CADMIUM TURNOVER AND CHANGES OF ZINC AND COPPER BODY STATUS OF RATS CONTINUOUSLY EXPOSED TO CADMIUM AND ETHANOL

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Abstract — The effects of continuous exposure to cadmium (Cd) and ethanol on Cd turnover and zinc (Zn) and copper (Cu) body status of male Wistar rats were studied. The animals received an aqueous solution of 10% (w/v) ethanol and/or 50 mg Cd/l as the only drinking fluid for 12 weeks. The concentrations of Zn, Cu and Cd in the serum (or blood), liver, kidneys, spleen, brain, heart, femoral muscle and femur as well as in 24-h urine and faeces specimens were assessed by atomic absorption spectrometry (AAS). Ethanol alone had no effect on Cd accumulation or excretion. By contrast, co-administration of ethanol with Cd influenced the turnover of this toxic metal. Long-term consumption of ethanol alone caused a decrease in femur Zn and liver Cu concentrations. Moreover, the urinary loss of both bioelements decreased, whereas their faecal excretion was increased. Exposure to Cd resulted in an increase in liver and kidney and in a decrease in femur 24-h urine Zn concentrations. An increase in Cu concentration in the kidney and a decrease in the brain were also noted. Moreover, Cd increased the total pool of Zn in organs (kidneys, liver, spleen, heart and brain), but did not influence that of Cu. Zn concentration in the liver, kidney and spleen of rats co-exposed to Cd and ethanol were increased, but were decreased in the brain and femur, compared to controls. The concentrations of Cu in livers and brains of these rats were decreased, whereas those in kidney, spleen and heart were increased. The urinary excretion of the elements was decreased, whereas their faecal excretion was increased. Moreover, the total amount of Cu in organs decreased below the control value and that of Zn was in the normal range. These changes in Zn and Cu levels could be explained by different effects of both toxic substances, differences in bioelement intakes (due to reduced consumption of drinking solutions and food), and the modifying effect of ethanol on Cd turnover. Our results suggest that alcoholics may be more susceptible to Cd accumulation and its effects on body Zn and Cu.

INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals. Exposure to this metal can occur in the workplace and in the natural environment because it is utilized in a number of industrial practices and is a ubiquitous contaminant of the environment and dietary products (World Health Organization, 1992). Cd toxicity in humans (World Health Organization, 1992; Jarup et al., 1998) and experimental animals (Sharma et al., 1991; World Health Organization, 1992; Moniuszko-Jakoniuk et al., 1999, 2001; Brzó ska et al., 2000, 2001) has been widely studied and reported. But in spite of that, not all aspects of its action have been sufficiently recognized.

It is well known that the metabolism and toxicity of Cd may be modified by many factors, including substances essential for life (Berglund et al., 1994; Moon, 1994; Brzó ska and Moniuszko-Jakoniuk, 1998; Brzó ska et al., 2001) as well as very toxic chemical compounds (Hopf et al., 1986, 1990; Sharma et al., 1991, 1992; Skoczyńska and Smolik, 1994; Brus et al., 1995; Galażyn-Sidorczuk et al., 1998; Moniuszko-Jakoniuk et al., 1999, 2001; Brzó ska et al., 2000). One of these substances is ethanol.

Interactions between Cd and ethanol are an important problem in modern toxicology. Because of the excessive consumption of ethanol by a large part of the population, especially smokers, exposed environmentally and/or occupationally to Cd (Grasmick and Huel, 1985; Schoeler, 1991; Samson and Harris, 1992). Previously we have reported that even short-term, low-level ethanol administration to Cd-exposed rats enhanced this heavy metal body burden and modified some of its toxic effects, including bioelements metabolism (Galażyn-Sidorczuk et al., 1998; Moniuszko-Jakoniuk et al., 1999, 2001; Brzó ska et al., 2000).

Microelements such as zinc (Zn) and copper (Cu) play an important role in metabolic pathways affected by Cd and ethanol. Disturbances in metabolism of these metals in humans and experimental animals have been observed after chronic Cd intoxication (Bonner et al., 1980; Mahaffey et al., 1981; Hopf et al., 1990; Sharma et al., 1991; Galażyn-Sidorczuk et al., 1998; Brzó ska et al., 2000, 2001; Oishi et al., 2000) as well as a consequence of excessive consumption of ethanol (Hopf et al., 1986; Sharma et al., 1991; Gonzalez-Reimers et al., 1993; Rodriguez-Moreno et al., 1997; Floriańczyk, 2000). However, up till now the metabolism of essential elements in conditions of long-term treatment with Cd and ethanol has not been successfully investigated.

Taking the above into account, it was considered of interest to investigate the effect of long-term Cd and ethanol consumption on Cd body burden and toxicity, including changes in bioelements metabolism. In this paper, we report the effect on Cd turnover and body status of Zn and Cu. Other results will be published in separate papers.

