IMPROVEMENT OF URINARY δ -AMINOLEVULINIC ACID DETERMINATION BY HPLC AND FLUORESCENCE DETECTION USING CONDENSING REACTION WITH ACETYLACETONE AND FORMALDEHYDE

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We improved the method for determining urinary δ -aminolevulinic acid (ALA) by HPLC-fluorometer after pre-column derivatization with acetylacetone and formaldehyde, and a stable ALA derivative was obtained without any effect from various urinary components as demonstrated by the complete recovery of ALA ($100.9 \pm 5.5\%$, $n=85$) from the urine samples. The modified procedure was as follows: Twenty μ l of urine sample, 5 ml of acetylacetone solution (acetylacetone/ethanol/distilled water containing 4 g/l of NaCl; 15/10/75), and 0.45 ml of 9.3% formaldehyde solution were mixed and boiled for 15 min. The fluorescent derivative of ALA was separated and analyzed by HPLC with the fluorometer at Ex 246 nm and Em 458 nm. Using a gradient program, the retention time of the ALA derivative was 7.3 min and the analysis could be repeated at 13 min intervals. Concentrations of ALA in urine samples measured by this method were significantly correlated with those measured by the Mauzerall-Granick (M-G) method ($n=85$, $r=0.993$, $p<0.001$). The values obtained by our method were, however, lower than those obtained by the M-G method. Urinary ALA concentrations of 40 non-lead workers ranged from 0.1 to 2.3 mg/g creatinine with the mean $\pm$ SD of 1.1 ± 0.4 mg/g creatinine as measured by the present method.

Key words: lead; urine; δ -aminolevulinic acid; HPLC; fluorometry; pre-column; formaldehyde; acetylacetone

INTRODUCTION

Recently, some new analytical procedures¹⁻⁵⁾ for measuring δ -aminolevulinic acid (ALA) in urine have been reported which can measure a large number of samples simpler and more accurately than by the conservative colorimetric methods. Among these procedures,¹⁻⁵⁾ the fluorometric HPLC method devised by Okayama et al.²⁾ (Okayama method) has the lowest detection limit and a wide range of linearity of 0.25-100 mg/l, and is suitable for automated analysis. However, as shown in our previous short com-

munication,⁶⁾ this method has some weak points in that the recovery rate of ALA from urine is relatively low and the recovery decreases with elevation of specific gravity of the urine.

Generally, the intensity of fluorescence is strongly affected by the components, analytical temperature, and other factors. However, when the worker's urine was checked by the HPLC method, the effect from co-existing substances was not theoretically significant because of the isolation of the ALA derivative. The thermal effect was also negligible because there was no space between the fluoreometer and the column oven.

It was presumed that co-existing substances interfered with ALA derivatization. Therefore, we studied the effect of urinary components on the reaction rate of the ALA derivatization in order to establish the optimum conditions for obtaining a recovery rate of more than 90% of all urine samples in this procedure. A summary

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of this method has been previously reported in a short communication.⁶⁾ This report describes in detail the interference from urinary co-existent substances and the optimization of the conditions.

The ALA values obtained by this method were compared with those obtained by the Mauzerall-Granick (M-G)⁷⁾ method, and the differences between the two methods were discussed. The normal range of urinary ALA concentrations by the fluorometric HPLC method was also described.

MATERIALS AND METHODS

The subjects were 45 male workers with moderate exposure to lead in a secondary lead refinery and 40 male pest control workers engaged in exterminating rats without occupational exposure to lead. The mean age of the 45 lead workers was 51.7 ± 13.2 yr, and that of the 40 pest control workers was 43.5 ± 11.2 yr. The blood lead concentration was 458 ± 273 $\mu\text{g/l}$ (mean $\pm$ SD; range 46–1,030 $\mu\text{g/l}$) for the lead workers and 47 ± 16 $\mu\text{g/l}$ (mean $\pm$ SD; range 18–93 $\mu\text{g/l}$) for the pest control workers.

