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Comparative study of effect of inorganic lead and cadmium on blood δ-aminolevulinate dehydratase in man

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Lauwerys, R. R., Buchet, J.-P., and Roels, H. A. (1973). *British Journal of Industrial Medicine*, **30**, 359-364. **Comparative study of effect of inorganic lead and cadmium on blood δ-aminolevulinate dehydratase in man.** δ-Aminolevulinate dehydratase (ALA[1]-D) of red blood cells, lead concentration in blood (Pb-B) and in urine (Pb-U), cadmium concentration in blood (Cd-B) and in urine (Cd-U), and ALA in urine (ALA-U) were measured in 77 workers occupationally exposed to cadmium, and in 73 control workers.

An excellent negative correlation was found between log ALA-D and Pb-B ($r = -0{\cdot}660$) or Pb-U ($r = -0{\cdot}501$), but no significant correlation was found between Cd-B and log ALA-D activity.

Unlike ALA-D, ALA-U is not correlated with Pb and Pb-U in the 'normal' range of Pb concentration investigated. Mean ALA-D activity in smokers is lower than in nonsmokers, and this is probably related to the fact that a higher mean Pb-B concentration is found in smokers than in nonsmokers.

It is clear from this investigation that in the general population, and even in Cd-exposed workers, Cd has no significant effect on ALA-D. Moreover, all the available evidence indicates that ALA-D activity of erythrocytes is a very sensitive and specific parameter of lead in blood.

The red blood cell enzyme δ-aminolevulinate dehydratase (5-aminolevulinate hydro-lyase: E.C. 4·2·1·24) (ALA-D) is extremely sensitive to inhibition by inorganic lead *in vitro* and *in vivo* (Lichtman and Feldman, 1963; Bonsignore, Calissano, and Cartasegna, 1965; Calissano, Cartasegna, and Bonsignore, 1965; de Bruin and Hoolboom, 1967; Nakao, Wada, and Yano, 1968; de Bruin, 1968; Hernberg and Nikkanen, 1970; Basecqz, Lauwerys, and Buchet, 1971). Several authors (Hernberg, Nikkanen, Mellin, and Lilius, 1970; Millar *et al.*, 1970; Basecqz *et al.*, 1971; Weissberg, Lipschutz, and Oski, 1971) have reported the existence of a good negative correlation between ALA-D activity and the concentration of lead in blood (Pb-B) even within the 'normal' range of Pb-B concentration (10–40 μg %) (Hernberg and Nikkanen, 1970; Millar *et al.*, 1970). ALA-D inhibition is considered to be a very early biological effect of exposure to lead (Hernberg, Tola, Nikkanen, and Valkonen, 1972), but it seems too sensitive to be of practical use for the control of workers exposed to lead (Basecqz *et al.*, 1971; Haeger-Aronsen, Abdulla, and Fristedt, 1971; Lauwerys, 1972; Hernberg *et al.*, 1972). However, the finding of a slight ALA-D inhibition in some urban populations has raised a still unsettled controversy on the importance of environmental pollution by lead.

The use of ALA-D activity measurement as a good quantitative parameter for the circulating lead in nonoccupationally exposed persons (Hernberg *et al.*, 1970 and 1972) requires not only that this method must be sensitive enough (which has already

ALA = δ-aminolevulinic acid

been demonstrated), but also that it must be specific for lead. If this were really so, the measurement of ALA-D activity would indeed be a very attractive procedure to control the degree of lead exposure of the general population, because, as is usually recognized, Pb-B measurement can give variable results in the range of low Pb concentration (Berlin, del Castilho, and Smeets, 1972). However, the application of ALA-D measurement to the prediction of specific lead effects has been extensively questioned in the last two years (NRC-NAS Report, 1971; CCE-EPA International Symposium, 1972; Goldwater, 1972). Ethanol consumption (Moore, Beattie, Thompson, and Goldberg, 1971) and cigarette smoking (Calandra, 1971, cited by Millar *et al.*, 1972) have been reported to inhibit ALA-D in the blood of man. The disturbing effect of other metal ions *in vivo* has been suggested (Haeger-Aronsen *et al.*, 1971; Goldwater, 1972), since ALA-D from human erythrocytes and from animal tissues is inhibited *in vitro* by various heavy metal ions, e.g., Cu^{2+}, Hg^{2+}, and Ag^{+} (Gibson, Neuberger, and Scott, 1955; Calissano *et al.*, 1965).

