

Mobilisation of heavy metals into the urine by CaEDTA: relation to erythrocyte and plasma concentrations and exposure indicators

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ABSTRACT To investigate the effects of calcium disodium ethylenediamine tetra-acetate (CaEDTA) on the urinary excretion, erythrocyte, and plasma concentrations and exposure indicators of seven heavy metals, CaEDTA was administered by intravenous infusion to 20 workers exposed to lead, zinc, and copper. The workers' blood lead concentrations ranged from 22 to 59 $\mu\text{g}/\text{dl}$ (mean 38 $\mu\text{g}/\text{dl}$ (1.8 $\mu\text{mol}/\text{l}$)). The 24 hour urinary excretion of metals after CaEDTA administration (mobilisation yield) was on average 13 times the background excretion for lead, 11 times for zinc, 3.8 times for manganese, 3.4 times for cadmium, 1.3 times for copper, and 1.1 times for chromium; no significant increase was found for mercury. The mobilisation yield of lead (MPb) was significantly correlated with whole blood and erythrocyte concentrations and the urinary excretion of lead but not with its plasma concentration; similarly, the mobilisation yield of cadmium was significantly correlated with its erythrocyte concentration. In addition, MPb was significantly correlated with intra-erythrocytic enzyme δ -aminolaevulinic acid dehydratase activity and urinary coproporphyrin excretion. The relation between the mobilisation yield of heavy metals and their body burden (and toxic signs) is discussed in the light of these findings.

It has recently been shown that plasma lead (PPb) concentration and urinary lead excretion are increased after the intravenous infusion of calcium disodium ethylenediamine tetra-acetate (CaEDTA) without a significant alteration in the lead concentration in either erythrocytes (EPb) or whole blood (BPb).¹ This finding suggests that urinary lead is mobilised for the most part from organs other than peripheral blood. The BPb concentration, on the other hand, is associated closely with the mobilisation yield of lead in urine by CaEDTA (MPb) in workers exposed to lead,²⁻⁵ indicating that BPb directly reflects the body burden of chelatable lead. As more than 90% of BPb exists in the erythrocytes, the EPb may also provide a better reflection of the lead body burden (as estimated by MPb)⁶ than the PPb.

The behaviour of zinc in plasma and erythrocytes (PZn and EZn) after CaEDTA infusion was entirely

different from that of lead; the PZn concentration fell rapidly, followed by a gradual rise in the EZn concentration during the first five hours after the infusion had begun.^{1,7} This observation suggests that chelatable zinc is mobilised mainly from the plasma into the urine shortly after CaEDTA infusion. The depleted PZn, however, was mostly compensated for by a redistribution of zinc from other organs during a 24 hour period.¹ It must be pointed out that the body burden of chelatable zinc is extremely large and only 0.1% of the body burden is excreted in the urine by CaEDTA per day.⁸ Our previous study has also shown that copper is mobilised into urine without a change in its concentration in either plasma (PCu) or erythrocytes (ECu) after CaEDTA infusion.⁷

In the present study we administered CaEDTA intravenously to workers exposed to lead, zinc, and copper; measured the mobilisation yields of these metals as well as four other toxic and essential heavy metals; and estimated their relation to erythrocyte and plasma concentrations and exposure indicators.

