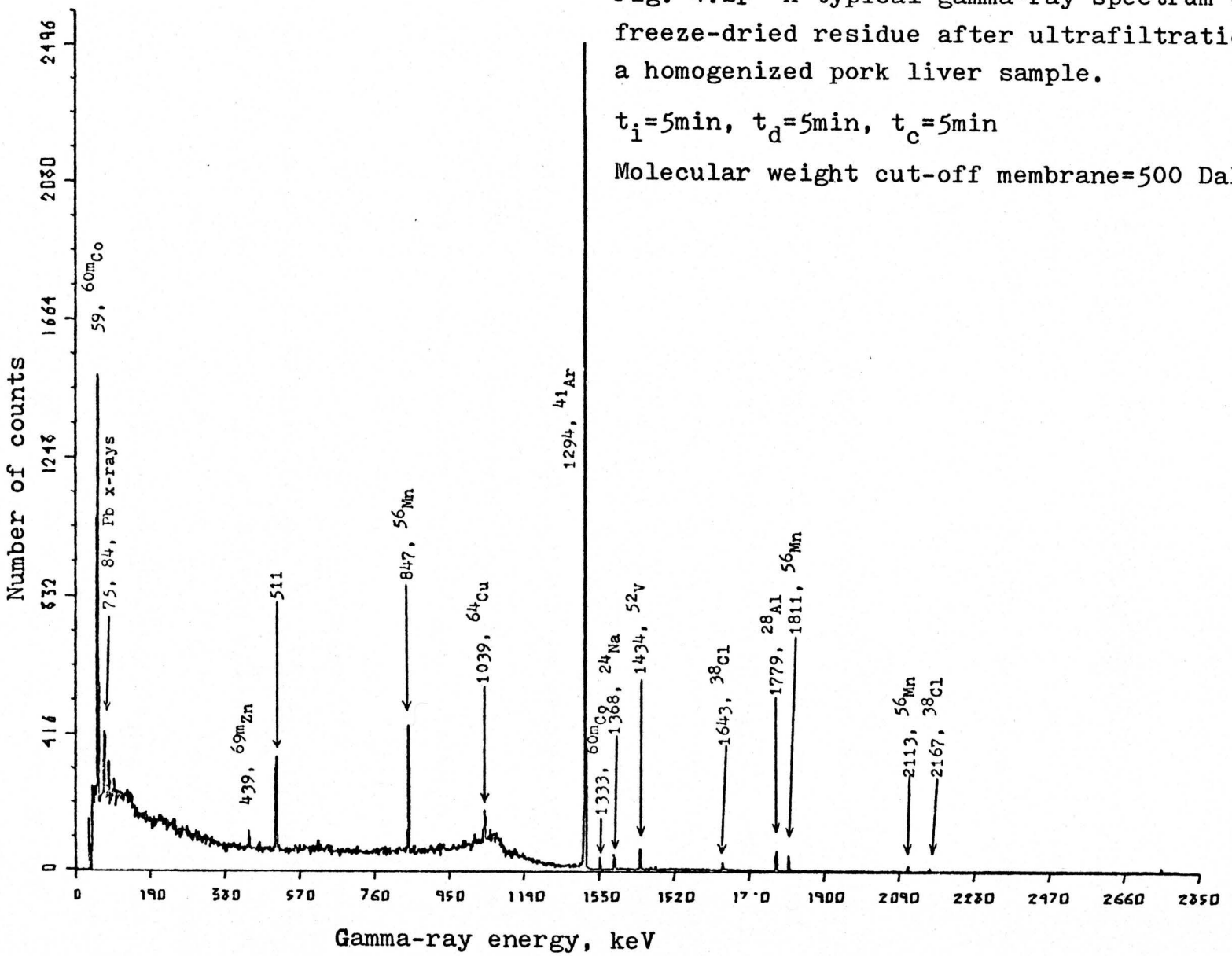


Fig. 4.21 A typical gamma-ray spectrum of a freeze-dried residue after ultrafiltration of a homogenized pork liver sample.

t_i=5min, t_d=5min, t_c=5min

Molecular weight cut-off membrane=500 Dalton

^{80}Br, ^{38}Cl and ^{24}Na were easily detected in Fig. 4.19 and Fig. 4.20, and only barely detected in Fig. 4.21. Therefore these elements were removed by ultrafiltration. On the other hand, the gamma-rays of ^{60m}Co were easily recorded in Fig. 4.20 and Fig. 4.21 indicating possible contamination by the probe of the ultrasonic homogenizer. Long irradiations followed by long decay and long counting periods also revealed the presence of the nuclides of Co, Eu, Ta and W.

Several blank experiments were carried out to identify any possible source of contamination. Finally, it was found that the probe of the Vertis ultrasonic homogenizer was releasing significant amounts of Co, and minor amounts of Eu, Ta and W. Several washing methods, such as with EDTA, water, etc., were applied to the probe. It was observed that the contamination by the probe could not be prevented under the experimental conditions used. Consequently, the ultrafiltration method was not subsequently used in this study.

Nevertheless, the advantages of using ultrafiltration as revealed by the above preliminary experiments should be noted here. Levels of Br, Cl, K, Na and Rb in livers were significantly (>80%) reduced by ultrafiltration with membranes of 500 MWC_o indicating their association with very low molecular weight proteins, or their presence as simple inorganic compounds or as dissociated ions. The reverse is possibly true for Al, Cu, Mg, Mn, Se and Zn which were mostly (>80%)

retained in the residue of the ultrafiltration cell. The ultrafiltration method is capable of removing highly active neutron activation products of Br, Cl, K and Na. These removals can be advantageously used for determining trace elements in tissues. For example, concentrations of Cu and Zn could be determined through 1039-keV peak of ^{66}Cu and 439-keV peak of ^{69m}Zn, respectively, after ultrafiltration and irradiation for 20min. The task of determining protein-bound trace elements can then be made relatively simple. It is recommended that in future experiments the very recently available (and rather expensive) cell homogenizers fitted with teflon probes be used to avoid contamination problems.

4.8 Summary

The various nuclear analytical method developed in this study and described in this chapter can be summarized under two headings depending on the type of facility available near the laboratory.

The first scheme (Section 4.1 through 4.4) consisting only of RNAA methods is ideal for radiochemical laboratories which are located fair distance (>1000km) away from the irradiation sites. The RNAA methods involve digestion of samples, and recovery of elements by distillation,iodide column chromatography, chloride column chromatography, and TOPO solvent extraction. Only long-lived nuclides need

to be assayed. The RNAA schemes are capable of determining Ag, As, Au, Br, Co, Cu, Fe, Hg, Mo, Pd, Pt, Ru, Sb, Sc, Se, Sn and Zn in tissues.

The second scheme (Section 4.5 and 4.6) is a combination of RNAA and INAA methods. The RNAA methods have been developed to make use of nuclides, with half-lives of a few days, which are very sensitive but cannot be detected because of severe interferences from either the matrix itself or the major elements present in the matrix. This scheme has been used in conjunction with an INAA method which is capable of determining several major and minor elements. Obviously, the combination of INAA and RNAA methods can be applied if an irradiation facility is available close to the radiochemical laboratories and counting facilities. This scheme requires less time and exposes the experimenter to very minimal radiation compared to the first scheme. Consequently, a combination of INAA and RNAA methods (the second scheme) have been used in the present study for the trace determination of Ag, Al, As, Au, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Mo, Na, Rb, Sb, Se, V and Zn in neoplastic and normal mice tissues.

5. RESULTS AND DISCUSSION

The concentrations of several elements in neoplastic and normal tissues, neoplastic cells, and in the materials related to mice's environment have been analyzed using the methods developed in this study. The results are discussed here within the author's limited knowledge on neoplasia. Differences in elemental content between tumor and control tissues of the same kind have been studied. The significances of the difference have been evaluated by student's t-test and Wilcoxon-two-sample test. The correlations between pairs of elements in the same type of samples are reported here. Multielement regression analyses have also been carried out wherever possible. Because of the small number of samples studied, care must be taken in deriving conclusions from them.

5.1 Types of Sample Studied

As described in Section 3.5, samples were collected from two kinds of mice, namely C57BL/6J and A/J strains. Some of the former type bore a lymphoma, namely EL4 tumor. And some of the latter bore a hepatoma, namely H6 tumor. Several

organs, namely livers, hearts, kidneys, lungs and spleens were collected from both tumor-bearing mice and control mice. In addition to these samples, the tumor cells of EL4 were collected. Due to the difficulties in sampling EL4 cells (explained in Section 3.5.1) several groups of them were collected under different conditions. In order to compare EL4 cell samples with control samples, matching thymus tissues (where part of lymphocytes came from) were collected from normal C57BL/6J mice. On the other hand, hepatoma H6 is a kind of solid tumor that grows in livers. Samples of it were simply collected from the tumor-bearing mice by cutting the tumor lumps from the corpses. Samples of the bedding, mouse chow and drinking water used by the animals were also collected and analyzed by INAA.

5.2 Cells and Tissues from C57BL/6J Mice

5.2.1 EL4 Cells and Thymus Tissues

Average values and standard deviations of elemental levels of four groups (viz. A, B, C, and D) of EL4 cells sampled under different conditions are presented in Table 5.1. A few elemental pairs, namely Ca-Na, Cl-Na, Cl-K, Cr-K, Mg-K, have been found to exhibit a correlation factor, R, greater than 0.80 and significance better than 0.05. Correlation coefficients and related statistics of all the element pairs of group "A" were tabulated and shown in appendix. The weighting scheme used to differentiate data with varied significance is shown in Table 5.2. Multielement regression

TABLE 5.1 ELEMENTAL CONTENT OF EL4 CELLS (IN PPM)[@]

Group		Age (month)	Al	As	Br	Ca	Cl	Co
EL4,A	Avg	5.16	28.5	n.d.	3.75	218	963	n.d.
	σ	0.70	5.26		2.80	100	884	
	N	9/9	9/9		9/9	4/9	9/9	
EL4,B	Avg	3.13	23.2	3.12*	69.1	1180	46800	<0.14
	σ	0.0	8.0	0.64	9.93	464	5700	
	N	3/3	2/3	3/3	3/3	2/3	3/3	
EL4,C	Avg	3.13	23.9	<0.42	20.5	419*	12000	<0.14
	σ	0.0	7.07		4.11	36.9	2520	
	N	4/4	2/4		4/4	2/4	4/4	
EL4,D	Avg	2.8	30.0	n.d.	5.47	376*	1400	n.d.
	σ	0.0	8.89		0.53	51	270	
	N	3/3	3/3		3/3	3/3	3/3	

* Values chosen to represent the concentration of the element in EL4 cells.

@ Weighted; n.d.- not determined.

