

Lead Turnover and Changes in the Body Status of Chosen Micro- and Macroelements in Rats Exposed to Lead and Ethanol

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Abstract

The effect of continuous exposure to lead (Pb) and ethanol on Pb turnover and zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) body status was studied in male Wistar rats. The animals received an aqueous solution of 10% (w/v) ethanol and/or 500 µg Pb/cm³ as the only drinking fluid for 12 weeks.

Exposure to Pb as well as ethanol alone influenced the body status of Zn, Cu, Fe, Ca and Mg. Disturbances in their metabolism were reflected in decreased bioavailability, changes in serum and/or tissue concentrations and urinary excretion. The most serious disorders under Pb influence were observed in Fe metabolism, while ethanol alone disturbed mainly Fe, Cu and Zn body status. In the conditions of co-exposure to Pb and ethanol some changes were more or less evident and sometimes they had different direction than at separate intoxication. Moreover, simultaneous exposure to Pb and ethanol led to changes in the concentrations of Cu, Fe and Mg, which were not observed in the case of separate administration. The independent action of Pb and/or ethanol as well as the interactive effect of both substances involving the modifying influence of ethanol on Pb turnover could explain the changes in the metabolism of bioelements under combined exposure to Pb and ethanol.

Our results seem to indicate that Pb-exposed human subjects abusing ethanol may be more vulnerable to the accumulation of Pb in body organs and metabolic disorders of some bioelements, which may in consequence enhance the risk of health injury.

Keywords: bioelements, lead, ethanol, metabolism, interaction

Introduction

Lead (Pb) is one of the most toxic heavy metals and a widespread natural and occupational environmental pollutant. Its toxicity in humans and experimental animals has been well known and widely reported [1-4]. Experimental, epidemiological and clinical data suggest that some toxic actions of Pb may be modified by excessive

ethanol consumption [5-11]. However, inter-dependence between Pb and ethanol and its consequences to health are not fully understood.

Both Pb and ethanol are known to change gastrointestinal absorption and tissue concentrations of essential micro- and macroelements, such as zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and magnesium (Mg) [7, 12-19]. Some of the salubrious health effects of Pb as well as ethanol action result from their influence on the metabolism and function of these elements. Moreover, dietary intake

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and body status of some bioelements have been reported to influence the metabolism and toxicity of Pb and ethanol [6, 13, 20, 21]. But so far little information is available concerning the body status of essential metals following combined intoxication with both xenobiotics [7].

Owing to increasing alcohol consumption [22, 23] and presence of Pb in the environmental or/and occupational conditions [1, 3, 8], the co-exposure to these can create serious medical health consequences. This prompted us to undertake the studies on interactions between Pb and ethanol using a rodent model. The present study was designed to examine the influence of long-term ethanol administration on Pb turnover and the body status of some necessary micro- and macroelements, such as Zn, Cu, Fe, Ca and Mg, under simultaneous exposure to Pb and ethanol.

Experimental Procedures

Chemicals

All reagents and chemicals were of analytical grade or higher purity. Ultra pure water (Milli-Q system, Millipore Corporation, USA), trace pure nitric (HNO_3) and hydrochloric (HCl) acids (Merck, Germany) as well as Pb, Zn, Cu, Fe, Ca and Mg standard solutions assigned for atomic absorption spectrometry (Sigma, USA) were used in the analysis.

Animals

Thirty-six inbred adult (10-week-old) male albino rats (Wistar strain) weighing 240-260 g were used. Since birth and during the course of experiment, the animals were kept, under controlled conditions, at a temperature of $22 \pm 1^\circ\text{C}$, with a relative humidity of $50 \pm 10\%$. They were allowed free access to drinking water and a standard pellet rodent laboratory LSM chow (Fodder Manufactures, Motycz, Poland). Pb and bioelement content in the diet is presented in Table 1.

Experimental Design

The rats were randomly divided into four groups of nine. Three groups of rats received water solution of lead acetate containing $500 \mu\text{g Pb/cm}^3$ or/and 10% (w/v) ethanol as the only drinking fluid, for 12 weeks. To eliminate various intake of Pb and bioelements between experimental groups, resulting from differences in fluid consumption, redistilled water was administered as drinking water for control rats and was used to prepare Pb and/or ethanol solutions. Drinking water and food consumption as well as body weight gain of rats were monitored throughout the study. In the last week of the experiment, 24-h faeces were collected in metabolic cages during five consecutive days and food consumption was evaluated during that time. On the last day of exposure, 24-h urine was collected. Next, following overnight starvation, blood was

taken from the heart (for heparin and for cloth) and liver, kidneys, spleen, heart, brain, femur and femoral muscle were removed under ether anaesthesia. The soft tissues were washed thoroughly in ice-cold physiological saline (0.9% NaCl), the femurs were cleansed of muscle tissue and weighed. A portion of blood was allowed to coagulate and serum was separated. The biological material not used immediately after sampling was frozen at -20°C until further analysis.

The study was approved by the Local Ethical Committee for animal experiments in Bialystok. Procedures involving the animals and their care conformed to the institutional guidelines, in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research [24].

Analytical Procedures

Ethanol Concentration in Blood

Samples of heparinized blood ($10 \mu\text{l}$) were analyzed by headspace gas chromatography (Hewlett-Packard; model 5890, series II) according to the manufacturer's recommendation with our own modification.

