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TRANSLATION:

Rausa, G., L. Diana and G. Perin: THE IN VITRO ACTION OF CARBON MONOXIDE AND LEAD ON THE HUMAN ERYTHROCYTE DEHYDRATASE ENZYME, DELTA-AMINOLEVULINIC ACID. (Azione in vitro dell'ossido di carbonio e del piumbo sull'attivita dell'enzima acido delta-amino-levulinico deidrastasi di eritrociti umani.) Institute of Hygiene, University of Padova, Director: Professor R. Vendramini.

ABSTRACT. Human red blood cells, suspended in physiological solution, are been subjected to treatment with carbon monoxide and lead. The ALAD activity of red blood cells has been tested.

The results pointed out a noticeable decrease of ALAD activity, that can result interesting if confirmed by tests in vivo.

INTRODUCTION

It has been observed for some time, that subjects exposed to lead exhibit an increased urinary excretion of delta-aminolevulinic acid (ALA) (Haeger 1957; Griggs and Harris 1958; Tanabe 1959; Haeger-Aronsen 1960; Rubino 1961; Stich 1961; Saita and Moreo 1961; Rubino 1962; Chiesura and Brugnone 1962; Balbo and Marucci 1962; Rasetti and Parigi 1962; Buskup and Mappes 1962; DeKretser and Waldron 1963; Sroczynski 1964; Cramér and Selander 1965 and 1967; Bonsignore 1966; De Bruin and Hoolbloom 1967; De Bruin 1968).

This metabolite is a precursor of porphobilinogen (PBC) and is, therefore, indispensible for the formation of hemin. The reduced ability of the subject, who has been poisoned by lead, to synthesize PBG from ALA might explain the increased urinary excretion of the latter, which, on the other hand, is closely related to the amount of lead that can be excreted by the organism during anti-lead poisoning therapy (Cramér and Selander 1965).

Since the conversion of ALA to PBG is an enzymatic process catalyzed by delta-aminolevulinic acid dehydrogenase (ALA dehydrogenase), some authors have

investigated the activity of this enzyme in erythrocytes of subjects afflicted by chronic lead poisoning (Goldberg and coworkers 1956; Rubino 1962; Heilmeyer 1963; Lichtman and Feldman 1963; Mole and Pesaresi 1964). But the results have been contradictory and have raised some doubt that they might have been affected by experimental factors, as suggested and demonstrated by Bonsignore (1966) and Bonsignore and coworkers (1965). These authors presented evidence that inhibition of ALA dehydrogenase is competitive in nature and is partly eliminated by cysteine, which contains sulfhydryl groups [4], and that the reduced enzymatic activity of erythrocyte ALA dehydrogenase during acute or chronic lead poisoning is substantial (about 80%) [3].

These interesting observations, which provide a plausible explanation for some of the biochemical aspects of lead poisoning, such as the increased urinary excretion of ALA, PBG, uro- and coproporphyrin, lead us to direct attention in toxicological problems to enzymatic activity, which has hitherto been investigated to a limited extent.

On the other hand, the complexity of intoxication relationships true and proper, or more simply the specific toxic aspects, is enough to remind one, that the individual study of a single toxin is hardly adequate to elucidate the effects on the organism.

Actually, by confining oneself to the area of specific aspects of atmospheric pollutants, it becomes evident that certain subjects will be exposed to more pollutants. A typical case in this connection is atmospheric pollution due to automobile traffic, which exposes a substantial number of persons to a wide range of pollutants.

In some cities, an alarming level may definitely develop in certain situations, e.g., garages, if the problem cannot be handled. Indeed, as we have

already reported in a previous paper [23], the pollution by exhaust products from automotive vehicles, particularly CO and Pb which may reach alarming levels in garages having inadequate ventilation.

Therefore, we have turned to a study of the effects of CO and Pb on the activity of the enzyme ALA dehydrogenase in human erythrocytes of subjects exhibiting no signs of carbon monoxide or lead poisoning, in order to establish whether simultaneous exposure to CO more or less further modified the activity of ALA dehydrogenase, already weakened by Pb.