TABLE 5.1 Cont'd

Group		Cr	Cu	Fe	I	K	Mg	Mn
EL4,A	Avg	1.24*	10.6*?	n.d.	3.71	2000	400	1.12
	σ	0.60	2.11		1.27	1650	161	0.29
	N	3/9	3/9		9/9	7/9	9/9	9/9
EL4,B	Avg	1.82*	<98	<100	<6	10700*	845*	<3.4
	σ	1.01				4170	52.9	
	N	2/3				2/3	2/3	
EL4,C	Avg	17.0	<26	<200	<1.7	11700*	708*	<0.9
	σ	24.0				3300	174	
	N	3/4				3/4	4/4	
EL4,D	Avg	3.00*	<10	n.d.	0.14*?	3160	256	0.627*?
	σ	0.14			0.02	620	122	0.081
	N	2/3			3/3	3/3	3/3	3/3

[a] Avg- average value; σ- standard deviation; N- no. of samples (#/& : #- weighted no. of samples; &- total no. of samples analyzed).

TABLE 5.1 Cont'd

Group		Mo	Na	Rb	Sb**	Se	V	Zn
EL4,A	Avg	<0.4	526	n.d.	223	0.97	n.d.	72.8
	σ		528		91	0.14		11.0
	N		9/9		2/9	9/9		9/9
EL4,B	Avg	<2.5	36400	14.0*	<200	3.38*?	<1.1	48.8*?
	σ		4340	0.0		0.27		1.50
	N		3/3	1/3		3/3		3/3
EL4,C	Avg	<1.5	7910	16.1*	<150	1.92*?	<0.32	93.9*?
	σ		1620	5.7		0.50		8.8
	N		4/4	3/4		4/4		4/4
EL4,D	Avg	<0.4	1230	n.d.	<110	1.15*?	n.d.	92.0*?
	σ		330			0.02		5.6
	N		3/3			3/3		3/3

?- the value may be chosen; ** in ppb

TABLE 5.2 WEIGHTING SCHEME FOR STATISTICAL EVALUATION

Value $>$ LQ, LD	weight = 1.0
LQ $>$ value $>$ LD	weight = 0.5
LQ, LD $>$ value	
$3\sigma > LD$	weight = 0.25
$LD > 3\sigma$	weight = 0.0

(i) 3σ was calculated by using the standard deviation of a prelimirary estimate of the average value of the element in a group of samples without any weighting.

(ii) value was the determined element content of an element of a sample.

equation has not been evaluated since its significance can be questioned because of small number of samples in a given group. Only group "A" of EL4 cells had enough data for regression analysis, but these samples had been immersed in PBS buffer before they were rinsed with ammonium acetate solution and analyzed. Being possibly contaminated by PBS, the elemental levels could only be used for qualitative interpretation.

From Table 5.1, it can be seen that the concentration of aluminum in the EL4 cells does not vary too much in presence of body fluids. Arsenic has been detected only in EL4 cells of "B" group.

The total amount of an element in group "B" can be represented by a simplified equation:

$$C_{total} = C_{bf} + C_{dc} + C_{nc}$$

where C_{total} is the total amount; c_{bf} is the amount present in body fluid; C_{dc} is the amount in the cells that is diffusable to the body fluid; and C_{nc} is the non-diffusable fraction in the cell. The amount remaining after washing the cells by ammonium acetate solution with a volume ratio (the volume of the cell to the volume of ammonium acetate) of $1/(k-1)$ can be described by

$$C_{re} = (1/k).C_{bf} + (1/k).K.C_{dc} + (1-K).C_{dc} + C_{nc}$$

where C_{re} is the amount remained after washing; K is the diffusion term which is constant under the same experimental conditions. It is based on the assumption that dilution efficiency is 100%. Using the value of chloride content and this equation, it can be said that more than 70% of the arsenic could come from the body fluid if $K < 0.5$, or most of the arsenic present is in a diffusable form and $K \doteq 1$.

Similar conclusions can be drawn for bromide and chloride. The level of calcium decreases tremendously from "B" to "C" while the decrease from "C" to "D" is not significant. This observation is consistent from the biochemical point of view that calcium exists mostly in the body fluid and attaches itself to the cell membranes.

Chromium levels have generally varied between 1 to 3ppm (Table 5.1). The significantly high value of 17.0ppm in group "D" is due to the high value of a single sample, as can be seen from the magnitude of the standard deviation. The high value of iodide in group "A" compared to that of group "D", indicates the possibility of contamination in group "A".

Due to the proximity of magnitudes of potassium determined in groups "B" and "C", both of them can be used as the potassium content of the EL4 cells. Magnesium has exhibited a similar behavior. These elements may mostly exist inside the cells. No reliable estimate can be made for manganese.

The manganese value obtained in group "A" may be influenced by the extent of contamination. The levels of sodium have been found to decrease with the degree of washing, which suggests that this ion exists mostly outside the cell or is very diffusable.

On the other hand, rubidium is likely to exist inside the cells as evident from the similar values in groups "B" and "C". The amount of antimony determined in group "A" is probably due to contamination. The magnitude of selenium decreases slowly with the extent of washing. The difference between selenium and sodium is that the extent of decrease is lesser for selenium than for sodium with the extent of washing. Thus selenium is very likely to exist inside the cells and in both dissociable and non-dissociable forms. An unexplanable situation exists in the magnitude of zinc content. The value in group "B" is very low compared to groups "A", "C" and "D". Contamination from ammonium acetate buffer is ruled out because the analyses of ammonium acetate buffer, showed no zinc in it.

Elemental content of thymus tissues from the C57BL/6J female mice are shown in Table 5.3. Samples of group "A" were collected from 17-month-old mice whereas those of group "B" were from 1.2-month-old mice. Since variation in elemental levels with age is possible, no attempt has been made to combine the values from these two groups. Thymus is only

TABLE 5.3 ELEMENTAL CONTENT OF THYMUS TISSUES OF C57BL/6J MICE (IN PPM)

Sample		Age (month)	Al	As	Au*	Br	Ca	Cl	Co
A-1	Value	17	7.5	n.d.	-	20.9	2020	486	1.10
	LD		0.5		10	1.5	280	5	0.30
	LQ		2.4		35	5.0	1400	18	1.0
A-2	Value	17	9.0	n.d.	-	21.6	2160	1030	0.93
	LD		0.5		10	1.5	280	10	0.30
	LQ		2.4		35	5.0	1400	37	1.0
B-1	Value	1.2	31.2	-	n.d.	10.7	1314	5490	0.168
	LD		5.4	0.20		0.45	197	80	0.21
	LQ		21	0.65		1.50	2200	330	1.0
B-2	Value	1.2	37.6	0.152	n.d.	8.95	360	5380	0.157
	LD		4.3	0.16		0.37	136	70	0.16
	LQ		17	0.53		1.20	1671	270	0.81

n.d.- not determined; *- in ppb; LD- qualitative detection limit;
LQ- quantitative determination limit.

TABLE 5.3 Cont'd

Sample		Cr	Cu	Fe	Hg	I	K	Mg	Mn
A-1	Value	1.38	3.0	29	n.d.	0.28	3040	327	0.39
	LD	0.4	3.2	11		0.15	150	150	0.04
	LQ	1.4	10	36		0.52	570	482	0.12
A-2	Value	0.87	3.1	18	n.d.	0.30	4900	296	0.49
	LD	0.4	3.2	11		0.15	150	150	0.04
	LQ	1.4	10	36		0.52	570	482	0.12
B-1	Value	3.76	3.1	129	-	-	19200	1015	1.98
	LD	1.6	23	131	0.52	1.3	2740	271	0.77
	LQ	5.8	87	572	1.8	5.0	10600	1537	2.90
B-2	Value	-	76.4	64	-	-	21200	1235	1.94
	LD	1.4	19	103	0.46	1.1	2360	2420	0.65
	LQ	5.0	71	447	1.6	4.2	8910	1260	2.40

TABLE 5.3 Cont'd

Sample		Mo	Na	Rb	Sb*	Se	V*	Zn
A-1	Value	n.d.	712	3.2	87	0.58	33	24
	LD		8	2.1	28	0.07	18	5
	LQ		26	8.4	90	0.32	59	20
A-2	Value	n.d.	822	8.5	44	0.55	19	36
	LD		8	2.1	28	0.07	18	5
	LQ		26	8.4	90	0.32	59	20
B-1	Value	-	2490	27.0	624	1.69	-	81.1
	LD	1.1	77	4.4	62	0.20	280	6.1
	LQ	3.4	283	19	200	0.83	1100	28
B-2	Value	-	2310	33.3	-	1.73	-	77.9
	LD	0.94	66	3.5	50	0.17	240	4.8
	LQ	2.9	239	15	160	0.70	920	22

a small piece of tissue and it reduces in size as the mouse grows older. To collect samples of reasonable dry-weight requires the sacrifice of quite a large number of mice. For this reason, only two samples (B-1, B-2) could be collected from baby mice. The elemental levels of these samples can be considered as those of controls. Among all the elements determined, concentrations of potassium, magnesium, manganese and rubidium are slightly higher and that of calcium is considerably higher in thymus samples compared to EL4 cells. Because of the small number of samples, no statistical evaluation of the significance of difference has been attempted. A lower value of calcium in tumor cells compared to controls was observed by some researchers (CAR44, SC043). The lower calcium content in cancer tissues was suggested to be related to the lessened adhesiveness of cells (COM44). It may also influence the extent of metastasis and local invasiveness.

5.2.2 Livers

Liver samples were collected from EL4 tumor-bearing mice and normal mice. Their elemental levels are presented in Table 5.4. The possibility of correlation between pairs of elements in the livers of the tumor-bearing mice has been studied. Twenty pairs of elements, e.g., As-Br, As-Rb, Mo-Br, Mo-Fe, Mo-Rb and Hg-Se, have been found to be correlated according to the following selected criteria, viz. for sample number less than ten a correlation coefficient, R, greater than 0.90 and significance, S, better than 0.01. For sample

TABLE 5.4 ELEMENTAL CONTENT OF LIVERS OF C57BL/6J MICE (IN PPM)[a]

	from mice with tumor			from control		
Element	average	σ	N	average	σ	N
Al	13.4	3.5	14/14	11.2	2.2	4/4
As(RNAA)*	39.7	11.4	5/7	73.7	3.6	4/4
Br	18.5	11.1	14/14	8.69	0.75	4/4
Ca	149	60	7/14	113	41	2/4
Cl	3420	1150	14/14	3030	340	4/4
Co*	353	175	7/14	282	39	2/4
Cu(INAA)	17.0	7.3	5/14	23.5	14.8	2/4
Cu(RNAA)	10.9	1.9	7/7	12.0	0.71	4/4
Fe	688	407	13/14	632	76	4/4
K	7780	1820	14/14	8280	820	4/4
Mg	579	103	14/14	562	64	4/4
Mn	2.13	0.77	14/14	2.13	0.39	4/4
Mo(INAA)	2.11	1.06	10/14	2.28	0.23	3/4
Mo(RNAA)	2.33	1.21	6/7	2.10	0.13	4/4
Na	2630	890	14/14	2560	340	4/4
Rb	18.4	5.2	12/14	15.3	1.7	4/4
Se	2.55	0.48	14/14	3.40	0.37	4/4
Zn	85.5	12.6	14/14	79.3	5.2	4/4
Hg(RNAA)*	152	72	6/7	40.2	11.9	2/4
V*	170			183	41	2/4
Age(months)	4.1 - 5.9		14/14	17		4/4

*- in ppb;

a- weighted.

numbers equal to or larger than ten, only 0.80 and 0.05 are required for R and S, respectively. Correlation table of all the element pairs of the liver of EL4 bearing mice was shown in appendix. Multielement regression has been studied for groups where the number of samples is greater than nine. These regression equation for liver of the tumor-bearing mice are shown in appendix.

