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Determination of Trace Elements in Human Whole Blood by Instrumental Neutron Activation Analysis

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The trace element composition of whole blood has been investigated by instrumental neutron activation analysis (INAA). The blood samples of 80 individuals of adult population in Taiwan were analyzed. The samples were lyophilized, irradiated together with synthetic standards, and determined γ-spectrometrically. The concentrations of 7 elements, Cr, Co, Fe, Rb, Sc, Se *and* Zn *were simultaneously determined. The reliability of the analysis was checked with NBS bovine liver reference standard material. The frequency distributions of all the elements measured are presented and the results are compared with available data.*

Key Words: human whole blood, trace elements, neutron activation analysis, gamma-spectrometry, NBS bovine liver reference standard

1. Introduction

During the last decade there has been a rapid increase in the number of trace elements shown to be essential and a remarkable surge of interest and activity in their significance in human health and disease[1)~3)]. The toxicity of some heavy metals is also well known[1)~3)]. Now, the analysis of trace elements, both essential and toxic, in human body has well been recognized to be of substantial importance in clinical laboratory for diagnosis of many diseases. For instance, diagnosis of Wilson's disease through determination of copper in serum and urine[4)], diagnosis of acute myocardial infarction through determination of serum nickel[5)], and diagnosis of lead poisoning through determination of lead in whole blood[6)].

Trace elements are continuously assimilated in human body through drinks, food and the ambient air. So, the level of these elements in a particular population will depend on local factors such as the geochemical variability of the environment and the dietary habits. A significant amount of data on trace elements in human blood in various regions of the world is now available in literature[3)]. But, no such data on Taiwan population are yet available. The aim of the present work was thus to establish the level of trace elements in whole blood of a "normal" adult population in Taiwan. It is believed that these data should be of importance in the hospital as a reference "normal range" to the clinical laboratory diagnosis for many diseases.

The concentrations of trace elements in human tissues and fluids are ordinarily very low, in the range between $\mu g \cdot ml^{-1}$ and $ng \cdot ml^{-1}$, and are normally maintained within very narrow limits. In order to determine the elements in such extremely low levels, obviously only modern instrumental methods characterized with high sensitivity can be of importance for this purpose. There are many instrumental techniques known to be important, among them graphite furnace atomic absorption spectrometry (GFAAS), particle-induced X-ray emission spectrometry (PIXE), inductively-coupled plasma atomic emission spectrometry (ICPAES) and neutron activation analysis (NAA) are frequently used for the determination of trace elements in the matrices of biological substances[7)]. The last technique more than other techniques has contributed to an outstanding of the role of trace elements in biological materials, primarily because of its high sensitivity and multielement capability.

In the extreme trace analysis, the systematic error inherent in the analytical methods frequently cause inaccurate analytical results, in

certain cases the errors even amounting to several orders of magnitude[8]. The sources of errors are complex and resulted primarily from impurities in the reagents, apparatus, surfaces and laboratory air throughout the analytical procedures, all of which should be meticulously controlled[9]. INAA is a direct instrumental method which basically involves no chemical treatment step prior to neutron activation and can therefore avoid introducing systematic error. However, in applying INAA, the possible effect of standard on the analytical results should be carefully evaluated. In most cases, INAA is used as a relative method in which the sample and a standard containing known amounts of elements of interest are irradiated simultaneously and their activities are measured under identical conditions. If the standard used is in different composition to the sample, the effect of matrix substances may possibly deteriorate the reliability of analytical results. It is therefore necessary to use a standard reference material which is very similar in composition to the sample in order to be able to recognize analytical errors. In our present case NBS bovine liver reference standard material is most suitable as it is the one which most resembles serum in its composition.

In the present work, INAA has been employed to determine trace elements in whole blood samples, and the analytical accuracy was routinely checked by bovine liver reference standard material.

2. Experimental

2·1 Sample preparation

Acids used were purified by sub-boiling distillation apparatus made of quartz. Water was first deionized and further purified by a two-stage quartz distillation apparatus (Bergforf Product, West Germany). The samples and standards for irradiation were prepared in a laminar flow clean bench (class 100).

The blood samples were supplied by Kaohsiung Blood Donation Center, Taiwan. About 2 ml blood sample was collected from each subject. The sample was drawn with a stainless steel needle to a polyethylene syringe and transferred to a clean and weighed 5 ml pyrex vial, and was then warmed in a water bath for 10 minutes to break the cell walls of the capsules.

Each sample was then lyophilized for 48 hours to a constant weight. The dried mass of blood was then ground to fine powder homogeneously. About 10 mg powder of each sample was sealed in a quartz tube for irradiation.