Concentration of Metals in Blood/Serum, Tissues, Urine and Faeces

The samples of blood collected in heparinized tubes (assigned for Pb determination) were wet-digested with 5% HNO_3 [25]. A known weight of whole organ (kidneys, spleen, heart, brain, femur), liver and femoral muscle samples of about 1 g as well as representative samples of faeces, after drying at 110°C , were dry mineralized in an electric oven at 450°C . The ash of soft tissues was dissolved in 1 M HNO_3 . Ashed femurs and faeces were digested in about 1 cm^3 of concentrated (36%) HCl and then adjusted with ultra

Table 1. Metals concentration in the LSM diet^{ab}.

Metal	Concentration
Zinc (Zn)	47.97 $\mu\text{g/g}$
Copper (Cu)	8.495 $\mu\text{g/g}$
Iron (Fe)	160.38 $\mu\text{g/g}$
Calcium (Ca)	11.08 mg/g
Magnesium (Mg)	1.90 mg/g
Lead (Pb)	0.529 $\mu\text{g/g}$

^a The LSM diet is prepared from corn, wheat, barley, wheat bran, soya-bruised grain, meat starch, skimmed powdered milk, phosphate, fodder-chalk, mineral and vitamin premix. It contains (%): total protein (15.9), raw fibre (3.5), raw fat (2.77), lysine (0.75), methionine and cystine (0.56). Metabolizable energy of the diet is 12.2 J/g.

^b The concentration of all metals, except Pb, in the diet is given according to the producer. Pb concentration was determined in our laboratory.

pure water to obtain 1 M HCl solution. Pb concentrations in the prepared samples of blood, tissues and faeces as well as in urine were measured (after appropriate dilution) at the resonance line of 283.3 nm by atomic absorption spectrometry (AAS) with flameless atomization in a graphite furnace and automatic dosage (Zeeman Atomic Absorption Spectrophotometer Z-5000, Hitachi, Japan). The concentrations of Zn, Cu, Fe, Ca and Mg in the tissue and faeces mineralizates as well as in serum and urine were determined (after appropriate dilution) using flame (air-acetylene burner) AAS (Z-5000, Hitachi). The cathode lamps of the respective elements were operated under standard conditions using their respective resonance lines: Zn, 213.9 nm; Cu, 324.75 nm; Fe, 248.3 nm; Ca, 422.7 nm and Mg, 285.2 nm. All metal concentrations were automatically read from calibration curves. Working standards were received after dilution of stock standards with HNO₃ (calibration curve for soft tissues and blood), HCl (for femur and faeces) and ultra pure water (for serum and urine).

Based on metal concentrations in soft tissues, the total pool of particular metals (Pb and bioelements) in all the organs examined was calculated (a sum of content in liver, spleen, kidneys, heart and brain).

Bioavailability and Total Pool of Metals

Bioavailability of particular bioelements was expressed as their apparent absorption calculated from the following equation:

$$\%A = I - E_F$$

where %A - apparent absorption during the balance period (5 days); I - bioelement intake during the balance period (5 days); E_F - amount of the bioelement excreted with faeces (5 days).

The total pool of particular metals (Pb and bioelements) in all the studied organs was calculated as a sum of their content in liver, spleen, kidneys, heart and brain.

Statistical Analysis

Differences between four experimental groups were evaluated using the nonparametric Mann-Whitney U test as data were not normally distributed according to the Kolmogorov-Smirnov test. $P < 0.05$ was considered significant. A linear Pearson's correlation analysis was performed for the relationship between Pb accumulation and bioelement tissue concentrations. All statistical calculations were done using the STATISTICA version 5.0 computer program.

Results

Food Consumption and Body Weight Gain

There were no statistically significant differences in LSM diet ingestion between all experimental groups (Table 2).

During the experiment an increase in body weight was noted in all rats (Table 2). Pb, irrespective of administration mode, caused retardation in weight gain. The gain in body weight of rats exposed to Pb alone and in conjunction with ethanol was lower by 16% ($P < 0.05$) and 27% ($P < 0.001$), respectively, compared to control animals. Ethanol had no effect on body weight gain.

Consumption of Fluids and Pb and Ethanol Intakes

The addition of Pb acetate reduced drinking solution consumption from the beginning of its administration. Fluid intake was reduced markedly when animals were given ethanol or Pb and ethanol in combination (Table 2).

Based on daily consumption of drinking solutions the intakes of Pb and ethanol were calculated (Table 2). In control rats, drinking redistilled water without contamination with Pb and ethanol, and in those receiving only ethanol solution the intake of Pb was assumed to be zero. As a result of decreased consumption of fluids caused by ethanol co-administration, Pb intake in the co-exposed rats was about half of that observed in those intoxicated with Pb alone.

Table 2. Body weight gain, food and drinking fluids consumption including Pb and ethanol intake^a.

Group	LSM diet consumption (g/24 h)	Body weight gain (g/12 weeks)	Drinking fluids consumption (cm ³ /24 h)	Pb intake		Ethanol intake ^b	
				Mean (mg/24 h)	Range (mg/kg body wt)	Mean (g/24 h)	Range (g/kg body wt)
Control	24.1 ± 1.1	117.0 ± 5.3	40.55 ± 0.51	-	-	-	-
Pb	22.8 ± 0.9	97.8 ± 4.6*	38.59 ± 0.52*	19.26 ± 0.26	54.8–75.9	-	-
Ethanol	21.2 ± 1.0	113.9 ± 3.0	20.78 ± 0.32 [†]	-	-	5.11 ± 0.08	56.1–81.1
Pb + ethanol	20.8 ± 0.9	85.9 ± 5.8**	19.72 ± 0.41 ^{††}	9.98 ± 0.17 [†]	29.5–39.5	4.92 ± 0.08	58.2–78.0

^a Control, control group; Pb, 500 µg Pb/cm³ in drinking water for 12 weeks; Ethanol, 10% (w/v) water solution of ethanol for 12 weeks; Pb + ethanol, Pb and ethanol simultaneously for 12 weeks. Values are means ± SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, [†]Pb, [‡]ethanol groups.

