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Correlations of trace element levels within and between different normal autopsy tissues analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES)

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Abstract

Imbalance in trace metal metabolism may lead to metal interactions that may be of patho-physiological importance. Knowledge of the relation between trace metals in normal tissues is needed to assess abnormal deviations associated with disease. In this study correlations between Cu, Co, Cr, Fe, Mn, Ni, Se, Zn, Al, Ba, Cd, Pb and Sr within the same and between 6 different, normal autopsy tissues were determined using Spearman rank correlation analysis based on analytical data obtained by inductively coupled plasma atomic emission spectrometry (ICP-AES). Fe-Co were correlated in most tissues. Cu-Mn, Zn-Cu, Zn-Mn and Zn-Cd were highly correlated in the kidney medulla. Ni-Ni, Sr-Sr and Cd-Cd were correlated between several tissues, while Fe-Fe, Zn-Zn and Cu-Cu were correlated between kidney cortex and medulla. Mn-Mn was highly correlated between the liver and brain front lobe, cerebellum and heart. High correlations were found for Ni-Co and for Se-Mn between the kidney cortex and brain front lobe and pancreas respectively. Inverse correlations were found for Se-Cd between kidney cortex and cerebellum, for Se-Cd and Cd-Zn between kidney medulla and heart, for Co-Sr and Fe-Sr between the liver and kidney cortex and heart respectively, and for Sr-Mn between kidney medulla and pancreas. A large number of trace elements are statistically correlated within and between different, normal tissues. Knowledge of these correlations may contribute to increase the understanding of kinetic interactions of trace metals in the body and the role of such interactions in normal and disturbed trace metal metabolism.

Introduction

Imbalance in amounts and composition of trace metals in the body may cause interactions which can lead to disease. Interactions can be synergistic which means that a metal may stimulate accumulation or the function of another, or antagonistic in which case uptake or function of another metal is inhibited. The interactive mechanisms at the molecular level between two or more metals may be complicated and our knowledge at this point is still very limited. Thirty years ago it was postulated that elements with similar chemical and physical properties interacted antagonistically by for instance competing for the same binding sites on transport proteins and enzymes (Hill & Matrone 1970). A well known example is the antagonism between Cu and Zn where excessive supply of Zn inhibits absorption of Cu leading to Cu deficiency and secondary to that, Fe deficiency (Davis 1980; O'Dell 1989; Bremner & Beattie 1995). Of special interest are the interactions between essential and non-essential, toxic trace elements. Toxic effects of Cd, Hg and Pb may be counteracted by essential metals like Se, Zn and in some cases by Mn (Telisman 1995; Milne 1999). In humans it was shown that an increase in Cd concentration in the kidney was accompanied by an equimolar increase in Zn probably attributed to induced biosynthesis of the transport protein metallothionein by these

metals (Piscator & Lind 1972; Elinder *et al.* 1977; Gerhardsson *et al.* 1986).

Knowledge about the quantitative relationships between trace metals in the body is fundamental to increase our understanding of trace metal metabolism and their mutual interactions. However, a major problem is to get reliable data on the concentration of trace metals in the different tissues. Blood tests can at the most give a rough indication of the total amount of a trace metal in the body, but tell little or nothing about the distribution of the metals between the different organs. A few organs like kidney, liver and gut are available for biopsy, and samples from several organs can be obtained during surgical operations. However, the most complete collection of tissue samples can only be obtained from autopsies.

The difficulties in this respect are reflected by the fact that in the literature most studies on humans are limited to a few trace metals in one or two organs at the time. There are very few if any, comprehensive reports of correlations of trace metals within and between tissues in healthy people.

Simultaneous determination of a whole range of trace metals in a tissue sample can be achieved by a multi-element technique such as the inductively coupled plasma atomic emission spectrometry (ICP-AES). In this report we present statistically significant metal – metal correlations calculated from the data of a recent study where we used ICP-AES to analyze 15 different trace metals in 6 different organs considered to be normal according to the pathology report (Rahil-Khazen *et al.* 2001)

Materials and methods

Tissue samples

Samples from brain (front lobe, both white and gray matter and cerebellum), heart muscle, kidney (cortex and medulla), liver, pancreas, and spleen were obtained from 30 autopsies taken from 17 women and 13 men with age range 17–96 years. The corpses were kept under refrigeration at the hospital's department of pathology and forensic medicine. The post-mortem time from death to autopsy ranged from 20 to 72 h with one sample after 96 h. Whenever post-mortem time had an effect on the trace element concentration, only samples autopsied within 48 h were included in the correlation study.

Samples were taken from normal organs according to the pathology report where 16 of the tissue donors (7 women and 9 men) were cases of sudden death or accidents and 14 were inpatients at the hospital. Tissue samples were removed in the mortuary with stainless steel scalpels, put into the acid pre-washed polypropylene vials and frozen at -20 °C until analysis. Before the digestion procedure, the samples were partially thawed and thick sections of the surrounding surface tissue, which could have been contaminated in an earlier stage, were cut off using an acid pre-washed plastic knife. Surrounding fatty tissues, blood vessels and membranes were also removed.

