Manganese, Copper, and Zinc Concentrations in Serum and Packed Blood Cells during Acute Hepatitis, Chronic Hepatitis, and Posthepatitic Cirrhosis

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Manganese, copper, and zinc concentrations were determined in serum and packed blood cells of normal controls, patients with acute and chronic (persistent or aggressive) hepatitis, and cases of postnecrotic cirrhosis. During the active phase of acute hepatitis, serum manganese concentrations are invariably increased; the difference between the mean value and the normal is highly significant, \( P < 0.001 \). The mean serum copper is also significantly increased \( (P < 0.01) \). The concentrations become normal during the subsiding phase. In chronic aggressive hepatitis and posthepatitic cirrhosis, the mean serum manganese concentration is increased, \( P < 0.001 \), whereas the serum zinc concentration is frequently decreased. There is a highly significant \( (P < 0.001) \) positive correlation between serum manganese concentration and the activity in serum of aminotransferases, in subjects with acute or chronic hepatitis or postnecrotic cirrhosis.

Additional Keyphrases: trace elements • liver disease • neutron-activation analysis • aminotransferases • normal values • bilirubin

We developed a blood-sampling technique and an analytical method for manganese, copper, and zinc that decreases contaminations to a negligible level. Some results obtained in control subjects were published elsewhere \( (1, 2) \).

Several reports concerning copper and zinc metabolism in liver disease have already been published \( (3-12) \), but we are not aware of data about manganese in serum and packed blood cells.

Here, we report manganese, copper, and zinc concentrations in serum and packed blood cells during the active and subsiding phase of acute, icteric viral hepatitis, during chronic persistent and chronic aggressive hepatitis, and during posthepatitic liver cirrhosis.

Materials and Methods

Forty-six controls (25 men and 21 women) were examined. Of these, 27 were hospitalized in the ophthalmologic ward, one in the orthopedic ward, and one in the internal medicine ward. Seventeen subjects were studied during a clinical examination for recruitment of university personnel. All were free of any obvious medical disease. Electrocardiogram, chest x-ray and laboratory examinations (including sedimentation rate, erythrocyte and leukocyte count, serum bilirubin, cholesterol, alkaline phosphatase \( (EC 3.1.3.1) \), thymol turbidity, aspartate and alanine aminotransferases \( (EC 2.6.1.1 \) and \( 2.6.1.2) \), serum total protein, albumin, and globulin) were normal. Subjects taking oral contraceptives were not included as it is known that these drugs influence copper and zinc concentrations in serum \( (6, 8, 13-16) \).

The patients with hepatic disease were hospitalized in the gastroenterology clinic. The diagnosis of acute icteric viral hepatitis was based on clinical and biochemical evidence. In one case the diagnosis of acute cholestatic viral hepatitis was made. There was one case of acute fulminant liver failure resulting in fatal hepatic coma. In four subjects the samples were taken during the active phase \( (17) \) of acute hepatitis, in four others during the subsiding phase \( (17) \), and in six patients both during the active and the subsiding phase. In all cases of chronic hepatitis the diagnosis was proved by peritoneoscopy and liver biopsy. Cases of chronic hepatitis were divided into a chronic persistent and a chronic aggressive form according to histological criteria \( (18, 19) \). One case of chronic aggressive hepatitis was studied shortly after she recovered from a hepatic coma provoked by gastrointestinal bleeding. The diagnosis of posthepatitic cirrhosis (five patients) was based on a documented clinical history and on biochemical, peritoneoscopic, and histological evidence.

Liver-function tests were done by standard techniques \( (20) \). Hepatitis B antigen (HB Ag) was determined by counterelectrophoresis or by the comple-
ment fixation reaction.

Of the 10 cases examined during the active phase of acute hepatitis, four were HB Ag positive. The four cases with chronic persistent hepatitis were HB Ag negative. Two of the four cases with chronic aggressive hepatitis and four of the five patients with postnecrotic cirrhosis were HB Ag positive.