^b Intake of 10% ethanol.

Table 3. Pb concentrations in whole blood and tissues of rats exposed to Pb and/or ethanol^a.

Group	Blood ($\mu\text{g}/100\text{ cm}^3$)	Liver ($\mu\text{g}/\text{g}$)	Kidney ($\mu\text{g}/\text{g}$)	Spleen ($\mu\text{g}/\text{g}$)	Heart ($\mu\text{g}/\text{g}$)	Brain ($\mu\text{g}/\text{g}$)	Femoral muscle ($\mu\text{g}/\text{g}$)	Femur ($\mu\text{g}/\text{g}$)
Control	6.19 \pm 0.70	0.18 \pm 0.03	0.65 \pm 0.12	0.69 \pm 0.06	0.13 \pm 0.01	0.18 \pm 0.03	0.22 \pm 0.03	0.04 \pm 0.01
Pb	59.65 \pm 2.32*	0.59 \pm 0.06*	7.56 \pm 0.80*	1.79 \pm 0.13*	0.19 \pm 0.02	0.47 \pm 0.04*	0.23 \pm 0.02	0.98 \pm 0.10*
Ethanol	7.02 \pm 0.48 [†]	0.36 \pm 0.04	1.42 \pm 0.20 [†]	0.69 \pm 0.06 [†]	0.14 \pm 0.02	0.19 \pm 0.02 [†]	0.16 \pm 0.03	0.07 \pm 0.01 [†]
Pb + ethanol	74.54 \pm 1.96***	0.89 \pm 0.05***	7.66 \pm 0.99**	2.08 \pm 0.09**	0.15 \pm 0.01	0.43 \pm 0.03**	0.21 \pm 0.03	0.67 \pm 0.07***

^a Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, [†]Pb, [‡]ethanol groups. Abbreviations are as in Table 2. Tissue Pb concentrations are expressed as $\mu\text{g}/\text{g}$ of wet tissue weight.

However, the intake of 10% ethanol in rats drinking ethanol alone and in those co-exposed to Pb was similar.

Blood-ethanol Concentration

Blood-ethanol concentration in the animals which were not given ethanol (control and Pb alone groups) was below 5 mg/dm³. It was significantly ($P < 0.001$) higher in those receiving ethanol alone (16.22 \pm 1.68 mg/dm³) or Pb and ethanol (14.93 \pm 2.05 mg/dm³).

Pb Body Burden

Pb concentrations in blood and tissues of rats exposed to Pb or/and ethanol are presented in Table 3. In the animals exposed to Pb alone a significant accumulation of the metal was noted in all tissues, except the heart and femoral muscle. The highest Pb levels, like in control, were observed in the kidney and spleen. But the main Pb accumulation was in bone tissue that was reflected in 24-fold ($P < 0.001$) increase in the femur Pb concentration. In the animals exposed to ethanol alone the concentrations of Pb in the whole blood and tissues were in the ranges of respective control values. In the rats simultaneously receiving Pb and ethanol, the concentrations of Pb in the blood and liver were higher by 25 and 50% ($P < 0.001$), respectively while in the femur – lower by 32% ($P < 0.01$) compared to the group intoxicated with Pb alone. There were no differences in the kidney, spleen, heart, brain and femoral muscle Pb concentrations between these groups. Pb or ethanol did not influence the heart and muscle tissue Pb concentrations.

As a result of Pb administration its total content in organs (liver, kidneys, spleen, heart and brain) was 6 times higher compared to control (24.65 \pm 2.46 vs. 4.21 \pm 0.67 μg , $P < 0.001$). In the rats drinking ethanol alone, Pb pool in organs was in the range of control group. There was no difference between the group exposed to Pb alone and that receiving a combination of Pb and ethanol. Total amount of Pb in the femur of the co-exposed rats (0.52 \pm 0.05 μg) was lower (by 32%, $P < 0.01$) than in those receiving Pb alone.

As a result of Pb administration its urinary and faecal excretions increased 3- and 311-fold ($P < 0.001$), respec-

tively, compared to those of control (Table 4). Ethanol alone had no influence on the urinary and faecal excretions of Pb. In the rats co-exposed to Pb and ethanol, the urinary Pb excretion was 3-fold ($P < 0.001$) lower, while its faecal excretion remained unchanged, compared to Pb alone.

Table 4. Urinary and faecal Pb excretions in rats exposed to Pb and/or ethanol^a.

Group	Urine ($\mu\text{g}/24\text{ h}$)	Faeces ($\mu\text{g}/\text{g}$ dry weight)
Control	1.61 \pm 0.12	5.79 \pm 2.01
Pb	5.05 \pm 0.79*	1799.96 \pm 172.54*
Ethanol	1.70 \pm 0.15 [†]	9.22 \pm 1.64 [†]
Pb + ethanol	1.64 \pm 0.18 [†]	1714.75 \pm 137.21**

^a Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, [†]Pb, [‡]ethanol groups. Abbreviations are as in Table 2.

Body Status of Bioelements

Zinc

The administration of Pb, ethanol and both substances simultaneously reduced ($P < 0.05$) the apparent Zn absorption by 20, 35 and 44%, respectively (Fig. 1). There were no statistically significant differences in Zn bioavailability between the groups exposed to Pb and ethanol separately and in combination.

