Changes in Serum Zn, Cu, Se, and Mn Levels in Patients with Chronic Liver Diseases and Hepatocellular Carcinoma

Sawako TAKIKAWA

*First Department of Internal Medicine, Iwate Medical
University, Morioka 020, Japan*

(Received December 20, 1989)

Summary Serum levels of Zn, Cu, Se, and Mn in 72 hospital patients suffering from liver cirrhosis (LC), hepatocellular carcinoma (HCC), or chronic hepatitis (CH) were determined by atomic absorption spectrometry, and the results were compared with those obtained from 151 healthy adults serving as controls. Serum Cu levels in subjects with HCC and LC were significantly elevated, whereas both Zn and Se levels were lowered. Subjects with CH also showed a decrease in Se level. Consequently, a marked increase (>40%) in the Cu/Zn ratio of the sera of both HCC and LC groups was observed. There were no significant differences in Mn levels between the controls and the diseased groups, except for the slight increase shown by the LC group. Changes in the level of these trace metals were found to be independent of the individual metals.

Key Words: serum trace metals, hepatocellular carcinoma, chronic liver disease, Cu/Zn ratio, atomic absorption spectrometry

Trace metals play an important roles in the body as components of metallo-proteins and metalloenzymes and as enzyme activator [1]. Since the biosynthesis and reactions of these compounds take place in the liver, studies on changes in body trace metals in patients with liver disorders have been of interest to many workers. Although an association of alcohol-dependent liver cirrhosis with Zn deficiency has been reported [2, 3], few studies have been conducted on the fate of this metal in relation to other liver diseases. Furthermore, little information is available concerning changes in the level of other trace metals in patients with liver disorders. One reason for this is the lack of suitable methods for analysis of serum trace metals. In recent years, marked progress has been made in trace element analytical techniques such as atomic absorption spectrometry, plasma atomic emission spectrometry, and neutron activation analysis. These techniques have enabled determination of various trace metals in small amounts of serum with

high sensitivity and precision [4-6].

It has long been known that trace metals in the body interact with each other. For example, administration of a large quantity of Zn in humans lead to Cu deficiency, and vice versa [7]. Thus, for study of the fate of trace metals in liver diseases, it is valuable to determine a number of elements in a sample simultaneously. In the present work, mainly by use of graphite-furnace atomic absorption spectrometry, we studied changes in serum levels of Zn, Cu, Se, and Mn in samples from both healthy adults and patients with chronic liver disease or hepatocellular carcinoma. The significance of these changes was examined in relation to the liver disorders.

MATERIALS AND METHODS

Serum sample. Blood samples were obtained from 72 hospital patients diagnosed as suffering from liver cirrhosis (LC, $n=33$), hepatocellular carcinoma (HCC, $n=28$), or chronic hepatitis (CH, $n=11$). Twenty-six of the HCC patients were shown to have liver cirrhosis as well. Also, excessive alcohol consumption was implicated in 4 of the HCC and in 10 of the LC patients. Control subjects comprised healthy males ($n=71$) and females ($n=80$), 20-69 years of age, who lived in areas with no known industrial pollution sources. They were not known to have been occupationally exposed to metals, and had no history of hepatic disorders. Fasting blood samples were taken in the morning, and the sera were separated by centrifugation. A portion of the resultant serum sample was used immediately for clinical laboratory tests, whereas the rest was frozen and maintained at -20°C until analyzed. Several basic clinical laboratory tests were run on serum samples from the diseased groups, and the results are shown in Table 1. Serum GOT, GPT, γ -GTP, and ALP were elevated in all three diseased groups, whereas serum albumin levels were decreased in HCC and LC. Total bilirubin was slightly increased in HCC and LC, but the standard deviation was high due to the presence of several cases of severe jaundice in these groups.

Analysis of metals. Analysis of the trace metals in the serum samples was performed by atomic absorption spectrometry. Zn was determined by a flame atomic absorption spectrophotometer, model AA 855 (Nippon Jarrel Ash, Kyoto). The sample, diluted 10 times with distilled water, was nebulized onto the flame

Table 1. Clinical laboratory findings in patients with liver diseases.