After measuring urine specific gravity and creatinine, the urine samples were centrifuged at 1,000 G for 10 min and kept at -20°C until the HPLC analysis was performed. The ALA in the urine of all workers was also measured by the M-G method.⁷⁾ The modified HPLC method was as follows: Twenty μl of urine or ALA standard solution, 5 ml of acetylacetone solution (acetylacetone/ethanol/distilled water containing 4 g/l of NaCl; 15/10/75, v/v), and 0.45 ml of 9.3% formaldehyde solution were put into a screw cap tube, mixed, boiled for 15 min, and cooled under water. Fifty microliters of the solution was injected into the HPLC system through an auto sampler (AS-8000, Tosoh, Japan), in which the sample was kept in the dark at 15°C . The injected sample was then separated through an octadecyl silica (ODS) column (TSK-80 TM, ϕ 4.6 $\times$ 150 mm, 5 μm particles, Tosoh, Japan) at 40°C , and eluted gradiently with two solutions (A) and (B) as shown in Fig. 1. Solution (A) consisted of acetonitrile, methanol, acetic acid and distilled water (100:350:10:540, v/v). Solution (B) consisted of 100% acetonitrile. The fluorescence intensity was measured at 458 nm with excitation

Fig. 1. Schematic diagram of gradient elution and a typical chromatogram for 50 μl injection of ALA standard solution (1.5 mg/l).

at 246 nm in a spectrofluorometer (HP 1046A, Hewlett-Packard, U.S.A.).

ALA hydrochloride (Sigma, U.S.A.) was dissolved in distilled water to give a concentration of 109.6 mg of ALA per liter and used as a stock solution. Methanol and acetonitrile were of the HPLC-grade (Wako Pure Chem., Japan), and the other chemicals were of the reagent-grade (Wako Pure Chem., Japan).

RESULTS

A typical chromatogram of the standard ALA solution and the gradient program of the mobile phase are shown in Fig. 1. The ALA derivative was eluted for 7.3 min. Using the gradient program, continuous analysis every 13 min with an auto-sampler could be carried out without elevation of the column pressure.

Since the recovery rate of ALA by the Okayama method⁹⁾ was not satisfactory, we examined the reaction under various conditions to obtain the highest reaction rate and the most stable derivative. Firstly, the reaction was performed under various concentrations of formaldehyde, but the volume of each solution in the mixture was the same as that of the Okayama method. The highest reaction rate was obtained when 9.3% formaldehyde solution was used, and the rate was twice of that obtained by the Okayama method (3.1% formaldehyde solution) as shown in Table 1. When 37% of formaldehyde solution was used as a reagent, many peaks were detected by the fluorometer and they were eluted earlier than the expected ALA derivative as shown in Fig. 2. When 0.45 ml of 9.3% formaldehyde solution was used and various volumes (2–7 ml) of the acetylacetone solution were tested, the highest reaction rate was

Table 1. Effect of formaldehyde concentration on ALA derivatization.

Formaldehyde conc.	Peak area	Ratio	FA/AA ratio
3.1 %	510,933	1.00	0.027
4.6	823,121	1.61	0.039
9.3	960,364	1.88	0.080
18.5	494,166	0.97	0.159
37.0	123,320	0.24	0.317

Reaction was performed by mixing and boiling for 15 min. The reaction mixture consisted of 50 μ l of 5.5 mg/l of standard solution, 3.5 ml of the acetylacetone solution and 0.45 ml of the various concentrations of formaldehyde solution. Fifty microliters of the mixture was injected into the HPLC system. FA/AA ratio expresses (final formaldehyde concentration)/(final acetylacetone concentration).

Table 2. Effect of various volumes of acetylacetone solution on ALA derivatization.

Acetylacetone (ml)	Peak area	Ratio (%)	Total vol (ml)	FA/AA ratio
2.0	3,239,478	62.4	2.47	0.140
3.5	3,231,968	100.0	3.97	0.080
5.0	2,391,355	101.9	5.47	0.056
7.0	1,601,926	93.3	7.47	0.040

Reaction was performed by mixing and boiling for 15 min. The reaction mixture consisted of 20 μ l of the 55.0 mg/l standard solution, various volume of the acetylacetone solution and 0.45 ml of 9.3% formaldehyde solution. Fifty microliters of the mixture was injected into the HPLC system. FA/AA ratio expresses (final formaldehyde concentration)/(final acetylacetone concentration).