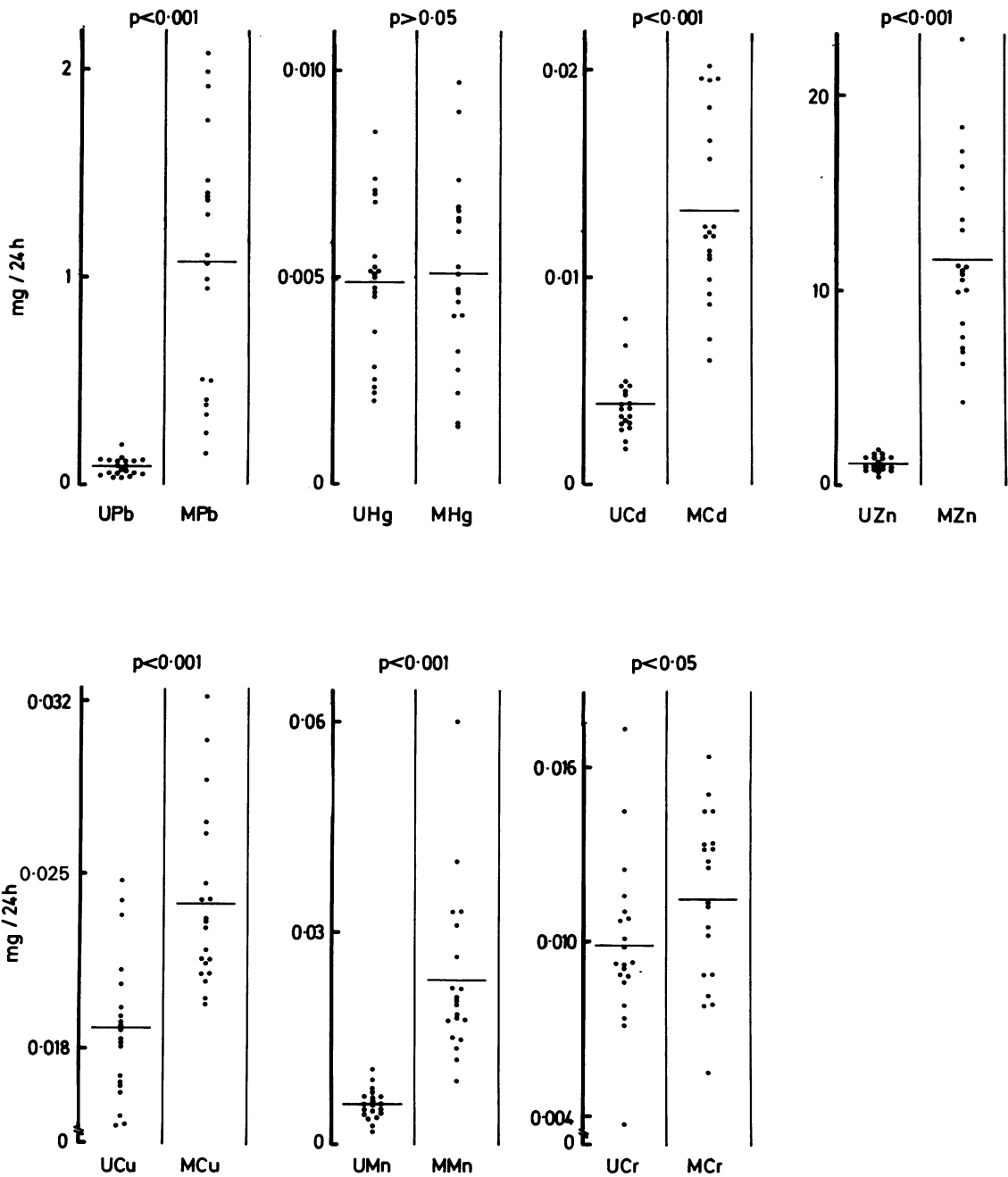


Fig 1 Spontaneous urinary excretion of lead, mercury (total mercury), cadmium, zinc, copper, manganese, and chromium (UPb, UHg, UCd, UZn, UCu, UMn, and UCr) and their mobilisation yield in urine by CaEDTA (MPb, MHg, MCd, MZn, MCu, MMn, and MCr) in 20 subjects. Transverse line in each column shows mean value. In addition, no significant difference was found between urinary excretion of inorganic mercury before and after CaEDTA infusion ($p > 0.05$). 1 mg/24 h for lead, mercury, cadmium, zinc, copper, manganese, and chromium correspond to 4.8, 5.0, 8.9, 15, 16, 18, and 19 $\mu\text{mol}/24\text{h}$, respectively.

