Trace Element Status in Alcoholism Before and During Disulfiram Treatment*

P. GRANDJEAN, M.D., PH.D.,†
K. KRISTENSEN, M.D.,‡ P. J. JØRGENSEN, M.Sc.,§
G. D. NIELSEN, M.Sc.,† and O. ANDERSEN, PH.D.†

†Department of Environmental Medicine,
Odense University,
5000 Odense C, Denmark,
and
‡Department of Psychiatry
and §Department of Clinical Chemistry,
Odense University Hospital,
5000 Odense, Denmark

ABSTRACT

Trace element status was ascertained in 19 alcoholic patients under long-term treatment with disulfiram and in 12 alcoholic patients treated for the first time; the latter group was re-examined after four weeks of treatment. Both groups were compared to matched controls with a moderate alcohol intake. The copper/zinc ratio in serum was increased in the patients under long-term treatment, and a significant decrease occurred during the first four weeks of treatment in the second group. Blood lead was slightly increased in the second group, but not in the patients under long-term treatment. Blood cadmium was high in both groups, perhaps related to excessive tobacco smoking. Blood mercury concentrations were uniformly low. Urinary nickel excretion in the first group was above reference values, and an increase was seen in the second group during the treatment period. Although trace element concentrations in body fluids may not reflect tissue levels, the results support the notion that trace element balances are influenced by alcoholism and disulfiram treatment.

Introduction

Trace element status depends both on intake levels and on metabolic factors. In this regard, the influence of high alcohol intake is of particular interest. Thus, wine may contain considerable amounts of lead, and the intake of other trace elements may be decreased owing to the nutritional changes which often accompany a high energy intake from alcohol. Further, alcohol itself may interfere
directly with trace element absorption or retention.\textsuperscript{10,30} For example, ingestion of small amounts of alcohol is related to a small increase in blood lead levels in the absence of alcohol-related disease.\textsuperscript{12} In patients with severe alcoholism, significant changes in trace element status have been documented with decreased serum levels of selenium\textsuperscript{2} and zinc (S-Zn),\textsuperscript{5,15,27} and increased concentrations of copper in serum (S-Cu)\textsuperscript{5} and lead in blood (B-Pb).\textsuperscript{9,18,32} Also, the ratio between copper and zinc concentrations in serum is increased in patients with alcoholism.\textsuperscript{5,10}

Disulfiram (tetraethylthiuram disulfide, Antabuse\textsuperscript{®}) is widely used for the alcohol aversion therapy. This compound is metabolized into two molecules of diethyldithiocarbamate\textsuperscript{25} that may chelate trace metals and change their distribution in the body. Experimental data show that the biokinetic behavior of mercury,\textsuperscript{22} copper and zinc,\textsuperscript{1} nickel,\textsuperscript{24} lead,\textsuperscript{23} and cadmium\textsuperscript{3} may be changed considerably by disulfiram or its metabolite. Increased nickel levels in body fluids have been demonstrated in disulfiram-treated patients,\textsuperscript{14} but available information on blood concentrations of other trace elements has not documented treatment-related effects.

Trace element levels have been examined in body fluids in alcoholics before and during disulfiram treatment and the levels compared to those seen in a reference group with a low intake of alcoholic beverages.

Materials and Methods

Human Subjects

Two groups of patients with alcohol abuse for at least two years were identified prospectively through the alcoholism treatment clinic at Odense University Hospital. Alcoholism in these patients was mainly a social and psychiatric problem, and cases of liver cirrhosis or other alcohol-associated disease were not included.

One group had been treated repeatedly over a long-term period (up to 10 years) and consisted of 18 males and one female (aged 28 to 54 years); all individuals were under current disulfiram treatment (800 mg twice a week) which had lasted for at least one month. Patients were excluded in case of other psychiatric disease, if drug abuse was suspected, and if occupational exposure to metals had occurred.