The investigations in our laboratory were, therefore, focused on whether ALA-D activity is affected by mercury and cadmium, which, like lead, are recognized as two contaminants of our environment. We have already reported that mercury at the blood concentration found in the general population does not inhibit ALA-D (Lauwerys and Buchet, 1973), and it was even observed that workers slightly exposed to mercury vapours and excreting less than 35 μg Hg/g creatinine exhibited a slight increase in ALA-D activity. Wada *et al.* (1969) found some inhibition of ALA-D by mercury, but only in workers excreting more than 200 μg Hg/g creatinine.

The present paper compares the action of lead and cadmium on ALA-D and is based on findings made during a survey of workers exposed to cadmium.

Materials and methods

Subjects

ALA-D activity of red blood cells, δ-aminolevulinic acid in urine (ALA-U), lead (Pb-U) and cadmium (Cd-U) in urine, and lead (Pb-B) and cadmium (Cd-B) content of blood were measured in 150 workers (90 men and 60 women) belonging to three different Belgian factories, i.e., an electronic workshop, a nickel-cadmium storage battery factory, and a cadmium-producing plant. Half of the employees in each factory were exposed to cadmium dust, and the other half constituted the control group.

The women were mainly employed in the electronic workshop where the exposure was significantly lower (about $\frac{1}{10}$ to $\frac{1}{20}$) than in the two other factories; there the employees were principally male and the average airborne concentration of Cd-dust was about 100 $\mu g/m^3$ (four workers in the cadmium-producing plant were also intermittently exposed to Cd-fumes).

A more detailed description of the different populations and the airborne concentrations of cadmium in the three factories is not relevant to the present report and will be published in other papers discussing the action of cadmium on lung function and on several biological parameters.

Analysis

Approximately 20 ml of blood was withdrawn by venepuncture and immediately transferred into tubes containing heparin; the tubes were kept at 4°C in a portable refrigerator.

ALA-D activity measurement was always performed 24 hours after blood withdrawal. To a test tube was added 1·3 ml of a 50 mmol/l phosphate buffer (pH 7) containing 0·2% Triton X-100 (Packard Instruments Company, Downers Grove, Ill., U.S.A.), 0·2 ml of whole blood, and 0·1 ml of a 20 mM δ-aminolevulinic acid solution (puriss., Fluka, Buchs, Switzerland) in phosphate buffer (50 mM, pH 7). After 30 minutes of incubation at 37°C the enzymatic reaction was stopped by the addition of 1 ml of a 10% trichloroacetic acid solution containing 10% $HgCl_2$ (UCB, Brussels, Belgium). After centrifugation at room temperature in a Sorvall GLC-1 (Sorvall Inc., Norwalk, Conn., U.S.A.) at 2500 rpm for 5 minutes, the supernatant was filtered on Whatman No. 54. To 1 ml of the filtrate was added 1 ml of modified Ehrlich's reagent (Bonsignore *et al.*, 1965). Exactly 5 minutes later the absorbance was read against water at 555 nm in cuvettes of 2 cm light path using a Zeiss spectrophotometer PMQ2 (Oberkochen, W.-Germany). A blank (addition of the substrate after trichloroacetic acid) was run for each blood sample. The ALA-D activity is expressed in units of absorbance for 2 cm light path: $(A_{analysis}—A_{blank}) \times 10/hour/ml$ red cells. The blood haematocrit was measured with an International Micro-capillary Centrifuge, Model MB (International Equipment Company, Needham, Mass., U.S.A.).