*Plasma and erythrocyte concentrations of heavy metals in 20 subjects (means with ranges in parentheses)**

	Plasma concentration ($\mu\text{g/dl}$)	Erythrocyte concentration ($\mu\text{g/dl}$)
Lead†	0.67 (0.30-1.00)	98 (50-138)
Mercury‡	0.32 (0.15-0.77)	0.49 (0.30-0.82)
Cadmium	0.07 (0.01-0.18)	0.68 (0.20-1.20)
Zinc	89 (77-111)	889 (613-1,327)
Copper	90 (64-126)	91 (75-124)
Manganese	0.2 (0.1-0.4)	0.2 (0.1-0.4)
Chromium	4.8 (3.4-6.6)	3.3 (1.8-4.7)

*1 $\mu\text{g/dl}$ for lead, mercury, cadmium, zinc, copper, manganese, and chromium correspond to 0.048, 0.050, 0.089, 0.15, 0.16, 0.18, and 0.19 $\mu\text{mol/l}$, respectively.

†Blood lead concentration ranged from 22 to 59 (mean 38) $\mu\text{g/dl}$.

‡Represents total (inorganic and organic) mercury (the plasma and erythrocyte concentrations of inorganic mercury were 0.23 (0.12-0.38) $\mu\text{g/dl}$ and 0.29 (0.15-0.42) $\mu\text{g/dl}$, respectively).

Subjects and methods

SUBJECTS

The 20 subjects, aged 34 to 59 (mean 49), were male gun metal foundry workers employed at a metal factory for 2-17 (mean 10) years. Their BPb and plasma and erythrocyte concentrations of lead, mercury

(total mercury), cadmium, zinc, copper, manganese, and chromium (PPb, EPb, PHg, EHg, PCd, ECD, PZn, EZn, PCu, ECu, PMn, EMn, PCr, and ECr) are shown in the table. The BPb, PZn, and PCu concentrations in these workers were significantly higher than those in 16 male steel foundry workers at the same factory, 8-20 (mean 13) $\mu\text{g/dl}$ (0.4-1.0 (mean 0.6) $\mu\text{mol/l}$) for BPb, 54-97 (mean 72) $\mu\text{g/dl}$ (8.3-14.8 (mean 11.0) $\mu\text{mol/l}$) for PZn, and 50-100 (mean 73) $\mu\text{g/dl}$ (7.9-15.7 (mean 11.5) $\mu\text{mol/l}$) for PCu, respectively ($p < 0.05$). No subject had ever suffered from renal disease; neither albuminuria nor glucosuria was found in any subject before and after this study.

COLLECTION AND ANALYSIS OF BLOOD AND URINE SAMPLES

Following collection of urine samples for 24 hours in 20 subjects, blood samples were collected; then CaEDTA was administered by intravenous infusion for one hour into the subjects in a dosage of 20 mg (53.4 μmol) per kg body weight in 250 ml of 5% glucose solution; and 24 hour urinary excretion of lead, mercury, cadmium, zinc, copper, manganese, and chromium before and after CaEDTA administration