The second group consisted of alcoholics treated for the first time or only treated very briefly in the past; this group included six males and six females (aged 31 to 49 years). The exclusion criteria were as in the first group. These patients were examined both before and after about four weeks of disulfiram treatment (500 mg twice a week). Seven additional patients were scheduled for inclusion in this group but did not complete the sampling protocol.

All members of the two patient groups had consumed 100 to 400 g of alcohol daily during the week before the initiation of aversion treatment. All patients were smokers (15 to 30 cigarettes per day).

Two different control groups were selected from the general population in Odense and two rural communities. These individuals were identified from a random sample of 200 individuals aged 20 to 69 years; the list was obtained through the county registry of residents. Members of the control groups were selected as age and sex matched individuals; if the first individual selected was unwilling to participate or had an alcohol use of more than one unit (corresponding to one beer) per day, the subsequent match was selected. If a matched individual could not be identified from the list, a deviation of ± one yr in age was allowed. Smoking and other possible
predictors were not considered as matching parameters. Of the 30 control subjects, 15 were cigarette smokers, four smoked a pipe only, and 11 were non-smokers. Eleven subjects consumed one to three units of alcohol (one unit corresponding to one beer) per day, while all other control subjects were abstainers or consumed alcoholic beverages only occasionally.

Informed consent was obtained from all individuals examined. The protocol was prepared in accordance with the Helsinki II convention and reported to the local ethical review committee.

**Methods**

Blood was drawn by venipuncture with a teflon i.v. catheter* into acid-washed 10 ml Minisorp® polyethylene tubes† under carefully controlled conditions to avoid sample contamination. Further, to assess the nickel excretion levels in the two patient groups, a morning spot urine was collected in acid-washed polyethylene containers. The samples were frozen at −80°C until analysis.

Electrothermal atomic absorption spectrometry was used to determine B-Pb, B-Cd and urine nickel (U-Ni) on a Perkin-Elmer model 5000 atomic absorption spectrometer with Zeeman background correction, HGA-500 graphite furnace, and an AS-40 autosampler.‡

The B-Pb results were read in duplicate against a blood-based standard curve. The total analytical imprecision was estimated to be 8.1, 2.1 and 1.9 percent at B-Pb levels of 0.24, 1.63, and 2.96 μmol per l, respectively. The accuracy of the lead determinations was ensured by using Seronorm® Trace Element batch 901, 902 and 903 as quality control material. The lead concentrations found in 150 determinations averaged 0.24, 1.63 and 2.96 μmol per l, respectively (assigned values were 0.3, 1.6 and 3.2 μmol per l).

The B-Cd results were read in duplicate against a blood-based standard curve. The total analytical imprecision was estimated to be 13.5, 7.3 and 4.8 percent at B-Cd concentrations of 0.008, 0.017 and 0.037 μmol per l, respectively. The accuracy of the cadmium determinations was ensured by using Seronorm® Trace Element batch 902 as quality control material; the cadmium concentrations averaged 0.0083 μmol per l in 19 determinations (assigned value, 0.008 μmol per l).

The U-Ni was determined by the method of standard addition. The total analytical imprecision was estimated to be 24.3, 11.7 and 7.6 percent at U-Ni levels of 0.035, 0.057 and 0.126 μmol per l, respectively. The accuracy of the nickel determinations was ensured by using Lanonorm® Metalle as quality control material; measured nickel concentrations averaged 0.057 μmol per l in 12 determinations (assigned nickel value, 0.06 ± 0.01 μmol per l).

The S-Zn and S-Cu concentrations were determined in undiluted serum by flame atomic absorption spectrophotometry on a Perkin-Elmer atomic absorption spectrometer model 403 using matrix-matched standards in 12 percent glycerol. The total analytical imprecision for S-Zn was estimated to be 4.9 and

---

* Venflon®, Viggo AB, Hälsingborg, Sweden.
† Nunc, Roskilde, Denmark.
‡ Perkin-Elmer, Norwalk, CT, USA.
§ Gaithersburg, MD, USA.
3.2 percent at S-Zn concentrations of 11.8 and 13.9 μmol per l, respectively. The corresponding values for S-Cu were 5.0 and 2.7 percent at S-Cu levels of 13.4 and 16.3 μmol per l, respectively. The accuracy of these determinations was ensured by using Seronorm® batch 1677 as quality control material. The measured zinc concentration (N = 12) averaged 11.8 μmol per l (assigned value, 11.5 μmol per l), while the copper concentrations (N = 15) averaged 16.3 μmol per l (assigned value, 17.1 μmol per l).