Zn concentration in the serum and soft tissues (Table 5), except for its increased level in the kidney of ethanol-only drinking rats (by 8%, $P < 0.05$), as well as the total Zn pool in organs (Fig. 2) were unchanged by any treatment. In the co-exposed animals the kidney Zn concentration was lower (by 19%, $P < 0.001$) than in those receiving only ethanol whereas in the spleen it was lower (by 4%, $P < 0.05$) compared to those drinking Pb alone. Femur level of the element (Table 5) in the rats receiving Pb and ethanol

alone was reduced ($P < 0.05$) by 14 and 13%, respectively, as compared to control. Simultaneous administration of both substances did not cause further change in the femur Zn concentration. The bone Zn concentration in those rats was lower by 16% ($P < 0.01$) as compared to control. The concentrations of Zn in the kidney ($r = -0.477$, $P = 0.003$) and femur ($r = -0.370$, $P = 0.026$) negatively correlated with Pb concentrations in these tissues. There was no correlation between Zn and Pb concentrations in other tissues.

The urinary excretion of Zn (Fig. 3) decreased by 54 ($P < 0.001$) and 29% ($P < 0.01$) in the ethanol and Pb + ethanol groups as compared to the control, respectively. The animals simultaneously treated with Pb and ethanol excreted less Zn than those given Pb alone (by 28%, $P < 0.01$) and more than those receiving only ethanol (by 52%, $P < 0.01$).

Copper

The administration of ethanol, but not Pb, alone decreased the apparent absorption of Cu by 30% ($P < 0.01$) while under their co-administration further reduction to 44% ($P < 0.001$) of the control value was observed (Fig. 1).

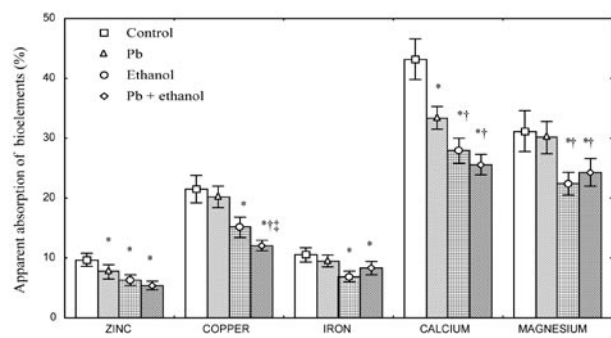


Fig. 1. Apparent absorption of bioelements in rats exposed to Pb and/or ethanol. Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, †Pb, ‡ethanol groups. Abbreviations are as in Table 2.

Pb and ethanol alone had no effect on Cu concentrations in the serum, femur and all soft tissues, except for the liver, as compared to control (Table 6). The liver Cu concentration was reduced under the influence of Pb, ethanol and both substances (by 13-16%, $P < 0.05$). Moreover, in the conditions of simultaneous administration of

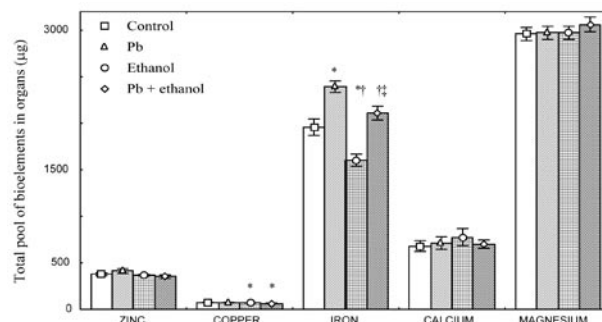


Fig. 2. Whole pool of bioelements in the organs of rats exposed to Pb and/or ethanol. Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, †Pb, ‡ethanol groups. Abbreviations are as in Table 2.

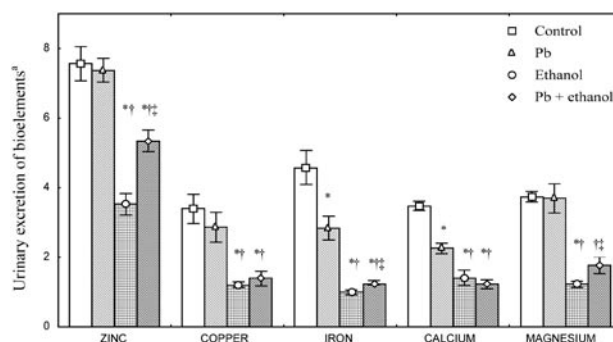


Fig. 3. Urinary excretion of bioelements in rats exposed to Pb and/or ethanol. Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, †Pb, ‡ethanol groups. Abbreviations are as in Table 2. ^a Zn, Cu and Fe excretions are expressed as $\mu\text{g}/24$ h while that of Ca and Mg - as $\text{mg}/24$ h.

Table 5. Zn concentrations in serum and tissues of rats exposed to Pb and/or ethanol^a.

