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Blood cell δ -aminolevulinic acid dehydratase activity in humans exposed to methylmercury

by ANDREJS SCHÜTZ, B.Sc.,¹ and STAFFAN SKERFVING, M.D.^{1, 2}

SCHÜTZ, A. and SKERFVING, S. Blood cell δ -aminolevulinic acid dehydratase activity in humans exposed to methylmercury. *Scand. j. work environ. & health* 1 (1975) 54—59. The δ -aminolevulinic acid dehydratase (ALA-D) activity in blood cells was studied in 15 subjects exposed to methylmercury through consumption of contaminated fish and 19 «unexposed» subjects with a similar sex and age distribution. The exposed subjects had a mean mercury level of 120 (range 15—370) ng/g blood cells while the controls had 9 (range 4—15) ng/g. Both groups had the same mean level of lead in whole blood (10 ± 1 μ g/100 ml). ALA-D activity decreased statistically significantly as both mercury and lead levels in the blood cells increased.

Key words: toxicology, human experiment, δ -aminolevulinic acid dehydratase, methylmercury, lead, blood.

Methylmercury in fish has grown into a major toxicological concern in many countries (3, 10, 20). Interest has so far been focused mainly on nervous tissue damage (2). It would be of great importance to know also if effects occur at lower degrees of exposure.

In the case of inorganic lead even a minor increase in the blood lead level causes a significant decrease in the activity of the blood cell δ -aminolevulinic acid dehydratase (ALA-D; E.C. 4.2.1.24) (5, 15, 16, 17, 18), an enzyme engaged in the heme synthesis. The sulfhydryl groups in ALA-D are necessary for enzymatic activity (14). Both inorganic lead and methylmercury bind to sulfhydryl groups in proteins and accumulate in the blood cells. The addition of inorganic mercury salt to hemolyzed human blood causes a decrease of ALA-D activity (7). Thus it was of interest to study whether ALA-D activity was depressed in subjects exposed to methylmercury through fish consumption.

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MATERIAL AND METHODS

Subjects

The exposed group contained 15 subjects (12 males aged 44—76 years and 3 females aged 53—56), who consumed various amounts of fish from four mercury-contaminated water areas in central Sweden. The average methylmercury levels in pike (*Esox lucius*) from those areas ranged from 0.5 to 6 mg of mercury per kilogram of wet weight. As reported in an earlier study no one had symptoms or signs of methylmercury poisoning (30).

Blood samples were also obtained from 19 «unexposed» subjects from urban areas of Stockholm and Lund. Their sex and age distribution was very similar to the exposed group. They had fish once a week or less. None of the unexposed had eaten fish from mercury-contaminated water areas.

None of the exposed or unexposed subjects had a history of occupational exposure to mercury or lead or of exposure to mercury-containing drugs. None had a record of alcohol abuse, and no one had consumed alcohol close to sampling.

Blood sampling

Blood was obtained through venipuncture into acid-washed polyethylene tubes containing heparine. The samples from the exposed group were transported to the laboratory by air. They were stored at room temperature and analyzed for ALA-D 4 to 6 hours after sampling. Most of the control samples were treated in the same way. However, seven samples from Lund were not sent by air but were kept in the laboratory at room temperature for a corresponding time before analysis.

ALA-D activity

ALA-D activity was determined in whole blood by the method of Bonsignore et al. (6) with the following modifications: The ALA-substrate solution was prepared by dissolving ALA·HCl in a commercially available (Svenska Finkemikalier, Altna, Sweden) phosphate buffer solution of pH 7.0 ± 0.025 (3.059 g KH_2PO_4 and 6.463 g $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$ per liter). The pH in the mixture of hemolyzed blood and ALA-substrate solution, as measured at 38°C in 10 different samples, was within the range of 6.88 to 6.93. No mercuric chloride was added to the trichloroacetic acid solution used for protein precipitation. A modified Erlich's reagent containing 1 g of paradimethylaminobenzaldehyde and 16 ml of perchloric acid (density 1.70) in 100 ml of glacial acetic acid was used for color development. Maximum absorbance was obtained after 10 minutes, and it remained stable for another 10 minutes. We calculated the porphobilinogen (PBG) concentration in the sample using the «apparent» molar extinction coefficient for PBG and the present Erlich's reagent ($6.2 \cdot 10^4 \text{ mole}^{-1} \cdot \text{cm}^{-1}$) reported by Mauzerall and Granick (24).

As ALA-D activity in blood is almost completely confined to the blood cells, the activity was expressed as the micromole increase of PBG per hour per liter of blood cells (RBC). The packed cell volume was obtained by use of a Coulter-counter (Mod. S).