Blood mercury (B-Hg) was analyzed as described by Magos and Clarkson19 using a UV-detector.**

To enable adjustment of urinary nickel excretion in morning spot urine, U-creatinine was measured on an AutoAnalyzer II by the procedure recommended by the manufacturer.†† Also, as S-Zn and S-Cu levels depend on the presence of carrier proteins, albumin was measured.13 Further, possible liver dysfunction owing to alcohol was assessed in the two patient groups by measuring alkaline phosphatase, alanine aminotransferase (ALAT) and gamma-glutamyl transpeptidase (GGT) according to the methods of the Scandinavian Committee on Enzymes.7,8

Results

Although alcoholic liver cirrhosis had not been diagnosed in the two patient groups, increased levels of liver enzymes were seen, in particular ALAT and GGT. Thus, four patients from the group under long-term disulfiram treatment (21 percent) and eight from the second group (67 percent) had an ALAT and/or GGT level above the reference interval. Except for two patients with an increase in ALAT, all members of the second group showed a decrease in enzyme levels when examined after four weeks of disulfiram treatment; the decrease in GGT was statistically significant (p < 0.01, Wilcoxon test). At the second examination, four patients (33 percent) still had an enzyme level above the reference interval.

When compared to the control group, the patients under long-term disulfiram treatment showed generally minor deviations in trace element status (table I).

The S-Zn concentrations were comparatively low, while S-Cu levels were relatively high. Thus, the copper/zinc ratio in the patient group was somewhat higher than in the controls, but this difference was not statistically significant. No obvious relationship with S-albumin was apparent (median, 742 μmol per l; range, 667 to 803 μmol per l), and all S-albumin results were within the reference interval.

The B-Cd was much higher in the patients under long-term treatment than in the controls. As all patients were smokers compared to only 10 of the controls, this difference could be at least partly owing to smoking habits. The B-Cd of the 10 smoking controls (median, 0.018 μmol per l; range, 0.001 to 0.044 μmol per l) was significantly lower when compared to the total patient group or to the 10 patients with

<table>
<thead>
<tr>
<th>Trace Element</th>
<th>Median</th>
<th>Range</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Cu</td>
<td>16.8</td>
<td>13.5</td>
<td>23.5</td>
<td>11.6</td>
</tr>
<tr>
<td>S-Zn</td>
<td>12.3</td>
<td>5.0</td>
<td>14.9</td>
<td>9.5</td>
</tr>
<tr>
<td>S-Cu/S-Zn</td>
<td>1.34</td>
<td>0.99</td>
<td>2.28</td>
<td>0.84</td>
</tr>
<tr>
<td>B-Pb</td>
<td>0.18</td>
<td>0.05</td>
<td>0.36</td>
<td>0.25</td>
</tr>
<tr>
<td>B-Hg</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.012</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>B-Cd</td>
<td>0.037*</td>
<td>0.017</td>
<td>0.071</td>
<td>0.009</td>
</tr>
<tr>
<td>U-Ni</td>
<td>22.2</td>
<td>8.3</td>
<td>48.4</td>
<td>-----</td>
</tr>
</tbody>
</table>

* LDC Mercury Monitor, Milton Roy, FL, USA.
†† Technicon, Tarrytown, NY, USA.

** p < 0.01 when compared to controls (one-tailed Mann-Whitney U-test).
whom they were matched by age (p < 0.01 in both cases).

The B-Pb levels were slightly lower in the patients than in the controls, while B-Hg concentrations were almost uniformly low. No control values were available for nickel, but the U-Ni excretion appeared relatively high when compared to recent findings recorded in the literature.