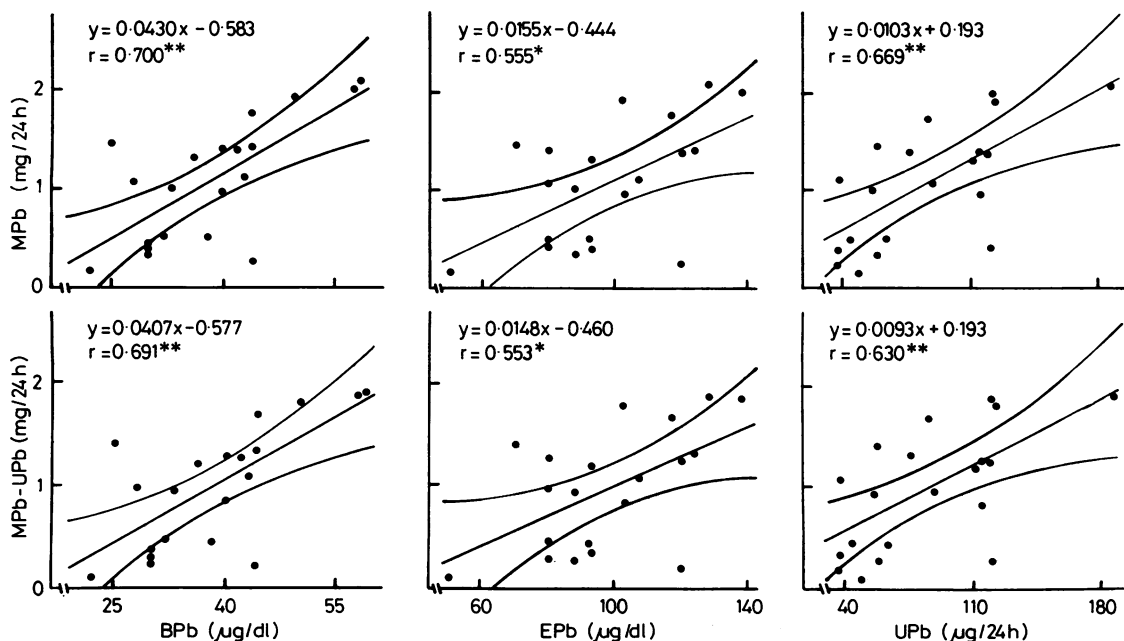


Fig 2 Correlations of mobilisation yield of lead in urine by CaEDTA (MPb) and MPb minus spontaneous urinary excretion of lead (UPb) with its whole blood and erythrocyte concentrations (BPb and EPb) and UPb in 20 subjects. * and ** indicate significant correlations at levels of $p < 0.05$ and 0.01, respectively (large sample conservative multiple significance test). Regression lines with 95% confidence limits are additionally shown. Conversion to SI units as in table and fig 1.

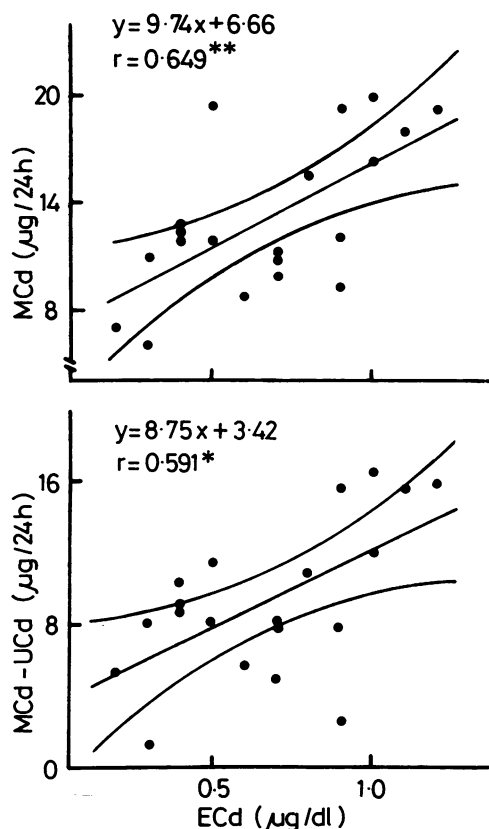


Fig 3 Correlations of mobilisation yield of cadmium in urine by CaEDTA (MCd) and MCd minus spontaneous urinary excretion of cadmium (UCd) with its erythrocyte concentration (ECd) in 20 subjects. * and ** indicate significant correlations at levels of $p < 0.05$ and 0.01 , respectively (large sample conservative multiple significance test). Regression lines with 95% confidence limits are additionally shown. Conversion to SI units as in table and fig 1.