Synthetic standards were prepared by dissolving known weights of spectroscopically pure metals or compounds into appropriate solvents, diluting to the desired concentration and transferring an appropriate aliquot into quartz tube for irradiation.

2·2 Neutron irradiation

The prepared sample, standard and NBS bovine liver standard were put together in an irradiation container for neutron irradiation. All irradiations were done in the National Tsing-Hua University Open Pool Reactor (THOR) at the vertical tube for 30 hours. The neutron flux at the irradiation position is about $2\times10^{13}\ n\cdot cm^{-2}\cdot s^{-1}$.

2·3 Activity measurement

After a decay period of 2 weeks, the irradiated samples along with the standards were washed with concentrated nitric acid solution to eliminate the surface contamination and then subject to γ spectral analysis under the identical counting condition for 3000-second counting. The counting instrument used was a 43 cc Ge(Li) detector in conjunction with a Tracor Northern TN-1710 model 4096-channel pulse height analyzer. The γ spectra of following nuclides ^{51}Cr, ^{60}Co, ^{59}Fe, ^{86}Rb, ^{46}Sc, ^{75}Se and ^{65}Zn were identified and measured.

3. Results and Discussion

In all, 80 blood samples were analyzed in this study. The subjects were randomly selected by the Kaohsiung Blood Donation Center from the voluntary blood donor. The ages of the donors are from 20 to 60.

Seven elements (Cr, Co, Fe, Rb, Sc, Se, Zn) are measured in this work. The ^{65}Zn determination is subjected to partial interference from ^{46}Sc. The 1120.3 keV line of ^{46}Sc partially overlaps the 1115.4 keV of ^{65}Zn which is the

only γ-photon suitable for measurement. In order to correct this, the 1120.3 keV/889.4 keV intensity ratio for ^{46}Sc was determined from a pure ^{46}Sc source. A correction, based on this ratio, was applied to remove the contribution of Sc in Zn measurement. The blank value of the quartz tube was also corrected.

The reliability of the analytical methods was checked with NBS bovine liver reference standard material. NBS has certified the concentrations of Co, Fe, Rb, Se, Zn and indicated the concentrations of Cr and Sc. Table 1 lists the analytical results of 7 trace elements in bovine liver obtained in this work and that from published works. For ease of comparison of analytical accuracy, the certified or indicated values given by NBS are also presented in the same table. Each datum indicated in our work is the average of 5 individual determinations. From the reasonably good agreement between our analytical results and that of NBS certified values as seen in the table, it may be concluded that the analytical technique employed in this work can be equally applicable to the analysis of blood samples.

Table 1 Trace element concentrations (ppm) in NBS bovine liver standard

Element	This study	Certified or indicated values given by NBS
Co	0.19±0.02	0.18
Cr	0.09±0.014	0.088±0.012
Fe	248±16	268±8
Rb	17.6±1.2	18.3±1.0
Sc	0.0011±0.0002	0.001±0.0001
Se	1.15±0.11	1.1±0.1
Zn	143±8	130±13

About 100 mg of dry blood sample was used for each analysis in this work. The average dry weight per ml of blood was found to be 0.218±0.017 g. This is in good agreement with the Khan's publication[10] and was used to the calculation of the concentrations of the elements in order to minimize the error arising from the uncertainty in the volume of individual blood sample.

The ranges, arithmetic means, and standard deviations of the human whole blood are given in Table 2. The frequency distribution of the concentrations of different elements are shown respectively in Fig. 1. The results are discussed below. Some of the literature values expressed in ppm or ppb are converted to $\mu g \cdot ml^{-1}$ or $ng \cdot ml^{-1}$ in Table 3 to Table 8.

Table 2 Trace element concentrations in human whole blood

Element	No. of samples	Mean ($\mu g \cdot ml^{-1}$)	S.D.* ($\mu g \cdot ml^{-1}$)	Range ($\mu g \cdot ml^{-1}$)
Cr**	78	29.9	16.7	4.0～66.8
Co**	13	58.3	17.1	28.9～84.3
Fe	80	494.4	43.1	347.2～545.5
Rb	80	3.4	1.2	0.6～6.1
Sc**	78	8.5	3.9	2.0～14.3
Se	80	0.095	0.028	0.041～0.151
Zn	76	6.8	2.4	3.3～16.6