MATERIALS AND METHODS

Animals

We used 68 locally bred adult male Wistar rats of initial body weight of ~250 g. All animals were kept under the same standard laboratory conditions. They were freely fed standard pelleted LSM diet (Fodder Factory, Motycz, Poland) and had unlimited access to drinking water. The LSM diet is designed for laboratory animals, especially rats and mice. It is prepared from: corn, wheat, barley, wheat bran, soya-bruised grain, meat starch, skimmed powdered milk, phosphate, fodder-chalk, mineral and vitamin premix. Metabolizable energy of the diet is 12.2 MJ/kg. Cd, Zn and Cu concentrations in the LSM diet were assessed by us to be 0.211, 63.6 and 5.28 mg/kg, respectively.

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Chemicals

All reagents and chemicals were of analytical grade or higher purity. Trace pure nitric (HNO₃) and hydrochloric (HCl) acids (Merck) as well as Cd, Zn and Cu standard solutions assigned for atomic absorption spectrometry (Sigma) were used in metals analysis.

Experimental design

The experiment was conducted up to 12 weeks. The animals (at the age of 10 weeks) were randomly divided into four experimental groups of 17 each: (1) a control group received redistilled water to drink during the whole period of the experiment; (2) an ethanol group received an aqueous solution of 10% (w/v) ethanol (rectified Spirit, POLMOS, Poland) as the only drinking fluid; (3) a Cd group was exposed to 50 mg Cd/l in the form of aqueous solution of CdCl₂ (POCh, Gliwice, Poland) administered as the only drink; (4) a Cd + ethanol group received as the only drinking fluid an aqueous solution containing simultaneously 50 mg Cd/l and 10% ethanol.

All fluids were administered ad libitum and their consumption was measured daily during the whole experiment. Food ingestion was also assessed. Twenty-four-hour urine collection (in glass metabolic cages) was done before, and every second week in the course of, the experiment. In the 12th week, 24-h faeces was collected during three consecutive days for Zn and Cu analysis. Seven animals of each group were killed after 8 weeks, and the remaining 10 after 12 weeks, of the intoxication. At the end of the experiment, the rats were weighed and 24-h urine and faeces were collected. Next, after overnight starvation, but with free access to drinking fluids, blood from the heart, and liver, kidneys, spleen, heart, brain, femur and femoral muscle were removed under ether anaesthesia. The animals were killed between 08.00 and 10.00.

Whole blood was centrifuged after coagulation and serum was separated immediately. The soft tissues were washed thoroughly in physiological saline, the femurs were cleansed of muscle tissue as much as possible. Next, they were weighed and submitted to dry mineralization. The biological material not used immediately after collection was frozen at –20°C until further analysis. In the material collected after 12 weeks of the experiment, concentrations of Cd, Zn and Cu were determined, whereas in that collected after 8 weeks, only Cd was analysed.

Analytical procedures

Preparation of tissues and biological fluids for metals analysis

The whole blood collected in anticoagulant was digested with 5% HNO₃, according to Raźniewska and Trzcinka-Ochocka (1995). The weighed hepatic and muscle tissue slices (~1 g) and the whole spleen, brain, heart, femur, right kidney and half of the left kidney as well as 24-h faeces were dry mineralized at 450°C in an electric oven. After ashing, the samples were dissolved in 10 ml of 1 M HNO₃, (soft tissues) or 1 M HCl (femur and faeces). The concentrations of metals (Cd, Zn and Cu) in such prepared mineralizates (after dilution with HNO₃, or HCl) as well as in serum and urine samples (after dilution with redistilled water) were determined by atomic absorption spectrometry (AAS) (Zeiss Jena AAS 30, Germany). Concentrations of metals in tissues were expressed as μg/g of wet weight.

Cd determination. Cd was determined by a flameless AAS method with electrothermal atomization in a graphite cuvette. The cathode lamp of Cd was operated under standard conditions using its respective resonance line 228.8 nm. Working standards containing 2.0, 4.0, 6.0, 8.0 and 10.0 ng Cd/ml were prepared from stock atomic absorption standard solutions containing 1005 μg Cd/ml.

Zn and Cu determinations. The concentrations of Zn and Cu were measured by a flame (an air–acetylene burner) AAS method. Working standards of Zn and Cu containing 0.2, 0.4, 0.6, 0.8 and 1.0 μg/ml were prepared from stock atomic absorption standard solutions containing 1000 μg Zn/ml or 1010 μg Cu/ml. The readings were recorded against suitable standards at 213.9 nm for Zn and at 324.75 nm for Cu.

Total Cd, Zn and Cu pools in organs. On the basis of assessed concentrations of metals in the soft tissues, their total contents in the liver and kidneys, the sum in both organs as well as the whole pool in all studied organs (kidneys + liver + spleen + heart + brain) were calculated.

Blood-ethanol concentration. The concentration of ethanol in the blood was analysed by headspace gas chromatography according to the manufacturer’s recommendation with our own modification. The Hewlett-Packard 5890 chromatograph (Series II) was used.

Statistical methods

To assess differences of all parameters studied between the four experimental groups, statistical analysis of data was conducted by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. P < 0.05 was considered to be statistically significant. Pearson’s correlation analysis was conducted for the relationship between Cd accumulation and Zn or Cu tissue concentrations.