Reagents and laboratory ware

Multi-element standards were prepared from Spectrascan single element stock solutions (1000 mg l^{-1}) purchased from Teknolab, Norway together with suprapure sodium chloride and potassium chloride from Merck. Suprapure nitric acid and hydrogen peroxide were from Merck. De-ionized water that was tapped from a Milli-Q system (Millipore Corporation) with a resistance close to 18 Ωcm^{-1} was used.

Tissue specimens were stored either in Nalgene Cryogenic vials or polypropylene vials with polyethylene screw caps.

Laboratory glassware, the vials for tissue storage and the digestion vessels and covers were all soaked in 10% (v/v) solution of nitric acid overnight (or at least half an hour for the vessels and covers) and then washed thoroughly with de-ionized water.

Both bovine liver SRM 1577b from the National Institute of Standards and Technology (NIST) and human hair GBW 09101 from Shanghai Institute of Nuclear Research were used as certified reference material.

Instrumentation

Microwave digestion and evaporation was carried out using the Milestone microwave system (MLS 1200 MEGA, Italy) with the VAC-60 vacuum module.

The ICP-AES (Ash IRIS/AP from Thermo Jarell) with a combination of charged injection device detector and an axial viewing mode was used for trace element analysis. The instrumental and the operating conditions were as described in a recent paper (Rahil-Khazen *et al.* 2000).

Trace element analysis

The procedure for the ICP-AES analysis of autopsy tissue samples was described previously (Rahil-

Khazen *et al.* 2001). Briefly, about 1 g of wet tissue was digested in a microwave oven using 5 ml HNO_3 and 2 ml of H_2O_2. Yttrium was added as an internal standard prior to digestion such that a final concentration of 1 mg l^{-1} was reached in the final solution. The samples were then evaporated to about 0.3–0.9 ml using the same microwave oven and diluted with deionized water to a volume of 3 ml. NIST bovine liver and blanks were digested, evaporated and analyzed with each batch of samples.

Ca, K, Mg and Na were first determined in the tissue samples, thereafter adequate matrix matched multi-element calibration standards were prepared.

Samples were analyzed for the following trace elements: Al, Ba, Be, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Se, Sr, and Zn. The trace elements were measured at the same wavelengths as given in the previous work (Rahil-Khazen *et al.* 2000). Due to interference from high concentration of iron, Se, Ni, Cr and Pb were not measured in the liver and spleen and Cd was not measured in the latter.

Analytical quality control

Repeated analysis of the bovine liver certified reference material from NIST gave a recovery that was $100 \pm 5\%$ of the target value for all elements except for Co and Sr where the recovery was 108% and 88%, respectively. The between days coefficient of variation for Cd, Co, Cu, Fe, Mn, Sr and Zn was $< 5\%$ and $< 10\%$ for Pb and Se. Recovery of Al from the bovine liver reference material was exceptionally low. However, by using human hair reference material a recovery of $100 \pm 5\%$ was obtained in repeated analyses. The human hair certified reference material was also used to assess the quality of analysing Cr and Ni that were not present in the bovine liver reference material from NIST. A recovery of $100 \pm 5\%$ for Cu, Co, Cr, Fe, Cd, Ni and Sr; and $100 \pm 10\%$ for Mn, Pb and Zn was obtained when using reference material weight of 1 g of human hair (Rahil-Khazen *et al.* 2001).

Statistics

Statistical analyses were performed using the SPSS 8.0 for Windows Microsoft. Correlations between the levels of the trace elements within the same and between different tissues were calculated using Spearman rank correlation analysis (non-parametric bivariate correlation test). Only results with a significance of $P \leq 0.01$ are reported.

Results

Trace elements profiles

In Figures 1 and 2 the median concentrations of the essential Cu, Co, Cr, Fe, Mn, Ni, Se, Zn and the non-essential Al, Ba, Cd, Pb, Sr in different tissues, are expressed on a logarithmic scale to outline the tissue profile of each element in the body. The spleen contained the highest level of Fe, which was about 1.5 times the level found in the liver. The highest level of the other essential trace elements was in general found in the liver. However, levels corresponding to those found in the liver were also found in the brain front lobe and cerebellum for Cu, in pancreas for Mn and in the spleen for Co. The highest level of Se was measured in the kidney cortex. Ni accumulated in heart and kidney medulla, while Cr was increased in pancreas, brain front lobe and cerebellum.

The non-essential elements showed a more incomplete distribution pattern with liver as the major storage organ for Al, and the kidney cortex for Cd, Pb and Ba. Sr was equally distributed between the kidney cortex and medulla and was the only non-essential metal that was detected in all the tissues.

