Delta-Aminolevulinic Acid and Lead in Urine of Lead Antiknock Workers

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Delta-aminolevulinic acid (ALA) and lead were measured in the urine of workers exposed to organic lead in the form of tetraethyl lead (TEL) and tetramethyl lead (TML). The levels of ALA and lead in urine were found to have a relatively low positive correlation of 0.52. These results are qualitatively similar to, but may be quantitatively different from, results reported by others for persons exposed to inorganic forms of lead. The mechanism through which the absorbed organic lead exerts its effect on urinary ALA is not indicated by this study. Urinary ALA was not considered to be an adequate replacement for urinary lead in a program of medical monitoring of workers exposed to organic lead in the form of TEL and TML.

Elevation of the delta-aminolevulinic acid (ALA) level in urine has been reported to be a sensitive and specific indication of increased absorption of lead,[1,2] except for certain rare disorders of porphyrin metabolism. The elevation is thought to result from a partial inhibition by lead of the conversion of ALA into porphobilinogen as one of the steps in the normal biosynthesis of hemoglobin. Such an elevation is an indication of a biological response to the absorbed material.

The usual clinical picture associated with intoxication due to inorganic lead[3] is quite different from that produced by organic lead in the form of tetraethyl lead (TEL).[4] The biochemical mechanisms underlying these differences are not understood. The reports that have been published concerning the effect of lead on urinary ALA levels have dealt with exposures to inorganic forms of lead. An investigation of the possible effects of absorption of an organic form of lead on urinary ALA levels was considered to be of interest.

Materials and Methods

Subjects.—One hundred twenty-three men of various ages and lengths of service, all working as operators or maintenance men in an area of production of lead alkyl antiknock compounds, provided the specimens of urine used in this study. Most of these men have a much greater relative potential for occupational exposure to organic lead in the form of TEL and tetramethyl lead (TML) than to inorganic forms of lead. Exposures must be consid-

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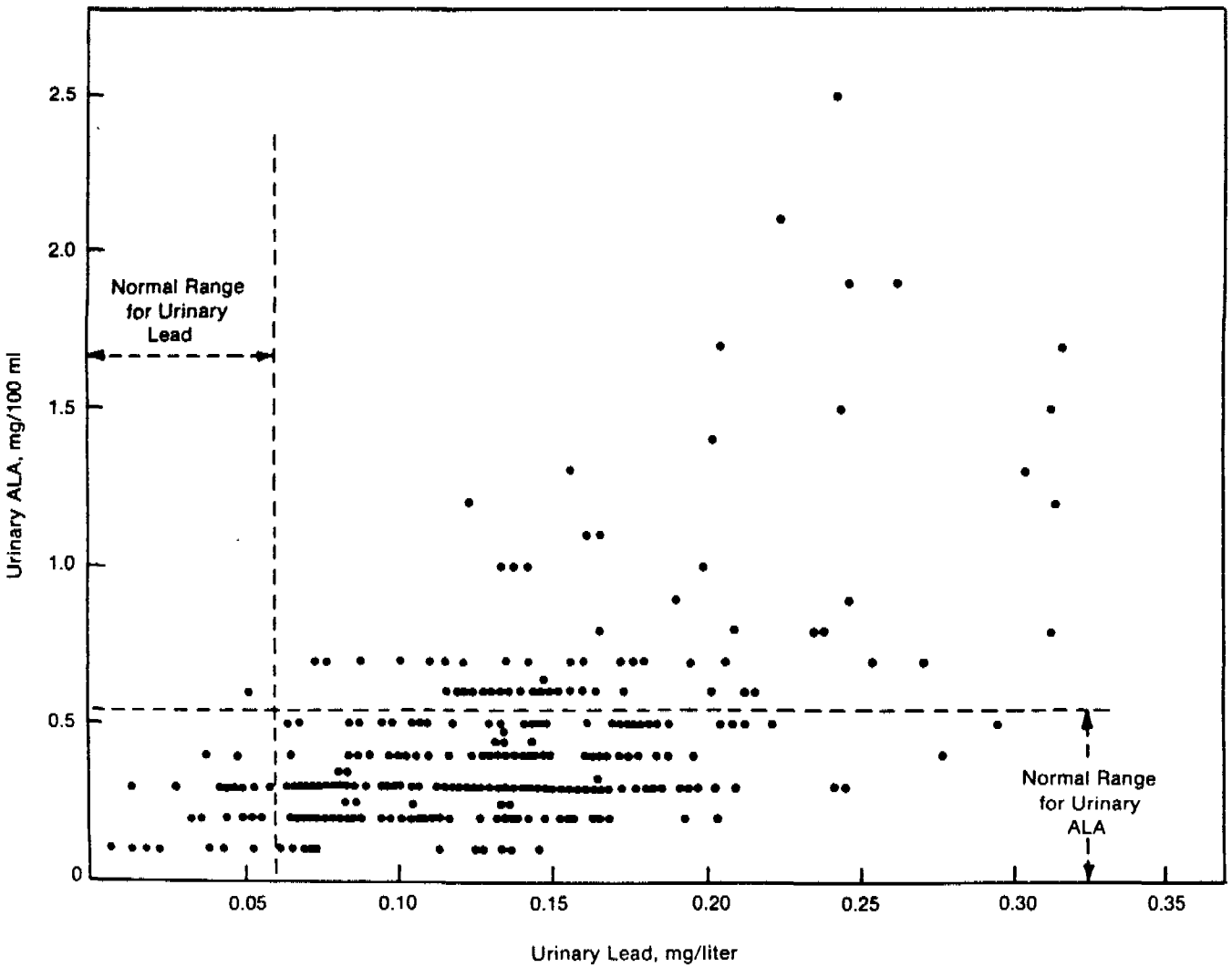

