An Application of Neutron Activation Analysis to Biological Materials. III. Natural Abundance of Small and Micro Amounts of Elements and Their Interactions in Human Tissues

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The natural abundance of the principal inorganic elements in five human tissues (brain, kidney, lung, liver, and heart) have been determined by neutron activation analysis. Cl, K, and Mn are equally distributed in all tissues. The highest concentration of Sc(0.22 ± 0.044 ppm) is found in the liver. V(0.64 ± 0.51 ppm) and Cr (0.19 ± 0.11 ppm) concentrations in the lung are significantly higher than in other tissues at a 95% confidence level. The lowest concentration of Br(2.7 ± 1.8 ppm) is found in the brain. The highest concentration of Cd (74 ± 11 ppm) appears in the kidney and differs significantly from that in other tissues. Significant correlations appear to exist between many pairs of elements in the kidney and the lung, whereas there are relatively few in the liver. The normal significant correlations between Na, Cl, K, Sc, Zn, Se, Cd, and other elements are given.

There are increasing reports concerning the physiological activity of small or micro amounts of metals contained in the cells or tissues of several organs. For example, micro amounts of Cu in the human body play an important role in hematopoitic activity.[1] Wilson's disease (hepato-lenticular degeneration) is at times characterized by an accumulation of Cu in the liver and brain tissues.[2] Schroeder[3] reported that Cd is related to the hypertension found in man. Hadjimarkos[4,5] suggested that trace amounts of Se appear capable of increasing the susceptibility to dental caries. Se is also reported to offer protection against human cancer.[6,7] Dick[8] has reported the physiological interactions between Cu, Mn, Mo, and sulfate. And Gather *et al.*[9] reported the role of Se in preventing the hazardous action of organic mercury. Sufficient information, however, concerning the effect of trace amounts of heavy metals in human tissues on physiological activities remains scant. Physiological functions in the human body are influenced by the heavy metals and chemical compounds are required further investigation. In this study, an attempt has been made to determine simultaneously the concentration of 15 elements in specified pathological conditions. The organs studied have been the brain, kidneys, lung, liver, and heart. The abundances and interactions of the elements in these tissues have been determined by neutron activation analysis.

Experimental

Collection and Preparation of the Samples. The human tissues analyzed were obtained from 9 autopsy cases examined in the Pathological Institute, Juntendo University, School of Medicine, Tokyo. Tissue samples were taken from minimally affected areas in the above mentioned organs, taking special care to avoid local neoplastic invention. All samples were dried in the desiccator (P_2O_5) for 7 days. The dried tissues (50 mg) were weighed and heat-sealed in clean polyethylene bags. The distribution of age, sex, and the causes of death are shown in Table 1.

Preparation of the Reference Samples. High purity metals, Mg, Cr, Mn, Fe, Co, Cu, Zn, and Cd, and special grade NaCl, KBr, Sc_2O_3, SeO_2, and V_2O_5 were used as standard material. A definite amount of each metal or compound was dissolved in nitric acid or pure water. A reference standard was prepared by pipetting an appropriate volume of the stock solution, impregnating it in a small sheet of filter paper and drying at room temperature. The amounts of Na, Mg, K, Cr, Mn, Fe, Cu, Zn, Se, and Cd in the reference standard were 1 μg, those of Co, Sc, and V were 0.1 μg and those of Cl and Br were 1.54 and 2.04 μg. The sheets of filter paper

TABLE 1. DISTRIBUTION OF AGE, SEX AND THE CAUSES OF DEATH FOR THE SUBJECTS

Age (years)	Sex	The causes of death
7	f	Pyothorax
40	f	Cerebello-pontine-angle tumor
61	f	Olivo-ponto-celebellar atrophy
34	m	3rd degree burn
51	m	Liver cirrhosis
61	m	Liver cirrhosis
62	m	Myocard infarction
63	m	Myocard infarction
70	m	Hodgkin's disease

a) f: female, m: male.