The significance of differences between the elemental content of the tumor-bearing and normal mice has been tested. Using the student's t-test, t-values have been evaluated either by pooled variance or separated variance estimates. The choice has been determined by the result of a F-test of the variances of the two groups. A value of probability equal to or less than 0.1 indicates the use of separated variance estimates. A weighting scheme (Table 5.2) and a two-tail probability calculations have been used in such tests. The results are listed in Table 5.5. In the t-test a normal distribution of the data is assumed; however, if no prior information on the distribution pattern of the data is available, as in the case of a few elements studied, Wilcoxon two-sample test may be more indicative of the real situation and reliable. The results of Wilcoxon test are shown in Table 5.6.

According to the results of these tests, the differences in the elemental contents of As, Br, and Se are significant with a confidence level of greater than 95%. A similar

TABLE 5.5 t-TEST OF SIGNIFICANCE OF DIFFERENCE IN ELEMENTAL CONTENT OF LIVERS OF C57BL/6J MICE[a]

	F-test		t-test			
Element	F-value	2-tail P	t-value	2-tail P	type	deg. of freedom
Al	2.54	0.48	1.15	0.27	p	16
As(RNAA)	10.1	0.09	-6.33	0.00	s	5.0
Br	219	0.00	3.31	0.01	s	13.4
Ca	2.12	0.96	0.77	0.47	p	7
Cl	11.6	0.07	1.14	0.27	s	15.8
Co	20.1	0.34	0.55	0.60	p	7
Cu(INAA)	4.18	0.22	-0.81	0.46	p	5
Cu(RNAA)	6.94	0.14	-1.04	0.33	p	9
Fe	29.1	0.02	0.47	0.65	s	14.1
K	5.01	0.21	-0.52	0.61	p	16
Mg	2.60	0.47	0.31	0.76	p	16
Mn	3.80	0.30	0.01	0.99	p	16
Mo(INAA)	21.5	0.09	-0.46	0.65	s	10.3
Mo(RNAA)	87.7	0.00	0.46	0.66	s	5.2
Na	6.95	0.14	0.15	0.88	p	16
Rb	9.04	0.10	1.79	0.10	s	13.5
Se	1.72	0.73	-3.27	0.01	p	16
Zn	5.93	0.17	0.95	0.36	p	16
Hg(RNAA)	36.3	0.25	2.08	0.08	p	6

a- weighted; P- probability; p- pooled variance estimation; s- separated variance estimation.

TABLE 5.6 WILCOXON TWO-SAMPLE TEST OF THE DIFFERENCE OF ELEMENTAL CONTENT BETWEEN LIVER SAMPLES OF C57BL/6J MICE

Element	average rank		probability of forming rank less than the smaller rank	P
	tumor mice	normal mice		
Al	10.1	7.25	0.18	0.37
As(INAA)	5.0	1.5	0.04	0.08
As(RNAA)	4.0	9.5	0.00	0.01
Br	11.1	4.0	0.01	0.02
Ca	10.2	7.13	0.17	0.34
Cl	10.0	7.75	0.25	0.49
Co	9.64	9.0	0.44	0.88
Cu(INAA)	8.21	14.0	0.03	0.06
Cu(RNAA)	5.07	7.63	0.13	0.26
Fe	8.79	12.0	0.16	0.32
K	9.11	10.9	0.30	0.60
Mg	9.79	8.5	0.36	0.71
Mn	9.43	9.75	0.48	0.96
Mo(INAA)	8.79	12.0	0.16	0.32
Mo(RNAA)	4.83	6.50	0.23	0.46
Na	9.36	10.0	0.44	0.88
Rb	9.71	8.75	0.40	0.79
Se	7.86	15.25	0.01	0.01
Zn	10.0	7.63	0.23	0.46
Hg	7.71	3.0	0.02	0.03

P = the probability of having both groups of samples drawn from the same population.

difference in mercury contents has been observed by Wilcoxon test. The probability of both groups of mercury levels drawn from the same population is higher by student's t-test than by Wilcoxon test. The lower levels of arsenic, bromine and selenium in the livers of tumor-bearing mice are significantly different from those of controls. These differences could explain a possible role for trace elements in neoplasia.

Another possible reason for the differences in elemental levels is the age difference between these two groups. However, two points should be noted. Firstly, the age difference among the samples of the same tumor-group is small. Secondly, there is no indication of any correlation between age and elemental content within the same group.

5.2.3 Hearts, Kidneys, Lungs, and Spleens

Hearts, kidneys, lungs and spleens of tumor-bearing and normal C57BL/6J mice were collected during the sampling of tumor cells and livers. They were combined, as before, to form samples of different types with reasonable weight. The results are given in Table 5.7, Table 5.8, Table 5.9 and Table 5.10. The data for samples from mice with tumor were weighted (Table 5.2) prior to statistical analysis. Because there was not enough material to form several samples of the control, only one control sample of each type was analyzed.

TABLE 5.7 ELEMENTAL CONTENT OF HEARTS OF C57BL/6J MICE (IN PPM)

	Mice with tumor[a]			Control		
Element	average	σ	N	value	LD	LQ
Al	13.3	1.7	4/4	10.3	1.7	9.3
As	<0.19			0.153	0.136	0.43
Br	15.8	1.6	4/4	10.2	0.32	1.0
Ca	154	-	1/4	107	98	1000
Cl	4480	680	4/4	3160	40	170
Co*	346	63	2/4	252	130	690
Cu	16.7	-	1/4	17.0	11	42
Fe	629	86	4/4	635	95	438
K	9760	640	4/4	8200	260	840
Mg	646	49	4/4	630	177	994
Mn	2.41	0.28	4/4	2.16	0.40	1.40
Mo	1.10	-	1/4	-	0.74	2.30
Na	3250	450	4/4	2630	40	150
Rb	18.9	4.0	4/4	13.4	3.6	18
Se	2.28	0.21	4/4	3.03	0.12	0.49
V	<0.2			0.265	0.15	0.56
Zn	84.2	3.1	4/4	67.9	4.1	20
Age(month)	4.1 - 5.9				17	

a- weighted;
*- in ppb.

TABLE 5.8 ELEMENTAL CONTENT OF KIDNEYS OF C57BL/6J MICE (IN PPM)

	Mice with tumor[a]			Control		
Element	average	σ	N	value	LD	LQ
Al	11.2	3.2	4/4	11.5	0.98	5.1
As	<0.3			0.231	0.10	0.32
Br	20.0	9.3	4/4	14.6	0.23	0.72
Ca	148	75	2/4	106	60	484
Cl	3260	1060	4/4	2560	30	100
Co*	504	141	3/4	230	56	29
Cu	14.6	-	1/4	15.5	6.5	23
Fe	399	54	4/4	632	43	184
I	1.04	0.07	2/4	-	0.43	1.5
K	6940	2180	4/4	6700	180	0.57
Mg	522	117	4/4	520	48	169
Mn	2.06	0.50	4/4	2.00	0.23	0.82
Mo	1.18	0.77	2/4	1.56	0.57	1.77
Na	2410	700	4/4	2080	26	89
Rb	17.2	4.0	4/4	13.9	2.3	9.6
Se	3.85	1.12	4/4	4.77	0.08	0.30
V	<0.20			0.202	0.08	0.31
Zn	70.6	23	4/4	67.0	2.0	9.0
Age (month)	4.1 - 5.9			17		

a- weighted

*- in ppb

TABLE 5.9 ELEMENTAL CONTENT OF LUNGS OF C57BL/6J MICE
(IN PPM)

	Mice with tumor[a]			Control		
Element	average	σ	N	value	LD	LQ
Al	18.3	4.3	4/4	12.7	1.2	5.6
As*	367	173	2/4	473	122	283
Br	33.7	7.6	4/4	20.4	0.28	0.87
Ca	199	85	2/4	131	64	489
Cl	4670	900	4/4	3010	30	100
Co*	291	50	2/4	242	60	350
Cu	<15			8.24	7.2	25
Fe	480	52	4/4	591	50	221
I	1.59	-	1/4	0.47	0.47	1.6
K	9750	1470	4/4	7580	210	670
Mg	727	37	4/4	500	52	180
Mn	2.19	0.47	4/4	2.15	0.25	0.87
Mo	1.27	-	1/4	1.48	0.70	2.1
Na	3340	490	4/4	2610	30	100
Rb	23.6	2.2	4/4	17.0	2.5	11.0
Se	2.31	0.16	4/4	3.31	0.10	0.35
V	<0.1			0.10	0.09	0.33
Zn	82.1	14.7	4/4	77.6	2.2	11
Age(month)	4.1 - 5.9				17	

a- weighted;
*- in ppb

TABLE 5.10 ELEMENTAL CONTENT OF SPLEENS OF C57BL/6J MICE
(IN PPM)

	Mice with tumor[a]			Control		
Element	average	σ	N	value	LD	LQ
Al	23.3	0.32	4/4	23.9	3.1	18
As	<0.30			<0.2		
Br	31.3[b]	30.9	4/4	9.33	0.45	1.45
Ca	263	62	2/4	216	145	2000
Cl	4040	660	4/4	3240	70	270
Co*	365	29	2/4	400	200	1500
Cu	37.8	-	1/4	18.6	18.8	73
Fe	430	106	3/4	3603	167	741
I	<1.6			<1.2	1.2	4.5
K	10000	930	4/4	8600	350	1160
Mg	1035	26	4/4	880	127	487
Mn	4.67	0.50	4/4	4.48	0.65	2.5
Mo	<2.0			<1.1		
Na	3330	420	4/4	2890	60	240
Rb	20.7	5.1	2/4	21.0	7.4	33
Se	2.62	0.41	4/4	4.0	0.30	0.95
V	<0.27			0.232	0.22	0.89
Zn	104	11	4/4	81.8	7.1	33
Age (month)	4.1 - 5.9				17	

a- weighted;

b- one datum was too high. Without it, average = 15.9

*- in ppb

Due to the small sample size, the differences in elemental contents have not been tested for significances. However, since the sample contained a reasonable amount of substance, the data may be used to give an indication of the elemental levels present in that kind of tissue. If maximum information is sought from these data, a test of 3σ as a rule of thumb may be used to indicate possible differences, assuming the value from the control is a data point in the group of data points of the mice with tumor. In this way, Br and Zn in hearts, Fe in kidneys, Mg and Se in lungs, Fe, Mg and Se in spleens have exhibited differences $>3\sigma$. Since these values are not the average values, no definite conclusion can still be drawn.