RESULTS

Food consumption and body weight gain

In the rats’ drinking water containing ethanol alone, a tendency to decreased food consumption, in comparison with control, was observed, but there was no statistically significant difference (Table 1). The same was true for the Cd intoxicated group (Table 1). The alcoholized animals co-exposed to Cd, however, ate less food. These data on food intake were reflected in body weight gain data (Table 1). Thus, administration of 50 mg Cd/l or 10% ethanol in drinking water for 12 weeks had no influence on rats’ body weight gain, whereas co-exposure to Cd and ethanol caused retardation in body weight. Body weight gain of animals simultaneously intoxicated with these substances in the course of the 12-week period of the experiment was 61% (P < 0.001) of that of controls.

Fluids consumption and Cd and ethanol intakes

The administration of Cd and ethanol alone as well as together reduced drinking fluid consumption (Table 1, Fig. 1). The rats exposed to these substances consumed less fluids from the first to the last day of their administration. In the animals drinking water containing only Cd or ethanol, fluids consumption was 63 and 48% (P < 0.001) of that of controls, respectively. Simultaneous administration of both substances further decreased fluid consumption. Drinking water ingestion
in the co-exposed animals was only 40% (P < 0.001) of that of controls.

As a result of decreased fluids consumption, the intake of Cd and ethanol in the Cd + ethanol group was only 62% (P < 0.001) and 82% (P < 0.01) of that in animals exposed separately to these substances, respectively (Table 1).

**Blood-ethanol concentration**

The concentration of ethanol in the blood of rats which did not drink ethanol (the control and Cd groups) was either zero or within the low physiological range (Table 1). Ethanol concentration in the ethanol and Cd + ethanol groups was also relatively low (Table 1), almost certainly because of the nocturnal drinking of small amounts of fluid several hours before the rats were killed.

**Cadmium**

*Cd concentration in the whole blood and tissues.* Ethanol administered alone as well as in combination with Cd for up to 12 weeks had no influence on blood Cd concentration (Table 2). Cd concentrations in all tissues of control rats were low. As for blood, the administration of ethanol alone had no significant effect on Cd concentration in various other tissues. Exposure to Cd resulted in its accumulation in all the organs and tissues studied. Most Cd was deposited in kidneys and liver, while the lowest levels were noted in brain and muscle tissue. In liver, kidney, heart and femoral muscle, but not in spleen, brain and femur, an increase in Cd concentration was noted between the 8th and 12th weeks of treatment (Table 2).

As a result of Cd administration for 12 weeks, its concentration in kidney, liver, spleen, heart, femur, muscle tissue and brain was increased (P < 0.001) by about 700-, 433-, 76-, 27-, 16-, 3- and 2-fold compared to the control values, respectively. After 8 weeks of co-exposure to Cd and ethanol, the concentration of Cd in the liver, kidney, spleen, heart, brain, muscle tissue and femur was in the range of values noted in the Cd-exposed group. On the other hand, after 12 weeks of simultaneous administration of both substances, Cd concentration in kidney, spleen, heart and femur was lower than in the rats intoxicated with Cd alone: about 72, 74, 70 and 66% of that in the Cd group, respectively (P < 0.001). But the longer co-administration of ethanol and Cd had no influence on Cd concentration in liver, brain or muscle tissue.

*Total Cd content in liver and kidneys and whole Cd pool in organs.* Almost all Cd (about 99.3–99.6%) accumulated in internal organs of rats exposed to Cd alone or in conjunction with ethanol was retained in the kidneys and liver. Higher amounts of this toxic metal were deposited in the liver than in the kidneys (data not presented).

Total Cd contents in the kidneys + liver as well as the whole pool of this toxic metal in organs in the Cd + ethanol group after 8 weeks of exposure were similar to those in the animals exposed to Cd alone (Fig. 2; only the whole pool of Cd is presented). By contrast, after 12 weeks of exposure, the total Cd contents in the kidneys + liver and its whole pool in the studied organs of co-exposed rats were lower (P < 0.001) by 31 and 31%, respectively compared to the group exposed to Cd alone (Fig. 2).

*Urinary and faecal Cd excretion.* In the Cd-exposed rats (the Cd and Cd + ethanol groups), this metal was excreted in urine (Fig. 3) and faeces (Fig. 4) in considerably higher amounts than in those drinking water without Cd (the control and ethanol groups). Administration of ethanol alone and with

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**Table 1.** Body weight gain, food and drinking fluids consumption including cadmium (Cd) and ethanol intakes, and blood-ethanol concentration

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean consumption of the LSM diet (g/24 h/rat)</th>
<th>Mean body weight gain (g/12 weeks/rat)</th>
<th>Mean consumption of drinking fluids (ml/24 h/rat)</th>
<th>Mean Cd intake (mg/24 h/rat)</th>
<th>Mean ethanol intake (g/24 h/rat)</th>
<th>Blood-ethanol concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.5 ± 3.2</td>
<td>118.7 ± 15.92</td>
<td>43.68 ± 2.16</td>
<td>0.000</td>
<td>0.000</td>
<td>0.003 ± 0.001</td>
</tr>
<tr>
<td>Ethanol</td>
<td>20.8 ± 2.7</td>
<td>116.2 ± 11.18</td>
<td>21.13 ± 1.79*†</td>
<td>1.388 ± 0.083*†</td>
<td>5.202 ± 0.441*</td>
<td>0.012 ± 0.005*</td>
</tr>
<tr>
<td>Cd</td>
<td>23.9 ± 3.4</td>
<td>110.7 ± 23.41</td>
<td>27.75 ± 1.66†</td>
<td>0.866 ± 0.079*†</td>
<td>4.261 ± 0.389*</td>
<td>0.002 ± 0.001†</td>
</tr>
<tr>
<td>Cd + ethanol</td>
<td>17.2 ± 2.9†‡</td>
<td>72.7 ± 10.29†‡</td>
<td>17.31 ± 1.58*†‡</td>
<td>0.000</td>
<td>0.000</td>
<td>0.003 ± 0.001†</td>
</tr>
</tbody>
</table>