Fig 1 —Lead and ALA levels in urine of organic lead (TEL, TML) workers.

ered to be a mixture of TEL and TML, with the relative amounts of each component being variable and unknown for a given individual. The subjects were seen as part of a routine program of medical surveillance of employees working in this area of production. None of the subjects were clinically ill and none were on any type of "deleading" therapy.

Urine Specimens.—Each urine specimen consisted of a single voiding of 75 ml or more obtained at some time during the employee's normal working day. Most of the specimens were obtained from those workers thought to have the greatest potential for increased absorption of organic lead. A few specimens were obtained from subjects whose work was thought to offer relatively little opportunity for occupational exposure to lead in any form. A small portion (about 2 ml) of each specimen was set aside for determination of ALA concentration and was stored at 15 C until analysis. No measurement or adjustment of urinary pH was done prior to storage. The remainder of each specimen was used for determination of specific gravity and of lead concentration. Specimens with a specific gravity of less than 1.011 were not used in this study (and only seven specimens had a specific gravity of less than 1.016). A total of 268 urine specimens were analyzed for both ALA and lead.

Analyses.—All ALA determinations were done in the clinical laboratory of the plant medical facility, using the modification by Davis et al[5] of the method of Mauzerall and Granick.[4] Disposable ion-exchange chromatographic columns and reagents were obtained commercially. Standards were run with each group of specimens analyzed. It should be recognized that the lower ("normal") values for urinary ALA indicated by the method used are only in part (10% to 20%) due to ALA, with the remainder presumably being due to a residue of amino ketones and glucosamine.[4] However, this method has been shown to be specific for elevated values of ALA found in subjects having a disorder of porphyrin metabolism,[1,6] and in subjects having excessive absorption of inorganic lead.[1]

All lead determinations were done by the Analytical Section of Research and Development Services at Ethyl Corporation's Baton Rouge, La, plant by a modification of the dithizone method of Snyder.[7] This method determines total lead in the urine.

Results

The values obtained in this study for ALA and lead in urine are shown in Fig 1. These values are not adjusted for urine specific gravity. The expected (normal) range of each com-