Fig. 2. Chromatograms of ALA derivatives prepared with 0.45 ml solution of several formaldehyde concentrations (37, 9.3, 3.1 %).

found between 3.5 and 5.0 ml of the acetylacetone solution, as shown in Table 2. When 0.45 ml of

Fig. 3. Stability of ALA derivative made from ALA standard solution (11 mg/l).

9.3% formaldehyde solution and 5 ml of acetylacetone solution were used for the reaction, the stability of the ALA derivative was very good as shown in Fig. 3. The decline was below 5% during 24 h in the dark at 15°C. When the sample was stored in a refrigerator with a glass door (MEDICOOL, SANYO, Japan) at 3°C for 24 h, the decrease was below 3% (data not shown). The within-day and between-day coefficients of variation were 1.1% ($n=5$) and 1.4% ($n=5$ for two days) for 7.6 mg/l of ALA in an urine sample,

Fig. 4. Effects of various substances on ALA derivatization using original method and the modified method. Original method: 50 μ l of standard solution containing the urinary component, 0.45 ml of 3.1% formaldehyde solution, and 3.5 ml acetylacetone solution. Modified method: 20 μ l of standard solution containing the urinary component, 0.45 ml of 9.3% formaldehyde solution, and 5.0 ml acetylacetone solution. "All" means addition of all the urinary components of NaCl, KCl, CaCl₂, urea, NaH₂PO₄, NH₄Cl and uric acid. * significantly different from the original method, $p < 0.05$. ** significantly different from original method, $p < 0.001$.

Fig. 5. Calibration curve of ALA standard with ($\blacktriangle$) or without ($\circ$) various substances in urine the concentrations of which are shown in Fig. 4.

respectively.

In order to study the effects of various urinary co-existing substances on ALA derivatization, we used the upper limit concentration of the normal urinary components in the Japanese people,⁸⁾ such

Fig. 6. Effect of boiling time on ALA derivatization. $\bullet$, aqueous ALA standard; $\circ$, aqueous ALA standard with various urinary components the concentrations of which are shown in Fig. 4.

as 15 g/l of NaCl, 3.0 g/l of KCl, 0.6 g/l of CaCl₂, 20 g/l of urea, 7.5 g/l of NaH₂PO₄, 3.0 g/l of NH₄Cl or 0.7 g/l of uric acid. Effects of the components added to the ALA standard solution on the recovery rate is shown in Fig. 4. Serious negative effects of NaH₂PO₄ and NH₄Cl were found in the Okayama method, but not in the modified method.

Figure 5 shows the calibration curves made from the ALA standard and the addition of all the components to the ALA standard solution. Both calibration curves showed complete agreement.

The relationship between the reaction rate of ALA derivatization and the boiling time is shown in Fig. 6. The highest reaction rate was obtained during 10–20 min of heating. In the standard solution containing all the components, no significant decrease was found from 5 to 45 min.

When urine samples from 45 lead workers were measured by the present method, the ALA concentration ranged from 0.4 to 72.4 mg/l. These values were well correlated with those by the Okayama method, but the values by the Okayama method (y) were 70% of those found by our method (x) ($y = 0.691x + 0.114$, $n = 45$, $r = 0.969$, $p < 0.01$). Recovery rates of ALA ($n = 45$) obtained by adding 1–2 times the amount of the urinary ALA concentrations were $84.6 \pm 7.6\%$ by the Okayama method and $101.3 \pm 5.5\%$ by our method. When measured by the Okayama method, the recovery rate significantly decreased with increase in specific gravity of the urine ($n = 45$, $r = -0.306$, $p = 0.0412$). Neither the urinary creatinine concentration nor the specific gravity of

Table 3. ALA concentration (mg/g creatinine) of workers.