Control: control rats; the other groups were exposed to 10% ethanol and/or 50 mg Cd/l for 12 weeks. Values are means ± SD of 10 animals per group. *In calculation on 40% ethanol.

*†‡Values are significantly different (P < 0.05; analysis of variance plus Tukey–Kramer’s test) compared to the control, ethanol and Cd groups, respectively.

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**Fig. 1.** Effects of cadmium (Cd), ethanol (Et), and their co-administration on fluid consumption.

Each point represents the mean value of 10 rats. The animals were exposed to 10% ethanol and 50 mg Cd/l separately (ethanol and Cd groups) and in combination (Cd + ethanol group) for 12 weeks. There were statistically significant (P < 0.05; analysis of variance plus Tukey–Kramer’s test) differences between all experimental groups at any time-point.
Table 2. Effects of cadmium (Cd), ethanol, and their co-administration on Cd concentration in the whole blood and tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood (μg/l in blood or μg/g of wet tissue weight)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
<th>Brain</th>
<th>Femoral muscle</th>
<th>Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 8 w (n = 7)</td>
<td>0.733 ± 0.134</td>
<td>0.032 ± 0.013</td>
<td>0.033 ± 0.012</td>
<td>0.010 ± 0.009</td>
<td>0.008 ± 0.005</td>
<td>0.017 ± 0.008</td>
<td>0.015 ± 0.008</td>
<td>0.02 ± 0.008</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.856 ± 0.186</td>
<td>0.041 ± 0.011</td>
<td>0.046 ± 0.010</td>
<td>0.020 ± 0.008</td>
<td>0.011 ± 0.007</td>
<td>0.012 ± 0.006</td>
<td>0.012 ± 0.008</td>
<td>0.019 ± 0.008</td>
</tr>
<tr>
<td>Cd</td>
<td>18.036 ± 2.030*†</td>
<td>10.234 ± 2.023**†</td>
<td>18.683 ± 3.248**†</td>
<td>0.786 ± 0.213**†</td>
<td>0.186 ± 0.024**†</td>
<td>0.028 ± 0.003*†</td>
<td>0.40 ± 0.006**†</td>
<td>0.315 ± 0.031*†</td>
</tr>
<tr>
<td>Cd + ethanol</td>
<td>16.626 ± 1.944***†</td>
<td>10.923 ± 1.248***†</td>
<td>20.821 ± 3.444***†</td>
<td>0.594 ± 0.198***†</td>
<td>0.203 ± 0.038***†</td>
<td>0.045 ± 0.015***†</td>
<td>0.324 ± 0.029***†</td>
<td>0.072 ± 0.008***†</td>
</tr>
</tbody>
</table>

Control: control rats; the other groups were exposed to 10% ethanol and/or 50 mg Cd/l for 8 and 12 weeks. Values are means ± SD of seven (after 8 weeks) or 10 animals (after 12 weeks).

*†‡Values were significantly different (P < 0.05; analysis of variance plus Tukey–Kramer’s test) compared to the control, ethanol, and Cd groups, respectively.

Fig. 2. Effects of cadmium (Cd), ethanol (Et), and their combination on the whole Cd pool in organs. Each point represents a mean value of seven (after 8 weeks of the experiment) or 10 rats (after 12 weeks). *†Values were significantly different (P < 0.05) compared to the control and ethanol groups, respectively.
Cd for 8 and 12 weeks had no influence on Cd urinary excretion. The faecal excretion of Cd in the rats exposed to this heavy metal in combination with ethanol was, however, lower ($P < 0.001$) by 46% after 8 weeks and by 30% after 12 weeks of the experiment, compared to its excretion in those receiving Cd alone.

### Zinc and copper

**Zn concentration in serum and tissues.** Administration of 10% ethanol alone had no influence on serum or tissue Zn concentrations, except femur, where an 11% ($P < 0.001$) reduction was noted (Table 3). Exposure to Cd alone resulted in an increase ($P < 0.001$) in the liver (by 28%) and kidney (by 37%) with a simultaneous decrease ($P < 0.01$) in femur (by 11%) Zn concentration. In other tissues, the concentrations of Zn were similar to control values (Table 3). Ethanol and Cd alone had no influence on spleen and brain Zn concentrations, whereas their co-administration resulted in a 22% ($P < 0.001$) increase in the spleen and a 15% ($P < 0.001$) decrease in the brain Zn concentration compared to the control group. On the other hand, Zn concentration in the serum, heart and femoral muscle remained unchanged even in conditions of simultaneous exposure to ethanol and Cd (Table 3). In the liver, kidney and bone of rats simultaneously receiving both substances, changes in Zn concentration were the same as in the rats exposed to Cd alone. In the liver, kidney and spleen of animals co-exposed to Cd and ethanol, Zn concentration was higher by 20% ($P < 0.05$), 29% ($P < 0.001$) and 22% ($P < 0.01$), respectively while in the brain and femur it was lower by 15 and 10% ($P < 0.05$), respectively compared to control values.