Among the four types of tissue studied here, the elemental levels fall almost in the same range except spleen, when variation between samples is considered. There are significant differences in magnesium and manganese content of spleen and other tissues. Zinc also shows a similar pattern. An interesting point is that these elements are involved in metalloenzymes.

5.3 Tissues from A/J Mice

Samples of different tissues and the solid tumor lumps of H6 were collected from A/J mice strain as described in Section 3.5.

5.3.1 H6 Solid Tumors

Since H6 is a solid tumor, the problem of sampling it was much simpler compared to that of the EL4 cells. The tumor samples were analyzed mainly by INAA primarily due to lack of time for carrying out extensive radiochemical separations. However, RNAA had to be used for determining copper because of its low levels in these samples. Concentrations of several elements in the H6 tumor are shown in Table 5.11. In an examination of correlationship between concentrations of elemental pairs, two pairs of elements namely Al-Mg and Cl-Na have been found to show values of $R \geq 0.8$ and $S \leq 0.01$. Among them, only one pair, viz. Cl-Na, satisfied the more strict requirements defined in the section of EL4 cells. Multielement regression equations have been calculated and are listed in appendix.

5.3.2 Livers

Livers from tumor-bearing and normal mice were analyzed by both INAA and RNAA. The results are shown in Table 5.12. Correlation tests between concentrations of elemental pairs have shown none with significance, in the livers of tumor-bearing mice and in the livers of normal mice. The lack of any correlation between elemental pairs in the livers of A/J mice is surprising. From Table 5.4 and Table 5.12,

TABLE 5.11 ELEMENT CONTENT OF H6 SOLID TUMOR OF A/J MICE

(IN PPM)[a]

Element	average element content	σ	N
Ag*	<55		
Al	24.9	1.54	9/9
Au*	<4		
Br	23.2	6.2	9/9
Ca	510	224	5/9
Cl	852	106	9/9
Cu(INAA)	<22		
Cu(RNAA)	5.72	0.54	9/9
K	1560	170	9/9
Mg	822	121	9/9
Mn	0.662	0.17	4/9
Na	673	120	9/9
Rb	23.5	2.76	9/9
Sb*	<45		
Se	1.91	0.11	9/9
V*	210	-	1/9
Zn	75.0	9.98	5/9

Age (month) 3.2 - 3.8

a- weighted;

*- in ppb

TABLE 5.12 ELEMENTAL CONTENT OF LIVERS OF A/J MICE
(IN PPM)[a]

	Mice with tumor			Control		
Element	average	σ	N	average	σ	N
Al	15.4	2.1	10/10	17.2	5.3	10/10
As(INAA)*	<160			218	90	3/10
As(RNAA)*	49.1	16.0	6/6	76.8	14.1	6/6
Br	7.65	0.94	10/10	9.14	1.26	10/10
Ca	182	74	5/10	129	22	3/10
Cl	2830	360	10/10	3590	140	10/10
Co*	172	43	5/10	245	60	5/10
Cr	<1.0			<1.0		
Cu(INAA)	24.7	13.5	5/10	16.6	4.2	5/10
Cu(RNAA)	11.0	1.4	6/6	13.2	1.0	5/5
Fe	391	76	9/10	457	62	10/10
I	<0.73			<0.74		
K	7980	740	10/10	10900	490	10/10
Mg	727	55	10/10	721	39	10/10
Mn	1.76	0.22	10/10	4.38	0.30	10/10
Mo(INAA)	2.20	0.58	7/10	3.70	0.61	9/10
Mo(RNAA)	2.54	0.23	6/6	4.35	0.46	5/5
Na	2240	200	10/10	2760	340	10/10
Rb	18.6	2.6	10/10	24.0	2.05	10/10
Sb*	<34			<60		
Se	2.87	0.31	10/10	5.49	0.26	10/10
V*	208	27	3/10	194	22	3/10
Zn	129	14	10/10	107	9.3	10/10
Hg(RNAA)*	64.6	12.9	5/6	44.7	8.4	4/6
Age(month)	3.2 - 3.8			4.25		

a- weighted;
*- in ppb.

it can be seen that the standard deviations of elemental levels in livers of the A/J mice are in general smaller than those of C57BL/6J mice. In other words, the concentrations of elements are fairly constant in the livers of the group of A/J mice studied. The small variations in the elemental content may be attributed to the random error of analysis and sampling. Multielement regressions for livers of A/J mice have been carried out. These equations for both tumor-bearing and control mice are shown in appendix.

Student's t-test and Wilcoxon two-sample test have been used to evaluate the significance of the differences between elemental contents of the tumor-bearing and control mice. The results are tabulated in Tables 5.13 and 5.14. Differences in concentrations of As, Br, Cl, Cu(RNAA), Fe, K, Mn, Mo(INAA), Mo(RNAA), Na, Rb, Se, Zn and Hg(RNAA) are significant with a confidence level greater than 95% for the t-test. Similar results have been obtained by Wilcoxon test for Ca and Co, in addition to the above elements. The concentrations of As, Br, Cl, Cu, Fe, K, Mn, Mo(INAA, RNAA), Na, Rb, Se are lower in the livers of tumor-bearing mice compared to control. However, the opposite situation exists with Hg and Zn.

Since H6 is a hepatoma, relationship may exist between the tumor tissues and the livers. Thus the differences in elemental contents have been tested again. Student's t-test has been used here for the sake of simplicity, and because of

TABLE 5.13 t-TEST OF SIGNIFICANCE OF DIFFERENCE IN ELEMENTAL CONTENT IN LIVERS OF A/J MICE[a]

	F-test		t-test			
Element	F-value	2-tail P	t-value	2-tail P	type	deg.of freedom
Al	6.66	0.01	-1.04	0.32	s	11.6
As(RNAA)	1.28	0.79	-3.19	0.01	p	10
Br	1.78	0.40	-3.00	0.01	p	18
Ca	10.9	0.17	1.19	0.28	p	6
Cl	6.75	0.01	-6.16	0.00	s	11.6
Co	1.92	0.54	-2.13	0.07	p	8
Cu(INAA)	10.2	0.05	1.22	0.29	s	4.2
Cu(RNAA)	1.83	0.58	-2.99	0.02	p	9
Fe	1.47	0.58	-2.08	0.05	p	17
K	2.27	0.24	-10.5	0.00	p	18
Mg	1.99	0.32	0.26	0.80	p	18
Mn	1.86	0.37	-22.1	0.00	p	18
Mo(INAA)	1.10	0.94	-4.90	0.00	p	14
Mo(RNAA)	4.24	0.15	-8.49	0.00	p	9
Na	3.03	0.11	-4.21	0.00	p	18
Rb	1.65	0.47	-5.19	0.00	p	18
Se	1.42	0.61	-20.5	0.00	p	18
V	1.50	0.80	0.69	0.53	p	4
Zn	2.17	0.27	4.03	0.00	p	18
Hg(RNAA)	2.38	0.50	2.48	0.05	p	6

a- weighted, P- probability; p- pooled variance estimation; s- separated variance estimation.

TABLE 5.14 WILCOXON TWO-SAMPLE TEST OF THE DIFFERENCE OF ELEMENTAL CONTENT BETWEEN LIVER SAMPLES OF A/J MICE

Element	average rank		probability of having smaller rank	P
	tumor mice	normal mice		
Al	9.85	11.15	0.33	0.65
As(RNAA)	4.0	9.0	0.01	0.02
Br	7.20	13.8	0.01	0.01
Ca	14.2	6.8	0.00	0.01
Cl	5.5	15.5	0.00	0.00
Co	7.1	13.9	0.01	0.01
Cu(INAA)	11.0	10.0	0.37	0.73
Cu(RNAA)	3.38	8.60	0.01	0.02
Fe	7.6	13.4	0.01	0.03
K	5.5	15.5	0.00	0.00
Mg	11.1	9.9	0.34	0.68
Mn	5.5	15.5	0.00	0.00
Mo(INAA)	5.7	15.3	0.00	0.00
Mo(RNAA)	3.5	9.0	0.00	0.01
Na	5.9	15.1	0.00	0.00
Rb	6.0	15.0	0.00	0.00
Se	5.5	15.5	0.00	0.00
V	8.0	9.33	0.32	0.63
Zn	14.3	6.7	0.00	0.00
Hg(RNAA)	8.67	4.33	0.02	0.04

P- the probability of both groups from the same population.

the magnitude of t-values calculated using pooled variance estimate are in general so large that no difference in results can be obtained using separated variance estimate. The results are shown in Table 5.15.

The results presented in Table 5.15 are likely to provide some information on how the H6 tumor can affect the metabolism of the living beings, particularly the A/J mice. From this table, it can be seen that for the elements studied (viz. Al, Br, Ca, Cl, Cu, K, Mg, Mn, Na, Rb, Se and Zn), only the differences in elemental content of Ca, Mg and Rb are not significant. The results in Table 5.15 indicate that the H6 tumor tissues are very different in comparison to the tissues they have been originally derived from in terms of their trace elemental composition.

If the presence of H6 neoplasia causes changes in metabolism of mice then a trend in elemental content of the H6 tumor, liver of tumor-bearing mice, and liver of normal mice may be expected. This relationship could be either linear, exponential or a combination of both. The trend can be recognized in the sign and magnitude of the t-values in Table 5.15. The elements such as Al, Cl, Cu, Mn and Se, in which this trend is significant, are grouped together in Table 5.16. Aluminum may be excluded from this group because the difference of its content between the tumor-bearing and normal liver is not that significant. It can therefore be concluded that the concentrations of Cl, Cu, Mn and Se are

TABLE 5.15 TEST OF SIGNIFICANCE IN THE DIFFERENCE OF ELEMENT CONTENTS OF H6 TUMORS AND LIVERS OF A/J MICE[a]

Element	tumor v s. liver from mice with tumor			tumor v s. livers from normal mice		
	t	deg. of freedom	P	t	deg. of freedom	P
Al	12.5	13	0.00	13.6	13	0.00
Br	6.15	13	0.00	5.64	13	0.00
Ca	2.28	6	0.06	2.25	5	0.07
Cl	-13.3	13	0.00	-19.5	13	0.00
Cu(RNAA)	-11.9	13	0.00	-18.4	12	0.00
K	-38.1	13	0.00	-37.6	13	0.00
Mg	1.53	13	0.15	1.56	13	0.14
Mn	-6.47	7	0.00	-12.7	8	0.00
Na	-12.6	13	0.00	-9.55	13	0.00
Rb	3.94	13	0.00	-0.02	13	0.98
Se	-4.57	13	0.00	-22.3	13	0.00
Zn	-11.6	9	0.00	-7.75	9	0.00

a- weighted; t- value of t in t-test;
P- the probability of both groups from the same population.