Manganese, copper, and zinc concentrations in serum and packed blood cells were determined by neutron-activation analysis. The collection of samples, the irradiation, chemical separations, and measurements were all done as described elsewhere (1). However, in most cases of the present study, blood was collected with a plastic cannula trocar (Intraneule 110 16; Vygon Sterile, Ecouen, France) instead of a disposable steel needle (18 G 1/2, Terumo). As proven by contamination studies according to a technique published previously (21) this method appeared to be preferable for the collection of the blood samples. Collection of blood was performed between 8 and 10 a.m. after an overnight fast.

Utmost care was taken to avoid all possible sources of contamination. Manipulations were carried out in a dust-free room. The samples were collected and irradiated in containers cleaned with extreme care (1). It should be noted that packed cells were irradiated at a reactor site with a thermal- to fast-neutron flux ratio of 90, as the iron content is very high. This decreases the interference caused by the $^{56}$Fe (n.p.) $^{56}$Mn reaction to about 7%. We applied a correction for this error. All samples were analyzed in duplicate. The percent standard error for the method is approximately 8% for manganese, 3% for copper, and 7% for zinc.

Means and standard deviations were calculated by using standard equations (22). The results for normal subjects were tested for outlying values by the methods of Grubbs (23). The normality of the distributions in normal subjects was checked by the chi-square test (24). For the comparison of two means, the usual equations were used for paired or for unpaired cases (22). The regression line and the correlation coefficients ($r$) between serum aminotransferases and serum manganese values were calculated after logarithmic transformation of both variables (22).

Results

The mean values, standard deviations, and ranges for manganese, copper, and zinc in serum and packed blood cells of normal subjects are set forth in Table 1. All distributions are normal, but the following values are outlying: 1.01 ng/ml (males) and 1.04 ng/ml (females) (serum manganese), 1.99 $\mu$g/ml (females) (serum copper), 36.9 ng/g (females) (packed cells manganese), 0.96 $\mu$g/g (females) (packed cells copper) and 15.5 $\mu$g/g (females) (packed cells zinc).

No difference of copper and zinc concentration in packed cells is found between men and women whereas the difference for manganese and copper in serum is not statistically significant. The difference between the manganese concentration in packed cells is slightly significant ($0.02 < P < 0.05$) and between the zinc concentration in serum it is significant ($0.001 < P < 0.01$). This last statement agrees with the findings of Lindeman et al. (25).

The mean values and standard deviations of serum manganese, copper, and zinc, determined in patients with acute hepatitis, chronic hepatitis, and posthepatitic cirrhosis, are also summarized in Table 1.

During the active phase of acute icteric viral hepatitis, we always observed an increased serum manganese concentration. The mean value is 2.32 ng/ml, highly significantly different from the normal values ($P < 0.001$). The highest value (4.09 ng/ml) was found in the patient with fulminant hepatic failure. There also is a slight increase of the mean serum copper concentration ($P < 0.01$), the mean value being 1.45 $\mu$g/ml and the highest 2.22 $\mu$g/ml (in the case of cholestatic viral hepatitis). The mean serum zinc concentration does not change significantly (mean ± SD: 0.90 ± 0.16 $\mu$g/ml). One serum zinc value, however, appears to be lowered (0.59 $\mu$g/ml).

During the subsiding phase of acute icteric viral hepatitis, the serum manganese concentrations normalize (mean ± SD: 0.60 ± 0.12 ng/ml). In one case of positive HB Ag + hepatitis, four months after a mitral valve prosthesis, the serum copper appeared to be markedly increased (2.32 $\mu$g/ml). The serum zinc concentration is normal in all cases (mean ± SD: 0.91 ± 0.07 $\mu$g/ml).

The paired values of serum manganese determined in six patients during the active and later during the subsiding phase of acute icteric viral hepatitis are shown in Figure 1. The difference of the two means is highly significant ($t = 4.885; 0.001 < P < 0.01$). For serum copper and zinc, no significant difference was found between the active and subsiding phase in these six patients (serum copper, $t = 1.230$; serum zinc, $t = 1.145$).