**Total Zn content in organs.** Long-term exposure to ethanol alone did not change the total Zn content in organs, whereas Cd administered alone resulted in an increase (by 16%, $P < 0.05$) in the total Zn pool (Fig. 5). On the other hand, in the animals exposed simultaneously to Cd and ethanol, the total amount of Zn in organs was in the range of the control group and was lower (by 14%, $P < 0.05$) compared to the group of rats exposed to Cd and ethanol alone.

### Table 3. Effects of Cd, ethanol, and their combination on Zn concentration in serum and tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
<th>Brain</th>
<th>Femoral muscle</th>
<th>Femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.60 ± 0.19</td>
<td>31.08 ± 4.64</td>
<td>24.54 ± 2.49</td>
<td>19.09 ± 2.25</td>
<td>14.74 ± 1.38</td>
<td>11.79 ± 1.47</td>
<td>9.75 ± 1.77</td>
<td>188.03 ± 13.14</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.57 ± 0.17</td>
<td>29.97 ± 3.48</td>
<td>26.61 ± 1.29</td>
<td>19.30 ± 2.72</td>
<td>14.86 ± 1.69</td>
<td>10.74 ± 0.85</td>
<td>10.16 ± 1.11</td>
<td>166.45 ± 14.42*</td>
</tr>
<tr>
<td>Cd</td>
<td>1.53 ± 0.10</td>
<td>39.87 ± 4.95††</td>
<td>33.67 ± 5.09††</td>
<td>20.82 ± 2.83</td>
<td>15.30 ± 2.06</td>
<td>12.17 ± 1.43</td>
<td>10.18 ± 0.99</td>
<td>166.42 ± 10.53*</td>
</tr>
<tr>
<td>Cd + ethanol</td>
<td>1.43 ± 0.15</td>
<td>37.28 ± 3.69††</td>
<td>31.77 ± 2.41††</td>
<td>23.25 ± 2.99††</td>
<td>17.66 ± 5.01</td>
<td>10.05 ± 1.72†‡</td>
<td>9.72 ± 1.69</td>
<td>169.97 ± 14.80*</td>
</tr>
</tbody>
</table>

Control: control rats; the other groups were exposed to 10% ethanol and/or 50 mg Cd/l for 12 weeks. Values are means ± SD of 10 animals.

*†‡Values were significantly different ($P < 0.05$; analysis of variance plus Tukey–Kramer’s test) compared to the control, ethanol, and Cd groups, respectively.

*†‡Values were significantly different ($P < 0.05$; analysis of variance plus Tukey–Kramer’s test) compared to the control, ethanol, and Cd groups, respectively.
exposed to Cd alone. Similar changes were observed regarding the content of Zn in the kidneys, liver, and kidneys + liver (data not presented).

**Cu concentration in serum and tissues.** Long-term ethanol consumption led to a decrease (by 13%, \( P < 0.05 \)) in the liver Cu concentration. In other tissues of these rats, the concentration of Cu was in the range of control values (Table 4). Treatment with Cd alone increased the kidney (by 47%, \( P < 0.001 \)) and decreased brain (by 21%, \( P < 0.01 \)) Cu concentrations, but it did not affect those in serum, liver, spleen, heart, muscle and femur (Table 4). In rats co-exposed to Cd and ethanol, the kidney Cu concentration was decreased (by 19%, \( P < 0.01 \)) compared to the Cd alone intoxicated rats, but the level was still higher than that in controls. Heart and spleen Cu concentrations in conditions of single exposure to these substances were unchanged, but, after their co-administration, it increased compared to the control group. Cu concentration in the kidney, spleen and heart of co-exposed animals was higher by 19% (\( P < 0.05 \)), 62% (\( P < 0.01 \)) and 34% (\( P < 0.01 \)), respectively, whereas in liver and brain it was lower by 18% (\( P < 0.001 \)) and 24% (\( P < 0.01 \)), respectively compared to the appropriate control values. The muscle and femur Cu concentrations were unchanged by any treatment (Table 4).

**Total Cu content in organs.** In the rats exposed to ethanol alone, a decrease in the total liver content of Cu was noted (data not given). The Cu contents in the kidneys and kidneys + liver as well as the whole pool of the bioelement in organs (in Fig. 5 only the whole pool of Cu is presented) were unchanged in these animals. Administration of Cd increased the kidneys and decreased the liver Cu contents, while the sum of both (kidneys + liver) as well as the whole pool in organs remained unchanged. In rats simultaneously receiving Cd and ethanol, the Cu content in the kidneys, liver and kidneys + liver was lower than in the group exposed to Cd alone. As a result, a 22 and 18% reduction (\( P < 0.001 \)) in the whole amount of Cu in organs of co-exposed animals compared to the control and Cd-alone groups, respectively, was observed.