Liver function test	HCC ($n=28$)	LC ($n=33$)	H ($n=11$)
Total bilirubin (mg/dl)	3.5 ± 7.7	2.8 ± 8.4	0.5 ± 0.1
GOT (IU/liter)	90.1 ± 68.5	49.7 ± 29.1	74.5 ± 67.7
GPT (IU/liter)	49.7 ± 35.6	38.2 ± 31.8	102.2 ± 88.8
ALP (IU/liter)	189.0 ± 88.0	174.0 ± 90.2	114.1 ± 33.0
γ -GTP (IU/liter)	110.1 ± 117.7	91.1 ± 72.1	74.5 ± 70.8
Albumin (g/dl)	3.5 ± 0.6	3.5 ± 0.4	4.3 ± 0.3

Values are means $\pm$ SD.

Table 2. Conditions employed for analysis of zinc by flame atomic absorption spectrometry.

Sample volume	1 ml
Wave length	213.9 nm
Lamp current	12 mA
Slit width	0.68 nm
Flow rate of air	12 liter/min
Flow rate of acetylene	2.5 liter/min

Table 3. Conditions employed for analysis of Cu, Se, and Mn by graphite furnace atomic absorption spectrometry.

	Metal		
	Cu	Se	Mn
Atomic absorption spectrophotometer			
Wavelength (nm)	324.8	196.03	279.4
Lamp current (mA)	8	20	10
Slit width (nm)	0.68	0.68	0.68
Graphite furnace atomizer			
Sample volume (μ l)	5	10	10
Drying (temperature/time)	150°C/30 s	150°C/30 s	132°C/30 s
Ashing	940°C/30 s	940°C/30 s	830°C/30 s
Atomizing	2,840°C/7 s	2,840°C/7 s	2,710°C/7 s

directly. Analysis of Cu, Se, and Mn was performed with an atomic absorption spectrophotometer and flameless atomizer, model FLA-100 (Nippon Jarrel Ash, Kyoto) with a graphite furnace, model C-51 (Mitsubishi Pencil Co., Ltd., Tokyo). Copper analysis was carried out using the platform [4], following dilution of the serum sample with 0.1% Triton X-100, and distilled water in a 1:1:8 ratio. Analysis of Se was performed by use of the platform and matrix with added Pd, and the final sample used in the analysis was prepared by mixing of the original serum sample with 0.1% Triton X-100 and distilled water in a 1:1:3 ratio. The sample used for the analysis of Mn was prepared by dilution of the original serum sample with an equal volume of 0.1% Triton X-100. To prevent diffusion of the sample in the graphite furnace and to improve the precision, we placed a tungsten bar in the furnace. The conditions employed for the determination of Zn and those of Cu, Se, and Mn are shown in Tables 2 and 3, respectively. Under the experimental conditions, the reproducibility given by the analysis was good, as in no cases did the coefficient of variation obtained from 10 runs exceed 5% (Table 4).

Reagents. Standard solutions (1,000 ppm) of Zn, Cu, Se, and Mn of AA spectrometric grade were obtained from Wako Pure Chemical Industries, Ltd., Tokyo.

Statistical analysis. Results are expressed as mean $\pm$ SD, except where indicated, and significant differences among the groups were determined by Student's *t* test.

Table 4. Reproducibility and limit of detection for serum Cu, Se, and Mn by atomic absorption spectrometry.

Metal	Coefficient of variation (%)		Limit of detection (g)
	Standard solution	Serum	
Cu	2.7	4.7	2.2×10^{-11}
Se	4.5	4.3	3.5×10^{-11}
Mn	1.7	2.9	2.0×10^{-12}

Table 5. Serum levels of Zn, Cu, Se, and Mn in healthy individuals according to different age groups.