MATERIALS AND METHODS

Heparinized blood samples: These were obtained from subjects not poisoned by CO and Pb and were subject to the action of Pb or CO and Pb at the same time.

Carbon monoxide, prepared by the action of sulfuric acid on formic acid, was bubbled through a suspension of erythrocytes in KCl solution (see below) long enough to obtain various degrees of saturation, which were established by determination of COHb according to Feldstein and Klendshoj [13].

Lead, in the form of lead acetate, was added as follows: various amounts of lead acetate solution of known strentgh are placed in the bottom of 50 ml flasks and allowed to evaporate on a water bath; the erythrocyte suspension in KCl is then added and the flasks allowed to stand 30 minutes, in order to reach equilibrium.

In the tests conducted with CO and Pb together, the procedure is the same as above, treatment with CO being carried out first.

As it follows from the above, we have preferred to add the CO and Pb directly to the erythrocyte suspensions and not to the red corpuscle hemolyzates, since this seems to correspond, to some extent, to more realistic conditions.

Preparation of the Erythrocyte Suspensions

After separating the plasma by centrifugation at 3000 rpm for ten minutes, the erythrocytes are washed three times with 0.15 M MCl and centrifuged ten minutes at 3000 rpm after each wash; the erythrocytes were then resuspended in 0.15 M KCl, for subjection to the above treatment.

Determination of ALA Dehydrogenase Enzyme Activity

After lysis of the erythrocytes with distilled water, enzyme activity was determined by the colorimetric method for PBG formed from ALA by the action of the enzyme, according to the method of Bonsignore et al. [2]. The results are expressed in U/ml of erythrocytes, taking the hematocrit value into account.

RESULTS

As far as the action of Pb is concerned, our studies have shown results similar to those obtained by Bonsignore et al. [3]. Indeed, as Table 1 shows, the activity of ALA dehydrogenase in erythrocytes subjected to the action of Pb at various concentrations and for different times indicates a reduction between 60-75% compared with the controls, while according to Bonsignore et al., reduction of enzyme activity should reach about 80%. This discrepancy can be explained on the basis of different experimental technique, since the authors tested this action of Pb on hemolyzed erythrocytes directly, thus allowing Pb to act on the "bare" enzyme.

TABLE 1. REDUCTION OF HUMAN ERYTHROCYTE ALA DEHYDROGENASE ENZYME ACTIVITY BY LEAD. (THE REPORTED VALUES ARE PERCENT REDUCTION OF ENZYMATIC ACTIVITY).

	Time	Pb µg % ml			
		50	100	200	400
Media E.S.*	30'	50 ± 1.1	56 ± 1.3	60 ± 2.0	67 ± 2.1
Media E.S.	60'	52 ± 1.3	56 ± 1.6	61 ± 1.5	72 ± 1.7
Media E.S.	90'	52 ± 1.6	57 ± 1.5	61 ± 2.3	75 ± 1.9
Media E.S.	120'	53 ± 1.7	57 ± 1.9	63 ± 1.7	75 ± 1.6

TABLE 2. REDUCTION OF HUMAN ERYTHROCYTE ALA DEHYDROGENASE ACTIVITY BY CO AND Pb (120' — CONTACT TIME). (THE REPORTED VALUES ARE PERCENT REDUCTION OF ENZYMATIC ACTIVITY).

	Pb µg % ml				
	50	100	200	400	
Media E.S.	52 ± 2.1	55 ± 1.3	65 ± 2.2	75 ± 1.9	COHb 10%
Media E.S.	54 ± 2.3	57 ± 2.1	67 ± 1.6	79 ± 1.5	COHb 20%
Media E.S.	56 ± 1.7	63 ± 1.3	72 ± 2.3	88 ± 3.5	COHb 40%
Media E.S.	58 ± 1.5	65 ± 2.2	77 ± 2.6	90 ± 2.3	COHb 80%

*E.S. -- Erythrocyte suspension?