**Relationship between Cd accumulation and Zn or Cu tissue concentrations.** A significant positive correlation was noted between Cd accumulation and Zn concentration in the kidney (\( r = 0.7599, P = 0.000 \)), liver (\( r = 0.7337, P = 0.000 \)) and spleen (\( r = 0.4261, P = 0.006 \)) as well as the whole pool of both metals in internal organs (\( r = 0.4500, P = 0.004 \)). There was also a positive correlation between Cd and Cu concentrations in kidney (\( r = 0.7613, P = 0.000 \)), spleen (\( r = 0.4204, P = 0.007 \)), heart (\( r = 0.3340, P = 0.035 \)) and femoral muscle (\( r = 0.3234, P = 0.042 \)). By contrast, in the brain (\( r = -0.4010, P = 0.010 \)) and femur (\( r = -0.3579, P = 0.023 \)) a negative relationship was observed.

**Zn and Cu urinary excretion.** There were no differences among all groups in Zn (Fig. 6) and Cu (Fig. 7) excretion in the 24-h urine collected before the beginning of the experiment. In rats exposed to Cd alone or ethanol alone, as well as in combination, urinary Zn excretion was decreased (\( P = 0.01–0.0001 \)) already after 2 weeks. After 12 weeks of the experiment the urinary excretion of this bioelement in the Cd, ethanol and Cd + ethanol groups was still decreased by 62, 52 and 64%, respectively (\( P < 0.001 \)), in comparison with the control group (Fig. 6). At the end of the exposure period, the urinary excretion of Cu in the Cd, ethanol and Cd + ethanol groups was also decreased (Fig. 7), by 68, 72 and 79%, respectively (\( P < 0.001 \)). Shorter administration of Cd or ethanol alone had no influence on urinary Cu loss. But in the case of their co-exposure, a 34% (\( P < 0.001 \)) decrease in Cu excretion was noted already after 4 weeks. There were no significant differences in Zn as well as Cu urinary excretion between animals exposed to Cd or ethanol alone and those co-exposed for the whole experiment. But the disturbances in bioelement excretion were most evident in the Cd + ethanol group.

**Zn and Cu faecal excretion.** Exposure to Cd had no effect on faecal Zn or Cu excretion (data not given). Ethanol administered alone led to a 15% increase (\( P < 0.05 \)) in faecal excretion of Zn and a 19% increase in that of Cu. In rats co-exposed to Cd and ethanol, the faecal loss of Zn and Cu was higher (\( P = 0.05–0.01 \)) than in controls (by 9 and 12%, respectively) and those receiving Cd alone (by 11 and 14%, respectively).

**DISCUSSION**

This study was undertaken to evaluate the effect of long-term ethanol ingestion on Cd turnover and Zn and Cu body status in rats. Cd consumption in rats drinking water containing 50 mg Cd/l was equivalent to intake that can occur in humans, especially smokers, occupationally exposed to this metal or living in heavily contaminated areas (World Health Organization, 1992). This is confirmed by Cd levels in blood, tissues and urine noted in the Cd-exposed group. The daily intake of ethanol in the animals drinking water containing 10% ethanol was equivalent to consumption ~0.7 l/day of 40% ethanol in man. Since the rate of ethanol oxidation in rats is three times faster than in humans (0.1 g/kg body wt/h), the animals need a higher dose of ethanol than humans for the same toxic effects (Wiśniewska-Knyp and Wrońska-Nofer,

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**Table 4. Effects of cadmium (Cd), ethanol, and their combination on Cu concentration in serum and tissues**

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum (µg/ml)</th>
<th>Liver (µg/g)</th>
<th>Kidney (µg/g)</th>
<th>Spleen (µg/g)</th>
<th>Heart (µg/g)</th>
<th>Brain (µg/g)</th>
<th>Femoral muscle (µg/g)</th>
<th>Femur (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.26 ± 0.12</td>
<td>5.55 ± 0.92</td>
<td>5.92 ± 0.69</td>
<td>2.52 ± 0.89</td>
<td>4.68 ± 0.32</td>
<td>3.65 ± 0.52</td>
<td>0.98 ± 0.15</td>
<td>4.18 ± 1.07</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.21 ± 0.14</td>
<td>4.83 ± 0.39*</td>
<td>5.65 ± 0.70</td>
<td>2.43 ± 0.57</td>
<td>5.37 ± 1.05</td>
<td>3.14 ± 0.30</td>
<td>1.04 ± 0.27</td>
<td>4.23 ± 0.64</td>
</tr>
<tr>
<td>Cd</td>
<td>1.41 ± 0.17*</td>
<td>5.08 ± 0.56</td>
<td>8.68 ± 1.35*</td>
<td>3.27 ± 0.59</td>
<td>5.40 ± 0.94</td>
<td>2.88 ± 0.53*</td>
<td>1.03 ± 0.22</td>
<td>3.40 ± 0.61</td>
</tr>
<tr>
<td>Cd + ethanol</td>
<td>1.25 ± 0.14</td>
<td>4.57 ± 0.31*</td>
<td>7.07 ± 0.72*</td>
<td>4.09 ± 1.70*</td>
<td>6.26 ± 1.51*</td>
<td>2.75 ± 0.36*</td>
<td>1.22 ± 0.28</td>
<td>3.43 ± 0.72</td>
</tr>
</tbody>
</table>

Control: control rats; the other groups were exposed to 10% ethanol and/or 50 mg Cd/l for 12 weeks. Values are means ± SD of 10 animals.

