

## MULTIELEMENTAL DETERMINATION IN NORMAL, BENIGN, AND CANCEROUS TISSUES OF THE HUMAN BRAIN

M. M. RAJADHYAKSHA, Z. R. TUREL

*Nuclear Chemistry Division, The Institute of Science,  
15, Madam Cama Road, Bombay-400 032 (India)*

(Received June 3, 1991)

Although the metal content of the human body is only about 3%, metals are very important for human lives. Diseases occur when an excess or deficiency of in-vivo metals appear, when other metal pollutants enter the body, or when poisons or viruses enter into the metal ligand competition. Cancer is caused by carcinogens, which are substances capable of producing tumors in any test species at any dose level. This paper discusses the determination of some elements in diseased tissues of the human brain. As the elements present are mostly at micro- or nano-gram levels, the very sensitive technique of neutron activation analysis involving radiochemical separation has been employed. Substoichiometric estimations were carried out wherever possible. The radiochemical separation procedure includes a solvent extraction and precipitation technique. The elements estimated in the tissue samples are Cu, Au, As, Se, Hg, Co, Zn, Ca, Fe, P, Cr, Na, and K. The accuracy, precision, and radiochemical purity of the method have been discussed. Two samples and a standard can be analyzed in four days.

### INTRODUCTION

Over the last 100 years, and specifically in the past 15 years, an increasing number of elements have been found to be constantly present in the living tissues. Because of recent world-wide interest in the role of elements in the pathological metabolic process, multi-elemental determination assumes great importance.

The present work describes a method for the determination of Cu, As, Se, Fe, Zn, Co, Na, K, Ca, Cr, Hg, and P in normal, benign, and cancerous tissues of the brain for the purpose of determining the significant difference in the elemental concentration with respect to diseased and normal tissues. The technique employed was neutron activation analysis, involving radiochemical separation of these elements followed by  $\gamma$ -ray spectrometry.

## EXPERIMENTAL

### Sampling:

Tissues were collected at autopsy in pretreated polythene bags to minimize contamination. The tissue samples after collection were dried by lyophilization. The dried, lyophilized samples were crushed in an agate mortar and paste.

### Reagents and Chemicals:

All reagent bottles and standard flasks were pretreated to avoid pre-irradiation contamination of the trace elements. Carrier solutions for all thirteen elements were prepared by dissolving appropriate amounts of A.R. grade salts in double distilled water to give the desired concentration. Acid was added wherever necessary. The solution was standardized by the method given in Vogel (1) and Scott (2). Standard solutions for irradiation were prepared by diluting the appropriate aliquot of the stock solution to give the required concentration.

### Instrumentation:

All  $\gamma$ -emitters were counted on a  $\gamma$ -ray spectrometer in conjunction with 3.5 cm x 3.5 cm (NaI(Tl)) well-type detector.  $\beta$ -emitters were counted on an end-window type G.M. counter having a timer, a decade scaler, and a high voltage unit. A gamma-ray spectrometer was calibrated using standard  $^{137}\text{Cs}$ ,  $^{203}\text{Hg}$ ,  $^{54}\text{Mn}$ , and  $^{65}\text{Zn}$  isotopes.

### Irradiation:

The three ampoules containing normal, benign, or cancerous tissues of the brain and standard ( $\mu\text{g}$  quantities) of the elements, were irradiated in the CIRUS reactor of the Bhabha Atomic Research Center, Trombay, at the self-serve position near the reflector with a thermal neutron flux of  $5 \times 10^{12} - 1 \times 10^{13} \text{ n cm}^{-2} \text{ sec}^{-1}$  for a week.

### Radiochemical Separation Procedure:

The radiochemical separation procedure for purifying the isotopes of the element is as given in Tables 1A, 1B, 1C, 1D, and 1E.