Age (year)	Sex	n	Zn ($\mu\text{g}/\text{dl}$)	Cu ($\mu\text{g}/\text{dl}$)	Se (ng/ml)	Mn (ng/ml)
20-39	M	15	88 ± 18.4	97 ± 17.4	120 ± 17.1	1.3 ± 0.4
	F	14	95 ± 15.5	95 ± 24.9	118 ± 9.9	1.3 ± 0.6
40-49	M	24	101 ± 24.0	100 ± 22.4	125 ± 21.7	1.3 ± 0.8
	F	32	95 ± 15.2	92 ± 14.6	122 ± 22.2	1.3 ± 0.6
50-59	M	15	101 ± 24.0	94 ± 19.9	123 ± 27.6	1.6 ± 0.8
	F	14	93 ± 19.9	88 ± 20.7	114 ± 24.4	1.4 ± 0.8
60-69	M	17	95 ± 20.3	87 ± 12.1	116 ± 23.9	1.2 ± 0.7
	F	20	95 ± 18.8	88 ± 20.3	118 ± 26.6	1.4 ± 0.6
Total	M	71	91 ± 20.6	95 ± 19.1	121 ± 22.6	1.3 ± 0.7
	F	80	95 ± 16.7	91 ± 19.1	119 ± 22.0	1.4 ± 0.6

Values are means $\pm$ SD.

Table 6. Correlation study of serum trace metals in healthy male subjects.

Metal	Zn	Cu	Se	Mn
Zn	—	-0.128	0.275	0.113
Cu		—	0.038	0.303
Se			—	0.083
Mn				—

Values are correlation coefficients.

RESULTS

Serum Zn, Cu, Se, and Mn levels of healthy adults

The serum Zn, Cu, Se, and Mn levels of the control groups are shown in Table 5 according to sex and age of the subjects. No significant differences were observed between any of the age groups or between the sexes. To study the correlation among the metals, we used the data for the male subjects. As shown in Table 6, little correlation was found among the metals. The data of the 151 control subjects were pooled and mean values recalculated irrespective of sex and age. The resultant means for serum Zn, Cu, Se, and Mn for the control group were 93.2 ± 19.1 , 93.3 ± 18.7 $\mu\text{g}/\text{dl}$, 120.4 ± 22.3 , and 1.4 ± 0.7 ng/ml (Table 7), respectively.

Table 7. Serum levels of Zn, Cu, Se, and Mn in patients with liver diseases.

Subjects	n	Zn ($\mu\text{g/dl}$)	Cu ($\mu\text{g/dl}$)	Se (ng/ml)	Mn (ng/ml)	Cu/Zn ratio
Control	151	93 $\pm$ 19.1	93 $\pm$ 18.7	120 $\pm$ 22.3	1.4 $\pm$ 0.7	1.0 $\pm$ 0.3
HCC	25	86 $\pm$ 27.8*	122 $\pm$ 41.8**	83 $\pm$ 26.5**	1.7 $\pm$ 1.1	1.5 $\pm$ 0.7*
LC	33	73 $\pm$ 17.6**	106 $\pm$ 41.4**	76 $\pm$ 19.6**	1.8 $\pm$ 1.2*	1.5 $\pm$ 0.7**
CH	11	99 $\pm$ 19.2	87 $\pm$ 17.7	92 $\pm$ 22.9**	1.1 $\pm$ 0.5	0.9 $\pm$ 0.3

Values are means $\pm$ SD. * p <0.05; ** p <0.01 vs. the corresponding control value.

Fig. 1. Distribution of serum selenium levels. $\square$, Control; $\square$ (hatched), HCC; $\blacksquare$, LC; $\square$ (cross-hatched), CH.

Serum Zn, Cu, Se, and Mn levels of patients with chronic liver diseases

The serum levels of Zn, Cu, Se, and Mn in patients suffering from HCC, LC, and CH are shown in Table 7. Compared with that of the healthy controls, the serum Zn was significantly lowered in patients with HCC and LC, whereas the level of Cu was markedly elevated. As a result, the Cu/Zn ratio of these two groups was significantly increased (p <0.01). No such differences were observed between CH and the control.

A significant decrease in serum Se (p <0.01) was observed in all three patient groups (Table 7). Compared with those of the control, the decreases were 30.9, 36.2, and 23.1% for HCC, LC, and CH, respectively. The distribution of serum Se level of the subjects was then studied. As shown in Fig. 1, the control group showed a normal distribution, peaking at 110–120 ng/ml. In contrast, the serum Se of the patient group, while exhibiting a normal distribution, tended to shift to lower concentrations than those of the controls, and 31% of the subjects in this group had levels lower than 61 ng/ml, the lowest level shown by the control group. This trend of shifting towards the left in distribution was most evident in HCC and LC, as the distribution for the LC group peaked around 70–80 ng/ml.