Methods	Lead workers (<i>n</i> =45)	Non-lead workers (<i>n</i> =40)
HPLC	6.9 $\pm$ 14.0	1.1 $\pm$ 0.4
(range)	(0.6-85.2)	(0.1-2.3)
M-G	8.3 $\pm$ 13.7	2.1 $\pm$ 0.5
(range)	(1.5-83.4)	(1.2-3.1)

Fig. 7. Relation between ALA concentrations (mg/g creatinine) by the M-G method (*X*) and those by the modified HPLC (*Y*). $\log Y = 1.241 \log X - 0.39$ (*n*=85, $r=0.997$, $p<0.001$).

the urine correlated with the recovery rate by our method. There was a significant positive correlation between the ALA concentrations by the M-G method (*x* mg/l) and those by our method (*y* mg/l) ($y = 1.005x - 1.668$, $n=45$, $r=0.991$, $p<0.001$). However, the concentrations by our method were lower than those by the M-G method among more than 95% of the samples.

When urine samples from 40 control workers were measured by the present method, ALA concentrations ranged from 0.4 to 3.9 mg/l (1.2 ± 0.6 mg/l). The recovery rate of ALA in urine was $100.3 \pm 5.5\%$ (range 90.9-111.8%, $n=40$) when 20 μ l of 1.5 mg/l ALA standard solution was added to each urine sample. A good recovery rate was also obtained in samples from non-lead workers. The correlation between the ALA concentrations by the M-G method (*x* mg/l) and those by our method (*y* mg/l) was significant ($y = 0.465x + 0.148$, $n=40$, $r=0.825$, $p<0.01$).

Fig. 8. Distribution of the differences between ALA concentrations (mg/g creatinine) by the M-G method and those by modified HPLC method. Open bars express the values found in lead workers (*n*=45), and shaded bars the values found in non-lead workers (*n*=40).

However, ALA concentrations obtained by this method were lower than those obtained by the M-G method in all samples from non-lead workers, and the former values were almost half of the latter, as shown in Table 3.

Although a significant correlation was found between ALA concentrations by our method and those by the M-G method in 85 workers as shown in Fig. 7, all workers, except two lead workers with high lead exposure, showed higher concentrations by the M-G method than those by our method. The difference was 1.2 ± 0.8 mg/g creatinine (range -1.7-3.7 mg/g creatinine, $n=85$), as shown in Fig. 8.

DISCUSSION

The automatic analytical system of ALA in urine has been reported by Lauwerys et al.,⁹⁾ but their system has not been widely employed because of such problems as use of large scale equipment and a standard addition procedure to calculate urinary concentrations. Since HPLC has recently become a common tool for selective analysis, some analytical methods of ALA by HPLC have been reported.^{1-5,10-12)} These reports can be divided in two ways: One is post-column procedure¹⁰⁻¹²⁾ in which ALA is isolated by HPLC, followed by

derivatization through a reaction tube and detected. The other is pre-column procedure¹⁻⁵⁾ in which the ALA is firstly derivatized and thereafter the derivative is isolated by HPLC and measured by a detector. Since ALA is derivatized after isolation by the post-column procedure, inhibition in the process of reaction can be negligible. However, the system needs not only a complicated apparatus such as pumps to pump solutions for the reaction and an oven for the reaction tube but also large quantities of reaction solvent are consumed. Thus the cost performance of the system seems to be low. On the other hand, the pre-column procedure does not need additional apparatus and is applicable to any laboratory if the simple pre-treatment is chosen. It is stated in some reports that ALA is derivatized using formaldehyde and acetylacetone,^{1,2,13,14)} dansyl chloride,³⁾ *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (ME),⁴⁾ or 2-amino-3-hydroxynaphthalene (AHN)⁵⁾ as derivatization reagents, then isolated by a HPLC and detected by a fluorometer. All the procedures have a sensitivity more than 10 times higher than the M-G method (colorimetric method). On the other hand, the procedure using dansyl chloride³⁾ needs toluene extraction to eliminate obstacles at the separation of ALA by HPLC. The derivatization using OPA and ME⁴⁾ requires a high temperature (97°C) and a long period (3 h) for the reaction, and the AHN procedure⁵⁾ needs ethyl ether extraction because AHN-ALA is unstable in aqueous solution.