TABLE 2. THE NUCLEAR DATA OF THE NUCLIDES USED IN THIS STUDY

Stable isotope	Natural abundance (%)	Nuclear reaction	Radio isotope	Half life	γ-Ray energy (keV)
^{23}Na	100	n, γ	^{24}Na	15.1 h	1367
^{26}Mg	11.7	n, γ	^{27}Mg	9.45 m	1014
^{37}Cl	24.47	n, γ	^{38}Cl	37.3 m	1642
^{41}K	6.68	n, γ	^{42}K	12.4 h	1525
^{45}Sc	100	n, γ	^{46}Sc	83.4 d	1120
^{51}V	99.75	n, γ	^{52}V	3.57 m	1434
^{50}Cr	4.31	n, γ	^{51}Cr	27.8 d	320
^{55}Mn	100	n, γ	^{56}Mn	2.57 h	1811
^{58}Fe	0.33	n, γ	^{59}Fe	45 d	1099
^{59}Co	100	n, γ	^{60}Co	5.26 y	1173
^{65}Cu	30.91	n, γ	^{66}Cu	5.1 m	1039
^{64}Zn	48.9	n, γ	^{65}Zn	245 d	1115
^{74}Se	0.87	n, γ	^{75}Se	120 d	265
^{79}Br	50.5	n, γ	^{80}Br	17.6 m	617
^{114}Cd	100	n, γ	^{115}Cd	2.69 d	412

TABLE 3. THE MEAN VALUES AND THE STANDARD DEVIATIONS OF THE ELEMENTS IN THE HUMAN TISSUES (ppm)

Elements	Brain M.V.±S.D.	Kidney M.V.±S.D.	Lung M.V.±S.D.	Liver M.V.±S.D.	Heart M.V.±S.D.
Na	1900±720	1000±710	2200±1800	970±670	1400±780
Mg	810±310	510±460	520±290	500±350	400±170
Cl	1000±550	990±570	1700±1200	870±480	1100±540
K	130±43	69±48	140±110	83±36	110±60
Sc	0.0069±0.0029	0.0065±0.0032	0.054±0.040	0.22±0.044	0.0087±0.0040
V	0.11±0.063	0.13±0.11	0.64±0.51	0.17±0.13	0.12±0.087
Cr	0.044±0.023	0.041±0.020	0.19±0.11	0.041±0.017	0.038±0.018
Mn	2.6±1.0	2.9±1.6	3.1±1.7	3.9±2.0	2.3±0.69
Fe	12±7.7	23±12	25±8.1	37±28	13±8.7
Co	0.0088±0.0049	0.034±0.039	0.018±0.0085	0.021±0.013	0.013±0.0076
Cu	37±11	14±10	9.4±5.5	22±14	16±7.2
Zn	87±23	140±45	100±34	140±63	99±23
Se	1.5±0.50	3.7±1.5	0.80±0.29	2.0±0.35	1.6±0.59
Br	2.7±1.8	35±26	44±40	7.7±3.6	19±17
Cd	4.1±3.0	74±11	6.2±3.8	12±8.9	4.7±2.0

a) M.V.: mean value, S.D.: standard deviation.

were then heat-sealed in clean polyethylene bags.

Neutron Activation and Determination of γ-Ray Spectra. Samples and reference standards were irradiated in a rotary specimen rack in a TRIGA MARK II reactor (Rikkyo University) with a thermal neutron flux of 5×10^{11} $n\cdot cm^{-2}\cdot s^{-1}$. The irradiation period was 3 min for short-lived nuclides, and 24 h(intermittent irradiation of 6 h a day for 4 days) for long-lived nuclides. Irradiated samples and reference sample were removed from the polyethylene bag and transferred to new one before measuring the γ-ray spectra.

The γ-ray spectra were determined on a Ge(Li) γ-ray detector and 4096 channel pulse height analyzer (Type 8100: Camberra Inductries Inc. USA).

The nuclear data of the nuclides used in this study, are shown in Table 2.