TABLE 5.16 t-VALUES OF TESTS AMONG H6 TUMORS, LIVERS FROM TUMOR MICE AND NORMAL LIVERS OF A/J MICE

Element	t-value		t-test of difference (livers from tumor mice vs. normal livers)	
	H6 vs. livers from tumor mice	H6 vs. normal livers	t-value	P
Al	12.5	13.6	1.04	0.32
Cl	-13.3	-19.5	-6.16	0.00
Cu	-11.9	-18.4	-2.99	0.02
Mn	-6.47	-12.7	-22.1	0.00
Se	-4.57	-22.3	-20.5	0.00

P- the probability of both groups from the same population.

very likely to be affected by the presence of the H6 tumor.

The lower chlorine levels of H6 and tumor-bearing liver may merely indicate lower salt content of these tissues, and/or lower intercellular fluid volume. On the other hand, the lower concentrations of manganese, copper and selenium, the essential elements, may be related to a decrease in the amounts of a few essential metalloenzymes. The lowering of copper and manganese levels in H6 tumor may also be due to the preference of glycolysis to oxidation in deriving energy needed for the growth of the tumor cells as mentioned in the literature survey. This decrease in copper and manganese concentrations of H6 tumor observed in this study is consistent with the results obtained by Oberley. He found no Mn superoxide dismutase activity and very low Cu-Zn superoxide dismutase activity in H6 hepatoma cells compared to normal and regenerating livers (OBE78). It is further supported by the fact that one of the major Cu enzymes, cytochrome c which exists in mitochondria, is lower in tumor-bearing mice compared to normals.

The role of selenium in neoplasia is not well understood at this stage. It has been reported that selenium could reduce liver necrosis. Selenium has been considered as an antioxidant, and suggested to have functions similar to vitamin E (SCH65, THO70). One of such seleno-enzymes is glutathione peroxidase. It has also been suggested that

selenium may have the possibility of being used in the prevention of cancer (YOU79). The decrease in selenium levels of tumor-bearing mice observed in this study indicates a possible role of this element in neoplasia.

The elements which exhibit a significant difference in levels between tumor tissues and livers (Table 5.12) but do not show any particular trend cannot be excluded from having a relationship with tumor. They may either be affected to a lesser extent or be involved in a more complicated mechanism. For example, zinc content is elevated in the liver of tumor-bearing A/J mice compared to control (Table 5.12), but lowered in the H6 tumor (Table 5.11 and 5.12). This finding is consistent with the results reported by Griffith for human beings (GRI73). His explanation was that the increase in liver zinc might reflect a wide spread pre-malignant change not peculiar to the liver; it might also be related to poor nutrition; or could be a feature of the chemical defense reaction of normal liver tissues on the invasion of malignant cells. The last possibility was preferred by Griffith. A lower zinc content in primary cancer of the liver in human was also found by Kew (KEW74). It may be related to the lower Cu-Zn superoxide dismutase in tumor-bearing species.

5.3.3 Hearts, Kidneys, Lungs, and Spleens

Samples of heart, kidney, lung and spleen of tumor-

bearing and normal A/J mice were also analyzed by INAA. The results are presented in Table 5.17 (heart), Table 5.19 (kidney), Table 5.21 (lung), and Table 5.23 (spleen). The differences in elemental content of these two groups have been evaluated with student's t-test for significance, and the respective results are listed in Table 5.18, Table 5.20, Table 5.22 and Table 5.24.

Concentrations of Mg and Zn are significantly lower in the hearts of H6 tumor-bearing A/J mice compared to normal mice with a confidence level of better than 95%. Similarly Al, Br, Cl, K, Mn, Na, and Zn contents are lower in the kidneys; Br, Cl, Fe, Na and Rb contents are significantly lower in the lungs; and Cl, K and Zn are lower in the spleens of the tumor-bearing mice in comparison to those controls. The elements, which exhibit significant difference in concentrations between the tumor-bearing and control mice groups, do not show similar differences in all types of tissue(Tables 5.18, 5.20, 5.22, and 5.24). It is very likely that they are subjected to the influences of many other factors. Among the four types of tissue studied here, lungs are not active in metabolic processes. The main function of lung is respiration. It consists of many small capsules and has a large surface area. It is surrounded by numerous capillaries . Thus the elemental content of lung may include the elemental levels of blood to certain extent. Such a statement can be substantiated by the higher sodium and chloride content, which are considered mostly extracellular, in lung tissues. The lower iron content in the lungs of tumor-

TABLE 5.17 ELEMENTAL CONTENT OF HEARTS OF A/J MICE
(IN PPM)[a]

	Mice with tumor			Control		
Element	average	σ	N	average	σ	N
Al	36	26	2/3	12.5	3.2	2/3
Br	9.01	3.72	3/3	9.33	1.02	3/3
Ca	1047	-	1/3	670	586	2/3
Cl	3020	180	3/3	3310	580	3/3
Co*	<600			<400		
Cu	412	338	2/3	37.2	4.2	2/3
Fe	443	65	2/3	541	94	2/3
K	7240	1750	2/3	9340	620	3/3
Mg	661	70	2/3	837	104	3/3
Mn	2.18	0.50	2/3	2.46	0.49	2/3
Mo	<1.7			<1.4		
Na	2050	290	3/3	2530	260	3/3
Rb	16.8	1.98	2/3	12.9	2.03	2/3
Se	3.09	1.14	3/3	1.38	0.06	3/3
Zn	112	10	3/3	68.8	2.9	3/3
Age(month)	3.2 - 3.8			4.25		

a- weighted;

*- in ppb.

TABLE 5.18 t-TEST OF SIGNIFICANCE OF THE DIFFERENCE IN ELEMENTAL CONTENT OF HEARTS OF A/J MICE

Element	t-value (pooled variance estimate)	P
Al	1.28	0.33
Br	-0.144	0.89
Cl	-0.64	0.56
Cu	1.57	0.26
Fe	-2.12	0.17
K	-1.92	0.15
Mg	-4.67	0.02
Mn	-0.46	0.69
Na	-1.42	0.23
Rb	2.26	0.15
Se	2.54	0.06
Zn	7.38	0.00

P- the probability of both groups from the same population.

TABLE 5.19 ELEMENTAL CONTENT OF KIDNEYS OF A/J MICE (IN PPM)[a]

Element	Mice with tumor			Control		
	average	σ	N	average	σ	N
Al	14.1	0.30	3/3	16.7	1.61	3/3
As*	<170			374	-	1/3
Br	12.7	3.4	3/3	20.6	1.2	3/3
Ca	217	93	2/3	172	16	2/3
Cl	2930	170	3/3	6130	800	3/3
Co*	<180			172	190	2/3
Cu	66.4	46.0	2/3	14.8	-	1/3
Fe	333	131	2/3	290	-	1/3
K	7300	680	3/3	9120	270	3/3
Mg	674	57	3/3	623	53	3/3
Mn	1.46	0.18	2/3	4.51	0.71	3/3
Mo	1.53	0.89	2/3	1.84	0.57	2/3
Na	2050	110	3/3	4570	590	3/3
Rb	18.5	3.98	2/3	14.1	2.41	3/3
Se	5.16	0.86	3/3	4.59	1.01	3/3
V*	<170			264	-	1/3
Zn	98.3	16.2	3/3	52.9	21.6	3/3
Age (month)	3.2 - 3.8			4.25		

a- weighted;

*- in ppb.

TABLE 5.20 t-TEST OF SIGNIFICANCE OF THE DIFFERENCE IN ELEMENTAL CONTENT OF KIDNEYS OF A/J MICE

Element	t-value (pooled variance estimate)	P
Al	-3.45	0.03
Br	-3.85	0.02
Ca	0.684	0.56
Cl	-6.09	0.00
K	-3.68	0.02
Mg	1.54	0.20
Mn	-4.80	0.02
Mo	-0.376	0.74
Na	-5.63	0.00
Rb	1.83	0.16
Se	0.75	0.50
Zn	4.67	0.01

P- the probability of both groups from the same population.

TABLE 5.21 ELEMENTAL CONTENT OF LUNGS OF A/J MICE
(IN PPM)[a]

Element	Mice with tumor			Control		
	average	σ	N	average	σ	N
Al	23.8	5.09	3/3	26.7	8.77	2/3
Br	25.6	0.46	3/3	35.3	2.2	3/3
Ca	316	261	2/3	479	-	1/3
Cl	3410	200	3/3	5220	170	3/3
Co*	<200			490	235	2/3
Cu	131	60	3/3	34.8	-	1/3
Fe	397	75	2/3	792	121	2/3
K	9180	1590	3/3	9460	1030	3/3
Mg	708	57	3/3	678	52	2/3
Mn	2.37	1.31	2/3	1.47	-	1/3
Na	2340	160	3/3	3720	50	3/3
Rb	21.4	0.87	2/3	12.6	3.08	2/3
Se	2.43	0.21	3/3	1.98	0.19	3/3
Zn	92.9	7.2	3/3	89	6.9	3/3
Age (month)	3.2 - 3.8			4.25		

a- weighted;
*- in ppb.

TABLE 5.22 t-TEST OF SIGNIFICANCE OF THE DIFFERENCE IN ELEMENTAL CONTENT OF LUNGS OF A/J MICE

Element	t-value (pooled variance estimate)	P
Al	-0.708	0.53
Br	-10.8	0.00
Cl	-6.84	0.00
Fe	-7.37	0.02
K	-0.257	0.81
Mg	0.703	0.53
Na	-9.03	0.00
Rb	6.35	0.02
Se	1.63	0.18
Zn	0.904	0.42

P- the probability of both groups from the same population.

TABLE 5.23 ELEMENTAL CONTENT OF SPLEENS OF A/J MICE (IN PPM)[a]

	Mice with tumor			Control		
Element	average	σ	N	average	σ	N
Al	25.3	3.5	2/3	27.2	0.31	3/3
Br	8.99	0.56	3/3	9.27	0.68	3/3
Ca	264	135	2/3	<300		
Cl	2870	150	3/3	4660	120	3/3
Co*	<400			<440		
Cu	375	389	3/3	<50		
Fe	1147	222	3/3	1993	372	3/3
K	8340	370	3/3	16400	830	3/3
Mg	1003	50	3/3	1024	58	3/3
Mn	1.85	0.68	2/3	1.07	-	1/3
Na	2360	140	3/3	2550	160	3/3
Rb	21.2	2.4	3/3	18.7	4.5	2/3
Se	2.20	0.39	3/3	2.45	0.46	3/3
V*	517	-	1/3	330	-	1/3
Zn	156	4.6	3/3	84.4	8.96	3/3
Age (month)	3.2 - 3.8			4.25		

a- weighted;
*- in ppb.

TABLE 5.24 t-TEST OF SIGNIFICANCE OF THE DIFFERENCE IN ELENENTAL CONTENT OF SPLEENS OF A/J MICE

Element	t-value (pooled variance estimate)	P
Al	-0.99	0.39
Br	-0.49	0.65
Cl	-8.21	0.00
Fe	-2.25	0.09
K	-14.2	0.00
Mg	-0.139	0.90
Na	-0.777	0.48
Rb	1.19	0.32
Se	-0.55	0.61
Zn	22.6	0.00

P- the probability of both groups from the same population.

bearing A/J mice may explain the occurrence of anemia which is frequently associated with neoplasia of living beings. The amount of residual blood in the tissue may also influence its iron levels.

