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

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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. Substocihiometric 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 γ-ray spectrometry.

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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 γ-emitters were counted on a r-ray spectrometer in conjunction with 3.5 cm x 3.5 cm (NaI(Tl) well-type detector. β-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 calculated using standard ^{137}Cs, ^{203}Hg, ^{54}Mn, and ^{65}Zn isotopes.

Irradiation:

The three ampoules containing normal, benign, or cancerous tissues of the brain and standard (μg 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 $5 \times 10^{12} - 1 \times 10^{13}$ n cm^{-2} $sec.^{-1}$ 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_2 water - scratched - centrifuged.

Precipitate - Se and Au + 2 ml aquaregia + conc. HCl - transferred to a separating funnel - made 2N with HCl - extracted with 10 + 10 ml of ethyl acetate.

Suparatent - remaining elements.

Organic phase - Au - taken in a breaker with 2N HCl - boiled to expell ethyl acetate - extracted Au with substoichiometric amounts of 2-HMBT at 2 pH with ammonia - extracted with 15 ml of chloroform for three mintues - 4 ml aliquot of organic phase taken for counting at the 0.412 MeV photopeak of ^{198}Au.

Aqueous phase - Se - boiled + holdback carriers of Cu(II), Fe(III), and Ag(I) - Se precipitated as metal with SO_2 water - centrifuged - washed with 5 + 5 ml of cold water and 5 ml of alcohol - transferred to a weighed counting tube - dried at 110°C and weighed - counted at the 0.27 MeV photopeak of ^{75}Se.

TABLE 1B

Supernatent containing Cu, As, Fe, Hg, Co, Zn, Ca, Cr, Na, and K - boiled - made 9N with respect to HCl - H_2S - diluted - precipitate of CuS, HgS, and As_2S_3 - centrifuged - washed with 5 + 5 ml of cold water.

Precipitate - Cu, Hg, As +1:10 NH_3 - centrifuged.

Supernatent - Fe, Co, Zn, Ca, Cr, P, Na, and K.

Precipitate - Cu + Hg - + 10 ml of 2N HNO_3 heated for three minutes - centrifuged.

Supernatent - As + 1 ml of HNO_3 - boiled - + 2 gm of sodium hypophosphite - precipitate of As metal contrifuged - washed with 10 + 10 ml of hot water - dried at the 110°C and weighed - counted at the 0.56 MeV photopeak of ^{76}As.

Precipitate - Hg - dissolved in 2 ml of HNO_3 + HCl - extracted with substoichiometric amount of 2-HMBT as a pH of 3 with 15 ml of chloroform - A 4 ml aliquot was counted at the channel corresponding to 0.28 MeV photopeak of ^{203}Hg.

Supernatent Cu - extracted with substoichiometric amount of 2-HMBT at a pH of 3.4 into 15 ml of chloroform - a 4 ml aliquot of the organic phase counted at the 0.51 MeV anihilation peak of ^{64}Cu.

TABLE 1C

Supernatent + 1 ml of conc HNO_3 - boiled - made 6.5N with HCl - extracted with 10 +.10 ml of ether saturated with 6.5N HCl.

Organic Phase - Fe and P 5 ml of 2N HCl - boiled + 1 g NH_4Cl 2 ml of NH_3 - centrifuged.	Supernatent - 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°C and weighed - counted on an end window Gm counter.	Supernatent - Fe - concentrated to 1 ml - made 1N with 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}Fe.

TABLE 1D

The supernatent was evaporated to dryness + 9N HCl - extracted with 10% TNOA in MIBK.

Organic phase - Co and Zn - equilibrated with 10 + 10 ml of 0.8N HNO_3 for five minutes to backextracted Co and Zn + 2N NaOH boiled - centrifuged.	Aqueous phase Ca, Cr, Na, and K.
Precipitate - Co - washed with 5 + 5 ml of hot water + 2 ml of 6N 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}Co.	Supernatent 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}Zn.

TABLE 1E

The supernatent was boiled for five minutes + NH_3 - precipitate centrifuged.

Precipitate - Ca, Cr - washed with 10 + 10 ml of hot water + 2N HCl + heated + Na_2O_2 - boiled - cooled - centrifuged.

Supernatent Na and K - evaporated to dryness + 4 ml of water + 4 ml of zinc uranyl acetate - centrifuged.

Precipitate Ca + HCl + ammonium oxalate - washed with 5 + 5 ml of hot water. Transferred to a weighed planchet - dried at 110°C and weighed. The Ca oxalate precipitate was beta counted.

Supernatent - CrO_4 + pH 4.0 with acetic acid - 5 ml of 1% $BaCl_2$ - precipitate - warmed - centrifuged & washed with 5 + 5 ml of hot water - transferred to a weighed counting tube - dried at 110°C and weighed - counted at the 0.32 MeV of ^{51}Cr.

Precipitate - Na + 5 ml hot water reprecipitated with Zn uranyl acetate - washed with 5 + 5 ml of alcohol - transferred to a weighed counting tube dried at 110°C and weighed - counted at the 1.37 MeV photopeak of ^{24}Na.

Supernatent - K - + chloro platinic acid. The precipitate of potassium chloroplatinate - centrifuged - 5 + 5 ml of alcohol - transferred to a counting tube - dried at 110°C and weighed - ^{42}K was counted at the 1.57 MeV photopeak.

TABLE 2

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

Thermal neutron flux = $5 \times 10^{12} - 1 \times 10^{13}$ n cm $^{-2}$ 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^{-2} sec^{-1}
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	N	22.	.107	63.	13.2	9.	17.1	120	320	.26	1.49	.25	.42	10
Waghmare	M	18.1	.52	26.2	2.4	6.4	38.7	247	120	.12	.7	.228	1.06	3.4
Age: 5 Sex: M Blood Type: B+ve Tumor Type: Cerebellear midline medulla blastoma														
Suresh	N	23.	.18	59.1	16.2	8.2	14.1	120	314	.22	.92	.39	.24	30.
Chavan	Be	20.	1.22	17.5	5.9	2.4	13.4	182	109	.176	.71	.078	1.3	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,γ) reaction on their stable isotopes. The radiochemical purity of the separated isotopes were determined by measuring the γ-energy, β-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.

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