Within tissue correlations

Table 1 presents the correlation coefficients between pairs of trace elements in the tissues. The P-values varied between 0.01 and 0.0005 with 54.5% of the P-values < 0.0005. The highest and most frequent correlations were found for Fe-Co. Most of the other significant correlations between essential elements were found in the kidney medulla with Zn-Cu, Zn-Mn and Cu-Mn being the highest. Interestingly, no correlations between essential metals were found in the heart.

Correlations between essential and non-essential metals were only found in the kidney except for the negative correlations between Sr and the essential metals Cu, Co and Fe. The highest coefficients were found for Zn-Cd in the medulla and the cortex respectively followed by Zn-Pb in both.

3 out of 5 significant correlations between non-essential metals were found in the kidney with the highest coefficient for Cd-Pb. Moderate correlation coefficient was also found for Cd-Sr in the cerebellum.

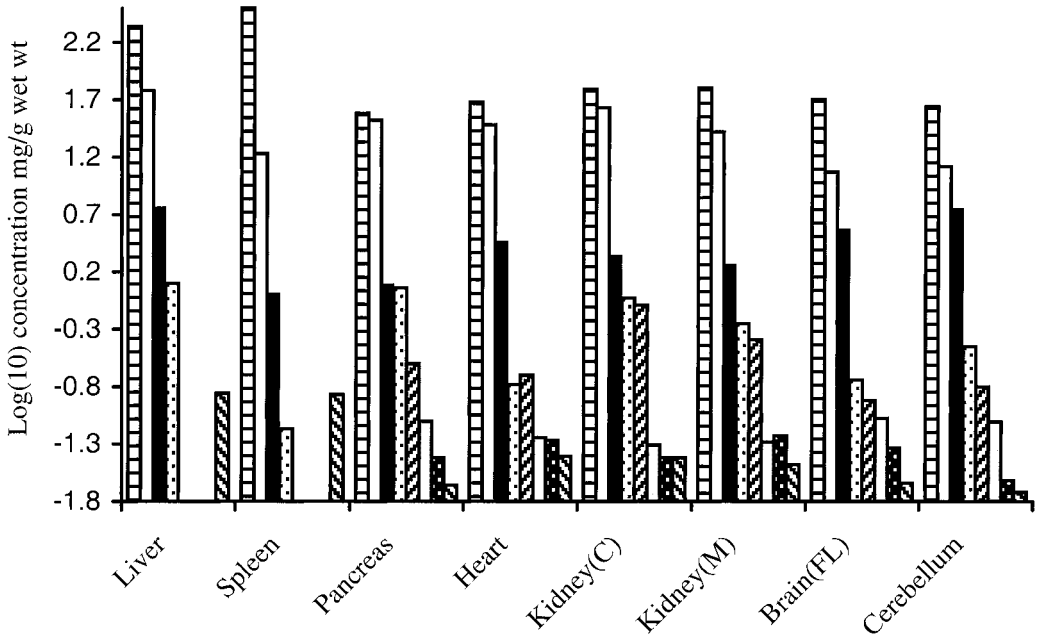

Fig. 1. Profiles of the essential trace elements in the liver, spleen, pancreas, heart, kidney cortex (C), kidney medulla (M), brain front lobe (FL) and cerebellum given in log (10) of the median concentrations in $\mu g\ g^{-1}$ wet tissue weight. The different trace elements are illustrated as follows: Fe (▭), Zn (□), Cu (■), Mn (▣), Co (▩), Se (▨), Cr (□), and Ni (▪).