(UPb, MPb, UHg, MHg, UCd, MCd, UZn, MZn, UCu, MCu, UMn, MMn, UCr, and MCr) was measured. The administration of CaEDTA was requested by the management and the workers at the factory. We explained to them that there was no medical indication for giving the chelating agent to them except for diagnostic purposes, and there is no evidence that in diagnostic and therapeutic doses this drug is hazardous to persons with "normal" renal function.⁹ They all elected to accept this test.

BPb, EPb, UPb, and MPb concentrations were measured by atomic absorption spectrophotometry (AAS) (Hitachi polarised zeeman atomic absorption

spectrophotometer 180–80) after wet ashing, chelation by sodium diethyldithiocarbamate (DDTC), and extraction into water saturated methyl-isobutyl ketone (MIBK); the PPb concentration was measured by the method of DeSilva.¹⁰ The total and inorganic mercury concentrations in plasma, erythrocytes, and urine were determined by the method of Magos (Sugiyama-gen mercury vapor meter MV-253R).^{11,12} PCd, ECd, PCu, and ECu concentrations were measured by the flameless AAS after deproteinisation by trichloroacetic acid (TCA); UCd and MCd by the method of Subramanian *et al.*¹³ PZn, UZn, and MZn concentrations were measured by the AAS after deproteinisation by TCA; EZn by the AAS after wet ashing. PMn, EMn, PCr, and ECr concentrations were measured by the flameless AAS using the standard addition technique after deproteinisation by TCA; UCu, MCu, UMn, MMn, UCr, and MCr concentrations by the AAS after wet ashing, chelation by DDTC, and extraction to MIBK. Urinary δ -aminolaevulinic acid (ALA) and coproporphyrin (CP) were measured by the methods of Tomokuni and Ogata¹⁴ and Soulsby and Smith,¹⁵ respectively; and the activity of the intra-erythrocytic enzyme ALA dehydratase (ALAD) was determined by the European standardised method.¹⁶

The lower limits of detection were 0.2, 0.05, 0.2, and 0.1 $\mu\text{g}/\text{dl}$ for BPb, PPb, EPb, and UPb (MPb) concentrations, respectively; 0.05 $\mu\text{g}/\text{dl}$ (0.002 $\mu\text{mol}/\text{l}$) for PHg, EHg, and UHg (MHg); 0.01 $\mu\text{g}/\text{dl}$ (0.0009 $\mu\text{mol}/\text{l}$) for PCd and ECd, and 0.05 $\mu\text{g}/\text{dl}$ for UCd (MCd); 2 $\mu\text{g}/\text{dl}$ for PZn, EZn, and UZn (MZn); 2.5 $\mu\text{g}/\text{dl}$ for PCu and ECu, and 0.2 $\mu\text{g}/\text{dl}$ for UCu (MCu); 0.1 $\mu\text{g}/\text{dl}$ (0.02 $\mu\text{mol}/\text{l}$) for PMn, EMn, and UMn (MMn); 0.2 $\mu\text{g}/\text{dl}$ (0.04 $\mu\text{mol}/\text{l}$) for PCr and ECr, and 0.1 $\mu\text{g}/\text{dl}$ for UCr (MCr); and 0.5 mg/l (4 $\mu\text{mol}/\text{l}$) and 5 $\mu\text{g}/\text{l}$ (0.008 $\mu\text{mol}/\text{l}$) for urinary ALA and CP, respectively.

The reproducibility of analysis for blood, plasma, erythrocyte or urinary lead, zinc, copper, ALA, and CP has been reported previously (the coefficients of variation for determination of BPb, PPb, EPb, and UPb were 3.3, 3.5, 3.5, and 3.4%, respectively).^{7,17} The coefficients of variation for determinations of other substances were 6.6% for PHg and EHg, and 7.1% for UHg (MHg); 3.4% for PCd and ECd, and 4.8% for UCd (MCd); 6.4% for PMn and EMn, and 6.8% for UMn (MMn); and 4.1% for PCr and ECr, and 5.8% for UCr (MCr).