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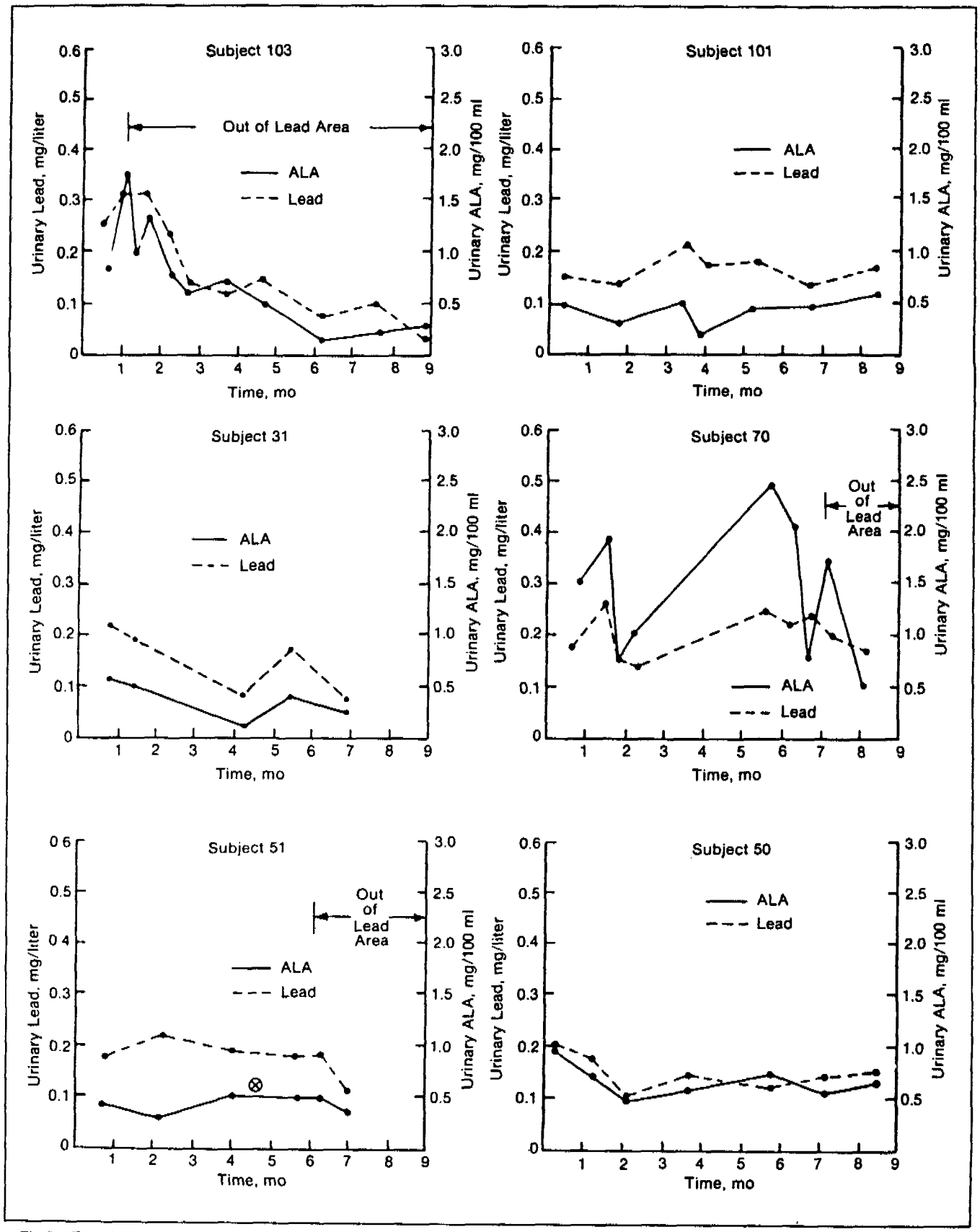

Fig 2.—Trends in urinary ALA and lead levels over time in certain organic lead (TEL, TML) workers. Encircled X (subject 51) indicates large urine sample with lead level of 0.11 mg/liter.