Group	Serum ($\mu\text{g}/100 \text{ cm}^3$)	Liver ($\mu\text{g}/\text{g}$)	Kidney ($\mu\text{g}/\text{g}$)	Spleen ($\mu\text{g}/\text{g}$)	Heart ($\mu\text{g}/\text{g}$)	Brain ($\mu\text{g}/\text{g}$)	Femoral muscle ($\mu\text{g}/\text{g}$)	Femur ($\mu\text{g}/\text{g}$)
Control	153.7 \pm 8.7	31.77 \pm 1.45	24.58 \pm 0.86	19.34 \pm 0.75	14.86 \pm 0.47	11.73 \pm 0.52	10.23 \pm 0.46	186.3 \pm 4.7
Pb	157.4 \pm 3.2	32.77 \pm 1.96	22.35 \pm 0.66	18.87 \pm 0.35	15.01 \pm 0.43	12.15 \pm 0.34	11.14 \pm 0.45	160.4 \pm 8.9*
Ethanol	157.4 \pm 6.0†	29.97 \pm 1.23	26.61 \pm 0.46††	19.30 \pm 0.96	14.86 \pm 0.60†	10.74 \pm 0.30	10.16 \pm 0.39	162.0 \pm 3.5*
Pb + ethanol	168.5 \pm 8.8	29.52 \pm 1.63	21.65 \pm 1.09‡	18.07 \pm 0.59†	14.16 \pm 0.56	11.26 \pm 0.80	10.07 \pm 0.51	156.4 \pm 6.4*

^a Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, †Pb, ‡ethanol groups. Abbreviations are as in Table 2. Tissue Zn concentrations are expressed as $\mu\text{g}/\text{g}$ of wet tissue weight.

Pb and ethanol a decrease in the kidney (by 25%, $P < 0.01$) with a simultaneous increase in the femoral muscle (by 51%, $P < 0.001$) Cu concentrations were observed. The kidney Cu concentration in the animals co-exposed to both substances was lower ($P < 0.01$) than in those under separate exposure, while that of femoral muscle was higher ($P < 0.01$). The serum Cu level in the co-exposed rats was lower (by 13%, $P < 0.05$) than in those given Pb alone. In the serum and other tissues of co-exposed animals Cu concentrations were in the ranges of respective control values. Only in the brain there was a correlation between Cu and Pb concentrations ($r = 0.394$, $P = 0.017$). The total Cu pool in organs (Fig. 2) was decreased under ethanol influence, irrespective of whether it was used alone (by 9%, $P < 0.05$) or in combination with Pb (by 12%, $P < 0.05$).

Exposure to ethanol alone and in combination with Pb decreased urinary Cu excretion (about 2.8- and 2.4-fold, $P < 0.001$ and $P < 0.01$, respectively) compared to control (Fig. 3).

Iron

The apparent Fe absorption (Fig. 1) decreased under ethanol influence by 34% ($P < 0.001$) while Pb alone had no effect on Fe bioavailability. In the rats co-administered Pb and ethanol, the absorption of Fe was reduced by 21% ($P < 0.001$) compared to control and it was similar to that observed in the ethanol-only treated animals.

Exposure to Pb alone resulted in an increase in the serum (by 76%, $P < 0.001$) and liver (by 41%, $P < 0.001$) Fe concentrations (Table 7). The spleen Fe concentration was decreased by both Pb (by 23%, $P < 0.05$) and ethanol (by 21%, $P < 0.01$) alone while the effect was not observed when they were co-administered. In the latter group the spleen Fe concentration tended to increase but the difference did not achieve statistical significance compared to control. Pb and ethanol alone had no effect on the femoral muscle Fe concentration, whereas simultaneous treatment with both resulted in an increase in Fe level (by 46%, $P < 0.01$). Fe concentrations in the kidney, heart, brain and femur were unchanged by any treatment compared to the control group. The total Fe pool in organs (Fig. 2) increased (by 22%, $P < 0.01$) in the rats exposed to Pb alone and decreased (by 18%, $P < 0.01$) in those receiving only ethanol compared to the control. On the other hand, in conditions of co-exposure, total Fe content was in the range of control group. A significant positive correlation ($r = 0.863$, $P = 0.0001$) between serum Fe concentration and blood Pb concentration was noted. Fe concentrations in the liver ($r = 0.518$, $P = 0.001$) and femoral muscle ($r = 0.344$, $P = 0.040$) correlated with Pb concentrations in these tissues. Moreover, a significant correlation ($r = 0.591$, $P < 0.0001$) between total Fe content in organs and blood Pb concentration was observed.

The urinary Fe excretion (Fig. 3) was reduced by the administration of Pb (by 38%, $P < 0.05$) and ethanol (by 78%, $P < 0.001$) alone and simultaneously (by 73%,

Table 6. Cu concentrations in serum and tissues of rats exposed to Pb and/or ethanol^a.

Group	Serum ($\mu\text{g}/100 \text{ cm}^3$)	Liver ($\mu\text{g}/\text{g}$)	Kidney ($\mu\text{g}/\text{g}$)	Spleen ($\mu\text{g}/\text{g}$)	Heart ($\mu\text{g}/\text{g}$)	Brain ($\mu\text{g}/\text{g}$)	Femoral muscle ($\mu\text{g}/\text{g}$)	Femur ($\mu\text{g}/\text{g}$)
Control	125.6 \pm 4.1	5.62 \pm 0.23	5.93 \pm 0.24	2.62 \pm 0.29	4.74 \pm 0.09	3.69 \pm 0.18	1.00 \pm 0.05	4.06 \pm 0.35
Pb	138.1 \pm 4.8	4.88 \pm 0.27*	5.71 \pm 0.28	2.41 \pm 0.3	5.51 \pm 0.31	3.67 \pm 0.10	1.15 \pm 0.06	4.30 \pm 0.18
Ethanol	121.4 \pm 4.8 [†]	4.83 \pm 0.14*	5.65 \pm 0.25	2.43 \pm 0.20	5.37 \pm 0.37 [†]	3.14 \pm 0.18 [†]	1.04 \pm 0.09	4.23 \pm 0.22
Pb + ethanol	119.7 \pm 3.5 [†]	4.71 \pm 0.17*	4.45 \pm 0.16** [†]	2.87 \pm 0.31 [†]	5.07 \pm 0.18	3.55 \pm 0.18	1.51 \pm 0.08** [†]	3.75 \pm 0.18

^a Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, [†]Pb, [‡]ethanol groups. Abbreviations are as in Table 2. Tissue Cu concentrations are expressed as $\mu\text{g}/\text{g}$ of wet tissue weight.