STATISTICAL ANALYSIS

The difference between the spontaneous urinary excretion of heavy metal and its mobilisation yield in urine by CaEDTA was compared with paired sample t tests. Correlations of MPb (and MPb minus UPb) with BPb, PPb, EPb, and UPb and with ALA, CP,

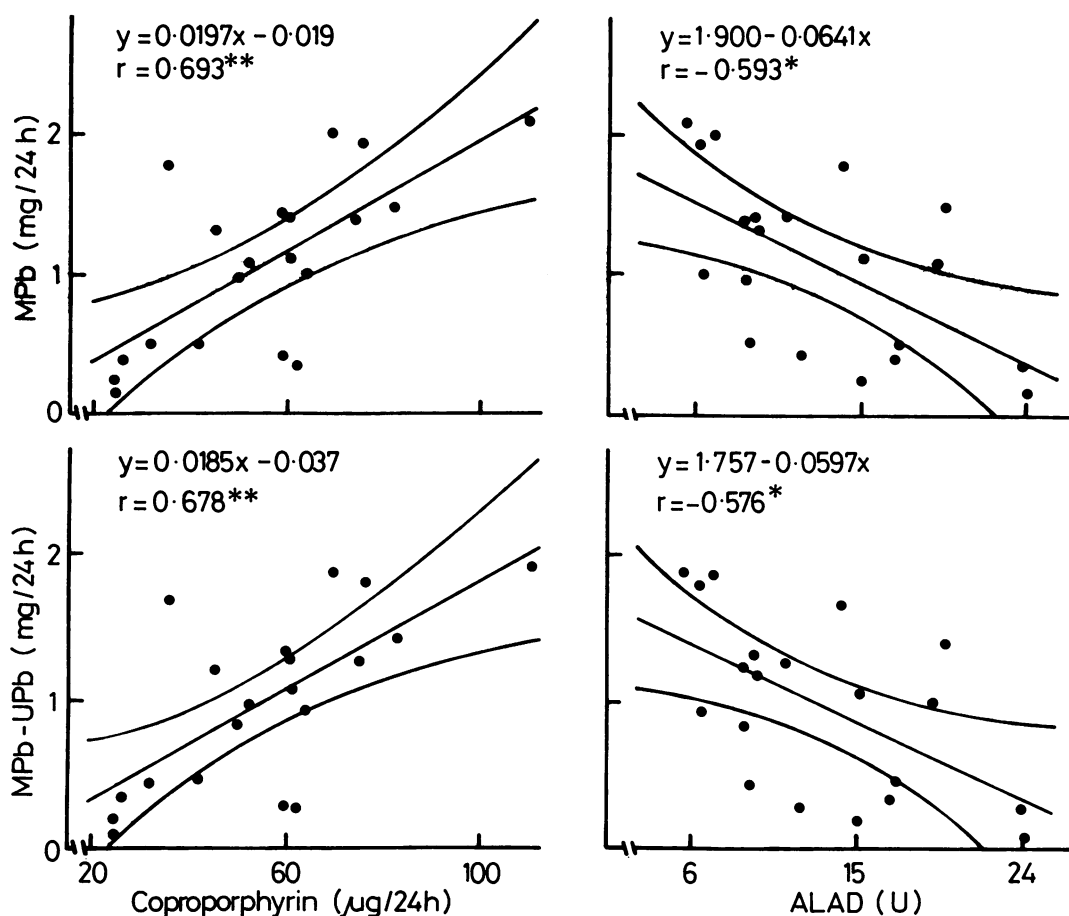


Fig 4 Correlations of mobilisation yield of lead in urine by CaEDTA (MPb) and MPb minus spontaneous urinary excretion of lead (UPb) with urinary excretion of coproporphyrin and intra-erythrocytic enzyme δ -aminolaevulinic acid dehydratase (ALAD) activity in 20 subjects. * and ** indicate significant correlations at levels of $p < 0.05$ and 0.01 , respectively (large sample conservative multiple significance test). Regression lines with 95% confidence limits are additionally shown. 1 $\mu\text{g}/24\text{h}$ for coproporphyrin corresponds to 1.5 nmol/24 h.