The derivatization method using formaldehyde and acetylacetone^{1,2,14)} is simple and forms a stable derivative without any extraction and thus the method is the most appropriate for routine analysis. However, the original Okayama method²⁾ produced relatively low recovery rate of added ALA and there was a significant negative correlation between the recovery rate and the specific gravity of the urine or urinary creatinine concentration. Tomokuni et al.¹³⁾ assumed that the low recovery was due to large quantities of components such as urea or NaCl, but significantly negative effects of NaH₂PO₄ and NH₄Cl, rather than of urea or NaCl, were observed, as shown in Fig. 4. Our modification based on the increase of acetylacetone solution from 3.5 ml to 5 ml, the increase of formaldehyde concentration in the formalin solution from 3.1% to 9.3%, and

the decrease of the sample volume from 50 μ l to 20 μ l overcomes the negative effects of these components of derivatization (Fig. 4).

Tomokuni et al.¹³⁾ reported that the ALA derivative was unstable and its fluorescence intensity decreased by almost 50% during 3 h at room temperature (18–21°C). On the other hand, the ALA derivative in our modified method is very stable as shown in Fig. 3, indicating that the formaldehyde concentration in this method is the most appropriate for making the fluorescent ALA derivative stable (Fig. 2). The recent paper by Tomokuni et al.¹⁴⁾ reported that the stability of the fluorescent ALA derivative was affected more by light than by temperature. We did not conduct the sample treatment with particular attention to light, but since the auto-sampler we used could keep a sample in the dark and at a relatively low temperature of 15°C, both conditions seemed to give good stability to the fluorescent ALA derivative. Under this procedure, continuous analysis using the auto-sampler can be performed after treating scores of samples at once.

Tomokuni et al.¹³⁾ reported that a fivefold dilution of the urine sample was needed to use aqueous ALA as a standard solution for a calibration curve because the intensity of the fluorescent ALA derivative in artificial urine was about 56% of that in a standard distilled water solution. In the present method, since the calibration curve made from the ALA and all the urinary components was almost the same as that from the aqueous ALA (Fig. 3), dilution of the urine was not necessary. This result agreed with the results where recoveries of added ALA were almost 100% in 85 samples and derivatization of ALA was not affected by the addition of components.

ALA concentration measured by this modified method was well correlated with that measured by the M-G method. However, urinary ALA concentrations measured by this modified method were lower than those measured by the M-G method in all workers except two with high lead exposure (Fig. 8). Since the differences were not correlated with ALA concentrations and were of an almost constant value (lower than 4 mg/g creatinine), they were not considered to be by-products or decomposition of the derivatization. Witting et al.¹⁰⁾ reported that urinary ALA concentrations obtained by the HPLC/UV method were lower than those obtained by the Davis

method¹⁵⁾ (a modification of the M-G method) and that in urine samples with the Davis values below 15 mg/l, the percentage of interfering compounds varied between 10% and 80%. Okayama et al.¹⁶⁾ reported that urinary ALA concentrations obtained by the post-column HPLC fluorometric method were lower than those obtained by the Sun method¹⁷⁾ (another modification of the M-G method). The differences between the values determined by the Sun method and those obtained by the HPLC method were not correlated with blood lead concentrations. The differences (almost two-thirds of the Sun's value) might have been caused by coexisting substances. These results agree with our results, indicating that the values determined by our modified method can give the true ALA concentrations in urine.

Urinary ALA concentrations in non-lead workers were found to be 1.1 ± 0.4 mg/g creatinine by this modified method and this value was almost 50% of the value found by the M-G method. Okayama et al.¹⁶⁾ reported that urinary ALA concentrations determined by the HPLC method were lower than those determined by the colorimetric method. An increase of urinary ALA was observed in the sample with lower blood lead levels as reported earlier, and the dose-response relationship between urinary ALA and blood lead levels changed from the earlier report as determined by the colorimetric method.

In conclusion, our modified HPLC method has some advantages: (1) urinary ALA can be determined without interference from the various components of urine without using a complicated system, (2) a stable derivative of ALA can easily be made, (3) a calibration curve can be made with the aqueous standard solution, and (4) the sensitivity is very high (≥ 0.01 mg/l ALA) with a wide range of linearity. Thus, this method is the most convenient for routine automated analysis. However, as ALA concentrations determined by the HPLC method were lower than those determined by the colorimetric method, it is suggested that the biological tolerance levels of urinary ALA concentrations by the HPLC method should be different from those obtained by the colorimetric method. In future studies, the effects of sex, age, and anemia on the normal range of ALA concentrations should be clarified.