![Fig. 1. Values for serum manganese determined in six subjects during the active and during the subsiding phase of acute icteric viral hepatitis](image-url)
In the four cases of chronic persistent hepatitis, serum manganese, copper, and zinc remain at normal values (Table 1).

In cases of chronic aggressive hepatitis, the mean serum manganese concentration (0.84 ng/ml) is significantly ($P < 0.001$) higher than the mean of the controls, the mean serum copper concentration (1.25 µg/ml) does not significantly differ from normal and the mean serum zinc concentration (0.79 µg/ml) is significantly ($0.02 < P < 0.05$) decreased.

In cases of postnecrotic cirrhosis, the mean serum manganese content (0.84 ng/ml) is significantly ($P < 0.001$) increased. The mean serum copper concentration (1.09 µg/ml) is normal. The mean of the serum zinc concentration (0.81 µg/ml) does not significantly differ ($0.05 < P < 0.10$) from the mean of the normal controls. However, in three cases of advanced cirrhosis, the serum zinc concentration was definitely low (respectively, 0.62 µg/ml, 0.47 µg/ml, and 0.65 µg/ml).

There is a significant positive correlation between serum bilirubin and serum manganese concentrations in acute and chronic hepatitis and postnecrotic cirrhosis ($r = +0.43; 0.01 < P < 0.02$). There also is a highly significant ($P < 0.001$) positive correlation between serum aminotransferase activities and serum manganese concentrations. If plotted in a double logarithmic diagram, the relationship is linear (Figure 2). The correlation coefficient calculated after double logarithmic transformation is +0.945 and +0.870 for aspartate aminotransferase and alanine aminotransferase, respectively, versus serum manganese. The equation of the regression line is $y = 0.434 x - 0.973$, $y$ being the log of serum manganese (in ng/ml) and $x$ the log of aspartate aminotransferase (in Karmen units).

Manganese, copper, and zinc concentrations in packed blood cells do not show significant variations. The results are summarized in Table 1. One exceptionally high value for manganese in packed cells was found during the active and subsiding phase in a 36-year-old woman with post-transfusion hepatitis (respectively, 39.6 and 45.2 ng/g).

### Table 1. Values of Manganese, Copper, and Zinc in Normal Controls and in Patients with Acute and Chronic Hepatitis and Posthepatic Cirrhosis

<table>
<thead>
<tr>
<th>Classification (and number)</th>
<th>Mn</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>ng/ml</td>
<td>µg/ml</td>
<td>µg/ml</td>
</tr>
<tr>
<td>Males (25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.45 - 1.01</td>
<td>0.73 - 1.51</td>
<td>0.72 - 1.21</td>
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<tr>
<td>Females (21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.38 - 1.04</td>
<td>0.77 - 1.99</td>
<td>0.69 - 1.15</td>
</tr>
<tr>
<td>Overall (46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.38 - 1.04</td>
<td>0.73 - 1.99</td>
<td>0.69 - 1.21</td>
</tr>
<tr>
<td>Acute viral hepatitis</td>
<td>2.32</td>
<td>1.43</td>
<td>0.90</td>
</tr>
<tr>
<td>Active phase (10)</td>
<td>±0.06</td>
<td>±0.36</td>
<td>±0.16</td>
</tr>
<tr>
<td>Acute viral hepatitis</td>
<td>0.60</td>
<td>1.27</td>
<td>0.91</td>
</tr>
<tr>
<td>Subsiding phase (10)</td>
<td>±0.12</td>
<td>±0.41</td>
<td>±0.07</td>
</tr>
<tr>
<td>Chronic persistent hepatitis (4)</td>
<td>0.58</td>
<td>1.04</td>
<td>1.02</td>
</tr>
<tr>
<td>Chronic aggressive hepatitis (4)</td>
<td>±0.13</td>
<td>±0.17</td>
<td>±0.16</td>
</tr>
<tr>
<td>Postnecrotic cirrhosis (5)</td>
<td>0.84</td>
<td>1.09</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>±0.20</td>
<td>±0.30</td>
<td>±0.33</td>
</tr>
</tbody>
</table>

Values are the mean ±1 SD. Many of these distributions are skewed.