Results and Discussion

The data have been analyzed by evaluating the mean values and the standard deviations for 15 elements in each tissue, after rejection of the widely different values according to the method of Smirnoff, the results of which are shown in Table 3. The results of the t-test for the elemnts for every two tissues are shown in Tables 4(1)—(8). In the Tables, the symbol "*" illustrates the significant difference in mean values between two tissues at a 95% confidence level. The coefficient of correlation for every two elements in each tissue are shown in Tables 5(1)—(3). In Table 5, the symbols

TABEL 4. THE SIGNIFICANT DIFFERENCE IN THE ABUNDANCE OF THE ELEMENTS IN HUMAN TISSUE AT 95% CONFIDENCE LEVEL

(1) Na and Mg

Na

		Bn	Ky	Lg	Lr	Ht
	Bn		**	--	**	--
	Ky	--		--	--	--
Mg	Lg	--	--		--	--
	Lr	--	--	--		--
	Ht	**	--	--	--	

(2) Cl and K

Cl

		Bn	Ky	Lg	Lr	Ht
	Bn		--	--	--	--
	Ky	--		--	--	--
K	Lg	--	--		--	--
	Lr	--	--	--		--
	Ht	--	--	--	--	

(3) Sc and V

Sc

		Bn	Ky	Lg	Lr	Ht
	Bn		--	**	**	--
	Ky	--		**	**	--
V	Lg	**	**		**	**
	Lr	--	--	**		**
	Ht	--	--	**	--	

(4) Cr and Mn

Cr

		Bn	Ky	Lg	Lr	Ht
	Bn		--	**	--	--
	Ky	--		**	--	--
Mn	Lg	--	--		**	**
	Lr	--	--	--		--
	Ht	--	--	--	--	

(5) Fe and Co

Fe

		Bn	Ky	Lg	Lr	Ht
	Bn		**	--	--	--
	Ky	--		--	--	--
Co	Lg	**	**		**	**
	Lr	--	--	**		**
	Ht	--	--	**	--	

(6) Cu and Zn

Cu

		Bn	Ky	Lg	Lr	Ht
	Bn		--	--	--	--
	Ky	**		--	--	--
Zn	Lg	--	--		--	--
	Lr	--	--	--		--
	Ht	--	--	--	--	

(7) Se and Br

Se

		Bn	Ky	Lg	Lr	Ht
	Bn		**	**	--	--
	Ky	**		**	**	**
Br	Lg	**	--		--	--
	Lr	**	--	--		--
	Ht	**	--	--	--	

(8) Cd

Cd

	Bn	Ky	Lg	Lr	Ht
Bn		**	--	--	--
Ky			**	**	**
Lg				--	--
Lr					--
Ht					

**: Significant difference at a 95% confidence level.
--: No difference.
Bn: Brain, Ky: Kidney, Lg: Lung, Lr: Liver, Ht: Heart.

"**" and "*" show the significant correlation at 95% and 99% confidence levels respectively. The symbols "-**" and "-*" show the reverse correlations at the same levels. The symbols "+" and "-" show the tendency of normal and reverse interactions.

The Abundances of the Elements in Human Tissue (Table 3). *Sodium*: The highest concentration of Na is found in the lung, but no significant difference exists in the average value of Na for any pair of tissues. Na and Cl are the main electrolytes in blood serum and therefore, it appears that the high content of Na and Cl in the lung are influenced by the blood volume existing at the time of death. The standard deviation of Na level is small in the brain and the mean value differs significantly from those in the kidney and liver at a 95% confidence level (Table 4 (1)).

Magnesium: The concentration of Mg in the brain is relatively higher than that in other tissues and is significantly different from the smallest level found in the heart at a 95% confidence level. The mean values of Mg were found to be small for all tissues except the brain (Table 4 (1)).

Chlorine: The highest concentration of Cl as well as Na is in the lung. No significant differences were found

TABLE 5-1. THE CORRELATIONS OF THE ELEMENTS IN THE HUMAN TISSUES

Brain

Kidney	Na	Mg	Cl	K	Sc	V	Cr	Mn	Fe	Co	Cu	Zn	Se	Br	Cd
Na		-**	*	+	*	*	-**	+	+	**	-	+	*	+	**
Mg	-		-*	-*	-	-	**	-	-	+	-	+	-	-	-**
Cl	*	+		+	*	+	-*	-	+	+	-	-	**	+	*
K	*	*	*		*	+	-	-	-	-	+	-*	+	-	*
Sc	*	+	+	+		*	-**	-*	+	+	-	-	*	+	*
V	-*	**	-**	-**	-**		-*	-**	-	**	-	-	-	-	**
Cr	+	-	+	+	+	+		+	+	-	**	+	-	-	-*
Mn	**	+	**	*	+	-	-		-**	-	+	-	-	+	-*
Fe	+	*	+	*	-	-	-	*		*	-	*	*	*	+
Co	**	*	+	*	+	**	-	*	*		-	*	*	*	+
Cu	+	+	-	+	-	-	+	+	**	+		-	-	-	-
Zn	**	+	**	**	*	-	**	**	**	+	*		+	*	-
Se	*	**	*	*	+	-	+	*	*	*	**	*		*	+
Br	+	+	**	+	**	+	*	+	-	-	-	+	+		+
Cd	*	+	*	*	+	-	*	+	+	+	+	*	**	*	