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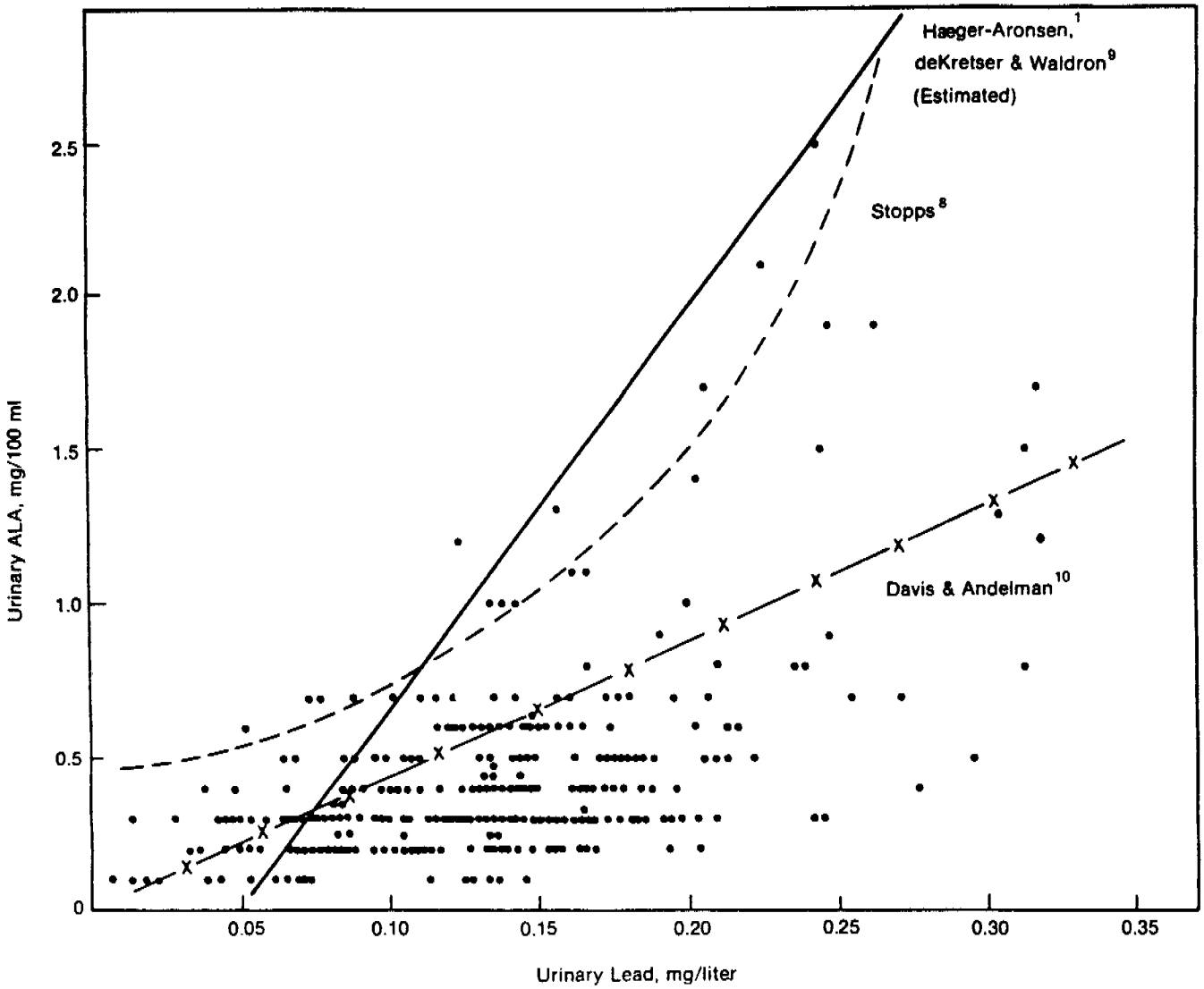

Fig 3.—Comparison of reported urinary ALA-Lead relationships for exposures to inorganic lead with results of present study involving organic lead (TEL, TML).

ponent for persons not occupationally exposed to lead is indicated. For purposes of calculation, the lower (normal) values for ALA were used as if they were due entirely to ALA. The resulting correlation coefficient is $r=0.52$, with a dispersion (standard deviation) of 0.38.

Similar calculations were made on values of ALA and lead, adjusted to a urine specific gravity of 1.024. No significant change in the relationships ($r=0.48$, SD=0.31) between urinary ALA and urinary lead resulted. Various combinations of normal and logarithmic values, adjusted and not adjusted for specific gravity, were investigated. None of these approaches yielded results significantly different from the initial one. These results, coupled with the absence of any known physiological reason for making an adjustment for urine specific gravity for the two materials involved (ALA and lead), led to the use of unadjusted values in this report.

Fig 2 shows the urinary ALA and lead values over time for each of six workers who have been observed for a number of months. Urinary ALA and lead values are seen to vary concomitantly over time in these workers. Of the men studied in this manner, one (Fig 2, No. 70) shows an apparent quantitative difference from the others in his urinary ALA-lead relationships.