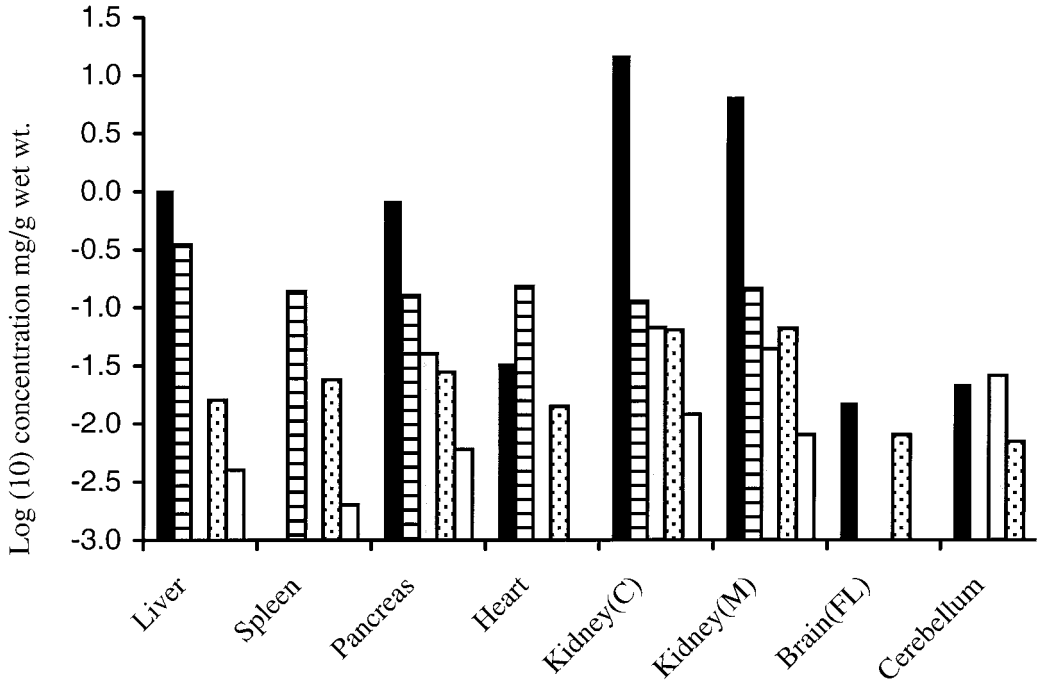

Fig. 2. Profiles of the non-essential trace elements in the liver, spleen, pancreas, heart, kidney cortex (C), kidney medulla (M), brain front lobe (FL) and cerebellum given in log (10) of the median concentrations in $\mu g\ g^{-1}$ wet tissue weight. The different trace elements are illustrated as follows: Cd (■), Al (▭), Pb (□), Sr (▣), and Ba (□).

Table 1 Correlations, given as Spearman's rho coefficients, between concentrations of different trace autopsy tissues in humans. The number of subjects (n) is given in brackets. Unless otherwise stated significance level is $P < 0.0005$. The dotted lines separate correlations between essential–essential, essential–non-essential and non-essential–non-essential elements, respectively.

Kidney				Brain				Heart		Liver		Pancreas		Spleen	
Cortex		Medulla		Front lobe		Cerebellum									
Elem(n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem(n)	Corr	Elem (n)	Corr	Elem (n)	Corr
Cu-Mn(28)	0.496[a]	Cu-Mn(28)	0.719	Co-Ni(22)	0.597[b]										
		Cu-Se(26)	0.665												
Fe-Co(26)	0.762	Fe-Co(26)	0.627[c]	Fe-Co(22)	0.741	Fe-Co(25)	0.731			Fe-Co(22)	0.873	Fe-Co(26)	0.843	Fe-Co(29)	0.838
		Mn-Se(26)	0.558[b]									Mn-Se(26)	0.571[b]	Fe-Mn(24)	−0.605[b]
		Zn-Cu(28)	0.814												
		Zn-Mn(28)	0.718												
		Zn-Se(26)	0.565[b]												
		Cu-Cd(28)	0.590[c]			Cu-Sr(25)	−0.591[b]	Cu-Sr(18)	−0.589[a]	Co-Sr(22)	−0.537[a]				
		Mn-Cd(28)	0.637							Fe-Sr(24)	−0.633[c]				
		Mn-Pb(28)	0.536[b]												
Zn-Pb(28)	0.677	Zn-Pb(28)	0.621												
Zn-Cd(28)	0.762	Zn-Cd(28)	0.817												
Al-Ba(21)	0.558[a]							Al-Sr(18)	0.654[b]						
Cd-Pb(28)	0.603[c]	Cd-Pb(28)	0.628			Cd-Sr(25)	0.665								

[a] $P \leq 0.01$, [b] $P < 0.005$, [c] $P \leq 0.001$

Between tissue correlations

Significant correlations between trace elements in the kidney (cortex and medulla) and other tissues are shown in Table 2. Most of the correlations between essential elements were found in the kidney cortex versus medulla followed by the kidney cortex versus pancreas. High correlations for Ni-Ni were found in kidney coupled with all the other organs except for the kidney cortex versus heart and spleen, and kidney medulla versus spleen. In addition Fe-Fe had high correlation coefficients in the kidney cortex versus medulla followed by Cu-Cu. Se-Mn had a high coefficient in the kidney cortex versus pancreas. Only a few metal pairs were correlated between kidney cortex and brain and spleen respectively.

Most of the mixed correlations between essential and non-essential metals were found in the kidney cortex versus cerebellum with 5 out of 7 correlations being negative. Only 4 correlations were found between kidney medulla versus heart, pancreas and cerebellum and all of them were negative. Of these Se-Cd had the highest correlation coefficient in both kidney cortex versus cerebellum and kidney medulla versus heart (Figure 3 and 4). Sr-Mn was prevalent with moderate coefficients between kidney cortex versus brain and pancreas and kidney medulla versus pancreas. Al-Co was the only significant correlation between essential and non-essential elements in the kidney cortex versus heart.

Correlations between non-essential elements were almost of even distribution between the kidney and the other organs. High correlation coefficients were found between Al and Ba in the kidney cortex versus Cd in heart. Al-Al, Sr-Sr and Cd-Cd were prevalent, with the highest correlation between kidney cortex and medulla for the last two pairs and between kidney medulla versus pancreas for Al-Al.