* Standard deviation $=\sqrt{\frac{\Sigma(X-\bar{X})^2}{N}}$

X=Individual value

$\bar{X}$=Mean of individual values

N=Number of determinations

** in $ng \cdot ml^{-1}$

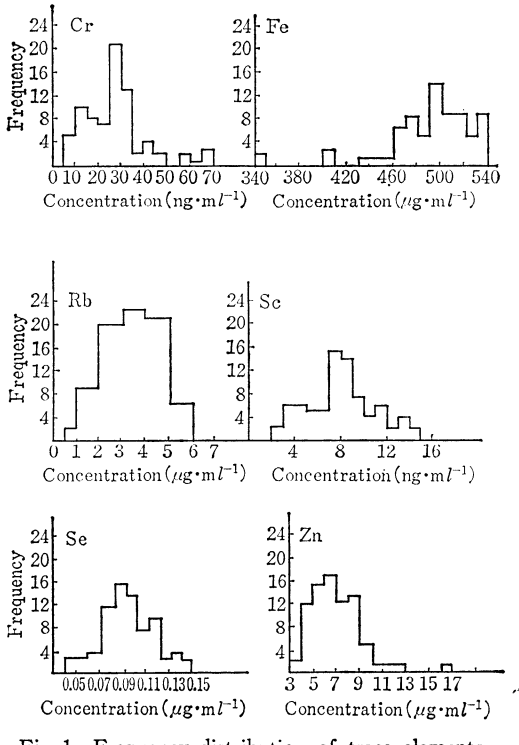

Fig. 1 Frequency distribution of trace elements in human blood.

Table 3 Whole blood chromium concentration

Authors		Analytical technique	Mean (ng·ml^{-1})	S.D. (ng·ml^{-1})	Range (ng·ml^{-1})	No. of subjects	Reference number
Brune	1950	Spect	12				11
Donev	1978	NAA	20			22	12
Imbus	1963	Spect	27.6		12.8~55.4	154	13
Maxia	1972	NAA	28		9~40	8	14
Ward	1979	NAA	28	9		Pooled	7
This work	1982	NAA	29.9	16.7	4.0~66.8	78	
Schroeder	1970		30				15
Babenco	1971		30				16
Anspaugh	1971		30				17
Pierce	1966	Spect	33		22~55	10	18
Pierce	1966	AAS	36		15~55	10	18
Bowen	1964	NAA	46			8	19
Schroeder	1966		50				20
Haller	1969	NAA	53	2.65		1	21
Bowen	1974	NAA	54		6~330		22

Table 4 Whole blood cobalt concentration

Authors		Analytical technique	Mean (ng·ml^{-1})	S.D. (ng·ml^{-1})	Range (ng·ml^{-1})	No. of subjects	Reference number
Brune	1963	NAA	3				30
Haller	1969	NAA	4.6	1.1			21
Curtis	1975	NAA			0.9~3.9	1	31
This work	1982	NAA	58.3	17.1	28.9~84.3	13	
Bowen	1974	NAA	58		0.2~200		22
Ward	1979	NAA	67	2		Pooled	7

Table 5 Whole blood iron concentration

Authors		Analytical technique	Mean (μg·ml^{-1})	S.D. (μg·ml^{-1})	Range (μg·ml^{-1})	No. of subjects	Reference number
Dyson	1978	PIXE	309			2	32
Lodhi	1979	PIXE	309	118	194~457		33
Khan	1980	PIXE	339	23	280~410	100	10
Bearse	1974	PIXE	384	27		27	34
Anspaugh	1971		390				17
Haller	1969	NAA	428	8.6		1	21
Iyengar	1978		447		301~530		3
Bowen	1974	NAA	464		350~525		22
Ward	1979	NAA	485	86		Pooled	7
This work	1982	NAA	494.4	43.1	347.2~545.5	80	
Tabellen	1971		500				35
Friberg	1979		500				36
Donev	1978	NAA	507	72		22	12
Donev	1972		543				37

3·1 Chromium

Chromium has an important role in the atherosclerosis, diabetes, and lung cancers[2]. But it is clearly a difficult element to be analyzed reliably. Factors influencing the determination of chromium in biological materials have been discussed by Parr[23] and investigated in detail by Behne, et al.[24] Investigations by Versieck[25] have shown that huge extraneous additions result from blood being drawn through a disposable steel needle. As borosilicate glass contains between 1 and 10 μg Cr·g^{-1} [26], it also