The effect of four weeks of disulfiram treatment was reflected in small changes in the trace element levels in the second patient group (table II). Zinc and copper levels changed only little, but the copper/zinc ratio decreased significantly during treatment. Only small changes in S-albumin occurred, and no important difference was seen when compared to the control group.

The median B-Cd remained about at the same level the four weeks of treatment, although one patient showed a decline from a high 0.044 to 0.020 μmol per l. No difference from the control group (where five were current smokers) was apparent.

The B-Pb levels decreased somewhat during the treatment period, but no significant difference from the control group was seen. Also in this group, the B-Hg concentrations were very low. In contrast, the U-Ni excretion increased significantly during the treatment period.

Although comparison of the levels seen in the two patient groups may not be warranted, the lead levels in the first patient group were significantly lower (p < 0.01, Mann-Whitney U-test) than the levels seen in the second group, both before and after four weeks of treatment. Further, the urinary excretion of nickel was somewhat higher in the first than in the second group, but this difference did not reach statistical significance.

Discussion

The study of alcohol interference with trace element metabolism is fraught with difficulties. Alcoholic beverages may contain some trace elements, notably lead, in high concentrations. Further, a high intake of liquids and the resulting increased urinary flow could influence the biokinetics of trace elements. Also, alcohol represents a considerable amount of energy, and nutrient intake is therefore likely to depend on the amount of alcoholic beverages consumed. As a result of alcohol aversion therapy with disulfiram, dietary habits and trace element intake will change. Some patients

<table>
<thead>
<tr>
<th>Trace Element</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Cu</td>
<td>17.2</td>
<td>16.5</td>
<td>15.7</td>
</tr>
<tr>
<td>S-Zn</td>
<td>12.1</td>
<td>12.6</td>
<td>12.1</td>
</tr>
<tr>
<td>S-Cu/S-Zn</td>
<td>1.53</td>
<td>1.27*</td>
<td>1.30</td>
</tr>
<tr>
<td>B-Pb</td>
<td>0.30</td>
<td>&lt; 0.005</td>
<td>0.17</td>
</tr>
<tr>
<td>B-Hg</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>0.006</td>
</tr>
<tr>
<td>B-Cd</td>
<td>0.024</td>
<td>0.025</td>
<td>0.007</td>
</tr>
<tr>
<td>U-Ni</td>
<td>14.8</td>
<td>20.8*</td>
<td>---</td>
</tr>
</tbody>
</table>

*p < 0.05 when compared to levels before treatment (one-tailed Wilcoxon test).
may also smoke more or otherwise change their habits with possible significance for the trace element balance. Although a control group has been included in this study, and one group of patients was examined both before and during disulfiram treatment, the caveats noted previously must be kept in mind when evaluating the results of the present study.

The effects of disulfiram can be examined both in the patients with long-term treatment (table I) and in the patients examined before and after four weeks of treatment (table II). In general, rather limited changes were seen.

The copper/zinc ratio tended to be higher than expected. This finding is in accordance with previous studies of patients with severe alcoholism. That S-Zn levels were not very low in the present study could perhaps be a reflection of a fairly adequate nutrition and normal S-albumin in this group of patients that cooperated fully in the study protocol and had no additional problems of substance abuse.

The increased B-Pb level in alcoholics would be expected from the earlier finding that B-Pb tends to increase with the amount of alcohol consumed. However, much higher lead levels have been seen in wine drinkers and in relation to liver cirrhosis. Not surprisingly, the B-Pb levels decreased during treatment, but the present study cannot distinguish between an effect of abstainment as such and that of the disulfiram treatment. The lack of a significant change in B-Pb levels during the four weeks of disulfiram treatment could well be owing to the long biological half-life of lead in the body.

The low mercury concentrations are most likely related to the diet being poor in fish. With the limited body burdens of this metal, any changes induced may be difficult to detect.