REFERENCES

- 1) Okayama A, Lim KM, Goto S. Fluorophotometric determination of δ -aminolevulinic acid in urine by reversed-phase high performance liquid chromatography. *Igaku no Ayumi* 1986; 139: 845-846 (in Japanese).
- 2) Okayama A, Fujii S, Miura R. Optimized fluorometric determination of urinary δ -aminolevulinic acid using pre-column derivatization, and identification of the derivative. *Clin Chem* 1990; 36: 1494-1497.
- 3) Minder EI. Measurement of 5-aminolevulinic acid by reversed phase HPLC and fluorescence detection. *Clin Chim Acta* 1986; 161: 11-18.
- 4) Meisch HU, Wannemacher B. Fluorometric determination of 5-aminolevulinic acid after derivatization with *o*-phthaldialdehyde and separation by reversed-phase high-performance liquid chromatography. *Anal Chem* 1986; 58: 1372-1375.
- 5) Meisch HU, Reinle W, Wolf U. Determination of 5-aminolevulinic acid in biological samples by high-performance liquid chromatography. *Anal Biochem* 1985; 149: 29-34.
- 6) Endo Y, Okayama A, Endo G, et al. Improvement of urinary δ -aminolevulinic acid determination by HPLC-fluorometry using pre-column derivatization. *Jpn J Ind Health* 1993; 35: 126-127 (in Japanese).
- 7) Mauzerall D, Granick S. The occurrence and determination of δ -aminolevulinic acid and porphobilinogen in urine. *J Biol Chem* 1956; 219: 435-446.
- 8) Kanai I, Kanai M, eds. Handbook of clinical chemistry, 26th ed. Tokyo: Kanehara Pub., 1972: 50-53 (in Japanese).
- 9) Lauwerys R, Delbroeck R, Vens M. Automated analysis of delta-aminolevulinic acid in urine. *Clin Chim Acta* 1972; 40: 443-447.
- 10) Witting U, Binding N, Muller G. Evaluation of a new specific analysis of urinary delta-aminolevulinic acid in man. *Int Arch Occup Environ Health* 1987; 59: 375-383.
- 11) Okayama A. Fluorimetric determination of urinary δ -aminolevulinic acid by high-performance liquid chromatography and post-column derivatization. *J Chromatogr* 1988; 426: 365-369.
- 12) Kondo M, Kimura H, Maekubo T, et al. Direct injection method for quantitation of δ -aminolevulinic acid in urine by high-performance liquid chromatography. *Chem Pharm Bull* 1992; 40: 1948-1950.
- 13) Tomokuni K, Ichiba M, Hirai Y, et al. Optimized liquid-chromatographic method for fluorometric determination of urinary δ -aminolevulinic

- acid in workers exposed to lead. *Clin Chem* 1987; 33: 1665-1667.
- 14) Tomokuni K, Ichiba M, Hirai Y. Measurement of urinary δ -aminolevulinic acid (ALA) by fluorometric HPLC and colorimetric methods. *Ind Health* 1992; 30: 119-128.
- 15) Davis JR, Andelman SL. Urinary delta-amino-levulinic acid (ALA) levels in lead poisoning. I. A modified method for the rapid determination of urinary delta-amino-levulinic acid using disposable ion-exchange chromatography columns. *Arch Environ Health* 1967; 15: 53-59.
- 16) Okayama A, Ogawa Y, Miyajima K, et al. A new HPLC fluorimetric method to monitor urinary delta-amino-levulinic acid (ALA-U) levels in workers exposed to lead. *Int Arch Occup Environ Health* 1989; 61: 297-302.
- 17) Sun MW, Stein E, Gruen FW. A single column method for the determination of urinary δ -amino-levulinic acid. *Clin Chem* 1969; 15: 183-189.