Table 7. Fe concentrations in serum and tissues of rats exposed to Pb and/or ethanol^a.

Group	Serum ($\mu\text{g}/100 \text{ cm}^3$)	Liver ($\mu\text{g}/\text{g}$)	Kidney ($\mu\text{g}/\text{g}$)	Spleen ($\mu\text{g}/\text{g}$)	Heart ($\mu\text{g}/\text{g}$)	Brain ($\mu\text{g}/\text{g}$)	Femoral muscle ($\mu\text{g}/\text{g}$)	Femur ($\mu\text{g}/\text{g}$)
Control	135.0 \pm 5.8	97.2 \pm 4.7	72.9 \pm 1.6	1256.3 \pm 60.8	63.6 \pm 2.0	23.5 \pm 1.7	16.4 \pm 1.4	69.1 \pm 2.2
Pb	237.3 \pm 4.3*	136.6 \pm 3.6*	79.6 \pm 3.2	966.5 \pm 64.2*	63.1 \pm 2.1	20.4 \pm 1.0	16.1 \pm 1.1	74.1 \pm 3.3
Ethanol	138.9 \pm 8.9 [†]	88.9 \pm 3.4 [†]	75.1 \pm 2.60	990.6 \pm 46.9*	66.8 \pm 2.2	19.3 \pm 1.1	15.3 \pm 0.9	64.1 \pm 3.3 [†]
Pb + ethanol	213.9 \pm 7.6** [†]	117.6 \pm 4.7** [†]	75.6 \pm 3.3	1492.2 \pm 93.6** [†]	66.0 \pm 2.1	20.6 \pm 1.0	23.9 \pm 1.7** [†]	64.6 \pm 2.7 [†]

^a Values are means \pm SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, [†]Pb, [‡]ethanol groups. Abbreviations are as in Table 2. Tissue Fe concentrations are expressed as $\mu\text{g}/\text{g}$ of wet tissue weight.

$P < 0.001$). In the rats co-exposed to Pb and ethanol the urinary Fe excretion was lower than in those receiving Pb alone (2.3-fold, $P < 0.001$) and higher than in animals drinking ethanol alone (by 26%, $P < 0.05$).

Calcium

Both Pb and ethanol irrespective of administration mode, reduced the apparent absorption of Ca (Fig. 1). The bioavailability of Ca in the animals receiving Pb alone was lower by 23% than in the control group and further decreased under co-administration with ethanol, reaching 60% of the control value. Ethanol alone decreased the apparent Ca absorption by 35% ($P < 0.001$).

Ca concentrations (Table 8) in the serum, heart, brain and femoral muscle as well as its total pool in organs were unchanged by any treatment. The liver Ca level was increased (by 31%, $P < 0.05$) only by ethanol alone. The administration of Pb alone resulted in an increase in the kidney (by 36%, $P < 0.01$) with a simultaneous decrease in spleen (by 32%, $P < 0.01$) Ca concentrations. Ethanol, irrespective of administration manner, had no effect on Ca concentrations in both organs. In the kidney and spleen of rats receiving Pb and ethanol in combination the directions of changes in Ca concentrations were similar to those observed in animals drinking only Pb. Ca concentration in the kidney was higher (by 26%, $P < 0.05$) while in the spleen lower (by 19%, $P < 0.05$), compared to control. Moreover, exposure to Pb and ethanol alone decreased (P

< 0.01 and $P < 0.05$, respectively) the femur Ca concentration (by 7.0 and 8.4%, respectively), although their combination did not cause further changes in the level of this bioelement. A significant correlation was noted between Ca and Pb concentrations in the kidney ($r = 0.540$, $P = 0.001$) and spleen ($r = -0.503$, $P = 0.002$).

The urinary Ca excretion (Fig. 3) decreased as a result of exposure to Pb and ethanol alone and in conjunction by 35, 59 and 65% ($P < 0.001$), respectively. The animals simultaneously exposed to both substances lost in urine similar amounts of Ca to those treated with ethanol alone.

Magnesium

Apparent Mg absorption (Fig. 1) was unchanged by exposure to Pb, whereas it was decreased (by 28%, $P < 0.001$) under ethanol influence. In the animals simultaneously receiving both substances, Mg bioavailability was similar to that in the ethanol group.

The administration of Pb alone had no influence on Mg concentrations (Table 9) in the serum and tissues. A tendency to an increase in the liver Mg level was observed in the animals exposed to Pb alone but the difference was not statistically significant ($P = 0.054$). Ethanol, neither alone nor in conjunction with Pb, influenced Mg concentrations in the serum and tissues, except for the liver and kidney. Both Pb and ethanol alone had no effect on the liver and kidney Mg concentrations, while their co-administration resulted in an increase in liver (by 8%,

Table 8. Ca concentrations in serum and tissues of rats exposed to Pb and/or ethanol^a.