Pb-B, Cd-B, Pb-U, and Cd-U were measured by the method of Vens and Lauwerys (1972). Pb-B and Cd-B are expressed in μg per 100 g of whole blood; Pb-U and Cd-U were expressed in $\mu g/g$ creatinine.

ALA-U was determined by the method of Lauwerys Delbroeck, and Vens (1972) and expressed in mg/g creatinine.

Results

Table 1 summarizes the principal characteristics of the study population. There are no striking differences between the Cd-exposed and corresponding nonexposed subgroups with regard to mean age and smoking habit. The smoking habit was taken into account, since it had been shown that Cd as well as Pb are present in cigarette smoke (Nandi *et al.*, 1969).

Table 2 gives the mean values of Pb-B, Cd-B, ALA-D, Pb-U, Cd-U, and ALA-U in the different subgroups. There is an increase in concentration of Cd-B and Cd-U in the exposed subgroups, but only in men exposed to Cd are both biological parameters significantly higher than in the control group. It is not surprising that Cd-B and Cd-U in exposed women were much lower than in exposed men, as

TABLE 1

PRINCIPAL CHARACTERISTICS OF DIFFERENT SUBGROUPS IN STUDY POPULATION
(mean ± SEM)

Subgroups of study population			*No. of subjects*	*Mean age (years)*	*No. of cigarettes smoked per day*	*Duration of smoking (years)*	*Duration of Cd-exposure (years)*
Women	Cd-exposed	S	13	31·6 ± 3·3	11·0 ± 2·0	9·5 ± 2·3	4·8 ± 0·9
		NS	18	29·7 ± 2·1	—	—	3·5 ± 0·4
		S + NS	31	30·5 ± 1·8	—	—	4·1 ± 0·5
	Nonexposed	S	14	29·6 ± 1·9	16·0 ± 2·1	9·3 ± 1·8	—
		NS	15	28·2 ± 2·3	—	—	—
		S + NS	29	28·9 ± 1·5	—	—	—
Men	Cd-exposed	S	36	48·7 + 1·7	13·9 + 1·1	27·3 ± 1·7	19·4 ± 2·0
		NS	10	31·3 ± 1·2	—	—	9·5 ± 3·9
		S + NS	46	44·9 ± 2·0	—	—	17·3 ± 1·8
	Nonexposed	S	33	47·7 ± 1·7	17·4 ± 1·2	25·6 ± 1·9	—
		NS	11	33·4 ± 4·3	—	—	—
		S + NS	44	44·1 ± 1·9	—	—	—

SEM = standard error of the mean
S = smokers; NS = nonsmokers

the women were working in the less polluted workshop. Furthermore, the results obtained in men indicate that Cd-U increases proportionally more than Cd-B upon exposure to Cd.

All the Pb-B and Pb-U means are also significantly higher in men than in women, but the mean ALA-U in men is not higher than in women (Table 2). This confirms that in the 'normal' range of Pb-B and Pb-U (as was the case with our study population) ALA-U is not correlated with Pb-B and with Pb-U (Table 3) and, therefore, is not a sensitive index of the Pb body burden of nonoccupationally exposed workers (Schubert and Sangl, 1972).

