Copy to [illegible]
Copy to Pb file
Analytical Techniques 1451

The Preservation of Urine Specimens for δ-Aminolevulinic Acid Determination

William F. Vincent[1] and William W. Ullman

The effect of light exposure and temperature on urinary δ-aminolevulinic acid recovery was studied. Addition of tartaric acid and storage of urine specimens in the dark results in excellent recovery of δ-aminolevulinic acid, even after several days at room temperature. Proper handling and storage of urine collected during surveys of lead poisoning are emphasized.

Additional Keyphrases *light exposure • storage temperature • tartaric acid preservative • lead poisoning*

DELTA-AMINOLEVULINIC ACID (ALA) is an intermediate metabolite in the synthesis of the heme moiety of hemoglobin, whereby two molecules of ALA condense to form porphobilinogen. This reaction is catalyzed by the enzyme δ-aminolevulinate dehydratase (5-aminolevulinate hydrolyase, EC 4.2.1.24), which is markedly inhibited by lead ions. Lead in the bones of patients with lead intoxication inhibits this condensation; consequently, more ALA is excreted in the urine.

Measurement of the amount of this metabolite in the urine has been shown to be a suitable screening procedure for the detection of lead intoxication in both children (*1*) and adults (*2, 3*). One drawback to this determination, however, is the instability of ALA. Unless the pH of the specimen is decreased and the sample refrigerated, the ALA concentration will rapidly decrease, particularly when the specimen is exposed to light (*3, 4*).

J. R. Davis (personal communication, 1969) has reported that a small amount of glacial acetic acid (0.1 ml/10 ml urine) satisfactorily preserves specimens. However, we found the use of acetic acid to be rather difficult since the urine specimens were collected by nonprofessionals or parents. The odor of glacial acetic acid in the urine specimen is disagreeable to the patient. More importantly, acetic acid eventually evaporates, even when screw-capped tubes are used.

We report here the use of dry tartaric acid as a preservative for urine specimens that are to be examined for ALA and also on the effect of light exposure and refrigeration on ALA recovery.

Materials and Methods

Determination of urinary ALA. This was essentially the single-column method of Sun *et al.* (*5*), in which ALA is adsorbed onto Dowex resin, converted to a pyrrole, and a colored complex is produced when modified Ehrlich's reagent is added. Prepacked, disposable columns for use in this test can be obtained from Bio-Rad Labs, Richmond, Calif.

Preservative. One-quarter ml of tartaric acid, 2 mol/liter, was placed in a 1.5 × 12 cm screw-capped glass tube and evaporated to dryness in an oven at 120 °C for 1 to 2 h. The small amount of dried tartaric acid in the bottom of the tube is almost invisible and rapidly goes into solution when the urine sample is added and shaken. This quantity of tartaric acid is sufficient to decrease the pH of 10 to 15 ml of urine to 2.4 to 2.7. For these studies, we used 10-ml aliquots of urine.

Urine specimens. Specimens with increased ALA concentrations were obtained from children suffering from lead intoxication.

Results

Preserved (acidified) and unpreserved specimens were held either at 4 °C or 25 °C and stored in the

From the Research Microbiology Laboratory, Laboratory Division, Connecticut State Department of Health, P.O. Box 1689, Hartford, Conn. 06101.

[1] Present address: Division of Laboratories and Research, New York State Department of Health, New Scotland Ave., Albany, N. Y. 12201.

Received March 16, 1970; accepted April 29, 1970.

Table 1. Recovery of δ-Aminolevulinic Acid from Urine Specimens Stored at 25°C

Specimen[a]	Time of Sampling	% of original ALA remaining			
		Acidified		Unpreserved	
		Light	Dark	Light	Dark
1	24 h	62.3	98.2	43.7	94.3
	48 h	38.3	98.0	21.3	82.5
	72 h	28.5	98.0	15.4	51.0
	2 wk	6.2	96.5	3.4	5.4
2	24 h	58.5	100.5	48.0	90.6
	48 h	42.5	98.0	19.8	80.6
	72 h	33.5	96.4	15.0	48.2
	2 wk	5.3	95.0	4.2	7.9
3	24 h	54.3	98.5	50.0	91.0
	48 h	51.5	97.0	23.0	77.0
	72 h	47.7	97.0	17.5	48.0
	2 wk	5.5	91.0	6.8	8.0

[a] Initial ALA concentrations of specimens were: No. 1, 23.4 μg/ml; No. 2, 32.8 μg/ml; and No. 3, 79.4 μg/ml.