The error of the method for duplicate analyses of 20 samples in the range of 700 to 1,800 $\mu\text{moles PBG/h/l RBC}$ was

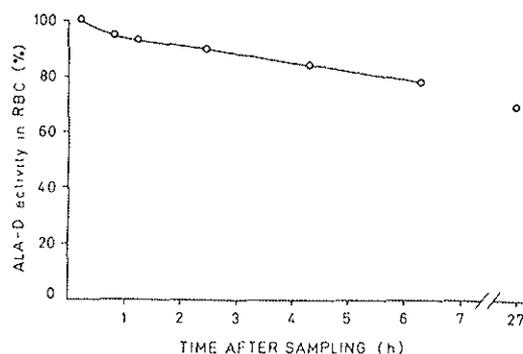


Fig. 1. Relation between the ALA-D activity in the blood cells and the time between blood sampling and the start of incubation in samples kept at room temperature ($\sim 20^\circ\text{C}$). The ALA-D activity at the first determination (incubation 13 minutes after sampling) has been used as 100 %.

$\pm 33 \mu\text{moles PBG/h/l RBC}$ (3 % of the mean). The effect of time between sampling and incubation of the activity was checked repeatedly. The average decrease during 6 hours was about 15 % (fig. 1).

Lead

Duplicate samples of 5 ml of whole blood were wet-ashed, and an ammonia-citrate buffer containing dithizone was added. Lead dithizonate was extracted with methyl isobutyl ketone (MIBK), and the concentration of lead in the MIBK phase was determined by atomic absorption spectrophotometry (Perkin-Elmer 403). The detection limit was about $1 \mu\text{g}/100 \text{ ml}$. The error of the method for the duplicate analyses of 20 samples in the range of 4 to $12 \mu\text{g}/100 \text{ ml}$ was $\pm 0.3 \mu\text{g}/100 \text{ ml}$ (4 % of the mean).

As lead is present in whole blood almost entirely in the blood cells (28), it was assumed in the statistical treatment that all lead was in the blood cells.

Mercury

In one part of each blood sample blood cells and plasma were separated by centrifugation at $200 \times g$ for 10 minutes. Mercury in blood cells and plasma was determined in duplicate samples by flameless atomic absorption spectrometry according

to Schütz (29). The limit of detection was 1 ng/g. The coefficient of variation at repeated analyses of a blood cell sample containing about 100 ng/g was 1 %.

The blood cell mercury level is a good indicator of methylmercury exposure (3). The mercury is present mainly as methylmercury (2, 4, 26).

Statistics

Conventional statistical methods were used (8).

RESULTS

The exposed group had much higher levels of mercury in their blood cells and plasma than the unexposed (table 1). The range of mercury levels within the exposed group was considerable. The average levels of lead in whole blood were identical in the two groups. The unexposed group had a slightly higher average ALA-D activity in their blood cells than the exposed group, but the difference was not significant ($t = 0.6$; $p > 0.5$).

When all subjects in the exposed and the unexposed groups were pooled, there was a slight, but statistically significant, decrease in both the linear (fig. 2; $t = 2.9$; $0.001 < p < 0.01$) and the logarithmic ($t = 3.6$; $p = 0.001$) value of the ALA-D activity as the blood cell mercury level increased.

There was no significant decrease in either the linear (fig. 3; $t = 1.8$; $0.05 < p < 0.1$) or the logarithmic ($t = 1.8$; $0.05 < p < 0.1$) value of ALA-D activity as the blood lead increased.

There was no correlation between

mercury and lead levels in blood cells ($r = -0.07$; $t = 0.4$; $p > 0.7$).

The relation between ALA-D activity on one hand and lead and mercury levels in blood cells on the other was studied by multiple regression analysis. The function arrived at was $ALA-D = 1,300 - 1,500 \cdot Hg - 1,100 \cdot Pb$, when concentrations of mercury (Hg) and lead (Pb) were expressed as mg/l RBC. The probability of obtaining these figures if no relation between ALA-D activity and levels of mercury and/or lead in blood cells existed was low ($F = 7.5$; $0.001 < p < 0.01$). The probability of obtaining the coefficient for mercury if the effect was entirely dependent on lead levels was also low ($t = -3.2$; $0.001 < p < 0.01$), as was the probability of arriving at the coefficient for lead if the effect was only dependent on mercury levels ($t = -2.3$; $0.01 < p < 0.05$). The impact of mercury was not statistically significantly larger than that of lead ($t = 0.6$; $p > 0.1$).

DISCUSSION

The lead levels of whole blood in both groups as well as blood cell and plasma mercury levels in the unexposed subjects fit well with results for «unexposed» subjects reported earlier by Haeger-Aronsen et al. (15) and Tejning (32). The mercury levels in the exposed group agreed with earlier reports on methylmercury-exposed Swedish «fisheaters» (4, 30) but were below the lowest level (about 400 ng/g blood cells) estimated to have been present at the onset of neurological symptoms in poisoned subjects (2, 3).

The decrease of about 15 % of the

Table 1. Mean (M), standard error of the mean (SE), and range of the levels of mercury, lead, and ALA-D in the blood of 15 subjects exposed to methylmercury and 19 unexposed subjects.