As in the case of Pb-B and Pb-U, there is also no

TABLE 2

BIOLOGICAL PARAMETERS OF CD-EXPOSED AND NONEXPOSED WORKERS
(mean ± SEM)

Subgroups in study population			*Pb-B (μg %)*	*Cd-B (μg %)*	*ALA-D*[1]	*Pb-U*[2]	*Cd-U*[2]	*ALA-U*[2]
Women	Cd-exposed	S	11·5 ± 1·6	2·3 ± 1·0	58·9 ± 8·2	8·8 ± 2·1	3·6 ± 0·8	5·1 ± 0·4
		NS	12·3 ± 1·3	1·2 ± 0·3	55·3 ± 6·1[3]	10·2 ± 1·8	7·3 ± 3·3	5·7 ± 0·6
		S + NS	12·0 ± 1·0	1·6 ± 0·5	56·8 ± 4·9	9·6 ± 1·3	5·3 ± 2·0	5·4 ± 0·4
	Nonexposed	S	11·5 ± 1·3	1·2 ± 0·4	61·9 ± 5·1	7·7 ± 2·0	2·6 ± 0·8	6·0 ± 0·8
		NS	13·3 ± 2·3	0·8 ± 0·3	73·5 ± 7·0	10·3 ± 2·9	1·5 ± 0·5	4·1 ± 0·5
		S + NS	12·4 ± 1·3	1·0 ± 0·2	67·9 ± 4·4	9·0 ± 1·8	2·0 ± 1·8	5·0 ± 0·5
Men	Cd-exposed	S	33·6 ± 1·9	3·0 ± 1·0[3]	15·9 ± 1·7	31·2 ± 3·1	29·1 ± 5·8[3]	4·6 ± 0·3
		NS	25·4 ± 2·3	2·7 ± 1·0[3]	18·1 ± 3·8	22·9 ± 6·0	16·3 ± 7·7[3]	4·5 ± 0·2
		S + NS	31·9 ± 1·7[3]	2·9 ± 0·9[3]	16·3 ± 1·6[3]	29·4 ± 2·8[3]	26·3 ± 4·9[3]	4·5 ± 0·2
	Nonexposed	S	28·4 ± 2·2	0·7 ± 0·1	19·6 ± 2·0	25·1 ± 3·1	2·0 ± 0·2	4·2 ± 0·3
		NS	24·0 ± 1·4	0·7 ± 0·1	25·2 ± 4·7	14·7 ± 1·3	1·0 ± 0·2	3·2 ± 0·3
		S + NS	27·3 ± 1·7	0·7 ± 0·1	21·0 ± 1·9	22·5 ± 2·5	1·8 ± 0·2	4·0 ± 0·3

SEM = standard error of the mean
S = smokers; NS = nonsmokers
[1]ALA-D activity expressed in absorbance units × 10/hour/ml red blood cells for 2 cm light path
[2]Concentration of Pb and Cd, in urine expressed in μg/g creatinine; ALA in urine in mg/g creatinine
[3]Mean significantly different ($P < 0.05$) from the mean of the corresponding nonexposed subgroup

TABLE 3

INTERRELATIONSHIPS BETWEEN DIFFERENT BIOLOGICAL PARAMETERS IN BLOOD (B) AND URINE (U) OF TOTAL STUDY POPULATION[1]

Simple and partial correlation coefficients				*Regression equation*
Factors correlated	*Factor held constant*	*r*	*P*	
log ALA-U. Pb-B	Cd-B	−0·130	>0·05	
log ALA-U. Pb-U	Cd-U	+0·051	>0·05	
log ALA-U. log Cd-B	Pb-B	+0·077	>0·05	
log ALA-U. log Cd-U	Pb-U	+0·010	>0·05	
log ALA-D. Pb-B	Cd-B	−0·660	<0·001	log ALA-D = 1·875−0·019 (Pb-B)
log ALA-D. log Cd-B	Pb-B	−0·123	>0·05	
Pb-B . Pb-U	—	+0·617	<0·001	Pb-B = 13·74+0·475 (Pb-U)
log Cd-B. log Cd-U	—	+0·433	<0·001	log Cd-B = 0·306 (log Cd-U)−0·178
log ALA-D. Pb-U	Cd-U	−0·501	<0·001	

[1]The total study population consists of 150 subjects, i.e., Cd-exposed men and women (n = 77) *plus* the corresponding non-exposed subgroups (n = 73).

significant correlation between ALA-U and Cd-B and between ALA-U and Cd-U for the range of Cd concentrations observed in our study population (Table 3).