The high B-Cd concentrations in the patient group are undoubtedly related to the heavy smoking. Although the patients in the first group that had undergone long-term disulfiram treatment tended to have the highest cadmium levels, no change in the average B-Cd was seen during the four-week treatment of the second group. However, some of the patients may have smoked more during the treatment period, thus masking any effects of the treatment. That smoking is a major risk indicator was confirmed in the control group where smokers had much higher blood cadmium concentrations than non-smokers.

The increased urinary nickel excretion as a result of the treatment deserves attention. Unfortunately, no control data were obtained in this study, but Sunderman et al. noted that individuals without excess nickel exposure normally show nickel excretion levels below 5 μg per g creatinine (9.6 μmol per mol creatinine), i.e., less than half of the median seen in the patients examined in the present study. Thus, these results tend to support the previous finding that disulfiram appears to cause an increased nickel excretion in urine.

The present study has dealt with trace element levels in body fluids only. However, experimental data have shown considerable effects on trace element retention, in particular in the brain. Despite the fact that levels in body fluids will tend to reflect major changes in trace element metabolism, increased retention in certain tissues may not necessarily be related to any change in the concentrations in blood or urine. Also, long-term changes may be less dramatic than those seen in experiments with single doses of trace metals. For example, chronic disulfiram treatment was unable to change the organ distribution of aged cadmium depots in mice.

Although limited, the results from the present study appear to support the evi-
dence that both alcohol intake and disulfiram treatment may interfere with trace element metabolism. In this regard, analysis of body fluids for trace elements may be of use, in particular B-Pb and U-Ni, although the changes seen may be much less than those occurring in the tissues. The interference caused by disulfiram will be of particular relevance to patients with current exposures to excess levels of toxic metals or deficiencies of essential trace metals.

Acknowledgments

The skillful assistance of Hanne Albæk, Brita Andersen, and Ranja Bjerring is greatly appreciated. The study was supported by grants from the Danish Medical Research Council and the Danish Health Foundation.

References

25. STÖRÖMME, H. E.: Metabolism of disulfiram and diethylthiocarbamate in rats with demonstra-

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION
(Act of October 23, 1962, Section 4369, Title 39, United States Code)

Date of Filing—September 20, 1989
Title of Publication—ANNALS OF CLINICAL AND LABORATORY SCIENCE
Frequency of Issue—Bimonthly
Location of Known Office of Publication—301 South Eighth Street, Philadelphia, PA 19106
Location of the Headquarters or General Business Offices of the Publisher—Same as above
Publisher—Institute for Clinical Sciences, Inc.
Editor—F. William Sunderman, M.D., Ph.D.
Managing Editor—Same as above
Owner—Institute for Clinical Sciences, Inc.

<table>
<thead>
<tr>
<th>Average No. Copies Each Issue During Preceding 12 Months</th>
<th>Actual Number of Copies of Single Issue Published Nearest to Filing Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Total No. Copies Printed (Net Press Run)</td>
<td>1100</td>
</tr>
<tr>
<td>B. Paid Circulation</td>
<td></td>
</tr>
<tr>
<td>1. Sales Through Dealers and Carriers, Street Vendors and Counter Sales</td>
<td>None</td>
</tr>
<tr>
<td>C. Total Paid Circulation</td>
<td>2005</td>
</tr>
<tr>
<td>D. Free Distribution by Mail, Carrier or Other Means, Samples, Complimentary, and Other Free Copies</td>
<td>None</td>
</tr>
<tr>
<td>E. Total Distribution (Sum of C and D)</td>
<td>2005</td>
</tr>
<tr>
<td>F. Copies not Distributed</td>
<td></td>
</tr>
<tr>
<td>1. Office Use, Left-over, Unaccounted Spoiled After Printing</td>
<td>95</td>
</tr>
<tr>
<td>2. Return from News Agents</td>
<td>None</td>
</tr>
<tr>
<td>G. Total (Sum of E and F)</td>
<td>1100</td>
</tr>
</tbody>
</table>

I certify that the statements made by me are correct and complete—F. WILLIAM SUNDERMAN, M. D., Ph. D.