Table 3 shows the interrelationship between liver and other tissues. The most prevalent essential-essential metal correlations with high coefficients were found for Mn-Mn in liver versus brain and liver versus heart. The correlation coefficient for Fe-Fe in liver versus spleen was also high. Correlations between mixed essential and non-essential elements were mostly negative. Correlations between non-essential elements were only found for pairs of the same element with Sr-Sr being the most prevalent with high correlation coefficients.

Table 4 shows correlations between the brain front lobe and cerebellum. Correlations between the same elements prevailed with the highest coefficients for the essential Ni-Ni and Cu-Cu and for the non-essential Sr-Sr.

Discussion

As shown in this study a high number of metal pairs were statistically correlated within and between tissues. From the present descriptive data we can not conclude about kinetic interactions between trace elements. However, solid knowledge about the distribution of trace metals in normal and diseased tissues and their mutual correlation is considered to be of fundamental importance in our effort to understand more of the complex interrelationships in trace element metabolism (O'Dell 1989; Elsenhans *et al.* 1991; Telisman 1995).

The capability of the ICP-AES technique to perform simultaneous analysis of many trace elements in the same sample is beneficial in studies like the present with the aim of examining correlations between several elements. In the literature we have not found any similar, comprehensive study that could be compared to ours and most of the smaller studies were performed using other methods. Due to the importance of liver, kidneys and lungs in the metabolism of trace elements and the vulnerability of these organs to toxic elements, they have been more extensively studied than other tissues. The concentrations of Cu, Fe, Mn, Ni, and Zn in the kidney and liver found in the present study, were within the range reported in the literature (Norheim & Aaseth 1980; Subramanian *et al.* 1985; Gerhardsson *et al.* 1986; Ringdal *et al.* 1986; Julshamn *et al.* 1989; Saltzman *et al.* 1990; Schuhmacher *et al.* 1992; Llobet *et al.* 1998; Benes *et al.* 2000). Kidney levels of Cr are similar to levels reported by some studies (Caroli *et al.* 1992; Schuhmacher *et al.* 1992; Llobet *et al.* 1998; Benes *et al.* 2000), but higher than others (Gerhardsson & Wester 1984; Lyon *et al.* 1989). Se concentrations in the kidney cortex were about 20–40% higher than the levels reported in Norway more than 10 years ago (Ringdal *et al.* 1986; Julshamn *et al.* 1989), but similar to those reported in Canada (Subramanian *et al.* 1985) and Germany (Oster *et al.* 1988) and about double those reported in Austria (Tiran *et al.* 1995) and the Czech Republic (Benes *et al.* 2000). Cd levels in the kidney cortex were in good agreement with those reported earlier in Norway (Julshamn *et al.* 1989), Spain (Schuhmacher *et al.* 1993; Torra *et al.* 1995; Llobet *et al.* 1998) and Canada (Benedetti

Table 2 Correlations, given as Spearman's rho coefficients, between concentrations of different trace elements in the kidney cortex (C) and kidney medulla (M) and those in the other autopsy tissues. The number of subjects (n) is given in brackets. Unless otherwise stated significance level is $P < 0.0005$. The dotted lines separate correlations between essential–essential, essential–non-essential and non-essential–non-essential elements, respectively.

Kidney (C)	Kidney medulla		Brain front lobe		Cerebellum		Heart		Pancreas		Spleen		Kidney (M)	Brain front lobe		Cerebellum		Heart		Pancreas		Spleen	
	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr		Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr
	Co-Co (26)	0.529[a]	Mn-Co (20)	−0.611[c]					Co-Co (22)	0.580[c]													
	Co-Fe (26)	0.560[c]	Ni-Co (15)	0.732[c]					Co-Fe (22)	0.547[a]													
	Cu-Cu (28)	0.709							Fe-Co (24)	0.558[c]	Fe-Co (26)	0.497[a]								Fe-Co (24)	0.628[b]		
	Cu-Cr (28)	0.504[a]			Zn-Cr (23)	0.538[a]																	
	Fe-Fe (28)	0.758									Fe-Fe (27)	0.513[a]										Fe-Zn (27)	−0.597[b]
	Mn-Mn (28)	0.557[c]							Mn-Mn (24)	0.595[b]													
	Ni-Ni (21)	0.913	Ni-Ni (15)	0.948	Ni-Ni (18)	0.892			Ni-Ni (17)	0.875				Ni-Ni (15)	0.924	Ni-Ni (18)	0.873	Ni-Ni (9)	0.911[b]	Ni-Ni (17)	0.858		
	Zn-Zn (28)	0.621							Se-Mn (17)	0.721[b]													
	Cd-Zn (28)	0.511[c]	Al-Se (20)	−0.581[a]	Al-Cu (23)	−0.559[a]	Al-Co (14)	0.863	Co-Pb (22)	0.547[a]						Pb-Cu (23)	−0.553[a]	Cd-Zn (14)	−0.701[c]				
			Fe-Sr (20)	−0.570[a]	Al-Fe (23)	−0.543[a]			Fe-Pb (24)	0.641[b]													
					Mn-Cd (23)	−0.571[c]																	
					Se-Cd (18)	−0.750												Se-Cd (8)	−0.881[c]				
					Se-Sr (18)	−0643[c]																	
			Sr-Mn (15)	0.639[a]	Sr-Mn (18)	0.641[c]			Sr-Mn (17)	−0.634[a]										Sr-Mn (17)	−0.716[b]		
	Zn-Cd (28)	0.644			Zn-Pb (23)	0.533[a]																	
	Al-Al (21)	0.587[c]					Al-Al (14)	0.773[b]	0.698 (24)											Al-Al (17)	0.803		
	Cd-Cd (28)	0.828	Cd-Cd (20)	0.608[c]	Al-Cd (23)	0.622[c]	Al-Cd (9)	0.912[b]	Cd-Cd (24)	0.668				Cd-Cd (20)	0.629[c]					Cd-Cd (24)	0.559[c]		
	Pb-Pb (28)	0.525[c]			Sr-Cd (18)	0.730[b]	Ba-Cd (9)	0.862[c]								Sr-Cd (18)	0.688[c]						
	Sr-Sr (21)	0.857			Sr-Sr (18)	0.746			Sr-Sr (17)	0.797				Sr-Sr (15)	0.693[c]	Sr-Sr (18)	0.695[b]			Sr-Sr (17)	0.738[b]		