* Per gram wet wt.

![Graph](image-url)
Discussion

Most of the reported manganese, copper, and zinc concentrations in packed blood cells (1, 3, 25–30) and copper and zinc concentrations in serum from normal subjects (1, 4–9, 13–16, 25–29, 31–44) show substantial agreement. Until recently, however, confusion has persisted concerning the normal serum manganese concentration (1, 29, 34, 44–52). Mean values published during the last decennium vary between 0.587 (45) and 24 ng/ml (51). It appears that the mean serum manganese value is very low and that the values in 46 control subjects approach a normal distribution with a quite small dispersion (0.57 ± 0.13 ng/ml) (2). The upper limit was found to be 1.04 ng/ml. Higher values described in normal subjects are apparently due to contaminations. The values reported in the present study for manganese in packed blood cells and for copper and zinc in packed blood cells and serum agree with other recent data (4, 7, 15, 25, 26, 29, 33, 37, 38). The concentration of manganese in packed blood cells is about 26-fold higher than in serum.

Our study shows that an increased serum manganese concentration is a constant finding during the active phase of acute viral hepatitis. A slight but statistically significant increase in the mean serum copper concentration was also observed, whereas the zinc concentration remained within normal limits except in one case with a low value. During the subsiding phase, normalization of the manganese and copper serum concentrations is observed.

We suggest that two hypotheses can be postulated to explain the invariably increased serum manganese during the active phase of acute viral hepatitis: necrosis of liver parenchyma, leading to an important release of hepatic manganese, or decreased hepatobiliary excretion. Indeed, the manganese content of the liver is very high as compared with its concentration in serum (1.5 ± 0.2 μg/g of wet liver (53) versus 0.57 ± 0.13 ng/ml of serum), and it has been demonstrated that the liver has an important role in the excretion of manganese (54, 55). Comparison of the liver copper content with serum concentrations also shows a difference (7 μg (56) or 11.6 ± 4 μg/g of wet liver (57) versus 1.16 ± 0.37 μg/ml of serum). Copper is also principally excreted into the bile (56). Thus, similar mechanisms can be postulated for the increase in serum copper during acute hepatitis. There is no full agreement concerning the plasma or serum zinc concentration during acute hepatitis (4, 7, 10, 11, 58). Our results agree with previous findings of some authors (4, 58) but disagree with those of others (7, 10, 11). The normal zinc concentration in liver is reported as being 67 ± 20 μg (56) or 70 μg/g of wet liver (27). During acute icteric viral hepatitis, however, an increased urinary zinc excretion has been reported (7, 11). It seems possible that the release of zinc during liver cell necrosis is counterbalanced by an increased urinary zinc excretion, resulting in a normal serum zinc value in most such patients.

In our cases of chronic persistent hepatitis, no abnormalities of serum manganese, copper, or zinc concentration were observed.

In cases of chronic aggressive hepatitis and postnecrotic cirrhosis, a statistically significantly increased serum manganese level was also found. The values for serum zinc are dispersed. Abnormally low values were found in one case of chronic aggressive hepatitis and in three cases of advanced cirrhosis. These observations are in harmony with previously reported data (10).

We believe that the statistically highly significant positive correlation between serum aminotransferasees and serum manganese permits us to postulate that an increased value of the latter can be an index to liver cell damage.

Although some manganese can be excreted by auxiliary gastrointestinal routes, especially in conditions of overloading, excretion by way of the bile constitutes the main regulatory route (54). Thus, determinations of serum manganese concentrations in various cholestatic syndromes are also needed.

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References