As for Mn, only the LC group showed an increase (p <0.05), 28% over the

Table 8. Correlation study of serum trace metals in male patients.

Metal	Zn	Cu	Se
Zn			
Cu	0.279		
Se	0.267	0.089	
Mn	-0.021	-0.055	-0.119

Values are correlation coefficients.

Fig. 2. Serum Cu/Zn ratio of individual patients with liver cirrhosis. ●, Value for each individual; ⊙, average value. Shaded area represents ranges of Cu/Zn ratios for the control.

controls, while no significant changes were observed in the level for HCC or CH (Table 7).

Additionally, to study whether changes in serum level of a trace metal in patients with any liver diseases are associated with changes of any other metals, we calculated correlation coefficients based on the data obtained from the male patients, as this group predominated in number. As shown in Table 8, no significant correlation was observed among any of the metals studied. Changes in the level of the trace metals were thus independent of each other.

Relationship between severity of liver diseases and serum trace metal levels

The association of the severity of LC with the level of serum trace metals was examined, based on the Child-Turcotte criteria (CTC) [8]. In these criteria, liver cirrhosis is grouped into three categories, i.e., A (mild), B (moderate), and C (severe), according to clinical observations on serum bilirubin and albumin, ascites, neurological disorder, and nutrition. Serum Zn level decreased with increasing severity of the disorder, as the means for CTC categories A, B, and C were 85.8 ± 12.3 , 73.0 ± 15.2 , and 61.0 ± 11.8 $\mu\text{g/dl}$, respectively.

In contrast, the Cu level increased significantly. Means for CTC categories A, B, and C were 97.7 ± 13.0 , 94.1 ± 35.1 , and 144.0 ± 45.3 $\mu\text{g/dl}$, respectively, with the value for category C being significantly higher than the values of the other two categories. No significant association was observed between the three categories

Fig. 3. Serum Cu/Zn ratio of individual patients with both hepatocellular carcinoma and liver cirrhosis. ●, Value for each individual; ○, average value. Shaded area represents ranges of Cu/Zn ratios for the control.

Table 9. Correlation between serum trace metals and liver function tests.

Liver function test	Metal			
	Zn	Cu	Se	Mn
Hemoglobin	0.530**	-0.025	0.445**	0.054
Albumin	0.530**	-0.043	0.506**	-0.016
GOT	-0.118	0.121	-0.479**	0.115
GPT	0.086	0.038	-0.343**	0.019
γ -GTP	-0.021	0.241	-0.374**	0.074

Values are correlation coefficients. ** $p < 0.01$.

and serum Se or Mn.

The relationship between the serum Cu/Zn ratio of the LC group with CTC categories is shown in Fig. 2. The Cu/Zn ratio of CTC category C (uncompensated liver cirrhosis) was 2.1 ± 0.7 , which was significantly ($p < 0.01$) higher than that of category A (1.2 ± 0.21) or B (1.3 ± 0.4). The Cu/Zn ratios of patients with both HCC and LC were grouped according to the three CTC categories and the results are shown in Fig. 3. Compared with the control, the ratios were significantly increased in all three categories. However, among the individual categories, no close association was found. Moreover, no significant differences were observed between alcohol-dependent and non-alcohol-dependent liver cirrhosis concerning serum Zn, Cu, Se, and Mn levels (data not shown).

The relationship between serum Se levels of HCC patients and tumor size was also studied. Average values for the two groups in which tumor size determined by ultrasonogram was below and above 6 cm in diameter were 78.9 ± 26.2 and 75.9 ± 13.3 ng/ml, respectively, and there were no significant differences between these two groups.

The correlation between results of liver function tests and each of the trace metals is shown in Table 9. Both Zn and Se were positively correlated with serum albumin and hemoglobin levels. In addition, Se levels were found to be negatively correlated with serum GOT, GPT, and γ -GTP.