*, ** : Significant correlation at a 99% and 95% levels

-*, -** : Reverse significant correlation at a 99% and 95% levels

TABLE 5-2. THE CORRELATIONS OF THE ELEMENTS IN THE HUMAN TISSUES.

Liver

Lung	Na	Mg	Cl	K	Sc	V	Cr	Mn	Fe	Co	Cu	Zn	Se	Br	Cd
Na		+	*	*	-	-	-	-	-	+	*	-	-	+	-
Mg	+		-	+	+	-	-	-	-	**	-	-	-	-	-
Cl	*	+		*	-	-*	+	+	-	+	*	-	+	+	+
K	*	*	*		-	-**	+	-	+	-	*	-	+	-	+
Sc	*	*	*	*		-	-	+	+	+	-	*	+	-	+
V	+	*	+	+	**		-	-	-	-	-	-	-	-	*
Cr	**	*	+	*	*	+		+	*	-**	+	-	+	-	-
Mn	+	-	+	-	+	-	-		+	-	-	+	*	-	*
Fe	**	**	**	*	+	+	+	+		-	-	-	+	-	-
Co	+	+	+	**	+	+	*	-	+		-	**	+	+	+
Cu	+	+	+	+	*	-	*	*	**	+		-	-	-	-
Zn	*	*	*	*	*	**	*	+	+	+	*		**	-	*
Se	8	*	**	*	*	+	-*	+	+	+	*	**		-	*
Br	**	*	*	+	*	*	+	-	-	-	-	*	+		-
Cd	+	*	+	+	*	**	+	*	+	-	*	**	*	**	

Table 5-3. The correlatlon of the elents in the human tissues

Heart

	Na	Mg	Cl	K	Sc	V	Cr	Mn	Fe	Co	Cu	Zn	Se	Br	Cd
Na		-	*	*	-*	+	-*	*	+	-	+	+	+	+	+
Mg			-	+	*	+	+	+	-	-	+	-*	+	-	-*
Cl				*	-	+	-*	**	+	-	+	**	+	+	+
K					-	**	-*	**	+	-	+	-	*	-	-
Sc						+	+	-	-	-	-	-*	-	-	-*
V							*	+	-	+	-	+	**	-	-**
Cr								-*	-	*	-*	+	-*	+	-
Mn									+	+	+	+	**	-	-
Fe										-	*	-	+	-**	+
Co											-	**	-	+	+
Cu												-	+	-	+
Zn													-	+	*
Se														-**	-
Br															+
Cd															

between the pairs of tissues analyzed (Table 4 (2)).

Potassium: The distribution of K is similar to that for Na and Cl (Table 4 (2)).

Scandium: Little data on the physiological activities and/or toxicities of Sc in human body exists.[10] Sc is widely distributed in all the organs but the highest concentration of Sc is observed in the liver. The concentrations of Sc decrease in the following order: lung, heart, brain and kidney (Table 4 (3)).

Vanadium: The highest mean value of V is found in the lung, corroborating the data of Tipton and Cook.[11] The average value of V in the lung significantly differs from that found in the other organs at a 95% confidence level, but in the other organs there are no significant differences in the V contents. Schroeder[12] found trace amounts of V in the lungs of sucklings and higher V levels in the adult lungs. In the lung of a 7 year old girl the V concentration was found to be 0.0083 ppm, although the average level in adult lungs is 0.72 ± 0.49 ppm. This suggests that the large amounts of V in the adult lung results from an accumulation of inhaled fly ash (Table 4 (3)).