In contrast to Pb-B and Pb-U, Cd-B and Cd-U values in the total population were not normally distributed, and a logarithmic transformation was therefore applied to the cadmium values to make their distribution normal.

ALA-D activity is lower in the Cd-exposed subgroups than in the control subgroups, but only in two exposed subgroups, i.e., women nonsmokers and men smokers *plus* nonsmokers, is the decrease in ALA-D activity statistically significant (Table 2), but the exposed men with inhibited ALA-D showed a significantly elevated Pb-B also. From these results above we cannot conclude whether ALA-D activity is significantly affected by exposure to Cd. Therefore the partial correlation coefficient between the logarithm of ALA-D activity and Pb-B (standardized for Cd-B) and also between the logarithm of ALA-D activity and Cd-B (standardized for Pb-B) were calculated (Table 3). The relationship between log ALA-D and Pb-B standardized for a Cd-B of 0·80 μg % (mean value of Cd-B in nonexposed workers) is illustrated in Fig. 1; Fig. 2 demonstrates the relationship between log ALA-D and Cd-B standardized for a Pb-B of 22·4 μg % (mean value of Pb-B in the total study population). It is evident that ALA-D activity is highly correlated with Pb-B (r = −0·660) but not with Cd-B.

The correlation coefficient that we observed

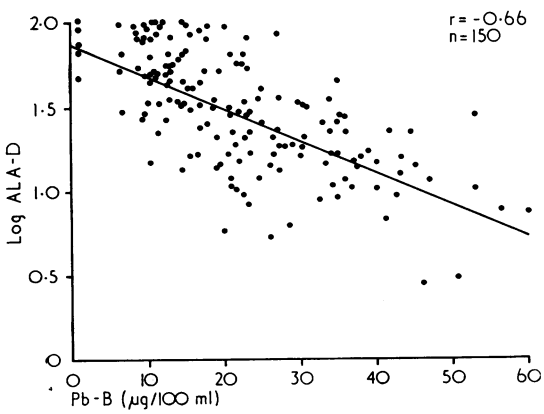

FIG. 1. Relationship in total study population (n = 150) between log ALA-D and lead in blood (Pb-B) standardized for a Cd-B (cadmium in blood) of 0·80 μg % (mean value of Cd-B in nonexposed workers).

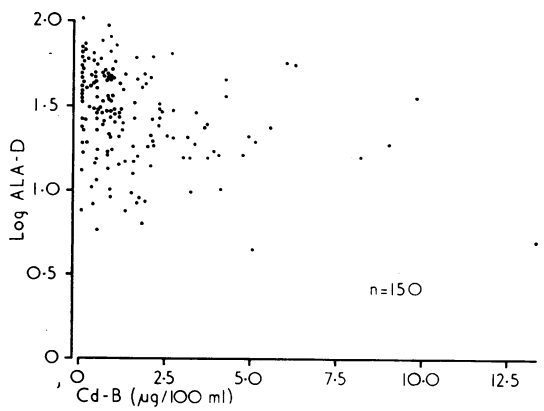

FIG. 2. Relationship in total study population (n = 150) between log ALA-D and cadmium in blood (Cd-B) standardized for a Pb-B (lead in blood) of 22·4 μg% (mean value of Pb-B in total study population).