Table 6 Whole blood rubidium concentration

Authors		Analytical technique	Mean (μg·ml^{-1})	S.D. (μg·ml^{-1})	Range (μg·ml^{-1})	No. of subjects	Reference number
Bearse	1974	PIXE	0.9	0.17		27	34
Haller	1969	NAA	1.4	0.03		1	21
Anspaugh	1971		1.5				17
Donev	1978	NAA	2.25			22	12
Bowen	1974	NAA	2.33		1.2~6		22
Hamilton	1973		2.7	0.04		165	38
Ward	1979	NAA	2.75	0.08		Pooled	7
Iyengar	1978		2.94		1.17~5.98		3
Lodhi	1979	PIXE	3.1	0.5	2.7~3.8		33
This work	1982	NAA	3.4	1.2	0.6~6.1	80	
Khan	1980	PIXE	4.18	0.71	2.5~6.0	100	10

Table 7 Whole blood selenium concentration

Authors		Analytical technique	Mean (μg·ml^{-1})	S.D. (μg·ml^{-1})	Range (μg·ml^{-1})	No. of subjects	Reference number
Maxia	1972	NAA	0.068		0.054~0.079	8	14
Griffiths	1974		0.068	0.013		170	43
Watkinson	1966		0.053		0.049~0.058		44
Hamilton	1973		0.08				38
Ward	1979	NAA	0.092	0.01		Pooled	7
This work	1982	NAA	0.095	0.028	0.041~0.151	80	
Brune	1966	NAA	0.120	0.020*		6	45
Tabellen	1971		0.12				35
Haller	1969	NAA	0.176	0.0088		1	21
Anspaugh	1971		0.18				17
Dickson	1967	NAA	0.182	0.036		254	46, 47
Bowen	1974	NAA	0.193		0.07~0.32		22
Bearse	1974	PIXE	0.20	0.10		27	34
Allaway	1968	Fluor.	0.206		0.10~0.34		48
Beteridge	1965		0.22				49
Burk	1967	Fluor.	0.22	0.02		12	50
Bowen	1963	NAA	0.27			8	51
Jaffe	1972		0.36				52

* Standard error

Table 8 Whole blood zinc concentration

Authors		Analytical technique	Mean (μg·ml^{-1})	S.D. (μg·ml^{-1})	Range (μg·ml^{-1})	No. of subjects	Reference number
Khan	1980	PIXE	4.39	0.96	3.0~10.8	100	10
Sunderman	1973		5				55
Dyson	1978	PIXE	5.3		2.7~7.9	2	32
Babenco	1971		6.21				16
Haller	1969	NAA	6.3	0.13		1	21
Bearse	1974	PIXE	6.4	0.45		27	34
Lodhi	1979	PIXE	6.4	1.2	4.9~7.5		33
Bowen	1974	NAA	6.53		3.4~13.7		22
Donev	1978	NAA	6.59	0.93		22	12
This work	1982	NAA	6.8	2.4	3.3~16.6	76	
Castieho	1977	AAS	7.0				56
Iyengar	1978		7.0		4.8~9.3		3
Anspaugh	1971		7.4				17
Stump	1977	XRF	7.76	0.88	4.80~10.23		57
Ward	1979	NAA	8.8	0.2		Pooled	7

remains open to question whether this has some bearing on the actual chromium content of the samples.

The concentration of Cr in this study has been found to be in the range of 4.0～66.8 ng·ml^{-1}. Compared to the values listed in Table 3, the range distribution is somewhat broad in the present work; but it is rather narrower than Bowen's publication[22]. However, the mean value of 29.9 ng·ml^{-1} obtained in this work is in good agreement with the other results[13)~18)].

In addition to the analytical factors, biogeographical differences in the distribution of chromium seem to be also of importance; they are currently under investigation[27),28)]. The chromium levels in tissues of the people in the United States of America decrease slowly throughout the life span, which may be related to the refinement of food, especially flour. This decrease in chromium levels may be an etiological factor in the high incidence of coronary heart disease in the population. Chromium will also competes with iron for the transferrin in the blood. Hence, the equilibrium between these 2 trace elements will be disturbed each other[29)].

3·2 Cobalt

Cobalt is an essential element to man and must be supplied in the diet entirely in the physiologically active form, vitamin B_{12}. But it is subjected to a variety of analytical problems which is mainly a consequence of the low concentration of this element in blood. However, the nuclear characteristics of cobalt are favourable for its determination by INAA because of the high cross-section, 100% abundance of the parent isotope, and long half-life (5.26 years) of the product nuclide.

In this study, we measured only 13 samples. The data are listed in Table 4 together with the existing results. The mean value of 58.3 ng·ml^{-1} agrees well with the literature values[7),22)]. But the other results[21),30),31)] are rather lower than ours. The range of cobalt has been found to be 28.9～84.3 ng·ml^{-1}. But the data in Bowen's publication[22)] spread even broader, from 0.2 to 200 ng·ml^{-1}.