Concentrations of zinc have been found to be elevated in all tissues of the tumor-bearing A/J mice. There are many important zinc metalloenzymes, such as, carbonic anhydrase, lactate dehydrogenase, D-glyceraldehyde 3 phosphate dehydrogenase, glutamate dehydregenase, etc. The higher value of Zn content of tumor-bearing mice may indicate high concentrations of some of the zinc enzymes mentioned above. The finding of this work is consistent with the observations of Griffith (GRI 73).

Concentrations of cadmium and gold were determined in some liver samples of both strains of mice. Since most samples did not contain detectable amounts of cadmium and gold, they have not been included in Table 5.4 and 5.12. Instead, they are grouped together in Table 5.28 for information only. No elaborate statistical treatment has been done on these data.

5.4 Environmental Samples

Samples related to the environment in which mice lived were collected at different intervals. Several samples of the same type were mixed to obtain composite samples. These

composite samples, namely mouse chow, bedding and potable water, were analyzed by INAA. Concentrations of several elements in mouse chow are given in Table 5.25, those of potable water in Table 5.26, and of mice bedding in Table 5.27. Since both tumor-bearing and control mice lived in the same environment, the contribution of these materials towards the total elemental burden of mice is expected to be the same. Consequently, the elemental levels of the above materials are of no particular significance in this study. However, they can be used if attempts are made to compare the absolute elemental levels in tissues obtained in this work with those of other researchers.

TABLE 5.25 ELEMENT CONTENT OF MOUSE CHOW

(IN PPM)[a]

Element	average	σ	N
Al	296	17	3/3
As*	571	159	3/3
Br	11.0	0.46	3/3
Ca	12500	1190	3/3
Cl	6580	170	3/3
Co*	316	54	3/3
Cr	0.910	-	1/3
Cu	<30		
Fe	320	29	3/3
I	<2.2		
K	10600	840	3/3
Mg	1770	140	3/3
Mn	50.2	3.1	3/3
Mo	2.01	0.20	3/3
Na	3380	110	3/3
Rb	7.85	1.24	2/3
Sb*	<40		
Se	0.564	-	1/3
V	1.71	0.09	2/3
Zn	51.0	3.3	3/3

a- weighted;

*- in ppb.

TABLE 5.26 ELEMENT CONTENT OF DRINKING WATER FOR MICE

(in μg/litre)

Element	sample one	sample two	LD	LQ
Al	23.9	10.8	1.6	5.3
As*	151	59	46	154
Br	4.40	3.95	0.08	0.26
Ca	3600	1770	40	180
Cl	3130	2690	10	40
Co*	17.5	20.0	12	65
Cr*	476	469	90	340
Cu	2.75	12.6	4.0	14
Fe	5.57	17	8.3	39
I *	619	468	193	627
K	92.7	198	367	1200
Mg	131	65.6	19.6	64.6
Mn	4.66	2.31	0.10	0.31
Mo*	<180	<93		
Na	1170	720	20	60
Rb*	562	707	333	1626
Sb*	14.7	39.6	13	46
Se*	<41	<23		
V *	90.7	37	65	257
Zn	7.69	1.26	0.34	1.75

*- in ng/litre.

TABLE 5.27 ELEMENTAL CONTENT OF MICE BEDDING

(IN PPM)[a]

Element	average	σ	N
Al	24.9	4.57	2/3
As*	<70		
Br	<0.16		
Ca	688	212	2/3
Cl	<135		
Co*	<220		
Cr	<1.6		
Cu	<34		
Fe	<144		
I	<3.2		
K	284	18.0	2/3
Mg	721	35.6	2/3
Mn	162	8.6	3/3
Mo	<0.4		
Na	<105		
Rb	<6.6		
Sb*	<20		
Se	<0.61		
V	<0.4		
Zn	9.43	1.08	2/3

a- weighted;

*- in ppb.

TABLE 5.28 Cd AND Au CONTENTS OF SOME LIVER SAMPLES

	Sample	Cd content (ppb)	Au content (ppb)
E	# 14	n.d.	0.88
	# 15	72.5	<LD
	# 16	120	<LD
	# 22	133	<LD
	LD ; LQ	88 ; 353	0.38 ; 1.4
EN	# 1	162	<LD
	# 2	124	
	# 4	136	
	# 5	277	
	LD ; LQ	86 ; 330	0.57 ; 1.9
HL	# 10	30.4	<LD
	# 11	80.8	
	# 12	45.3	
	# 13	33.7	0. 71
	# 14	52.2	<LD
	# 15	33.9	
	LD ; LQ	30 ; 110	0.56 ; 1.8
HNL	# 1	86.3	<LD
	# 3	40.8	
	# 4	98.1	
	# 5	75.5	
	# 6	69.7	
	LD ; LQ	86 ; 325	0.46 ; 1.6

E- from EL4-bearing C57BL/6J mice; EN- from C57BL/6J mice;
HL- from H6-bearing A/J mice; HNL- from A/J mice.

6. CONCLUSIONS AND RECOMMENDATIONS

The primary objectives of this research project were essentially all achieved. Radiochemical and instrumental neutron activation analysis techniques were developed and used in a comprehensive study of the behaviour of some inorganic elements in tissues of mice with tumor, and control mice.

There are many existing INAA and RNAA schemes for the analysis of biological materials. Different methods may be used in these schemes, and each of the methods has its advantages and disadvantages in some aspects of an analysis. The combination of the methods used in a scheme depends on the elements of interest, and the availability of the apparatus and chemicals, etc.

Platinum complexes, copper complexes and possibly palladium complexes can be applied in chemotherapy to cancer patients. Analytical methods for these metals may be useful in studying the distribution of the metals in

tissues after such applications. On the other hand, metal ions which participate in the biological activities in the living things can be affected by the existence of neoplasia, as described in the literature survey. And it is very likely that those elements which take part in metalloenzyme systems will have a higher probability of being affected. Copper, magnesium, molybdenum and zinc are examples of such elements.

With the availability of on-site counting and in order to minimize the exposure to gamma radiation, an INAA scheme (t_i=30s, t_d=10s, t_c=60s; t_i=5 min, t_d=3 min, t_c=5 min; t_i=8 h, t_d=2 d, 14 d, t_c=1 h, 2 h) which had been optimized for all experimental factors was used to analyze the tissues of mice with tumor, and control. Twenty-three elements were possibly detected by the INAA scheme mentioned. Among those elements mentioned above, magnesium and zinc were determined with gamma peaks of reasonable signal to back - ground ratios, while there were difficulties in determining copper and molydenum and a few other elements because of the presence of the Compton background of ^{24}Na and ^{38}Cl from the activated matrix of the samples.

Several RNAA methods were developed in this work to determine those elements which cannot be analyzed by INAA. One of them may be used to analyze platinum metals. Since

the mice sacrificed were not treated with these metals, and these elements were usually present in very low quantities in tissues, much lower than the detection limits achieved in the method developed, they were not determined in this work. Instead, a RNAA scheme developed for analyzing a few elements of interest, such as Cu and Mo, in neoplastic tissues was used in the part of analyses in this work.

The development of such a method was based on a few considerations. Firstly since the half-lives of the nuclides, ^{64}Cu and ^{99}Mo, of copper and molybdenum are 12.8h and 67h respectively, rapid radiochemical separation after irradiation seemed to be a better approach for analyzing these two elements, than a time consuming RNAA scheme. And the nuclide, ^{64}Cu, is known as a positron emitter. The gamma-peak of 511-keV from the annihilation is about two hundred times more sensitive than the 1346-keV gamma emitted in the decay of ^{64}Cu. The former peak, 511-keV gamma, is usually not used because of the presence of other positron emitters and the existence of pair production by high energy gamma-rays. In RNAA, if copper is separated alone in one fraction the more sensitive gamma-peak of ^{64}Cu is then useful. Thus the detection limit of copper will be lowered at least two hundred times. Similarly, the gamma of ^{99}Mo emitted is low in gamma energy, 140-keV. Thus it is seriously interfered with by those active matrix nuclides. The application of

RNAA will improve the sensitivity of determination of this element if active nuclides of other elements are removed. A RNNA scheme was thus developed for this purpose.

After a long decay (40d), some of the samples analyzed by INAA were re-irradiated again for 8-16h. The tissue samples were then digested by oxidizing agents. Volatile elements were then distilled with hydrobromic acid. After that, copper and molybdenum were separated into two different fractions by use of an iodide-conditioned anion exchange column. As indicated by the results, the qualities of the data of those elements determined by RNAA are much better than those obtained by INAA, if it is possible by the latter.

After the distillation process, medium-lived nuclides ($t_{\frac{1}{2}} < 3d$) such as As, Hg and Sb were used in the determination of these elements. The sensitivity was improved. Selenium and zinc were also separated. The former was group-separated in the sulfide precipitate of the distillate and the latter was found in the fraction with molybdenum. Both of the elements could be determined by INAA using long-lived nuclides.

The reliability of the activation methods were tested by analyzing some standard reference materials (SRM) such as Bovine Liver (NBS SRM-1577), Gelatin (Kodak TEG-50-B),

Orchard Leaves (NBS SRM-1571), Pine Needles (NBS SRM-1575), Spinach (NBS SRM-1570), and Tomato Leaves (NBS SRM-1573). Both the precision and accuracy of measurements were found to be 10% or better.

If only the elemental contents are of interest, as indicated by the result, spleen was found to have much higher Mg and Zn contents than in other tissues in both strains of mice. It may reveal the active role of spleen in metabolism, as magnesium and zinc are essential metal ions, and bind to a lot of metalloenzymes which are important in metabolism.

That metal contents can be influenced by age is well known (KEL77). Between the two tumor models, samples obtained from A/J mice are better, since the age difference is negligible. On the other hand, the age difference between mice with tumor and control samples is not small in the model of EL4. Another factor which may affect the elemental level is sex. In this work only female mice were used. Thus this problem does not exist.

The number of mice used was 54 in model EL4 and 30 in model H6. Because the design of sampling was governed by the availability of C57BL/6J mice, the samples of this mice strain were collected in a way that was not too good. Thus in the giving data for statistic analysis, reliability

of inferences drawn from the results obtained from the former model is not as good as the latter. Eventhough the number of A/J mice used is less in model H6, than that of EL4 yet the samples of model H6 were from similar age groups, same sex, and in better arrangement. Thus the inferences drawn from the results are likely to reflect the behavior of the population.

As indicated by the results, copper content seems to be affected by the presence of tumor H6. Selenium, which was determined via the short-lived nuclide ^{77m}Se, was also found to have similar behavior. Thus it can be seen that with the cooperation of the INAA and RNAA methods developed, a few significant elements in these kind of tissues can be determined in all samples.