DISCUSSION

With the recent advancements in analytical techniques, it has become possible to determine many trace metals in biological samples. As a result, there has been a growing interest in relating changes occurring in trace elements to a number of human disorders. However, while the essentiality of many trace metals has been established, our knowledge concerning the normal levels of some trace metals is still limited because of the difficulties involved in their analysis. We have studied suitable analytical methods for the determination of several trace metals in biological samples, especially in serum samples. For the analysis of Se, in particular, a new method has been developed through the use of the platform method and of Pd as a matrix modifier (unpublished results). The coefficient of variation given by use of this method was 4.3%, which is superior to the 6.7–8.1% obtainable by conventional methods [9, 10].

Reported normal levels of serum trace metals in healthy individuals vary with workers and the methods used for their analysis. Many workers have reported average Zn and Cu levels to be between 80 and 120 $\mu\text{g/dl}$. Our data for Zn and Cu levels were 93.2 ± 19.1 and 93.3 ± 18.7 $\mu\text{g/dl}$, respectively. Concerning serum Se levels, the reported average ranged from 42–190 $\mu\text{g/liter}$ [11]. Such a wide variation may be attributed to several factors, including difficulties inherent in the analysis itself, regional differences in Se content in the soil, and varying intakes of dietary Se [12]. The experimental data obtained from this study gave a mean value of 120.4 ± 22.3 $\mu\text{g/liter}$, in line with the normal distribution, and are considered satisfactory in view of the good reproducibility and high sensitivity (Table 4).

Reported normal values for serum Mn have tended to become lower with advancement in the analytical methods, but they range widely, as values of 0.54–34.3 ng/ml have been reported [11]. The present study gave a value of 1.4 ± 0.7 ng/ml. According to Versieck and Cornelis [11], Mn levels in healthy subjects are normally distributed around a mean value of 0.5–0.6 ng/ml, whereas the upper limit is approximately 1.0–1.2 ng/ml.

As shown in Table 5, the levels of Zn, Cu, Se, and Mn found in control subjects composed of both sexes and different age groups were not significantly different from each other. For this reason, when we studied the serum level of these metals in normal healthy individuals and patients with liver diseases, sex and age were excluded as variables. Emphasis was thus placed on significant differences shown by the two groups themselves.

Vallee *et al.* [13], in 1956, reported lowered serum Zn levels and enhanced urinary Zn excretion in patients suffering from liver cirrhosis. Since then, research on changes in trace metal levels in patients with liver diseases has mainly focused on the fate of Zn in LC. Decreases in serum Zn levels in LC patients have been attributed to (i.) lowered intake of dietary Zn; (ii.) interference with intestinal absorption of Zn; and (iii.) an increased urinary excretion of Zn as a result of

decreased serum albumin acting as a carrier of Zn [14]. In this present study, a decrease in serum Zn levels was also observed in patients with LC and in those with HCC accompanied by LC. A decreased Zn level was particularly evident in patients with critically low serum albumin. It has been shown that, in LC patients, the Zn-binding ability of albumin may be diminished as a result of decreased affinity between serum albumin and Zn [15]. Further work is necessary to clarify this particular aspect.

As shown in Table 7, the serum Cu levels of patients with LC or HCC were significantly higher than those of the control. Since Cu is excreted mainly through bile, and since in LC patients an increase in serum estrogen levels occurs, stimulating the biosynthesis of ceruloplasmin [16], an enhanced level of serum Cu may be expected. However, an elevated level of non-ceruloplasmin Cu has also been reported in LC [17].

An increase in serum Cu levels has been reported in patients with lung cancer, breast cancer, and lymphoma [18–20]. Miatto *et al.* [21] showed that the serum Cu levels of HCC were higher than those of LC patients. In particular, since the only patients having serum Cu levels above 170 $\mu\text{g}/\text{dl}$ were those with HCC, these authors noted that serum Cu levels served as a valuable diagnostic tool. In contrast, the data obtained from our study failed to show any significant difference in the average serum Cu level between HCC and LC patients. However, 10 out of the 13 patients showing serum Cu levels above 160 $\mu\text{g}/\text{dl}$ had HCC, while the remaining 3 had LC ($p < 0.05$). In addition, among the 48 cases whose serum Cu levels were below 160 $\mu\text{g}/\text{dl}$, there were 18 HCC and 30 LC.