TABLE 4

COMPARISON BETWEEN BIOLOGICAL PARAMETERS IN SMOKERS AND NON-SMOKERS OCCUPATIONALLY EXPOSED TO Cd AND NONEXPOSED
(mean ± SEM)[1]

Subgroups in study population		*No. of subjects*	*Pb-B* (μg %)	*Cd-B* (μg %)	*ALA-D*[3]	*Pb-U*[4]	*Cd-U*[4]
Cd-exposed	S	49	27·7 ± 2·0[2]	2·8 ± 0·8	27·3 ± 3·7	25·2 ± 2·7	22·1 ± 4·6
	NS	28	17·0 ± 1·7	1·7 ± 0·4	42·0 ± 5·4	14·7 ± 2·6	10·5 ± 3·5
	S + NS	77	23·8 ± 1·5	2·4 ± 0·5	32·6 ± 3·1	21·4 ± 2·1	21·1 ± 3·3
Non-exposed	S	47	23·4 ± 2·0	0·8 ± 0·1	32·2 ± 3·5	19·9 ± 2·5	2·2 ± 0·3
	NS	26	17·9 ± 1·8	0·7 ± 0·2	53·0 ± 6·5	12·1 ± 1·8	1·3 ± 0·3
	S + NS	73	21·4 ± 1·4	0·8 ± 0·1	39·6 ± 3·4	17·1 ± 1·8	1·9 ± 0·2

SEM = standard error of the mean; S = smokers; NS = nonsmokers
[1]Mean ± SEM is calculated by combining the results obtained for men and women
[2]Statistically different means are indicated
[3]ALA-D activity is expressed in absorbance units × 10/hour/ml red blood cells for 2 cm light path
[4]Concentrations of Pb and Cd in urine are expressed in μg/g creatinine

between Pb-B and Pb-U ($r = 0{\cdot}617$; $P < 0{\cdot}001$) in our study population of workers nonoccupationally exposed to lead is very close to that ($r = 0{\cdot}66$) reported by Selander and Cramér (1970) on workers occupationally exposed to lead; similarly, we found for the total population also a significant correlation between Cd-B and Cd-U ($r = 0{\cdot}433$; $P < 0{\cdot}001$) (Table 3).

Furthermore, we also found a good negative correlation between log ALA-D and Pb-U ($r = -0{\cdot}501$) (Table 3).

Table 4 shows the relationship between the smoking habit and Pb-B, Pb-U, Cd-B, Cd-U, and ALA-D of Cd-exposed and nonexposed workers; the mean values of the biological parameters were calculated by combining the results obtained for men and women. It is not surprising that Cd-B and Cd-U in the Cd-exposed smokers and nonsmokers are significantly higher than in the respective nonexposed groups. ALA-D, however, does not parallel at all this relationship, emphasizing again the fact that ALA-D is not much affected by exposure to Cd. By comparing smokers and nonsmokers in each subgroup (i.e., Cd-exposed and nonexposed) we observed in smokers significantly higher mean values for Pb-B, Pb-U, and Cd-U, confirming the presence of Cd and Pb in cigarettes. Although Cd-B is higher in smokers than in nonsmokers (Table 4), the difference is not statistically significant. The increase of Pb-B in smokers is accompanied by a statistically significant depression of ALA-D.

Discussion

This study confirms the existence of a good negative correlation between ALA-D activity in red blood cells and Pb-B even within a 'normal' Pb-B range (10–40 μg %). The correlation between ALA-D and Pb-B ($r = -0{\cdot}66$) is lower than that, i.e. $r = -0{\cdot}82$, reported in an earlier paper (Basecqz *et al.*, 1971), but the study population exhibited then a larger range of Pb-B as printshop workers, occupationally exposed to lead, were also included.

It is not surprising that there is a good correlation between ALA-D and Pb-U because there are good correlations between ALA-D and Pb-B and between Pb-B and Pb-U.

ALA-D activity is not correlated with Cd-B. The potential effect of Cd on ALA-D in the general population can therefore be considered negligible compared to the effect of lead, of which the blood concentration is normally 10 to 40 times that of Cd-B (Table 3).

Furthermore, on the basis of the comparison between smokers and nonsmokers (Cd-exposed and nonexposed) (Table 4) we are strongly inclined to conclude that the reduction of ALA-D activity described above for two Cd-exposed subgroups (Table 2) is only fortuitous and does not reflect a real and specific effect of exposure to Cd.