Group	Serum (mg/100 cm ³)	Liver (µg/g)	Kidney (µg/g)	Spleen (µg/g)	Heart (µg/g)	Brain (µg/g)	Femoral muscle (µg/g)	Femur (mg/g)
Control	9.31 ± 0.27	38.86 ± 3.68	45.56 ± 1.60	38.41 ± 2.18	19.78 ± 1.18	59.19 ± 4.50	38.51 ± 1.80	182.7 ± 5.0
Pb	9.41 ± 0.23	41.07 ± 5.99	61.78 ± 3.11*	26.07 ± 1.21*	20.20 ± 1.13	58.95 ± 4.52	40.95 ± 1.74	168.8 ± 5.6*
Ethanol	9.53 ± 0.21	51.00 ± 5.31*	48.58 ± 2.12†	36.68 ± 2.07†	17.52 ± 0.43	64.36 ± 4.06	40.90 ± 2.81	166.4 ± 5.0*
Pb + ethanol	9.34 ± 0.25	46.94 ± 4.46	57.17 ± 3.30**	31.15 ± 1.34**†	18.36 ± 1.21	62.45 ± 4.29	44.24 ± 3.67	169.2 ± 2.9*

^a Values are means ± SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, †Pb, ‡ethanol groups. Abbreviations are as in Table 2. Tissue Ca concentrations are expressed as µg/g or mg/g of wet tissue weight.

Table 9. Mg concentrations in serum and tissues of rats exposed to Pb and/or ethanol^a.

Group	Serum (mg/100 cm ³)	Liver (µg/g)	Kidney (µg/g)	Spleen (µg/g)	Heart (µg/g)	Brain (µg/g)	Femoral muscle (µg/g)	Femur (mg/g)
Control	4.50 ± 0.13	210.1 ± 4.6	210.0 ± 4.0	230.1 ± 9.6	236.3 ± 5.4	139.9 ± 4.6	252.6 ± 7.9	5.06 ± 0.13
Pb	4.22 ± 0.16	243.1 ± 12.6	203.3 ± 4.7	244.7 ± 6.9	249.4 ± 3.9	138.9 ± 4.6	256.0 ± 3.7	5.33 ± 0.15
Ethanol	4.41 ± 0.13	223.2 ± 3.6	209.5 ± 4.2	235.3 ± 9.9	227.2 ± 3.1†	129.9 ± 5.8	259.8 ± 6.2	4.68 ± 0.11†
Pb + ethanol	4.64 ± 0.28	226.5 ± 5.5*	193.5 ± 3.6**	262.2 ± 23.3	246.4 ± 6.1‡	132.2 ± 5.2	236.9 ± 7.3	4.80 ± 0.12†

^a Values are means ± SEM for groups of 9 rats. Marked values differ significantly ($P < 0.05$; Mann-Whitney U test) from *control, †Pb, ‡ethanol groups. Abbreviations are as in Table 2. Tissue Mg concentrations are expressed as µg/g or mg/g of wet tissue weight.

$P < 0.05$) and a decrease in kidney (by 8%, $P < 0.05$) concentrations of the metal. The total Mg pool in organs was unchanged by any treatment. The serum and tissue Mg concentrations did not correlate with Pb accumulation.

The urinary Mg excretion (Fig. 3) was unchanged by Pb alone, whereas it was reduced by 67% ($P < 0.001$) under ethanol influence. In the animals simultaneously exposed to both substances, Mg urinary excretion was lower than that in the Pb group ($P < 0.001$) and slightly higher ($P < 0.05$) than in those receiving ethanol alone.

Discussion

We have already reported that ethanol consumption influences cadmium turnover and toxicity in rats, including the effect of this heavy metal on the metabolism of bioelements, especially Zn and Cu [18, 19, 26, 27]. In the present report we have presented some of the results of our study on the interaction between ethanol and Pb, which refer to the influence of simultaneous exposure to Pb and ethanol on Pb turnover and the body status of important micro- and macroelements such as Zn, Cu, Fe, Ca and Mg.

The level of rat intoxication with Pb ($500 \mu\text{g Pb}/\text{cm}^3$) used corresponds to human occupational exposure. The blood Pb concentration in the Pb-exposed rats at the end of the experiment reached the values comparable with those noted in Pb workers [28, 29]. The level of ethanol treatment (10%, w/v) may be tantamount to alcohol abuse in man [30].

The addition of Pb acetate to drinking water caused a slight decrease in its consumption, which was further reduced when animals simultaneously received ethanol. Because rats administered ethanol (alone or in combination with Pb) developed a stronger aversion to drinking than those which did not receive ethanol, the intake of Pb in the co-exposed animals was considerably lower than in those receiving Pb alone. On the other hand, there was no difference in ethanol consumption between rats drinking water containing ethanol alone and those receiving it in combination with Pb. The difference in Pb intake is very important and has to be taken into account in the interpretation of the results. We have used such an experimental model to create conditions by all means similar to those that can take place in human life. Alcoholics may have lower Pb intake than their non-alcoholic counterparts, since they consume small amounts of food being the main source of general population exposure to this heavy metal [1].

Exposure to Pb (but not ethanol) alone resulted in a decrease in body weight gain, which further declined in rats simultaneously intoxicated with Pb and ethanol. The effect of co-exposure is supported by other studies [11, 31]. As there were no discrepancies in food ingestion between experimental groups, the reduction in body weight gain could be a result of Pb-ethanol action. The loss in body weight due to heavy metals has been recognized as an effect of enhanced synthesis of glucose from non-carbohydrate sources leading to mobilisation of fat from body deposits [32].

Our results confirm the well-known fact that bone tissue is the main site of Pb accumulation in the body [33, 34]. It was reflected in an approximately 24-fold increase in the femur Pb concentration that was considerably higher than in other tissues. Although Pb concentrations in the kidney and spleen were higher than in the femur but as bone tissue has larger weight than soft tissues the total amount of Pb accumulated in the whole skeleton was comparatively higher.