[a] $P \leq 0.01$, [b] $P \leq 0.001$, [c] $P \leq 0.005$

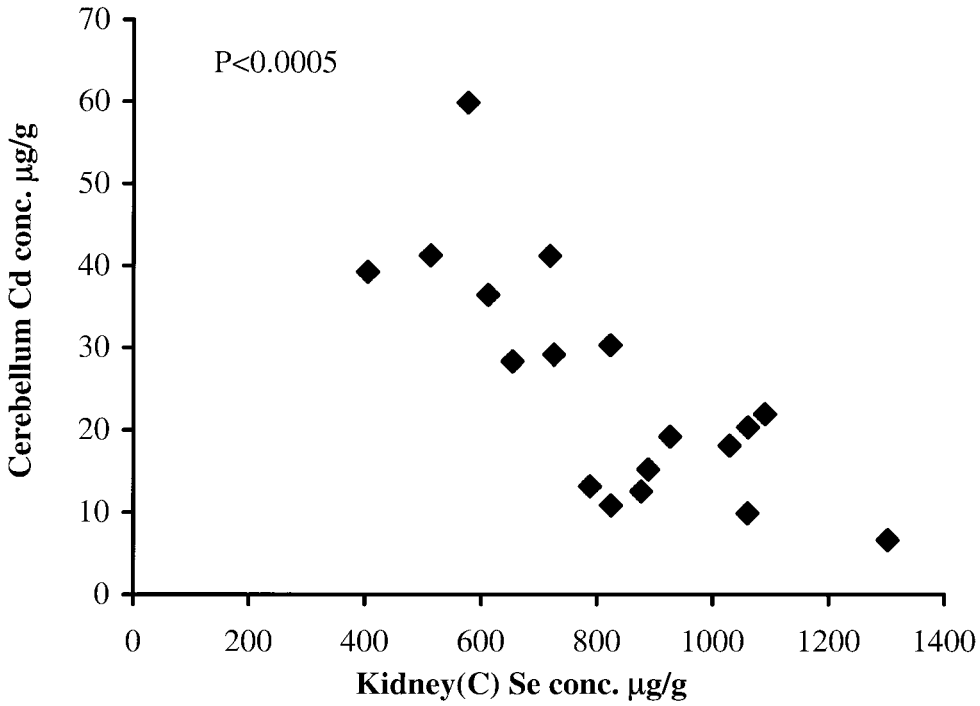

Fig. 3. Correlation between Se in the kidney cortex (C) versus Cd in the cerebellum given as concentrations in μg g^{-1} wet tissue weight. The significance of the correlation calculated from Spearman's correlation is given on the graph.