It has been reported that, in HCC patients, serum Cu/Zn ratios may serve as a tumor marker, or as a valuable marker showing the extent of tumor growth or metastasis [22]. Since almost all of the patients with HCC had LC as well in our study, patients suffering mild LC and yet manifesting an increase in Cu/Zn ratios may need to be considered as having HCC at the same time.

Se is a component of glutathione peroxidase, an enzyme involved in the prevention of cellular membrane damage induced by lipid peroxidation. In laboratory experiments, liver necrosis has been observed in rats fed Se-deficient diets [23]. A study on the association of Se-deficiency with liver disease would be of interest, but very limited information is available concerning serum Se levels in relation to liver diseases. There are several reports showing decreased serum Se levels in patients with alcohol-dependent liver cirrhosis [24, 25]. As shown in Table 7, serum Se levels were found to be significantly lowered in patients with LC, HCC, or CH, compared with the control value. An examination of serum Se levels of alcohol- and non-alcohol-dependent liver cirrhosis showed no significant differences between them. Välimäki *et al.* [26] considered that alcohol might not have an influence on serum Se, since decreases in serum Se levels were observed in LC regardless of the causes, and since the values remained unchanged in individuals exhibiting varying serum alcohol levels or in individuals who had given up alcohol consumption.

The experimental data suggest that serum Se levels may be related to other liver functions, as negative correlation coefficients were shown between serum Se levels and GOT, GPT, and γ -GTP (Table 9). But serum Se, in contrast to serum Zn, was not found to be related to the severity of liver cirrhosis. Further work is necessary to study the mechanism involved in the decline of Se levels in the serum.

Concerning the relationship between serum Se and cancer, it has been demonstrated that administration of Se was effective in controlling tumor growth in experimental animals [27]. In human clinical observations, a decrease in serum Se levels was also shown in patients with cancer of the gastrointestinal tract, particularly in those where metastasis was manifest [28]. The data from our study also showed a significant decrease in Se levels in HCC patients (Table 7). However, there were no significant differences between HCC and LC, and no correlation was observed between Se levels and tumor size. The question of whether decreased serum Se levels might be a risk factor remains controversial, as results of various epidemiological studies have conflicted with each other [29–32]. Further study is needed for an answer to this question.

Manganese in the serum is a component of pyruvate carboxylase and superoxide dismutase and is an essential trace metal involved in the metabolism of carbohydrates and bone [33]. Manganese is excreted mainly through the bile. The liver and the pancreas play a predominant role in the homeostasis of Mn. Detailed studies on changes occurring in Mn levels in patients with hepatic disorders are still lacking, however. Published reports on serum Mn levels of LC patients include those by Nakahara *et al.* [34] and Sullivan *et al.* [35], who found no significant differences when they compared these values with the control one. In this study, a significant difference was observed only between the control and LC group, whereas neither HCC nor CH was significantly different from the control (Table 7). Recent reports show that serum Mn may not reflect the body burden of this metal [36]. It may be necessary, therefore, to use such samples as erythrocyte or hair in order to study the fate of Mn in patients with hepatic disorders.

The content of this paper was presented at the Second Meeting of the International Society for Trace Element Research in Humans, held Aug. 28–Sept. 1, 1989, in Tokyo, Japan.

My sincere thanks are due to Professor Shunichi Sato (First Department of Internal Medicine, Iwate Medical University), Professor Humio Tsunoda (Department of Hygiene and Public Health, Iwate Medical University), and Professor Ming-Ho Yu (Huxley College of Environmental Studies, Western Washington University, U.S.A.) for their guidance in this work and assistance with the manuscript. I would like also to express my appreciation to Dr. Kazuyoshi Itai (Department of Hygiene and Public Health, Iwate Medical University) for his assistance with the chemical analysis, and to Dr. Kazuyuki Suzuki and others involved in liver disease research at the First Department of Internal Medicine, Iwate Medical University, for their support during the course of this work.