These results suggest also that the reduction of ALA-D activity by cigarette smoking, reported previously (Calandra, 1971, cited by Millar *et al.*, 1972), could be due to lead since smokers have a higher Pb-B than nonsmokers (Table 4). This hypothesis should, however, be confirmed by comparing ALA-D activity and Pb-B in smokers and nonsmokers of the same age.

The results of our previous studies concerning the effect of mercury on ALA-D (Lauwerys and Buchet, 1973) and those presented here indicate that among three heavy metals (Hg, Pb, Cd), usually recognized as contaminants of our environment, only lead influences significantly the ALA-D activity of red blood cells. It is worth mentioning here that no decrease in ALA-D activity has been found in the

erythrocytes obtained from patients with various kinds of neurological and haematological diseases (Nakao *et al.*, 1968; Battistini *et al.*, 1971). Therefore, all the available evidence strongly suggests that depression of ALA-D activity in red blood cells can be considered not only a very sensitive but also a specific warning of exposure to lead. However, further investigations on various parameters (e.g., age, pregnancy, drugs) which may influence ALA-D activity are justified as well as on the biological significance (if any) of the inhibition of this enzyme.

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References

Basecqz, J.-M., Lauwerys, R., and Buchet, J.-P. (1971). Étude comparative de divers tests biologiques d'exposition au plomb. *Archives des Maladies Professionnelles*, **32**, 453-463.

Battistini, V., Morrow, J. J., Ginsburg, D., Thompson, G., Moore, M. R., and Goldberg, A. (1971). Erythrocyte delta-aminolaevulinic acid dehydratase activity in anaemia. *British Journal of Haematology*, **20**, 177-184.

Berlin, A., del Castilho, P., and Smeets, J. (1972). European intercomparison programme (communication No. 92). International symposium on environmental health aspects of lead. CCE-EPA, Amsterdam, October 2-6, 1972.

Bonsignore, D., Calissano, P., and Cartasegna, C. (1965). Un semplice metodo per la determinazione della δ-amino-levulinico-deidratasi nel sangue. Comportamento dell'enzima nell'intossicazione saturnina. *Medicina del Lavoro*, **56**, 199-205.

Calissano, P., Cartasegna, C., and Bonsignore, D. (1965). Azione di alcuni metalli sull'ALA-deidratasi eritrocitaria purificata de sangue umano. *Lavoro Umano*, **17**, 493-497.

CCE-EPA (1972). International symposium on environmental health aspects of lead organized by the Commission des Communautés Européennes and the United States Environmental Protection Agency, Amsterdam, October 2-6, 1972.

de Bruin, A. (1968). Effect of lead exposure on the level of δ-aminolevulinic-dehydratase activity. *Medicina del Lavoro*, **59**, 411-418.

——, and Hoolboom, H. (1967). Early signs of lead-exposure. A comparative study of laboratory tests. *British Journal of Industrial Medicine*, **24**, 203-212.

Gibson, K. D., Neuberger, A., and Scott, J. J. (1955). The purification and properties of δ-aminolaevulinic acid dehydrase. *Biochemical Journal*, **61**, 618-629.

Goldwater, L. J. (1972). An assessment of the scientific justification for establishing 2 $\mu g/m^3$ as the maximum safe level for airborne lead. *Industrial Medicine and Surgery*, **41**, No. 7, 13-18.

Haeger-Aronsen, B., Abdulla, M., and Fristedt, B. I. (1971). Effect of lead on δ-aminolevulinic acid dehydrase activity in red blood cells. *Archives of Environmental Health*, **23**, 440-445.

Hernberg, S., and Nikkanen, J. (1970). Enzyme inhibition by lead under normal urban conditions. *Lancet*, **1**, 63-64.