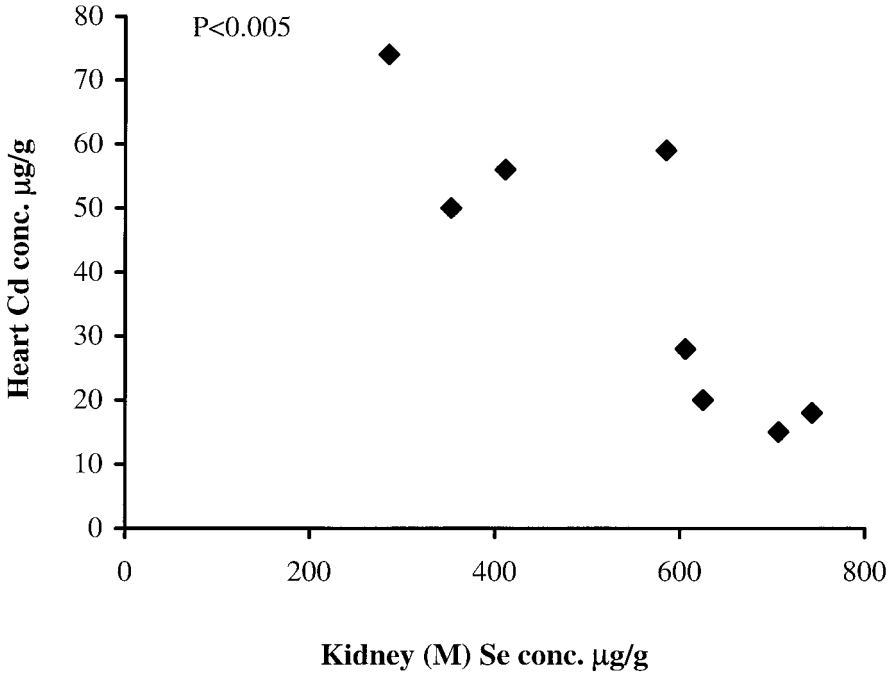

Fig. 4. Correlation between Se in the kidney medulla (M) versus Cd in the heart given as concentrations in μg g^{-1} wet tissue weight. The significance of the correlation calculated from Spearman's correlation is given on the graph.

Table 3. Correlations, given as Spearman's rho coefficient, between concentrations of the different trace elements in the liver and those in the other autopsy tissues. The number of subjects (n) is given in brackets. Unless otherwise stated significance level is $P \leq 0.005$. The dotted lines separate correlations between essential–essential, essential–non-essential and non-essential–non-essential elements, respectively.

Liver	Kidney cortex		Kidney medulla		brain front lobe		Cerebellum		Heart		Pancreas		Spleen	
	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr	Elem (n)	Corr
					Co-Cu(17)	0.623[a]							Co-Fe(22)	0.605
					Mn-Mn(18)	0.773[c]	Mn-Mn(18)	0.723[b]	Mn-Mn(13)	0.736	Mn-Fe(20)	−0.565[a]	Cu-Zn(24)	0.549
			Zn-Co(19)	−0.616									Fe-Co(23)	0.641[b]
	Zn-Fe(21)	−0.562[a]	Zn-Fe(21)	−0.688[b]			Zn-Zn(18)	0.620					Fe-Fe(24)	0.690[c]
	Co-Sr(14)	−0.713	Ba-Co(19)	−0.668							Ba-Mn(20)	−0.596[a]		
			Zn-Sr(15)	0.704					Fe-Sr(13)	−0.743				
											Zn-Pb(20)	−0.617		
			Al-Al(15)	0.776[b]							Al-Al(20)	0.564[a]		
	Cd-Cd(20)	0.655	Cd-Cd(20)	0.660										
	Sr-Sr(15)	0.659[a]			Sr-Sr(18)	0.656			Sr-Sr(13)	0.823[b]	Sr-Sr(20)	0.819[c]		

[a] $P \leq 0.01$, [b] $P \leq 0.001$, [c] $P < 0.0005$

Table 4. Correlations, given as Spearman's rho coefficient, between concentrations of the different trace elements in the brain front lobe and those in the cerebellum. The number of subjects (n) is given in brackets. Unless otherwise stated significance level is $P < 0.0005$. The dotted lines separate correlations between essential–essential, essential–non-essential and non-essential–non-essential elements, respectively.

Brain (FL)	Cerebellum	
	Elem (n)	Corr
	Co-Co(22)	0.637[a]
	Cu-Cu(22)	0.736
	Mn-Mn(22)	0.609[b]
	Ni-Ni(22)	0.955
	Zn-Zn(22)	0.659[a]
	Co-Cd(22)	0.606[b]
	Cd-Cd(22)	0.764
	Cd-Sr(22)	0.553[c]
	Sr-Sr(22)	0.851

et al. 1999), yet lower than the levels reported in some other parts of Canada (Subramanian *et al.* 1985), USA (Saltzman *et al.* 1990), Poland (Orlowski *et al.* 1996), Czech Republic (Benes *et al.* 2000) and China (Xia *et al.* 1989). However, they were higher than those reported by others (Caroli *et al.* 1992; Tiran *et al.* 1995) including studies reported from Sweden (Elinder *et al.* 1976; Gerhardsson *et al.* 1986). The Pb levels were within the levels reported by some studies (Subramanian *et al.* 1985; Llobet *et al.* 1998; Benes *et al.* 2000), yet much lower than those reported by others (Gerhardsson *et al.* 1986; Saltzman *et al.* 1990; Caroli *et al.* 1992; Schuhmacher *et al.* 1993).