REFERENCES

1. Jarres, J.D.K. (1987): Clinical indications for trace element analyses. *J. Trace Elem. Electrolytes Health Dis.*, **1**, 5-19.
2. Kiilerich, S., Dietrichson, O., Loud, F.B., Naestoft, J., Christoffersen, P., Juhl, E., Kjems, G., and Christiansen, C. (1980): Zinc depletion in alcoholic liver cirrhosis. *Scand. J. Gastroenterol.*, **15**, 363-367.
3. Schölmerich, J., Becher, M.S., Köttgen, E., Rauch, N., Häussinger, D., Löhle, E., Vuilleumier, J.P., and Gerok, W. (1983): The influence of portosystemic shunting on zinc and vitamin A metabolism in liver cirrhosis. *Hepatogastroenterology*, **30**, 143-147.
4. Delves, H.T. (1987): Atomic absorption spectroscopy in clinical analysis. *Ann. Clin. Biochem.*, **24**, 529-551.
5. Karen, A.W. (1988): Inductively coupled plasma-emission spectrometry. *Methods Enzymol.*, **158**, 190-205.
6. Versieck, J. (1988): Neutron activation analysis. *Methods Enzymol.*, **158**, 267-286.
7. Hill, C.H. (1988): Interactions among trace elements, in *Essential and Toxic Elements in Health and Disease*, ed. by Prasad, A.S., Alan, R. Liss, Inc., New York, pp. 491-500.
8. Child, C.G., and Turcotte, J.G. (1964): Surgery and portal hypertension, in *The Liver and Portal Hypertension*, ed. by Child, C.G., W.B. Saunders Co., Philadelphia and London, pp. 48-55.
9. Nève, J., and Molle, L. (1987): Direct determination of selenium in human serum by graphite furnace atomic absorption spectroscopy. *Acta Pharmacol. Toxicol.*, **59**, 606-609.
10. Imura, T., and Kuroda, M. (1987): Determination of serum selenium using graphite furnace atomic absorption spectrophotometry and the levels in diseases. *Trace Metal Metabolism*, **15**, 53-61.
11. Versieck, J., and Cornelis, R. (1980): Normal levels of trace elements in human blood plasma or serum. *Anal. Chim. Acta*, **116**, 217-254.
12. Levander, A.O. (1988): Selenium, in *Modern Nutrition in Health and Disease*, ed. by Shils, M.E., and Young, V.R., Lea and Febiger, Philadelphia, pp. 263-267.
13. Vallee, B.L., Warren, E.C., Wacker, W.E.C., Bartholomay, A.F., and Robin, E.D. (1956): Zinc metabolism in hepatic dysfunction I. Serum zinc concentrations in Laënnec's cirrhosis and their validation by sequential analysis. *N. Engl. J. Med.*, **255**, 403-408.
14. McClain, C.J., Adams, L., and Shedlofsky, S. (1988): Zinc and the gastrointestinal system, in *Essential and Toxic Elements in Health and Disease*, ed. by Prasad, A.S., Alan, R., Liss, Inc., New York, pp. 55-73.
15. Giroux, E., Schecter, P.J., Schoun, J., and Sjoerdsma, A. (1977): Reduced binding of added zinc in serum of patients with decompensated hepatic cirrhosis. *Eur. J. Clin. Invest.*, **7**, 71-73.
16. Smallwood, R.A. (1978): Other diseases associated with increased liver copper concentration, in *Metals and Liver*, ed. by Powell, L.W., Marcel Dekker, Inc., New York, pp. 313-330.
17. Frommer, D.J. (1976): Direct measurement of serum non-caeruloplasmin copper in liver disease. *Clin. Chim. Acta*, **68**, 303-307.
18. Diez, M., Cerdan, F.J., Arroyo, M., and Balibrea, J.L. (1989): Use of the copper/zinc ratio in the diagnosis of lung cancer. *Cancer*, **63**, 726-730.
19. Garofalo, J.A., Ashikari, H., Lesser, M.L., Botet, C.M., Rundles, S.C., Schwartz, M.K., and Good, R.A. (1980): Serum zinc, copper, and the Cu/Zn ratio in patients with benign and malignant breast lesions. *Cancer*, **46**, 2682-2685.
20. Reddy, I.S., Khilanani, P., and Bishop, C.R. (1980): Serum copper levels in non-Hodgkin's lymphoma. *Cancer*, **45**, 2156-2159.
21. Miatto, O., Casaril, M., Gabrielli, G.B., Nicoli, N., Bellisola, G., and Corrocher, R. (1985): Diagnostic and prognostic value of serum copper and plasma fibrinogen in hepatic car-