Analysis	Exposed		Unexposed	
	M ± SE	Range	M ± SE	Range
Mercury in blood cells (ng/g)	120 ± 25	15—340	9.4 ± 0.7	4.5—15
Mercury in plasma (ng/g)	14 ± 2.6	1.7—34	3.1 ± 0.4	1.5—7.6
Lead in whole blood (μg/100 ml)	10 ± 1	4—18	10 ± 1	5—22
ALA-D activity in blood cells (μmoles PBG/h/1 RBC)	810 ± 63	360—1,300	1,000 ± 63	620—1,800

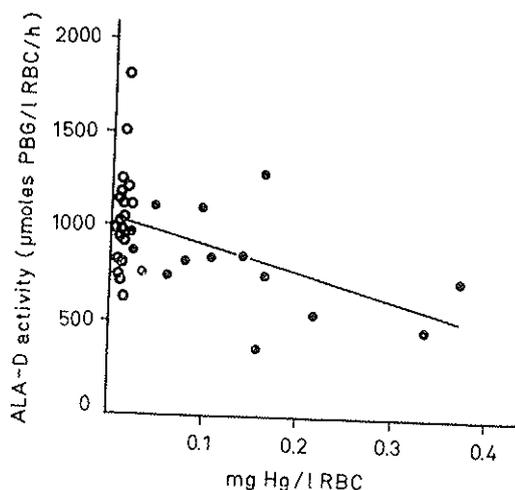


Fig. 2. Relation between the mercury (Hg) level and ALA-D activity in the blood cells (RBC) of 15 subjects exposed to methylmercury (dots) and 19 unexposed subjects (circles). ($Y = 1,000 - 1,400 X$)

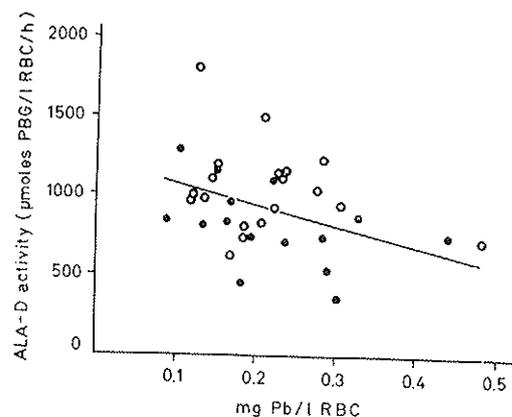


Fig. 3. Relation between the lead (Pb) level and the ALA-D activity in the blood cells (RBC) of 15 subjects exposed to methylmercury (dots) and 19 unexposed subjects (circles). ($Y = 1,200 - 1,300 X$)

ALA-D activity that was assumed to have occurred between sampling and analysis — and which is in fair agreement with earlier studies (27) — should not have affected the results significantly, as samples from exposed and unexposed subjects were treated similarly. It might, however, have contributed to some extent to the interindividual variation in ALA-D activity.

The average ALA-D activity in the exposed group was lower than in the unexposed, but the difference did not reach the limit of statistical significance. However, when the results of the two groups were pooled and studied, a clear decrease in ALA-D activity with increasing mercury levels was revealed. This effect was further supported by the multiple regression analysis. The latter calculation also displayed an effect of lead, which on a molar basis was of about the same strength as that of mercury.

Wada et al. (36) reported a negative correlation between mercury levels in urine and blood cell ALA-D activity in workers exposed to elemental mercury vapor. They did, however, not report the blood lead levels, and the result is thus difficult to interpret. Hernberg et al. (17) and Lauwerys and Buchet (21) made

similar studies, but the subjects were less exposed. In addition Hernberg et al. determined the blood lead levels. In neither study was there any effect of elemental mercury vapor exposure on the blood cell ALA-D activity. The mercury levels in blood were, however, much lower than in the exposed group in the present study.

There is at present no reason to assume any deleterious effect from a depression of ALA-D activity in blood cells. Methylmercury is, however, widely distributed in the body (3, 26), and there are some indications that the effect of methylmercury on ALA-D might not be restricted to the blood cells.

In animal experiments the in vivo administration of p-chloromercuribenzoate (PCMB) and mercaptomerin inhibited heme synthesis (19). Exposure to methylmercury increases ALA and coproporphyrin excretion in the urine of man (23, 33), while elemental mercury vapor and inorganic and phenyl mercury salts increase coproporphyrins (13, 31, 35, 36), but not ALA (13, 36), in the urine.

Despite the effect of methylmercury on ALA-D activity (at least in blood cells), anemia is not a typical sign in methylmercury poisoning. However, even in the case of lead exposure, the ALA-D activity

in blood cells may be almost completely inhibited for long periods without evidence of anemia (15).

Inorganic mercuric salt inhibits ALA-D in liver when added in vitro (11). Methylmercury has been shown to affect a heme-containing cytochrome in vivo in the liver microzome detoxification system (1, 9, 22).

Experimental administration of lead causes a depression of ALA-D in brain tissue (12, 25). If a similar depression of ALA-D activity in nervous tissue does occur with methylmercury exposure, it might be of importance in the pathogenesis of the nervous tissue damage in methylmercury poisoning.

ALA-D activity is used as an index of lead exposure in groups (34). The present results raise no serious objections towards that screening method since methylmercury exposure heavy enough to give blood levels comparable to those of lead is extremely uncommon in most areas. Even in the most exposed of our subjects, the levels of mercury and lead in blood cells were about equal. It is important, however, to keep in mind that ALA-D is not affected by lead only.

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