Effect of Urea on the Simple Determination of δ -Aminolevulinic Acid in Urine

Key words: Lead exposure—Urinary ALA—Determination—Urea—Interference

The level of urinary δ -aminolevulinic acid (ALA) has been widely used as a measure of the biological effect of lead. The determination of urinary ALA has been carried out using ion-exchange column chromatography which is necessary for removal of interfering substances present in the urine, namely, porphobilinogen, urobilinogen and urea.¹⁻³⁾ These methods are more accurate for determining ALA in urine, but are time-consuming in large-scale routine analysis. A simpler and more rapid method for the determination of urinary ALA using liquid-liquid extraction instead of column chromatography was developed in 1972.⁴⁾ Presently, this simple method is widely used for the routine screening of workers exposed to lead. In this procedure, ALA-pyrrole is separated from most of the interfering substances present in the urine by extraction with ethyl acetate.

Recently, we found that the recovery of ALA from normal urine is inversely correlated with the urine density, when urinary ALA is determined by this simple

Fig. 1. Effect of added urea concentration on the apparent ALA concentration

The urea was added to an ALA standard solution (10 mg/l) (—●—) and the urine of a lead worker (ALA; 12.8 mg/l) (—○—). Each point shows the mean value obtained in duplicate analyses.

Fig. 2. Absorption spectra for an ALA standard solution (5 mg/l) (—), the same ALA solution containing 50 g/l of urea (---), and a urea standard solution (50 g/l) (- - - -)

method. As urine density is well known to depend on the concentrations of urea and sodium chloride in the urine, we examined the effect of interference by urinary urea on ALA analysis, and the results are reported in the present paper.

The relationships between the amounts of urea added to an ALA standard solution (10 mg/l) or a urine sample from a lead worker whose urinary ALA was 12.8 mg/l and the apparent ALA concentration are shown in Fig. 1. It was indicated that urea depresses the apparent concentration of ALA, the degree of depression being dependent on the concentration of urea added to the sample. The depression apparent in urine samples from 5 lead workers (including the worker mentioned above) was $10 \pm 2\%$ (mean $\pm$ SD) for 25 g/l of added urea, and $16 \pm \%$ for 50 g/l.

The absorption spectra obtained from an ALA standard solution (5 mg/l), the same ALA solution containing urea (50 g/l) and a urea standard solution (50 g/l) are shown in Fig. 2. It was found that urea may be complexed with modified Ehrlich's reagent to produce a yellow-colored product showing a maximum absorbance at 425 nm. However, the absorbances at 425 nm were almost constant, irrespective of the concentrations of urea added. Therefore, urea may prevent not the color reaction of ALA-pyrrole with Ehrlich's reagent, but the condensation of ALA with ethyl acetoacetate to produce ALA-pyrrole.

In healthy Japanese adults, the excretion of urea in urine has been reported

to be 15 to 30 g per day.⁵⁾ Accordingly, the concentration of urea in normal urine is estimated to be 10 to 20 g/l. In addition, no data have been reported on any significant correlation between urinary excretion of urea and lead exposure. Judging from these data, it is suggested that the interference of urea is comparatively mild, when the simple method is applied to the quantitative analysis of ALA in the urine of lead workers.

ACKNOWLEDGEMENT

This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education of Japan (No. 60570243).

REFERENCES

- 1) Mauzerall, D. and Granick, S. (1956). The occurrence and determination of δ -aminolaevulinic acid and porphobilinogen in urine, *J. Biol. Chem.*, **219**, 435.
- 2) Davis, J. R. and Andelman, S. L. (1967). 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.
- 3) Sun, M. W., Stein, E. and Gruen, F. W. (1969). A single column method for the determination of urinary δ -aminolevulinic acid, *Clin. Chem.*, **15**, 183.
- 4) Tomokuni, K. and Ogata, M. (1972). Simple method for determination of urinary δ -aminolevulinic acid as an index of lead exposure, *Clin. Chem.*, **18**, 1534.
- 5) Kanai, I. and Kanai, M., Eds. (1983). *Rinshokensaho Teiyo*, 29th. ed., P. 152, Kanehara Publications, Tokyo (in Japanese).

*Department of Community Health Science,
Saga Medical School, Nabeshima,
Saga 840-91, Japan*

**Katsumaro TOMOKUNI
Yukio HIRAI**

(Received August 13, 1985)