ALAD, and duration of lead exposure were analysed by a large sample conservative multiple significance test¹⁸; multiple correlations for other metals were also analysed by this test.

Results

The urinary excretion of all metals, with the exception of mercury, significantly exceeded the pretreatment urinary excretion values (fig 1). MPb and the value for MPb minus UPb were significantly correlated with the BPb and EPb concentrations and UPb excretion (fig 2) but not with the PPb concentration ($r = 0.28$ and 0.28 , respectively, $p > 0.05$). MCd and the value for MCd minus UCd were also significantly correlated with the ECd concentration (fig 3) but not with the PCd concentration ($r = 0.14$ and 0.01 ,

respectively) and UCd excretion ($r = 0.21$ and -0.13) ($p > 0.05$). Furthermore, MPb and the value for MPb minus UPb were significantly correlated with urinary CP excretion and ALAD activity (fig 4). No significant correlation was found between the mobilisation yield of any two heavy metals.

Discussion

The 24 hour urinary excretion after CaEDTA infusion was on average 13 times the spontaneous excretion for lead, 11 times for zinc, 3.8 times for manganese, 3.4 times for cadmium, 1.3 times for copper, and 1.1 times for chromium; no significant increase was found for mercury. Our previous studies have shown that the ratio of MPb to UPb was on

average 4.8 for non-exposed "healthy" men and 9.8 for lead workers (their UPb averaged 20 and 61 $\mu\text{g}/24\text{ hr}$, respectively).^{2,19} In this study the UPb averaged 85 $\mu\text{g}/24\text{ hr}$. It is thus suggested that the higher the pretreatment UPb the higher the rise in MPb and MPb minus UPb. The same evidence has been given for the relation between BPb and MPb in lead workers by us^{2,6} and in children by Chisolm *et al.*²⁰

In 1959 Perry and Perry injected 3 g CaEDTA intravenously into six patients with hypercholesterolaemia, and found a pronounced increase in 24 hour urinary zinc excretion (the ratio of MZn to UZn ($\mu\text{g}/\text{l}$) was 11) and lesser but consistent increases in lead, cadmium, and manganese excretion.²¹ In 1958 Teisinger and Fiserová-Bergerová found that 2.8 g CaEDTA increased the urinary secretion of copper from 0–124 $\mu\text{g}/24\text{ hr}$ to 62–249 $\mu\text{g}/24\text{ hr}$.²² In 1955 Bell *et al* did not find an increase in urinary mercury excretion by CaEDTA in a patient of chronic mercury poisoning²³ whereas Woodcock did.²⁴ Our present and past¹⁹ results do not differ substantially from those observations; furthermore, the present study indicated a slight but significant increase in chromium excretion and a non-significant increase in mercury excretion in men free from occupational exposure to these metals.

MPb, together with the value for MPb minus UPb, was significantly correlated with CP and ALAD. This confirms previous findings^{2,5} and indicates that MPb reflects the body burden of chelatable lead on which toxicity depends. Similarly, the high correlations of BPb, EPb, and UPb with MPb and MPb minus UPb indicate that BPb, EPb, and UPb, but not PPb, closely reflect the body burden of chelatable lead; the correlations of ECd with MCd and MCd minus UCd suggest that ECd, but not PCd and UCd, directly reflects the body burden of chelatable cadmium.

On the other hand, MZn did not correlate with either PZn or EZn ($r = 0.31$ and 0.22 , respectively, $p > 0.05$). Similarly, no significant correlation was found between MCu and PCu or ECu, between MMn and PMn or EMn and between MCr and PCr or ECr. These findings suggest that zinc, copper, manganese, and chromium are mainly mobilised from organs other than the peripheral blood.

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