Results of the present study indicate that ethanol influences Pb turnover in rats. There was no difference in the total Pb content in internal organs in the rats co-exposed to Pb and ethanol and in those receiving Pb alone in spite of 2-fold lower Pb intake in the co-exposure. However, blood and liver Pb concentrations in animals receiving Pb and ethanol were found to be significantly higher than in those exposed to Pb alone. This suggests that ethanol may enhance retention of Pb in soft tissues and especially in the liver. This may result from the increased gastrointestinal absorption of Pb as a consequence of increased permeability of cellular membranes under ethanol influence [35] as well as from ethanol influence on Pb binding and releasing from bone tissue [36, 37]. Pb absorbed from gastrointestinal tract and released into circulation from bones is first transported into liver and this can explain the ethanol-induced increased Pb concentrations in the blood and liver of rats co-exposed to both substances. In the absence of any modifying effect of ethanol, Pb concentrations in animals simultaneously exposed to ethanol ought to be lower than in those receiving Pb alone because of its 2-fold lower intake. The ethanol-induced increase in Pb body burden under exposure to this heavy metal may have deleterious consequences for health, increasing the risk of its toxic action, including the effect on the body status of bioelements and their biological functions.

Blood concentration of Pb has been recognized as the best parameter to monitor Pb exposure [4, 38]. Elevated blood Pb levels have been observed in Pb-exposed alcoholic industrial workers compared to non-alcoholic [8] and in acute alcohol poisoning [9]. Excessive ethanol consumption has also been reported to increase Pb concentration in the blood of human subjects exposed to Pb from environmental sources [5, 6, 9, 21]. The effect of ethanol has been thought to be a consequence of increased gastrointestinal absorption of Pb and its enhanced release from the skeleton. Our results allow the conclusion that alcoholics who consume less Pb can have the same or even higher concentrations of this metal in the blood and soft tissues compared to non-alcoholics ingesting higher amounts of Pb.

Exposure to Pb, ethanol or both caused, less or more serious, disturbances in the metabolism of bioelements reflected in changes in their bioavailability, tissue concentrations and urinary excretion. After treatment with Pb the most serious disturbances were observed in Fe while the slightest were in Mg body status. Disorders in the metabolism of bioelements under Pb influence have also been reported by other authors [7, 12]. A decrease in the

femur Zn and Fe concentrations and no changes in the femur, liver and brain Ca and Mg levels in rats treated with 200 mg Pb/kg of diet for 10 weeks were noted [12]. But at this level of exposure, Pb did not affect the liver and kidney levels of Fe and kidney Ca, while reducing brain Fe concentration and increasing the renal Mg level. Some of these changes are inconsistent with our results, as we have noted an increase in liver Fe and kidney Ca as well as no change in kidney Mg concentration.

Ethanol, similarly to Pb, had only a slight effect on Mg body status and caused less or more advanced changes in the metabolism of other elements. Various directions of changes (decrease, increase or no change) in the body status of bioelements have been reported as a result of ethanol consumption [7, 14, 16, 17, 39-41].

Although treatment with Pb or ethanol did not cause statistically significant changes in the LSM diet consumption, the tendency to decrease food ingestion was evident, especially in rats co-exposed to Pb and ethanol. Thus, changes in the body status of essential metals could be caused by differences in their consumption. Moreover, they might result from the influence of Pb and/or ethanol on absorption of these metals, tissue turnover (uptake and releasing) and excretion from the body. Due to increased cellular membranes permeability under ethanol influence, absorbed bioelements could be transported from circulation into the lumen of the gastrointestinal tract.

Some of the changes in tissue element concentrations (including reduced Cu and Mg concentrations in the kidney as well as enhanced liver Mg and muscle Cu and Fe levels) were noted only under simultaneous exposure to Pb and ethanol in spite of lower Pb intake than during its separate administration. It seems to indicate that the interaction between Pb and ethanol affects metabolism of these bioelements. In some cases, the body status of necessary metals in rats co-exposed to Pb and ethanol differed considerably not only from the control but also from the animals receiving each xenobiotics alone. It is true especially for Fe and results from various directions of disorders caused by Pb and ethanol.

The changes in the metabolism of bioelements in rats co-exposed to Pb and ethanol were caused by the independent action of Pb and/or ethanol, as well as by the interactive effect of both substances including the modifying influence of ethanol on Pb body turnover. It is also important to note that the body dehydration resulting from reduced fluid consumption in rats exposed to ethanol alone and in conjunction with Pb may be a possible factor influencing the changes in Pb turnover and bioelement metabolism. Lack of literature data concerning the metabolism of bioelements in conditions of simultaneous exposure to Pb and ethanol does not allow wider discussion of our results.

The present study clearly indicates that ethanol changes the metabolism of Pb and interacts with this toxic metal to influence the metabolism of bioelements in rats. Ethanol can modify the action of Pb, changing its turnover in the body. Because of differences in Pb intake

in the Pb and Pb + ethanol groups, in the experiment it is difficult to evaluate to what extent ethanol modified the influence of Pb on the body status of the bioelements examined. However, the fact that the intake of ethanol in the two groups receiving alcohol (ethanol and Pb + ethanol groups) was at the same level allows the conclusion that Pb can change some effects of ethanol action concerning tissue concentrations of chosen biometals. Ethanol can influence body turnover and action of Pb while Pb is able to modify some of the ethanol effects. This indicates the existence of multidirectional mutual interactions between Pb and ethanol. We hypothesize that Pb-exposed ethanol abusers are more vulnerable to accumulation of Pb and toxic action of both substances, including disturbances in the metabolism of bioelements. Further studies are needed to clarify mutual interdependence of Pb and ethanol and health risk resulting from their interactions.

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