As the tumor tissues in this work were found to have lower copper and selenium contents which were believed to participate in certain metalloenzymes, the enzymes which are affected can be further specifically determined using the technique of protein separation such as ultrafiltration. Analysis in this approach have been attempted. Due to the existence of contamination from the ultrasonic homogenizer used, no significant work has been done. The problem of contamination from the homogenizer may be removed if some better designed devices such as a dismembrator

for trace element analysis (AME80) or a better made homogenizer is used. If the specific enzymes or proteins involved are determined, a better understanding of the challenge problem,cancer, may result .

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8. APPENDICES

THE REDUCTION OF Cu(II) TO Cu(I) BY I^-

$$2\left[Cu^{2+}\right] + 3\left[I^-\right] \rightleftharpoons 2\left[Cu^{+}\right] + \left[I_3^-\right] \quad ; \quad E_o = -0.382$$

$$E = E_o - \frac{0.059}{2}\log\frac{\left[Cu^{+}\right]^2\left[I_3^-\right]}{\left[Cu^{2+}\right]^2\left[I^-\right]^3} \quad \ldots.(1)$$

$$\left[Cu^{+}\right] + 2\left[I^-\right] \rightleftharpoons 2\left[CuI_2^-\right] \quad ; \quad K \doteq 10^9$$

$$\frac{\left[Cu^{+}\right]\left[I^-\right]^2}{\left[CuI_2^-\right]} = 10^{-9} \quad \ldots.(2)$$

$$\left[I^-\right] \doteq 10M \qquad \text{(RIE70)} \quad \ldots.(3)$$

$$\text{Assuming } \left[I_3^-\right] = 0.01\left[I^-\right] = 0.1M \quad \ldots.(4)$$

$$\left[Cu^{2+}\right] \leq 10^{-3}\left[CuI_2^-\right] \quad \ldots.(5)$$

Put (2), (3), (4), (5) into (1)

$$E = -0.38 - \frac{0.059}{2}\log(10^{-16} \cdot 10^{-4})$$

$$= 0.21$$

Therefore, the reduction of copper(II) to copper(I) is a spontaneous reaction.

Assumption (5) is obtained by tracer study and the instabilities of Cu(II) and (I) with I^- and Cl^-.

Alternatively, from (1) and (2)

$$E = -0.382 - \frac{0.059}{2} \log \frac{\left[CuI_2^-\right]^2 \left[I_3^-\right]}{\left[Cu^{2+}\right]^2 \left[I^-\right]^7 \; K^2}$$

Let $\left[Cu^{2+}\right] = 10^{-6}$ at start, an equilibrium may be established as

$$\underset{10^{-6}-2x}{2\,Cu^{2+}} + 7\,I^- \rightleftharpoons \underset{2x}{2\,CuI_2^-} + \underset{x}{I_3^-}$$

Since $K = 7.08 \times 10^8$

$$\frac{\left[CuI_2^-\right]^2 \left[I_3^-\right]}{\left[Cu^{2+}\right]^2 \left[I^-\right]^7} = 5.63 \times 10^4 \qquad \ldots.(6)$$

Putting (3) into (6)

$$\frac{(2x)^2 \, x}{(10^{-6}-2x)^2 \; 10^7} = 5.63 \times 10^4$$

$$4x^3 - 2.25 \times 10^{12} x^2 + 2.25 \times 10^6 x - 0.563 = 0 \qquad \ldots.(7)$$

By Newton-Raphson's Method, using $\geqslant 10^{-9}$ as an initial estimate of x, the solution is

$$x = 0.52 \times 10^{-6}; \text{ or } \quad 2x = 1.04 \times 10^{-6}$$

The result indicates that $\left[Cu^{2+}\right] \leq 0.01 \times 10^{-6}$ at equilibrium, within calculation errors. More than 99% of the element is in the complexed form, CuI_2^-.

CORRELATION TABLE OF EL4, A

	age	Al	Br	Ca	Cl	Cr	Cu	I
age	-[a] (-)[b]	-.51 (.08)	.67 (.03)	-.01 (.49)	.51 (.08)	.24 (.27)	-.19 (.33)	-.12 (.38)
Al		- (-)	-.13 (.37)	.34 (.19)	-.07 (.43)	.20 (.31)	.24 (.29)	.33 (.19)
Br			- (-)	.02 (.48)	.40 (.14)	.36 (.17)	-.34 (.21)	-.58 (.05)
Ca				- (-)	.73 (.01)	.34 (.18)	-.08 (.43)	.16 (.34)
Cl					- (-)	.65 (.03)	-.36 (.19)	.01 (.49)
Cr						- (-)	-.72 (.02)	-.19 (.31)
Cu							- (-)	.60 (.06)
I								- (-)
K								
Mg								
Mn								
Mo								
Na								
Sb								
Se								
Zn								

a - correlation coefficient
b - significance

CORRELATION TABLE OF EL4, A cont'd

	K	Mg	Mn	Mo	Na	Sb	Se	Zn
age	.75	.63	-.39	.08	.28	.60	.20	.10
	(.01)	(.04)	(.15)	(.44)	(.23)	(.11)	(.31)	(.40)
Al	-.18	-.03	.42	.37	-.02	-.17	.20	-.40
	(.32)	(.47)	(.13)	(.24)	(.48)	(.37)	(.30)	(.14)
Br	.65	.73	-.40	.27	.22	.25	.35	-.21
	(.03)	(.01)	(.15)	(.30)	(.29)	(.31)	(.18)	(.29)
Ca	.30	.27	-.54	.57	.83	.56	.44	-.37
	(.22)	(.24)	(.07)	(.12)	(.00)	(.11)	(.12)	(.16)
Cl	.80	.59	-.70	.79	.93	.87	.46	-.30
	(.01)	(.05)	(.02)	(.03)	(.00)	(.01)	(.11)	(.22)
Cr	.81	.68	-.25	.64	.42	.77	.69	-.68
	(.00)	(.02)	(.26)	(.09)	(.13)	(.04)	(.02)	(.02)
Cu	-.61	-.54	.30	-.11	-.13	-.45	-.65	.42
	(.06)	(.08)	(.24)	(.43)	(.38)	(.18)	(.04)	(.15)
I	-.20	-.50	.28	-.14	.13	.08	-.16	.27
	(.30)	(.09)	(.24)	(.40)	(.37)	(.44)	(.34)	(.24)
K	-	.81	-.50	.50	.53	.90	.63	-.39
	(-)	(.00)	(.09)	(.16)	(.07)	(.00)	(.03)	(.15)
Mg		-	-.27	.40	.29	.66	.43	-.49
		(-)	(.24)	(.22)	(.22)	(.08)	(.12)	(.09)
Mn			-	-.76	-.77	-.26	-.46	.25
			(-)	(.04)	(.01)	(.31)	(.11)	(.26)
Mo				-	.81	.44	.41	-.81
				(-)	(.03)	(.28)	(.21)	(.03)
Na					-	.63	.34	-.20
					(-)	(.09)	(.18)	(.30)
Sb						-	.39	-.06
						(-)	(.23)	(.46)
Se							-	-.67
							(-)	(.02)
Zn								-
								(-)

CORRELATION TABLE OF LIVERS OF C57BL/6J (EL4)

	Al	As	Br	Cl	Cu	Fe	K
Al	- [a]	-.01	-.20	.08	.52	.24	.30
	(-)[b]	(.49)	(.25)	(.39)	(.12)	(.20)	(.15)
As		-	.86	.86	-.27	.90	.80
		(-)	(.01)	(.01)	(.28)	(.00)	(.02)
Br			-	.12	-.44	.56	.23
			(-)	(.34)	(.16)	(.02)	(.22)
Cl				-	-.31	.55	.88
				(-)	(.25)	(.02)	(.00)
Cu					-	-.29	-.16
					(-)	(.26)	(.36)
Fe						-	.64
						(-)	(.01)
K							-
							(-)

Mg

a- correlation coefficient

Mn b- significance

Na

Rb

Se

Zn

CORRELATION TABLE OF LIVERS OF C57BL/6J (EL4) cont'd

	Mg	Mn	Na	Rb	Se	Zn
Al	.59	.09	.12	.12	.42	.51
	(.01)	(.38)	(.35)	(.34)	(.07)	(.03)
As	.52	.78	.84	.90	.73	.74
	(.11)	(.02)	(.01)	(.00)	(.03)	(.03)
Br	-.12	.34	.17	.41	.08	.00
	(.34)	(.12)	(.28)	(.08)	(.40)	(.50)
Cl	.68	.52	.97	.80	.83	.71
	(.00)	(.03)	(.00)	(.00)	(.00)	(.00)
Cu	-.11	.13	-.38	-.19	-.16	.24
	(.41)	(.39)	(,20)	(.34)	(.36)	(.31)
Fe	.43	.53	.58	.74	.57	.46
	(.06)	(.03)	(.02)	(.00)	(.02)	(.05)
K	.79	.51	.86	.85	.90	.86
	(.00)	(.03)	(.00)	(.00)	(.00)	(.00)
Mg	-	.39	.73	.63	.88	.80
	(-)	(.08)	(.00)	(.01)	(.00)	(.00)
Mn		-	.60	.60	.43	.27
		(-)	(.01)	(.01)	(.06)	(.18)
Na			-	.78	.81	.65
			(-)	(.00)	(.00)	(.01)
Rb				-	.85	.74
				(-)	(.00)	(.00)
Se					-	.85
					(-)	(.00)
Zn						-
						(-)

MULTIPLE REGRESSION OF ELEMENT CONTENTS OF LIVERS OF TUMORED C57BL/6J MICE

	Al	Br	Cl*	Fe	K*	Mg	Mn	Na*	Rb	Se	Zn
approx. av. value	13	19	3.4	660	7.8	580	2.1	2.6	17	2.5	86

$$Cl = -0.186 + 1.32\,[Na] + 0.0271\,[Zn] - 0.00348\,[Mg] - 0.009\,[Br]$$

R = 0.99 S = 0.00

$$K = -1.92 + 0.860\,[Se] + 0.619\,[Cl] + 0.057\,[Zn] + 0.027\,[Br]$$

R = 0.96 S = 0.00

$$Mg = 74 + 145\,[Se] + 7.6\,[Al] + 112\,[Na] - 75\,[Cl]$$

R = 0.94 S = 0.00

$$Na = 0.095 + 0.71\,[Cl] + 0.109\,[Mn] + 0.00214\,[Mg] - 0.0161\,[Zn]$$

R = 0.99 S = 0.00

$$Rb = -1.17 + 0.352\,[K] + 0.00462\,[Fe] + 7.1\,[Se] - 0.405\,[Al]$$

R = 0.94 S = 0.00

$$Se = 0.29 + 0.050\,[K] + 0.0023\,[Mg] + 0.041\,[Rb] - 0.076\,[Mn]$$

R = 0.96 S = 0.00

$$Zn = 34 + 4.5\,[K] + 1.03\,[Al] - 4.6\,[Mn] + 0.77\,[Rb]$$

R = 0.93 S = 0.00

* specified values are in parts per thousand; others in ppm.