3·3 Iron

The iron value of 494.4±43.1 μg·ml^{-1} in the present work compares well with some of the reported values, but it is obviously higher than some others as can be seen in Table 5. The range of Fe concentration has been found to be 347～545 μg·ml^{-1}. Of the 80 samples analyzed, 75 are included in this range. Lower values were obtained in 5 cases, with a minimum of 347.2 μg·ml^{-1}. The 5 cases may be due to iron deficiency as the ubiquitous problem of iron deficiency is familiar to all physicians.

3·4 Rubidium

Biological interest in Rb has been stimulated by its close physicochemical relationship to potassium and its presence in living tissues in higher concentrations, relative to those of potassium, than in the terrestrial environment. A growing body of experimental evidence indicates that the element possesses unique neurophysiological characteristics[39)].

All the soft tissues of the body carry Rb concentrations that are high compared with many trace elements, with a total body content approximating 360 mg in adult man[40)]. It might act to some extent as a nutritional substitute for potassium. The plasma or serum rubidium concentration has been reviewed by Versieck and Cornelis[41)]. A relatively large variability with time of the plasma rubidium concentration in healthy adults was noted by Wood[42)], results in a male varied from 0.09 to 0.20 μg·ml^{-1} over a 3 month period. The mean concentration of Rb in this work was found to be 3.4±1.2 μg·ml^{-1}. This is in good agreement with the most values of previous studies(Table 6). But the value reported by Bearse, et al.[34)] seems to be improbably low in comparison with the other results.

3·5 Scandium

Scandium is one of the elements whose essentiality in biological function has not yet been established. Very few data of whole blood scandium concentration have been published.

Scandium concentration has been found to be 2.0～14.3 ng·ml^{-1}. The mean value is 8.5 ng·ml^{-1}, and is very close to the value investigated by Ward and Ryan[7)].

3·6 Selenium

Selenium is an essential constituent of glutathione peroxidase and has the biological effects on vitamin E[2]. Increased growth in children suffering from Kwashiorkor from the administration of Se as selenite had been reported earlier. Selenium is a cancer protecting element. It also exerts a beneficial effect on the incidence of dental caries[2].

Table 7 gives some existing data. The mean concentration of Se obtained in this work is $0.095 \pm 0.028\ \mu g \cdot ml^{-1}$. The published means varied from $0.068\ \mu g \cdot ml^{-1}$ to $0.36\ \mu g \cdot ml^{-1}$. Selenium is a volatile element, it will volatilize during sample digestion, but no loss could be detected during lyophilization[53].

Experimental evidence presented by Lombeck, et al.[54] suggests that the selenium concentration in blood is age-dependent. Furthermore, geographical differences appear exist. New Zealand adults are reported to have a low selenium status[2]. Inhabitants of the central, eastern, and south-eastern districts of Finland were found to have lower selenium levels than other Finns.

3·7 Zinc

Zinc is an essential constituent of about 70 metalloenzymes. The prominent deficient effects of zinc on human are growth retardation, hyposmia and hypogeusia[2].

The concentration of Zn has been found to be in the range of $3.3 \sim 12.6\ \mu g \cdot ml^{-1}$, with only one exception where the value was $16.6\ \mu g \cdot ml^{-1}$ (Table 8). This value was not considered for the calculation of the mean. Of the 80 samples analyzed, 74 are included in the range of $4 \sim 10\ \mu g \cdot ml^{-1}$. The mean value is $6.8\ \mu g \cdot ml^{-1}$ in this work and is compared with other data listed in Table 8. These data are in good agreement with each other except that Khan's, et al.[10] has a significantly lower value in the Bangladeshi population having low intake of animal protein.

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要　旨

機器中性子放射化分析によるヒト全血の微量元素の定量

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台湾人80名の全血の微量元素の機器中性子放射化分析を行った。血液サンプルは高雄血液センターより無作意に得られた20歳より60歳までの80検体で，中性子照射は国立 Tsing-Hua 大学原子炉の中性子密度 $2\times10^{13}\,n\cdot cm^{-2}\cdot s^{-1}$ で，NBS の片肝臓標準参考試料と同時に照射し，ガンマスペクトロメータで測定した。^{51}Cr, ^{60}Co, ^{59}Fe, ^{86}Rb, ^{46}Sc, ^{75}Se および ^{65}Zn の測定結果を文献データと比較検討した。