It is evident that while there is no clear increase in inhibition of ALA dehydrogenase activity as a function of time, for Pb concentrations between 50 and 200 mcg/ml, there is, on the contrary, increased enzymatic inhibition as contact time increases, at the 400 mcg/ml concentration (see Figure 1).

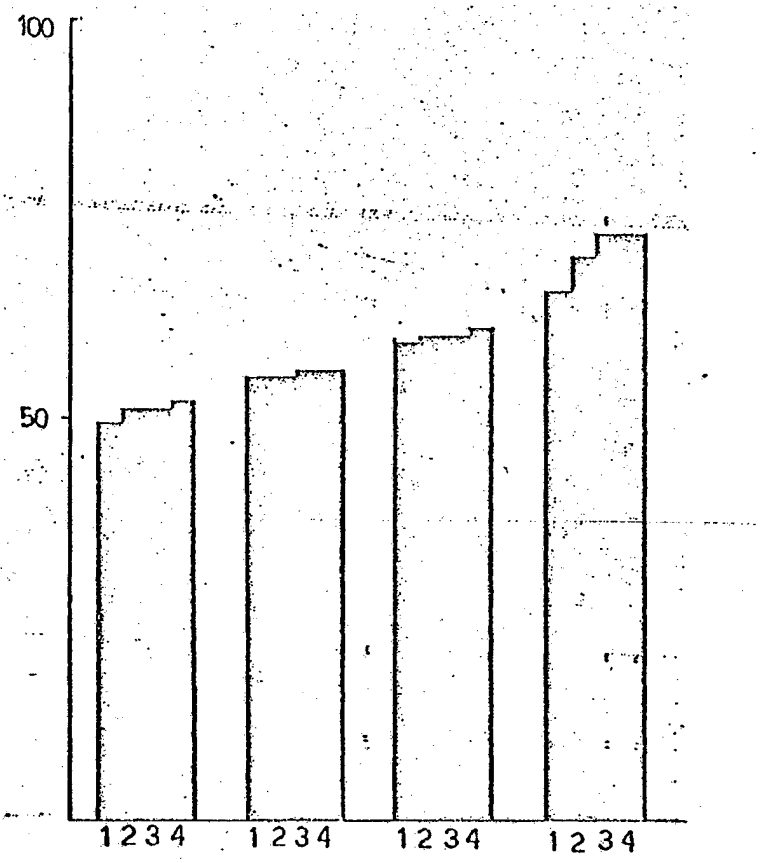

Figure 1. Numbers 1, 2, 3 and 4 refer to 30, 60, 90 and 120 min. treatment times respectively; the first series of columns corresponds to tests made with 50 mcg%/ml Pb; the second, third and fourth refer to tests with 100, 200 and 400 mcg%/ml respectively. Percent enzymatic inhibition is shown on the ordinate.

The tests dealing with the combined effect of CO and Pb are reported in Table 2, which shows how the experiments, on the basis of results from the preceding tests, can be carried out with varying concentrations of CO (COHb equal to: 10, 20, 40 and 80% respectively) and Pb (50, 100, 200 and 400 mcg/ml respectively) after a 120-minute contact period.

The results clearly indicate, that while percentages of COHb on the order of 10% produce practically the same inhibition of ALA dehydrogenase activity as that obtained without CO, 20% COHb, on the other hand, more markedly and in a statistically significantly manner inhibits the enzyme, starting with Pb-concentrations of 200 and 400 mcg%/ml, the value of t being greater than that required for $P = 0.05$. At COHb concentrations of 60 and 80% the inhibition becomes quite marked and reaches the value 90%, for Pb-concentrations of 400 mcg%/ml.