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英文論文の和文要旨

IMPROVEMENT OF URINARY δ -AMINOLEVULINIC ACID DETERMINATION
BY HPLC AND FLUORESCENCE DETECTION USING CONDENSING REACTION
WITH ACETYLACETONE AND FORMALDEHYDEYoko ENDO, Akira OKAYAMA, Ginji ENDO, Teruko UEDA, Naoki NAKAZONO,
and Shun'ichi HORIGUCHI.....49-56アセチルアセトンとホルムアルデヒドによる縮合反応を用いた HPLC/蛍光検出法による
尿中 δ -アミノレブリン酸定量法の改良

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我々は尿中デルタアミノレブリン酸 (δ -ALA) をアセチルアセトンとホルムアルデヒドを用いたプレカラム誘導体法による HPLC-蛍光光度計で定量する方法が有用であることを先に報告した。しかしながら、その方法による δ -ALA の回収率は尿中共存物質の影響を強く受け、尿中 δ -ALA の回収率はやや低くなっていた ($84.6 \pm 7.6\%$, $n=45$)。本報告者は、我々は尿中 δ -ALA の回収率がほぼ 100% となるように方法を改善した ($101.3 \pm 5.5\%$, $n=45$)。この改訂法は以下の方法で行った：尿 20 μ l, アセチルアセトン溶液 (アセチルアセトン：エチルアルコール：4 g/l の塩化ナトリウムを含有する蒸留水=15：10：75) 5 ml, および 9.3% ホルムアルデヒド溶液 0.45 ml を混合し沸騰浴で 15 分間反応させた後、流水で冷却し、その 50 μ l を HPLC-蛍光光度計で測定した (励起波長 246 nm, 測定波長 458 nm)。 δ -ALA の保持時間は 7.3 分であった。溶液グラジエント法を用い

ることによりカラム圧の上昇を避けられ、13 分間隔での連続分析が可能となった。この方法によれば、種々の尿中共存物は δ -ALA 誘導体の生成に影響を与えず、生成された誘導体はほぼ 24 時間安定であった。この方法で測定された尿中 δ -ALA 濃度はモーゼル-グラニック (M-G) 法で測定した値と有意に相関した ($n=85$, $r=0.993$, $p<0.001$)。しかしこの方法では δ -ALA の類似物質を分離しているため、M-G 法の値よりもこの方法による値のほうが低く、この方法による非鉛曝露作業員 40 名の尿中 δ -ALA 濃度は $0.1 \sim 2.3$ mg/g creatinine (1.1 ± 0.4 mg/g creatinine) と、M-G 法による濃度 ($1.2 \sim 3.1$ mg/g creatinine (2.1 ± 0.5 mg/g creatinine)) の約半分の値であった。HPLC 法による尿中 ALA 濃度の許容値は従来の比色法による数値とは異なった値を設定する必要があると考えられる。

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EFFECTS OF REGULAR HEALTH PRACTICES ON SUBJECTIVE EVALUATION OF
HEALTH

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主観的健康に及ぼす規則的な健康習慣の影響

川田智之, 新明ローザ怜美, 鈴木庄亮

Breslow の 7 つの健康に関連する好ましい行動あるいは状態として、喫煙しない・ほとんどの飲酒・定期的運動・適正体重の維持・適度な睡眠時間・朝食の摂取・間食しない、が挙げられ、これら 7 項目を守ることの重要性は西洋諸国で広く認められ、またそれらに対する保健対策も計画実施されつつある。これらの提言は、米合衆国で行われたコホート調査を基に策定されたものであるが、もともと死亡や疾病発症に対する危険度に関する結果であるため、予防医学あるいは健康増進の観点から、

身体的精神的な不具合の主観的愁訴と健康習慣との関連性については十分な検討がなされているとは言えない。著者らは東大式健康調査票 (THI) の 130 項目の質問中に、Breslow の 7 つの健康習慣が含まれていることを確認し、しかも本調査票が近年広く国内で使用されていることを踏まえて、主観的精神身体愁訴への良い健康習慣の影響を断面調査として実施した。

対象は東京都北区の某製造会社の男子工員および事務員とその妻各 115 人で、夫婦共に回答した各 95 人