Table 2. Recovery of δ-Aminolevulinic Acid from Urine Specimens Stored at 4°C in the Dark[a]

Specimen	Time of sampling	% of original ALA remaining	
		Acidified	Unpreserved
1	24 h	99.3	93.2
	48 h	100.0	88.4
	72 h	99.0	74.2
	2 wk	96.0	42.5
2	24 h	100.0	96.8
	48 h	98.4	86.2
	72 h	97.8	71.0
	2 wk	97.5	46.5
3	24 h	98.0	90.4
	48 h	98.5	86.2
	72 h	99.0	68.4
	2 wk	96.5	51.5

[a] See footnote to Table 1.

dark or exposed to artificial light (110 footcandles, achieved with an 100-watt bulb at 12 inches). At 24-h intervals, duplicate 1.0-ml aliquots were removed and the ALA concentration was determined. The results obtained with specimens stored at room temperature are shown in Table 1 and those obtained with specimens stored in the cold are shown in Table 2.

Discussion

The results clearly demonstrate the adverse effects of temperature and light exposure on ALA recovery and the need to decrease the pH of urine specimens if laboratory findings are to be accurate. If ALA is to be estimated immediately after the specimens are collected, the influence of the conditions is decreased. It was recently reported in a field study that, with tartaric acid as a preservative, 48% of the children with increased urinary ALA (10 μg/ml or more) also had increased blood lead (*6*). These findings compare favorably with those of Davis and coworkers (*1*), who assayed for ALA almost immediately after collection of specimens (J. R. Davis, personal communication, 1969). When large numbers of specimens are collected and mailed to a central laboratory, it is imperative that all concerned be instructed in the proper handling and preservation of urine specimens.

The necessity for acidifying urine specimens cannot be overemphasized. During the warm July weather, duplicate sets (acidified and unpreserved) of positive urine specimens were mailed on Friday morning and delivered to the laboratory the following Monday morning. ALA recovery in preserved samples ranged from 92% to 98%; in unpreserved samples, from 42% to 60%. Three of the five unpreserved specimens handled in this manner would have been reported to the physician as having less than 10 μg ALA/ml when in fact all specimens contained abnormally high amounts.

The authors are grateful to Dr. Joseph R. Davis for his advice and encouragement during the course of this study and preparation of the manuscript.

References

1. Davis, J. R., Abrahams, R. H., Fishbein, W. I., and Fabrega, E. A., Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning. II. Correlation of ALA levels with clinical findings in 250 children with suspected lead ingestion. *Arch. Environ. Health* **17**, 164 (1968).

2. Cramer, K., and Selander, S., Control of lead workers by determination of urinary delta-aminolevulinic acid. *Brit. J. Ind. Med.* **24**, 283 (1967).

3. Haeger-Aronsen, B., Studies on urinary excretion of delta-aminolevulinic acid and other haem precursors in lead workers and lead-intoxicated rabbits. *Scand. J. Clin. Lab. Invest.* **12** (Suppl. 47), 1 (1967).

4. Bossenmaier, I., and Cardinal, R., Stability of δ-aminolevulinic acid and porphobilinogen in urine under varying conditions. CLIN. CHEM. **14**, 610 (1968).

5. Sun, M.-W., Stein, E., and Gruen, F. W., A single column method for the determination of urinary δ-aminolevulinic acid. CLIN. CHEM. **15**, 183 (1969).

6. Vincent, W. F., Ullmann, W. W., and Weidner, G. L., The measurement of urinary Δ-aminolevulinic acid in detection of childhood lead poisoning (letter to editor). *Amer. J. Clin. Pathol.* (accepted for publication).