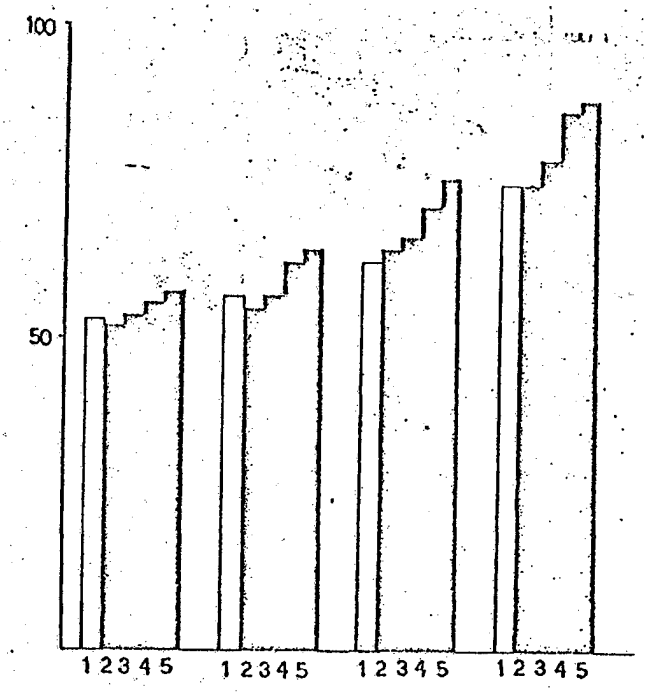

Figure 2. The four series of columns correspond to tests conducted with 50, 100, 200 and 400 mcg%/ml Pb respectively; the number 1 in each series (white column) represents samples treated with Pb alone; the numbers 2, 3, 4 and 5 represent samples treated with Pb at the above concentrations, and with CO for final COHb concentrations equal to 10, 20, 40 and 80% respectively. Percent Enzymatic inhibition is shown on the ordinate.

TABLE 3. RESULTS OF APPLYING STUDENT'S t TO DIFFERENCES OF MEAN LOSS IN TESTS CONDUCTED WITH Pb ALONE (COLUMN DX) AND WITH CO AND Pb TOGETHER (COLUMN SN).

	t	Pb µg % ml
COHb 10%		
Pb µg % ml	N.S.	50
50	N.S.	50
100	N.S.	100
200	N.S.	200
400	N.S.	400
COHb 20%		
Pb µg % ml		
50	N.S.	50
100	N.S.	100
200	M.S. (2.35)	200
400	M.S. (2.44)	400
COHb 40%		
Pb µg % ml		
50	M.S. (2.49)	50
100	M.S. (2.87)	100
200	M.S. (2.57)	200
400	M.S. (2.79)	400
COHb 80%		
Pb µg % ml		
50	M.S. (2.78)	50
100	M.S. (2.98)	100
200	M.S. (3.11)	200
400	M.S. (3.01)	400

N.S. = not significant; M.S. = very significant; (P = 0.05).

CONCLUSIONS

The results of our in vitro tests appear to suggest the following conclusions:

-- The inhibition of ALA dehydrogenase enzyme in erythrocytes of subjects not affected by CO and Pb intoxication, obtained by in vitro exposure to these toxic agents is obviously quite good and reaches very high values, especially when the action of Pb is combined with that of CO:

-- The increased inhibition, at the same Pb-concentration, is present in tests conducted with CO and Pb, compared to that found in tests carried out with Pb alone, although it is evident that starting with non-indifferent concentrations of COHb (20%) a definite interest may be revived in these situations, which are then the predominating ones, where there may be a simultaneous exposure of the organism to more pollutants. If it is indeed true that hematic concentrations of Pb of 400 mcg%/ml and of CO equal to 20% COHb are not readily detectable outside of strictly occupational and hence limited toxicological situations, it is also well to consider that in in vitro tests, for obvious reasons, the contact time is always limited, while under actual conditions, CO and Pb may have a way of acting on the organism at low concentrations, for much longer times and by mechanisms not always superimposable on the simplified ones of the in vitro tests;

-- Therefore, it is advisable to extend the experimentation to laboratory animals.

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