Chromium: The abundances of Cr are similar to V. As Cr is one of the essential elements in living cells, it is widely distributed in all organs. The higher abundance in the lung may be attributed to the accumulation of fly ash deposition in the pulmonary tissues through inhalation. The Cr concentration gradually enriches in the lung due to ageing[13] (Table 4 (4)).

Manganese: Manganese is widely distributed in all the tissues. No significant differences however were found in any two tissues and the results are slightly higher than that reported by Sumino *et al.*[14] and Tipton *et al.*[11] The weight loss of the tissues after drying for 7 days is *ca.* 80% for the wet weight, and consequently the figures cannot be compared without a calibration (Table 4 (4)).

Iron: The mean value of Fe is slightly lower in the brain and the heart than in other tissues (Table 4 (5)).

Cobalt: The highest value of Co is found in the kidney and the lowest in the brain. The average content of Co is widely distributed in each tissue (Table 4 (5)).

Copper: The highest content of Cu is present in the brain and significant differences exist between some pairs of tissues such as the brain and the kidney, the lung and the heart, the lung and the liver at 95% confidence level. The value of 22 ppm in the liver is lower than the value of 35 ppm reported by Bruckmann and Zondek[15] (Table 4 (6)).

Zinc: Similar abundances of Zn were detected in the kidney and the liver and these results agree with the values of Tipton and Cook.[11] The Zn content in the kidney is significantly higher than in the brain (Table 4 (6)).

Selenium: There are few reports on the Se contents in human tissue.[6,16] The Se concentration in the kidney is significantly higher than in the other tissues. Allaway *et al.*[6] have reported that the Se contents in blood serum inversely correlate with the cancer death rate. Such a trend was not found in the present work (Table 4 (7)).

Bromine: The highest content of Br is found in the lung and the lowest in the brain which amounts to only 6.1% of the lung. One of the authors has reported that the abundance of Br in the optic nerve is smaller than in the other eye organs. Suggesting that Br interferes with the actions of the nervous organs (Table 4 (7)).

Cadmium: An extremely high value of Cd was found in the kidney (74 ± 11 ppm). Several reports[17,18] have suggested that Cd accumulates in the kidney and is not excreted. The Cd content of the standard American has been found to be 30—40 ppm in the kidney and 2—3 ppm in the liver,[19] Whereas in the Japanese it is twice this value.[17] These results therefore are in good agreement[19] (Table 4 (8)).

Interaction of the Elements in Human Tissue. Several interesting facts have emerged from the present study and are shown in the Tables 5 (1)—(3). The principal points are:

Characteristics in the Brain: Several elements have been correlated with other elements, for example, Na normally correlates with Cl, Sc, V, Co, Se, Cd, and inversely with Mg, Cr. Significant inverse correlations were found between (Mg and Na, Cl, K, Cd), (Cr and Na, Cl, Sc, V, Cd) and (Mn and Sc, V, Fe, Cd), and a significantly normal correlation between (Na and Cl, Sc, V, Co, Se, Cd), (Sc and Na, Cl, K, V, Se Cd), (Co and Na, V, Fe, Zn, Se, Br), and (Se and Na, Cl, Sc, Fe, Co, Br). Cu in the brain normally correlates with only Cr and other pairs of elements including Cu have inverse interactions.

Characteristics in the Kidney and the Lung: Similar tendencies of correlation of the elements are found in the kidney and the lung. Normal significant correlations exist for each of the pairs of elements in both tissues, and significant inverse correlations in four pairs in the kidney and one pair in the lung. Inverse interactions between V and other elements except Mg, Cr, Co, and Br at the kidney exist.

Sc significantly correlates with Na, Mg, Cl, K, V, Cr, Cu, Zn, Se, Br, and Cd in the lung. A more characteristic factor is that there is a significant inverse correlation between Cr and Se, and there appears inverse interactions in the lung.

Characteristic in the Liver: Inverse interactions exist although no significant correlations are present in the liver.

Characteristics in the Heart: Significant inverse correlations are found between (Cr and Na, Cl, K, Mn, Cu, Se) and (Sc and Na, Zn, Cd). Normal significant correlations are also found between (Cl and Na, K, Mn, Zn) and (K and Na, V, Mn, Se). Such tendencies as reported above are almost uniformly distributed in the elements in the heart.

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