*‡ Values were significantly different (\( P < 0.05 \)); analysis of variance plus Tukey–Kramer’s test) compared to the control, ethanol, and Cd groups, respectively.
substances to ether have a bad taste, and thus animals develop aversion to drinking them (Gur et al., 1995; Srubak et al., 1998; Brus et al., 1999). Moreover, ethanol is known to cause anorexia and weight loss (Srubak et al., 1998; Gupta and Gill, 2000). The effect of co-exposure to Cd and ethanol on the body weight gain observed in this study is supported by the results of other studies (Tandon and Tewari, 1987; Gupta and Gill, 2000).

Because rats simultaneously treated with Cd and ethanol developed stronger aversion to drink than those exposed separately to each substance, they also ingested less Cd and ethanol. As a result, ethanol concentration in the whole blood in the Cd + ethanol group was also lower than in the ethanol group. The difference in Cd intake is especially noteworthy and has been taken into account in interpretation of the results of this study. Alcoholics can also have lower Cd intake than their non-alcoholic counterparts, because they very often consume less food, which is the main source of exposure of the general population to this toxic metal.

Observations made in the present study suggest that ethanol may be a factor affecting Cd metabolism, but our results do not give definite proof. The same level of the Cd body burden in rats simultaneously receiving Cd and ethanol for 8 weeks as in those intoxicated with Cd alone, in spite of reduced Cd intake by ethanol co-administration, suggests that ethanol influences Cd turnover. In the absence of a modifying effect of ethanol, Cd concentration in whole blood and tissues as well as its whole pool in internal organs of co-exposed rats should be lower (because of its lower level of intake) than in the Cd-alone intoxicated animals. The unfavourable effect of ethanol is also visible when we compare the results obtained after 8 and 12 weeks of the experiment. In the liver and kidneys of rats co-exposed to Cd and ethanol, the concentration of this metal did not increase between the 8th and 12th weeks of the experiment, whereas in the animals receiving Cd alone, a further augmentation in its concentration in these organs was noted. It is difficult to explain why in the co-exposed rats the concentration of Cd in the liver and kidneys, the main sites of storage of this metal, did not increase during these extra 4-week period. In the first phase of exposure (perhaps up to the 5th week), ethanol probably enhanced the absorption of ingested Cd, but absorption of this metal might subsequently be lower. This conclusion is based on differences in Cd levels in blood and tissues after 8 and 12 weeks and in faecal Cd excretion.

It is important to note that the results obtained after 12 weeks of intoxication did not clearly delineate the effect of ethanol on Cd accumulation. At first glance, it can be concluded that the lower tissue levels of this heavy metal in the co-exposed rats were a simple consequence of its decreased intake. But, after this period, rats receiving Cd alone excreted 93.2% of ingested Cd in faeces, while those co-exposed to ethanol lost only 80.3%, thus suggesting increased absorption of Cd.

Ethanol is able to increase the permeability of biological membranes to various substances, including toxic metals (Pal et al., 1993) and was reported to increase Cd retention (Sharma et al., 1991; Gałąźyn-Sidorczuk et al., 1998; Brzóska et al., 2000; Moniuszko-Jakoniak et al., 2001). The latter effect is connected with the ability of both substances to induce metallothionein synthesis (Sharma et al., 1991; Ebadi et al.,

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Fig. 6. Effects of cadmium (Cd), ethanol (Et), and their combination on urinary copper (Cu) excretion.

Each value is the mean of 10 animals. *Values were significantly different ($P < 0.05$; analysis of variance plus Tukey–Kramer’s test) compared to the control group.

Fig. 7. Effects of cadmium (Cd), ethanol (Et), and their combination on urinary zinc (Zn) excretion.

Each value is the mean of 10 animals. *Values were significantly different ($P < 0.05$; analysis of variance plus Tukey–Kramer’s test) compared to the control group.
It is proposed that ethanol induces synthesis of this protein indirectly by increasing the body burden of Cd and by altering Zn and glucocorticoid homeostasis (Sharma et al., 1992; Gałążyn-Sidorczuk et al., 1998; Brzóska et al., 2000). However, reduced liver metallothionein concentration after long-term ethanol administration (Hopf et al., 1986, 1990), in contrast to short-term treatment (Sharma et al., 1991, 1992; Gałążyn-Sidorczuk et al., 1998; Brzóska et al., 2000), has been reported.

It is known that long-term ethanol consumption damages the gastrointestinal tract and influences absorption of various metals (Fairweather-Tait et al., 1988; Bode and Bode, 1997). We cannot exclude the possibility that the results regarding the effect of ethanol on Cd turnover observed in the Cd + ethanol rats after 12 weeks of treatment were, at least partly, connected with changes in Cd absorption from the gastrointestinal tract and in its tissue accumulation. Because of structural and functional changes noted in the main organs of Cd storage (data to be published), their ability to further accumulate the metal could be reduced, resulting in greater amounts of the metal being released into the bloodstream by damaged cellular membranes. This may be why there was no difference in tissue (especially liver and kidneys) Cd concentration in the Cd + ethanol rats between the 8th and 12th weeks in spite of further Cd intake, or in blood Cd concentration between the rats intoxicated with Cd alone and in combination with ethanol after the longer treatment in spite of lower Cd intake in the last group.