TABLE 1A

Radiochemical Separation Procedure

7 mg each of Cu(II), Co(II), Zn(II), Hg(II), Fe(III), and 10 mg each of As(III), Ca(II), Cr(III), P(V), Na(I), and K(I) + irradiated sample + aquaregia - boiled for 10 minutes - nitrate expelled with HCl + SO<sub>2</sub> water - scratched - centrifuged.

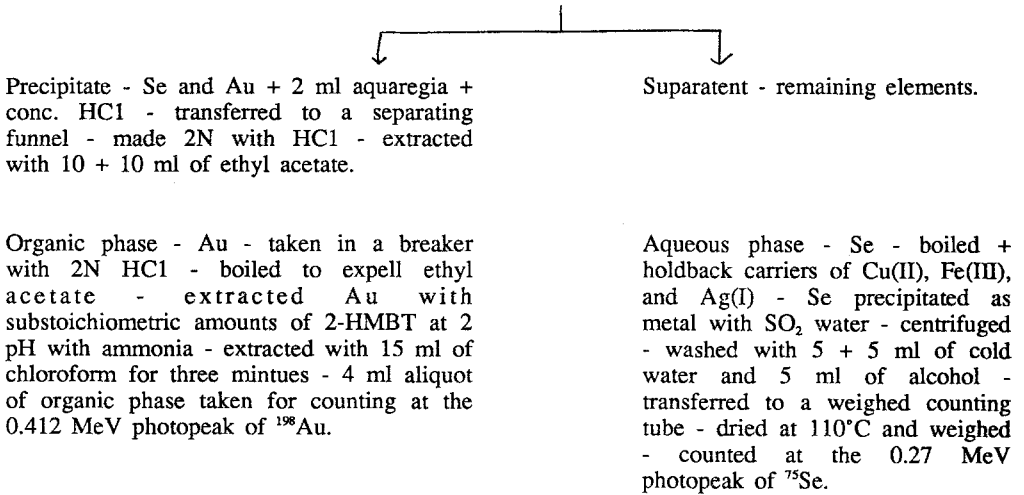


TABLE 1B

Supernatant containing Cu, As, Fe, Hg, Co, Zn, Ca, Cr, Na, and K - boiled - made 9N with respect to HCl - H<sub>2</sub>S - diluted - precipitate of CuS, HgS, and As<sub>2</sub>S<sub>3</sub> - centrifuged - washed with 5 + 5 ml of cold water.

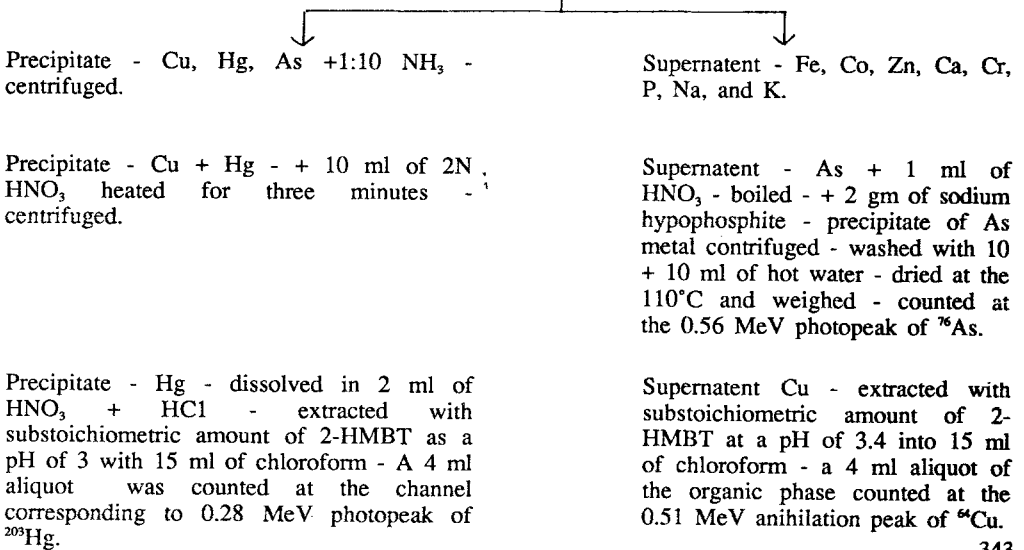


TABLE 1C

Supernatant + 1 ml of conc  $\text{HNO}_3$  - boiled - made 6.5N with  $\text{HCl}$  - extracted with 10 + 10 ml of ether saturated with 6.5N  $\text{HCl}$ .