CORRELATION TABLE OF H6 TUMOR

	Al	Br	Cl	Cu	K	Mg	Na	Rb	Se	Zn
Al	- [a]	-.03	-.68	.05	.22	.86	-.15	.45	-.31	.25
	(-)[b]	(.47)	(.02)	(.45)	(.29)	(.00)	(.35)	(.11)	(.21)	(.26)
Br		-	.21	-.18	-.56	.09	-.01	.13	.54	-.08
		(-)	(.29)	(.32)	(.06)	(.41)	(.49)	(.37)	(.07)	(.42)
Cl			-	.03	-.46	-.71	.95	-.42	.03	-.07
			(-)	(.47)	(.11)	(.02)	(.00)	(.13)	(.47)	(.43)
Cu				-	.06	.01	.02	-.09	-.25	.31
				(-)	(.44)	(.49)	(.48)	.41)	(.26)	(.21)
K					-	.05	-.46	-.17	-.14	-.10
					(-)	(.45)	(.11)	(.34)	(.36)	(.40)
Mg						-	-.72	.39	-.28	.15
						(-)	(.02)	(.15)	(.23)	(.35)
Na							-	-.46	-.15	-.06
							(-)	(.11)	(.35)	(.44)
Rb								-	.08	.73
								(-)	(.42)	(.01)
Se									-	-.42
									(-)	(.13)
Zn										-
										(-)

a- correlation coefficient

b- significance

MULTIPLE REGRESSION OF ELEMENT CONTENTS OF H6 TUMOR

	Al	Br	Cl*	Cu	K*	Mg	Na*	Rb	Se	Zn
approx. avg. value	25	23	0.85	5.7	1.6	820	0.67	24	1.9	75

$$Al = 11.3 + 0.0099\,[Mg] + 1.97\,[K] + 0.102\,[Rb]$$

$$R = 0.90 \qquad S = 0.03$$

$$Cl = -0.32 + 0.99\,[Na] + 0.0062\,[Br] + 0.174\,[K] + 0.0038\,[Rb]$$

$$R = 0.99 \qquad S = 0.00$$

$$Mg = 242 + 51.3\,[Al] - 449\,[Cl] - 203[K]$$

$$R = 0.91 \qquad S = 0.02$$

$$Na = 0.385 + 0.97\,[Cl] - 0.0063\,[Br] - 0.187[K] - 0.0044\,[Rb]$$

$$R = 0.99 \qquad S = 0.00$$

$$Rb = -21.9 + 0.286\,[Zn] + 12.7\,[Se] + 0.0090\,[Mg] - 1.36\,[Cu]$$

$$R = 0.96 \qquad S = 0.02$$

$$Zn = 79.2 + 3.17\,[Rb] - 42.9\,[Se] - 0.028\,[Mg] + 4.68\,[Cu]$$

$$R = 0.96 \qquad S = 0.02$$

* specified values are in parts per thousand; others in ppm.

CORRELATION TABLE OF LIVERS OF A/J MICE (H6)

	Al	As	Br	Cl	Cu	Fe	K
Al	-	.41	.57	.57	.61	.68	.38
	(-)	(.21)	(.04)	(.03)	(.07)	(.02)	(.14)
As		-	.71	.21	.62	.11	.62
		(-)	(.06)	(.35)	(.10)	(.42)	(.09)
Br			-	.59	.17	.33	.72
			(-)	(.04)	(.37)	(.18)	(.01)
Cl				-	-.16	.03	.49
				(-)	(.38)	(.47)	(.07)
Cu					-	.60	.21
					(-)	(.10)	(.35)
Fe						-	.21
						(-)	(.28)
K							-
							(-)
Mg							
Mn							
Na							
Rb							
Se							
Zn							

a - correlation coefficient

b - significance

CORRELATION TABLE OF LIVERS OF A/J MICE (H6) cont'd

	Mg	Mn	Na	Rb	Se	Zn
Al	.37	.40	.69	.34	-.13	.41
	(.15)	(.13)	(.01)	(.17)	(.36)	(.12)
As	.21	-.78	.48	-.56	.45	.66
	(.35)	(.03)	(.17)	(.13)	(.18)	(.08)
Br	.07	-.01	.59	.44	.28	-.05
	(.43)	(.49)	(.04)	(.10)	(.22)	(.45)
Cl	.60	.56	.75	.71	-.37	-.29
	(.03)	(.05)	(.01)	(.01)	(.15)	(.21)
Cu	.63	-.37	-.06	-.34	.44	.88
	(.09)	(.24)	(.45)	(.25)	(.19)	(.01)
Fe	-.09	.01	.11	.16	.10	.57
	(.40)	(.49)	(.38)	(.33)	(.39)	(.04)
K	.30	-.16	.52	.71	.50	-.24
	(.20)	(.33)	(.06)	(.01)	(.07)	(.26)
Mg	-	.51	.46	.52	-.04	-.04
	(-)	(.07)	(.09)	(.06)	(.46)	(.45)
Mn		-	.21	.28	-.64	-.28
		(-)	(.28)	(.22)	(.02)	(.22)
Na			-	.49	.00	.06
			(-)	(.07)	(.50)	(.44)
Rb				-	-.01	-.48
				(-)	(.49)	(.08)
Se					-	.19
					(-)	(.30)
Zn						-
						(-)

MULTIPLE REGRESSION OF ELEMENT CONTENTS OF LIVERS OF TUMORED A/J MICE

average value

Al	Br	Cl	Fe	K*	Mg	Mn	Na*	Rb	Se	Zn
15	7.7	2800	380	8.0	730	1.8	2.2	19	2.9	130

$$Al = -8.99 + 6.8\,[Na] + 0.0167\,[Fe] + 2.92\,[Mn] - 0{,}0132\,[Rb]$$

R = 0.97 S = 0.00

$$Cl = -33.8 + 948\,[Na] + 298\,[Mn] + 56.7\,[Rb] - 294\,[Se]$$

R = 0.93 S = 0.02

$$Fe = 504 + 51.8\,[Al] - 350\,[Na] - 153\,[Mn] + 7.4\,[Rb]$$

R = 0.93 S = 0.02

$$K = 1.06 + 0.295\,[Br] + 0.163\,[Rb] + 0.796\,[Se] - 0.369\,[Mn]$$

R = 0.93 S = 0.02

$$Mg = -72.2 + 0.203\,[Cl] - 58.2\,[Br] + 173\,[Se] + 99\,[Mn]$$

$$Na = 1.14 + 0.000180\,[Cl] + 0.101\,[Al] - 0.00156\,[Fe] - 0.361\,[Mn]$$

R = 0.92 S = 0.03

$$Se = 1.45 - 0.342\,[Mn] + 0.323[K] - 0.00070\,[Cl] + 0.00197\,[Mg]$$

R = 0.91 S = 0.04

$$Zn = 82.2 + 0.135\,[Fe] - 4.37\,[Rb] + 0.165\,[Mg] - 24.9\,[Mn]$$

R = 0.95 S = 0.01

* specified values are in parts per thousand; others in ppm.

CORRELATION TABLE OF LIVERS OF A/J MICE (NORMAL)

	Al	Br	Cl	Fe	K	Mg
Al	-[a]	-.29	.02	-.10	-.16	-.56
	(- [b])	(.21)	(.48)	(.39)	(.33)	(.05)
Br		-	.01	-.25	.20	-.15
		(-)	(.49)	(.25)	(.29)	(.34)
Cl			-	.04	.09	-.04
			(-)	(.45)	(.40)	(.46)
Fe				-	-.00	.23
				(-)	(.50)	(.27)
K					-	.15
					(-)	(.34)
Mg						-
						(-)
Mn						
Mo						
Na						
Rb						
Se						
Zn						

a- correlation coefficient
b- significance

CORRELATION TABLE OF LIVERS OF A/L MICE (NORMAL) cont'd

	Mn	Mo	Na	Rb	Se	Zn
Al	.03	.42	.16	-.08	.20	-.41
	(.47)	(.11)	(.33)	(.41)	(.22)	(.12)
Br	.31	.15	-.17	.46	.17	.58
	(.20)	(.34)	(.32)	(.09)	(.32)	(.04)
Cl	.35	-.17	.64	-.24	.21	-.10
	(.16)	(.32)	(.02)	(.25)	(.28)	(.39)
Fe	-.62	-.33	-.14	-.71	.14	.02
	(.03)	(.18)	(.35)	(.01)	(.35)	(.48)
K	.18	-.01	.70	.22	.38	.15
	(.30)	(.49)	(.01)	(.27)	(.14)	(.34)
Mg	.04	-.17	.03	.14	-.15	.32
	(.45)	(.32)	(.47)	(.35)	(.34)	(.19)
Mn	-	.33	.40	.57	.34	.47
	(-)	(.18)	(.13)	(.04)	(.17)	(.09)
Mo		-	-.04	.38	.65	.34
		(-)	(.45)	(.14)	(.02)	(.17)
Na			-	.02	.34	-.22
			(-)	(.48)	(.17)	(.27)
Rb				-	-.10	-.48
				(-)	(.39)	(.08)
Se					-	.38
					(-)	(.14)
Zn						-
						(-)

MULTIPLE REGRESSION OF ELEMENT CONTENTS OF LIVER OF NORMAL A/J MICE

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approx. avg. value

Al	Br	Cl*	Fe	K*	Mg	Mn	Mo	Na*	Rb	Se	Zn
17	9.1	3.6	460	11	720	4.4	3.6	2.8	24	5.5	110

$$Cl = 4.3 + 0.65\,[Na] - 0.32\,[K] + 0.066\,[Br] + 0.0092\,[Fe]$$
$$R = 0.96 \qquad S = 0.01$$

$$Fe = 907 - 20\,[Rb] + 4.9\,[Zn] - 150\,[Mn] + 58\,[Na]$$
$$R = 0.94 \qquad S = 0.01$$

$$K = 12 + 1.9\,[Na] - 2.7\,[Cl] + 0.20\,[Br] + 0.0027\,[Fe]$$
$$R = 0.97 \qquad S = 0.00$$

$$Mn = 4.4 - 0.0025\,[Fe] + 0.0024\,[Zn] + 0.74\,[Na] - 0.31\,[K]$$
$$R = 0.95 \qquad S = 0.01$$

$$Mo = -5.9 + 2.7\,[Se] + 0.27\,[Rb] - 0.69\,[K] - 0.92\,[Mn]$$
$$R = 0.95 \qquad S = 0.01$$

$$Na = -6.2 + 0.50\,[K] + 1.4\,[Cl] - 0.10\,[Br] - 0.0014\,[Fe]$$
$$R = 0.99 \qquad S = 0.00$$

$$Rb = 23 - 0.022\,[Fe] + 0.13\,[Zn] - 2.5\,[Se] + 1.1\,[K]$$
$$R = 0.92 \qquad S = 0.03$$

$$Se = 2.3 + 0.33\,[Mo] + 0.25\,[K] - 0.095\,[Rb] + 0.36\,[Mn]$$

*specified values are in parts per thousand; others in ppm.