Other investigations reported no effect, or an increased Cd accumulation in various organs by ethanol (Hopf et al., 1990; Sharma et al., 1991).

Until now, little has been known about the Zn and Cu status in conditions of simultaneous exposure to both Cd and ethanol (Hopf et al., 1990; Sharma et al., 1991; Gałążyn-Sidorczuk et al., 1998; Brzóska et al., 2000). Exposure to Cd caused disturbances in Zn and Cu metabolism reflected by changes in these bioelement concentrations and contents in tissues and biological fluids. Similar results were reported previously by us (Gałążyn-Sidorczuk et al., 1998; Brzóska et al., 2000) and other investigators (Bonner et al., 1980; Mahaffey et al., 1981; Sharma et al., 1991; Oishi et al., 2000; Brzóska et al., 2001).

The increased Zn concentration and its total content in liver and kidneys with the simultaneous decrease in bone tissue concentration suggest a Zn redistribution under the influence of Cd. The Cd-induced Zn redistribution in the body and kidney Cu deposition were a result of Cd accumulation and its ability to induce metallothionein synthesis, especially in the liver and kidneys (Hopf et al., 1990; Gałążyn-Sidorczuk et al., 1998; Brzóska et al., 2001). Metallothionein plays an important role, not only in Cd retention and detoxification, but also in the homeostasis and storage of Zn and Cu (Funk et al., 1987; Hopf et al., 1990; Sharma et al., 1991; Kelly et al., 1996; Brzóska and Monuszko-Jakoniuk, 2001).

The ethanol-induced disturbances in Zn and Cu distribution were not as pronounced as those of Cd. The body deficit of Zn and Cu caused by ethanol, reflected by the decrease in bone Zn and liver Cu concentrations as well as reduced urinary excretion of both metals, might result from their decreased gastrointestinal absorption. This mechanism of ethanol action is likely, because ethanol is known to damage the gastrointestinal tract (Bode and Bode, 1997), and in view of the enhanced faecal excretion of Zn and Cu noted in this study. Various directions of changes (decrease, increase, no change) in Zn and Cu concentrations in tissues and biological fluids have been reported by others (Hopf et al., 1986; Flora et al., 1991; Sharma et al., 1991; Floriańczyk, 2000).

The changes in Zn and Cu status noted in the co-exposed animals compared to the groups intoxicated separately with Cd or ethanol can result from many causes. They reflect, on the one hand, the differences in Cd and ethanol as well as Zn and Cu intakes, and, on the other, the influence of ethanol on the turnover and action of Cd. The modifying effect of ethanol can be explained by its direct and indirect actions. The mechanism of the direct effect of ethanol may be related to reduced Zn and Cu ingestion (as a result of aversion to food), disturbances in metallothionein synthesis (Hopf et al., 1986) and increased permeability of biological membranes to metals (Pal et al., 1993). It has been reported that long-term treatment with 5% ethanol inhibits metallothionein synthesis and at the same time reduces Zn and Cu contents in liver, but not in kidneys, of experimental animals (Hopf et al., 1986). The indirect action of ethanol can be connected with the decrease in Cd intake (as a result of reduced drinking fluid consumption) and influence on the turnover of consumed Cd.

The decreased urinary Zn and Cu excretion may be secondary to the effect of Cd and/or ethanol on tissue concentrations of these elements, and also a consequence of reduction in urine output (due to decreased fluids consumption and possibly also kidney insufficiency). Reduced fluids consumption can cause dehydration and thus mask a real Cd and/or ethanol effect on serum, and tissue, Zn and Cu concentrations.

The modifying influence of prolonged ethanol consumption on Cd-induced changes in Zn and Cu tissue concentrations in rats was reported by Sharma et al. (1991). However, the current literature shows that there are insufficient studies evaluating Cd turnover under ethanol influence and few data on the effect of both substances on Zn and Cu body status.

On the basis of this study, due to variations in ingestion of Cd and ethanol as well as Zn and Cu, we cannot correctly interpret an interactive effect of Cd and ethanol on both bioelements body status. Nevertheless our results show that co-exposure to Cd and ethanol can cause stronger or different disturbances in Zn and Cu status than those observed in conditions of exposure to even higher Cd levels but without simultaneous intake of ethanol. We speculate that, in the case of the same Cd and ethanol intake in co-exposed rats as in those receiving both substances separately, changes observed in Zn and Cu status will be more serious in the combined group. Although Cd and ethanol individually pose a risk to Zn and Cu homeostasis, co-exposure can create a major threat. We conclude that alcoholics may be more susceptible to Cd accumulation and the consequences of its actions, including Zn and Cu body status. Further studies are needed for the better understanding of the effect of ethanol on Cd turnover as well as Cd and ethanol interactions with Zn and Cu metabolism.

REFERENCES