Organic Phase - Fe and P  
- boiled + 1 g  $\text{NH}_4\text{Cl}$  - centrifuged.  
5 ml of 2N  $\text{HCl}$   
2 ml of  $\text{NH}_3$  -

Supernatant - Co, Zn, Ca, Cr, Na, and K.

Precipitate - P - washed with 10 + 10 ml of hot water - transferred to a weighed planchet - dried at  $110^\circ\text{C}$  and weighed - counted on an end window  $\text{Gm}$  counter.

Supernatant - Fe - concentrated to 1 ml - made 1N with  $\text{HCl}$  - extracted substoichiometrically with BPHA in 15 ml of chloroform - a 4 ml aliquot of organic phase was taken for counting at the 1.29 photopeak of  $^{59}\text{Fe}$ .

TABLE 1D

The supernatant was evaporated to dryness + 9N  $\text{HCl}$  - extracted with 10% TNOA in MIBK.

Organic phase - Co and Zn - equilibrated with 10 + 10 ml of 0.8N  $\text{HNO}_3$  for five minutes to backextracted Co and Zn + 2N  $\text{NaOH}$  boiled - centrifuged.

Aqueous phase Ca, Cr, Na, and K.

Precipitate - Co - washed with 5 + 5 ml of hot water + 2 ml of 6N  $\text{HCl}$  - extracted with substoichiometric amounts of 2-HMBT at a pH of 8.5 into chloroform - 4 ml aliquot of the organic phase was counted at the 1.33 MeV photopeak of  $^{60}\text{Co}$ .

Supernatant Zn - extracted with substoichiometric amount of 2-HMBT at a pH of 9.5 into chloroform - 4 ml aliquot of the organic phase was counted at 1.11 MeV photopeak of  $^{65}\text{Zn}$ .

TABLE 1E

The supernatant was boiled for five minutes + NH<sub>3</sub> - precipitate centrifuged.