Comment

The degree of dispersion of the results reported here is such that no single line or curve is thought to be justified as portraying the relationship between ALA and lead in the urine of these workers. It is not clear whether this dispersion is primarily due to innate variability of response of individuals to absorbed organic lead, to differences in the composition of the TEL-TML mixture to which individuals were exposed, to differences in the exposure/no-exposure time relationships, to differences in intervals between exposure and sampling, or to

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other possible variable factors. Because of this degree of dispersion, precise quantitative comparison of these results with the results others have reported for exposures involving inorganic lead[8-10] cannot be made. However, comparison of such results by simple inspection (Fig 3) suggests that a given elevation of urinary lead excretion probably is associated with a lower level of urinary ALA excretion when exposure is to organic lead (TEL, TML) than when exposure is to inorganic lead. It is not clear whether this difference might be related primarily to differences in circumstances of exposure or to differences in metabolism (biotransformation) of the different forms of lead to which exposure occurred.

In vitro and in vivo studies by Cremer[11] and in vivo studies by Bolanowska[12] suggest that TEL is converted, at least in part, in the body to triethyl lead and that this latter form of lead produces a disturbance in central nervous system function similar to that observed in intoxication due to TEL. Unconfirmed results by Bolanowska indicate that triethyl lead may be excreted as such in urine and feces quite slowly and without evidence of further degradation by the body. A portion of Bolanowska's work suggests that some part (less than 20%) of absorbed TEL may be converted directly to inorganic lead by the body. This portion of lead presumably would behave in a manner physiologically the same as lead derived from inorganic lead exposures, including effects on porphyrin metabolism and urinary ALA excretion. This suggests also that the urine of persons who have absorbed TEL contains organic as well as inorganic lead.

In vivo and in vitro studies by Cremer and Callaway[11] suggest that TML may undergo a relatively slow conversion in the body to (the more toxic) trimethyl lead. No evidence has been reported to indicate whether or not some portion of absorbed TML might be converted directly to inorganic lead within the body. The excretion product(s) of TML is (are) not known.

There have been no studies reported to indicate what effect, if any, triethyl lead or trimethyl lead per se might have on porphyrin metabolism and, hence, on urinary ALA excretion.

It is impossible at present, therefore, to assess the relative importance of TEL and TML (and possible metabolites, triethyl lead, trimethyl lead, and inorganic lead) in producing the deviations from normal of urinary ALA values observed in the present study. Further investigation must be done if the roles of the various compounds mentioned are to be understood.

An increase in urinary excretion of porphyrins has not been noted following exposure to TEL,[4] in contrast to the elevation found following absorption of inorganic lead. The results of the present study suggest that porphyrin metabolism is affected at the ALA level, ie, prior to the formation of porphyrins, by absorption of one or the other (or perhaps both) of the forms of organic lead (TEL, TML) involved in this study.

Subject 70, whose urinary ALA-lead relationship seems to differ from the other subjects in this study (Fig 2), works in the lead recovery (furnaces) area of the plant. His job may entail a greater potential for exposure to inorganic lead than other job assignments in the organic lead production area. This subject's urinary ALA-lead pattern is quantitatively quite similar to those reported by others for workers exposed to inorganic lead.[1,8,9] None of the other furnace workers seen during the course of the present study exhibited a significant elevation of either urinary lead or urinary ALA levels and thus do not furnish information to evaluate this point more definitely.

Determination of ALA in urine has been considered by some authors to be of considerable value in the routine monitoring of workers exposed to inorganic lead.[1,14,15] The present study indicates that urinary ALA determinations would be of uncertain value in a monitoring program where exposure is primarily to organic lead (TEL, TML). Programs of preventive medicine have been in existence for many years at facilities for the production of lead antiknock compounds. These programs have relied heavily on urinary lead determinations. Long experience has shown such programs to be adequate for the prevention of overt intoxication by organic lead. Recent studies (T.R.R., unpublished data) have provided mortality and morbidity data and information on certain other health factors that strongly indicate that at least one such program has been adequate over a period of 20 or more years to prevent any detectable significant adverse effects on health. Programs of such proven effectiveness should be significantly altered only for compelling reasons.