——, ——, Mellin, G., and Lilius, H. (1970). δ-Aminolevulinic acid dehydrase as a measure of lead exposure. *Archives of Environmental Health*, **21**, 140-145.

——, Tola, S., Nikkanen, J., and Valkonen, S. (1972) Erythrocyte δ-aminolevulinic acid dehydratase in new lead exposure. *Archives of Environmental Health*, **25**, 109-113.

Lauwerys, R. (1972). Le diagnostic biologique d'exposition excessive au plomb. *Louvain Médical*, **91**, 19-30.

——, and Buchet, J. P. (1973). Relationship between occupational exposure to mercury vapors and biological action. *Archives of Environmental Health*, **27**, 65-68.

——, Delbroeck, R., and Vens, M. D. (1972). Automated analysis of delta-aminolaevulinic acid in urine. *Clinica Chimica Acta*, **40**, 443,447.

Lichtman, H. C., and Feldman, F. (1963). In vitro pyrrole and porphyrin synthesis in lead poisoning and iron deficiency. *Journal of Clinical Investigation*, **42**, 830-839.

Millar, J. A., Battistini, V., Cumming, R. L. C., Carswell, F., and Goldberg, A. (1970). Lead and δ-aminolaevulinic acid dehydratase levels in mentally retarded children and in lead-poisoned suckling rats. *Lancet*, **2**, 695-698.

——, Thompson, G. G., Goldberg, A., Barry, P. S. I., and Lowe, E. H. (1972). δ-Aminolevulinic acid dehydrase activity in the blood of men working with lead alkyls. *British Journal of Industrial Medicine*, **29**, 317-320.

Moore, M. R., Beattie, A. D., Thompson, G. G., and Goldberg, A. (1971). Depression of δ-aminolaevulic acid dehydrase activity by ethanol in man and rat. *Clinical Science*, **40**, 81-88.

Nakao, K., Wada, O., and Yano, Y. (1968). δ-Aminolevulinic acid dehydratase activity in erythrocytes for the evaluation of lead poisoning. *Clinica Chimica Acta*, **19**, 319-325.

Nandi, M., Jick, H., Slone, D., Shapiro, S., and Lewis, G. P. (1969). Cadmium content of cigarettes. *Lancet*, **2**, 1329-1330.

NRC-NAS report prepared by Committee on Biologic Effects of Atmospheric Pollutants, Division of Medical Sciences (1971). *Airborne Lead in Perspective*. National Research Council—National Academy of Sciences, Washington D.C.

Schubert, R. H. W., and Sangl, H. (1972). The problem of lead exposure caused by the exhaust gases of motor vehicles. A contribution to the applicability of the determination of delta-aminolaevulinic acid in urine as a criterion for the lead exposure caused by environmental factors. *Zentralblatt für Bakteriologie, Parasitenkunde, Infectionskrankheiten und Hygiene*, 1. *Abteilung Originale Reihe B.*, **155**, 470-475. (In German, with English abstract).

Selander, S., and Cramér, K. (1970). Interrelationships between lead in blood, lead in urine, and ALA in urine during lead work. *British Journal of Industrial Medicine*, **27**, 28-39.

Vens, M. D., and Lauwerys, R. (1972). Détermination simultanée du plomb et du cadmium dans le sang et l'urine par le couplage des techniques de chromatographie sur résine échangeuse d'ions et de spectrophotométrie d'absorption atomique. *Archives des Maladies Professionnelles*, **33**, 97-105.

Wada, O., Toyokawa, K., Suzuki, T., Suzuki, S., Yano, Y., and Nakao, K. (1969). Response to low concentration of mercury vapor. *Archives of Environmental Health*, **19**, 485-488.

Weissberg, J. B., Lipschutz, F., and Oski, F. A. (1971). δ-Aminolevulinic acid dehydratase activity in circulating blood cells. A sensitive laboratory test for the detection of childhood lead poisoning. *New England Journal of Medicine*, **284**, 565-569.

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