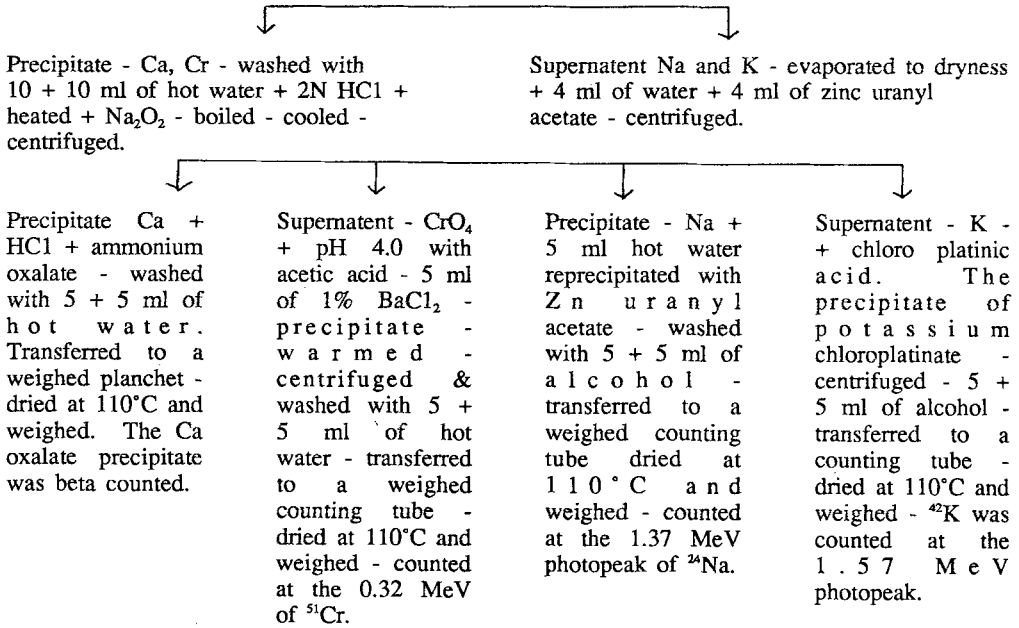


TABLE 2

Determination of Accuracy of the Method Employed for the Estimation of the Elements in Tissue Samples by Neutron Activation Analysis

Thermal neutron flux =  $5 \times 10^{12} - 1 \times 10^{13} \text{ n cm}^{-2} \text{ sec}^{-1}$   
 Duration of irradiation = 7 - 10 days.

Elements	μg	
	Taken	Found
Cu	8.32	8.56 ± 0.2
Se	0.92	0.95 ± 0.02
Fe	170.5	176.1 ± 4.4
P	38.3	37.6 ± 2.4
Zn	19.3	19.3 ± 0.5
Mo	0.76	0.78 ± 0.04
Co	1.74	1.84 ± 0.1
Ca	18.3	19.0 ± 2.0
Na	101	105.0 ± 5.0
K	105	111.0 ± 8.0

TABLE 3  
 Determination of the Elemental Concentration (Cu, Se, Fe, Zn, Co, Hg, Cr, Ca, P, Na, Ag, K, and As) in Normal, Benign, and Cancerous Tissue of the Human Brain

Thermal Neutron Flux =  $5 \times 10^{13} - 1 \times 10^{12}$  n cm<sup>-2</sup> sec<sup>-1</sup>  
 Duration of Irradiation = 8 days.

Patient Name	Tissue Type	Cu µg/g	Se µg/g	Fe µg/g	Zn µg/g	Co ng/g	Hg ng/g	Cr ng/g	Ca µg/g	P %	Na %	K %	Au ng/g	As ng/g
Suresh Waghmare	N M	22. 18.1	.107 .52	63. 26.2	13.2 2.4	9. 6.4	17.1 38.7	120 247	320 120	.26 .12	1.49 .7	.25 .228	.42 1.06	10 3.4
Age: 5 Sex: M Blood Type: B+ve Tumor Type: Cerebellar midline medulla blastoma														
Suresh Chavan	N Be	23. 20.	.18 1.22	59.1 17.5	16.2 5.9	8.2 2.4	14.1 13.4	120 182	314 109	.22 .176	.92 .71	.39 .078	.24 1.3	30. 49.
Age: 29 Sex: M Blood Type: B+ve Tumor Type: Suprascalar sub-frontal craniopharyngioma														

## RESULTS AND DISCUSSION

Copper, As, Au, Se, Fe, P, Co, Zn, Hg, Ca, Cr, Na, and K have been estimated by the  $(n,\gamma)$  reaction on their stable isotopes. The radiochemical purity of the separated isotopes were determined by measuring the  $\gamma$ -energy,  $\beta$ -energy, and half-life of the isotopes wherever possible. The values are in agreement with those reported in literature. The chemical recovery varied between 50% and 80%.

The ages of the patients whose tissues were analyzed ranged between 3 and 72 years. Different types of tumors from different locations of the brain have been analyzed. The accuracy and precision of the method were determined and are given in Table 2. A representative analysis of the samples is given in Table 3. Overall, 20 samples have been analyzed.

## CONCLUSIONS

The blood groups of the patients, their case histories, premedications, etc. have been recorded for drawing pre-cause conclusions in the future after more samples have been analyzed.

\*

## References

1. A. I. VOGEL, Textbook of Quantitative Inorganic Analysis, Longmans Green and Co. Ltd., London, 1975.
2. W. W. SCOTT, Standard Methods of Chemical Analysis, I. D. Van Nostrand and Co., New York, 1939.