As can be seen in the group results (Fig 1) and in results for individuals (Fig 2, subjects 51 and 101), levels of urinary lead shown by long experience in the above programs to indicate the desirability for reduction in continued occupational exposure to lead (ie, a consistent urinary lead level of 0.18 mg/liter or more) may be associated with levels of urinary ALA that are within normal limits. It cannot categorically be denied that normal urinary ALA levels in such instances might be an indication that an adequate safety margin still exists for the individual, but there is no obvious reason to presume this to be the case. It appears very unlikely that this (relatively mild) response of the hemopoietic system would be related in any direct manner to the CNS dysfunction that is characteristic of intoxication by organic lead (TEL, TML). Due to the serious nature of such intoxication, workers should not be allowed to continue exposures that produce urinary lead values in excess of the established guidelines for the purpose of testing such a possibility. For the present, then, the determination of lead in urine must continue to be considered as the most useful laboratory tool in the routine medical surveillance (monitoring) of workers who have a potential for exposure to organic lead in the form of TEL and TML.

The clinical implication, if any, of an elevation of urinary ALA level is not known at the present time. It does not appear per se to indicate the existence of a disease state. In order

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to help clarify this matter, and as a relatively early objective indication of a response to increased absorption of (organic or inorganic) lead, consideration should be given to the inclusion of urinary ALA determinations as a part of the continuing medical surveillance necessary to protect the health of workers who have a potential exposure to lead in any form. Information obtained thereby should be of value in long-term clinical and epidemiological studies and may be useful in the evaluation of such additional indicators of biological response as may be developed in the future.

In summary, the present study shows that absorption of organic lead in the form of TEL and TML, as indicated by increased urinary lead levels, is associated with an increase in urinary ALA levels. The mechanism through which this effect is produced is not revealed by this study. Urinary ALA determinations should be considered for inclusion in any program of medical monitoring of organic or inorganic lead workers in order to clarify the clinical significance of this biological response to lead absorption. Urinary lead determinations continue to be the primary laboratory tool in the medical monitoring of workers having a potential for exposure to organic lead in the forms of TEL and TML.

References

1. Hæger-Aronsen B: Studies on urinary excretion of δ-aminolaevulic acid and other haem precursors in lead workers and lead-intoxicated rabbits. *Scan J Clin Lab Invest* 12(suppl 47):1-128, 1960.
2. Chisolm JJ Jr: Disturbances in the biosynthesis of heme in lead intoxication. *J Pediatr* 64:174-187, 1964.
3. Johnstone RT: Clinical inorganic lead intoxication. *Arch Environ Health* 8:250-255, 1964.
4. Sanders LW: Tetraethyllead Intoxication. *Arch Environ Health* 8:270-277, 1964.
5. Davis JR, et al: Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning: II. Correlation of ALA values with clinical findings in 250 children with suspected lead ingestion. *Arch Environ Health* 17:164-171, 1968.
6. Mauzerall D, Granick S: The occurrence and determination of delta-aminolevulinic acid and porphobilinogen in urine. *J Biol Chem* 219:435-446, 1956.
7. Snyder LJ: Improved dithizone method for determination of lead: Mixed-color micromethod at high pH. *Ind Eng Chem Anal Ed* 19:684-687, 1947.
8. Stopps GJ: Symposium on air quality criteria: lead. *J Occup Med* 10:550-564, 1968.
9. deKretser AJ, Waldron HA: Urinary delta amino-laevulinic acid and porphobilinogen in lead-exposed workers. *Br J Ind Med* 20:35-40, 1963.
10. Davis JR, Andelman SL: Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning: I. A modified method for the rapid determination of urinary delta-aminolevulinic acid using disposable ion-exchange chromatography columns. *Arch Environ Health* 15:53-59, 1967.
11. Cremer JE: Biochemical studies on the toxicity of tetraethyl lead and other organo-lead compounds. *Br J Ind Med* 16:191-199, 1959.
12. Bolanowska W: Distribution and excretion of triethyllead in rats. *Br J Ind Med* 25:203-208, 1968.
13. Cremer JE, Callaway S: Further studies on the toxicity of some tetra and trialkyl lead compounds. *Br J Ind Med* 18:277-282, 1961.
14. Gibson SL, Mackenzie JC, Goldberg A: The diagnosis of industrial lead poisoning. *Br J Ind Med* 25:40-51, 1968.
15. Cramér K, Selander S: Detection of industrial lead poisoning. *Lancet* 1:544-545, 1966.

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