- cinoma. *Cancer*, **55**, 774-778.
22. Inutsuka, S., and Araki, S. (1978): Plasma copper levels in patients with malignant tumors of digestive organs. *Cancer*, **42**, 626-631.
 23. Schwarz, F., and Foltz, C.M. (1957): Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.*, **79**, 3292-3293.
 24. Aaseth, J., Alexander, J., Thomassen, Y., Blomhoff, J.P., and Skrede, S. (1982): Serum selenium levels in liver diseases. *Clin. Biochem.*, **15**, 281-283.
 25. Dworkin, B., Rosenthal, W.S., Jankowski, R.H., Gorden, G.G., and Haldea, D. (1985): Low blood selenium levels in alcoholics with and without advanced liver disease. *Dig. Dis. Sci.*, **30**, 838-844.
 26. Välimäki, M.J., Harju, K.J., and Vlikahti, R.H. (1983): Decreased serum selenium in alcoholics—a consequence of liver dysfunction. *Clin. Chim. Acta*, **130**, 291-296.
 27. Ip, C. (1985): Selenium inhibition of chemical carcinogenesis. *Fed. Proc.*, **44**, 2573-2578.
 28. Pothier, L., Lane, W.W., Bhargava, A., Michielson, C., and Douglas, H.O. (1987): Plasma selenium levels in patients with advanced upper gastrointestinal cancer. *Cancer*, **60**, 2251-2260.
 29. Salonen, J.T., Salonen, R., Lappeteläinen, R., Mäenpää, P.H., Alfthan, G., and Puska, P. (1985): Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: Matched control analysis of prospective data. *Br. Med. J.*, **290**, 417-420.
 30. Willet, W.C., Peto, B.F., Morris, J.S., Stampfer, J.J., Pressel, S., Rosner, B., and Taylor, J.O. (1983): Prediagnostic serum selenium and risk of cancer. *Lancet*, **2**, 130-134.
 31. Ringstad, J., Jacobsen, B.K., Tretli, S., and Thomassen, Y. (1988): Serum selenium concentration associated with risk of cancer. *J. Clin. Pathol.*, **41**, 454-457.
 32. Coates, R.J., Weiss, N.S., Daling, J.R., Morris, J.S., and Labbe, R.F. (1988): Serum levels of selenium and retinol and the subsequent risk of cancer. *Am. J. Epidemiol.*, **128**, 515-523.
 33. Kord, M. (1988): Manganese homeostasis in humans and its role in disease states, in *Essential and Toxic Elements in Health and Disease*, ed. by Prasad, A.S., Alan, R. Liss, Inc., New York, pp. 253-273.
 34. Nakahara, H., Kudo, H., Muramatsu, H., Masago, K., and Murakami, Y. (1980): Activation analysis of trace elements in the human blood serum of some specific diseases, in *Trace Element Analytical Chemistry in Medicine and Biology*, ed. by Bratter, P., Walter de Gruyter & Co., Berlin and New York, pp. 365-380.
 35. Sullivan, J.F., Blotcky, A.J., Jetton, M.M., Hahn, H.K.J., and Burch, R.E. (1979): Serum levels of selenium, calcium copper, magnesium, manganese and zinc in various human diseases. *J. Nutr.*, **109**, 1432-1437.
 36. Matsuda, A., Kimura, M., Kataoka, M., Ohkuma, S., Sato, M., and Itokawa, Y. (1989): Quantifying manganese in lymphocytes to assess manganese nutritional status. *Clin. Chem.*, **35**, 1939-1941.