The highest number of significant correlations between pairs of trace elements was found in the kidney medulla. Also, many essential and non-essential metals were positively correlated between cortex and medulla (Table 2). This may reflect a dominating role of the kidney in trace element metabolism (Caroli *et al.* 1994; Taylor 1996).

There was a highly significant correlation of Zn-Cu and Zn-Cd in kidney medulla and Zn-Cd in the kidney cortex. Mutual interactions of Cu, Zn and Cd are described in the literature (Davis 1980; O'Dell 1989; Elsenhans *et al.* 1991; Telisman 1995; Goyer 1997). A similar correlation was not found in any of the other organs that were examined. Contrary to other studies which also found correlation between Cu and Cd in the kidney cortex (Aalbers & Houtman 1985; Tanaka *et al.* 1987), we only found a significant positive correlation in the kidney medulla. Opposite to Benes *et al.* (2000) who found that Cu was inversely correlated to Mn in the kidney, we found that these metals were positively correlated in kidney cortex and medulla in agreement with a previous study (Carvalho *et al.* 1998). Like Kollmeier *et al.* (1992) we found a positive correlation for Zn-Pb in the kidney, but opposite to the finding of Kido *et al.* (1988) we did not find a positive correlation for Cu-Se in the kidney cortex, only in the medulla (Table 1).

The correlations between kidney cortex and medulla for Cu, Fe, Zn, Mn, Cd and Ni (Table 2) were quite comparable with results obtained by Tanvaka *et al.* (1987), who found significant positive correlations for Cu, Fe, and Mn and less significant correlations for Zn, Ni and Cd.

Liver and kidney are important in trace metal metabolism, and as shown here several metals were correlated between the two organs. While the correlation coefficients were positive for toxic metals like Cd and Al, they were negative for Zn-Fe and Zn-Co. The negative correlation of Zn-Fe between liver and kidney may be of significance in clinical conditions due to iron overload or zinc deficiency.

Cd-Cd and Sr-Sr were the most frequently correlated non-essential metals between organs, while Ni-Ni was the most frequently correlated of the essential metals, followed by Mn-Mn. We consider manganese as an essential metal even though it does not fulfil all the criteria for essentiality since signs of deficiency are not yet proven in humans. However, essential functions have been described (Milne 1999).

Of particular interest are the mixed correlations between essential and non-essential metals since some essential metals are known to be antagonistic to toxic metals and therefore may protect the tissues against damage (Telisman 1995; Patriarca *et al.* 1998; Milne 1999). In the kidney both Zn and Mn were positively correlated to Cd and Pb, and Cu was positively correlated to Cd. Also, the highly significant inverse correlations of Se-Cd between kidney cortex and cerebellum (Figure 3) and of Se-Cd between kidney medulla and heart (Figure 4) should be noted.

The differences between the tissue levels of the trace elements in different studies, may be related to (1) different study designs with respect to age, gender,

cause of death at the time of autopsies, and life-style habits such as smoking (2) different exposure to toxic metals caused for instance by air pollution and (3) methodological differences in the sampling procedure and analysis of the samples. Due to the small sample size that arises when correlating trace elements in tissues taken from the same persons, the present study design did not include stratification with respect to age and gender. The results must therefore be considered as average values in subjects with a mean age which ranged from 61 to 64 years in different organs. However, in a previous report the effect of age and gender on the concentrations of trace elements in the same tissues were presented (Rahil-Khazen *et al.* 2001). Furthermore, individual data on alcohol or cigarette smoking were not available. Smoking in particular can influence the content of Cd in liver, kidneys (Lewis *et al.* 1972a, b; Elinder *et al.* 1976) and lungs (Lewis *et al.* 1972a, b; Gerhardsson *et al.* 1986, 1988). From 1980 to 1993, the prevalence of smoking in Norway declined by only 2% units as compared to about 10% in other European countries including Sweden. The prevalence of smoking in Norway in 1993 was 35% compared to 23% in Sweden (Kraft & Svendsen 1996; Thelle 2000). This may at least partly, explain the lower tissue levels of Cd in the Swedish study (Gerhardsson *et al.* 1986).

The analytical quality control of our method was sufficient according to the results obtained from analysing the certified reference materials of bovine liver and human hair. Contamination during sampling is especially relevant for metals such as Ni, Cr and Co. Use of stainless steel was found to increase the concentration of Cr, Ni and to a lower extent Co and Mn (Aitio & Järvisalo 1994; Cornelis *et al.* 1996). We used stainless steel during sampling, but avoided contamination by using acid washed plastic knives to cut big portions of the surrounding tissue, thus isolating the central part which finally was analysed.

In conclusion, a large number of trace elements are statistically correlated within and between different, normal tissues. The knowledge of these correlations may contribute to increase the understanding of kinetic interactions of trace elements in the body and the role of such interactions in normal and disturbed trace metal metabolism.

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