Technical methods

A simple and rapid one-tube method for the determination of urinary delta-aminolaevulinic acid

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The method of Mauzerall and Granick (1956) has been widely used as the reference method for the determination of urinary delta-aminolaevulinic acid (ALA). The procedure is lengthy and involves the use of two ion exchange columns. The eluted ALA is condensed with acetylacetone to form a pyrrole which is then reacted with a modified Ehrlich's reagent to give a pink colour.

Other workers have omitted the ion exchange columns and treated the urine directly with acetylacetone (Grabecki, Haduch, and Urbanowicz, 1967; Basecqz, Lauwerys, and Buchet, 1971). We have observed that both these procedures do not obey Beer's law when a low concentration of urinary ALA is being measured. The use of a standard curve derived by adding different amounts of ALA to pooled urine as suggested by Grabecki *et al* (1967) is unreliable as this curve showed varying slopes for different urines.

We have developed a one-tube method for the direct measurement of urinary ALA which shows good linearity between absorbance and added ALA over a wide range of concentrations. Our method minimizes colour suppression due to the urine and can measure low levels of ALA which makes it advantageous for discriminating normal persons from those suffering mild lead toxicity.

Materials and Methods

REAGENTS

Unless otherwise specified, all reagents are of analytical grade.

Acetate buffer, pH 4.6

Dissolve 272 g $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ in 108 ml glacial acetic acid and make up to 2 litres with water. Check the pH.

Acetate/acetylacetone buffer

Dissolve 12.5 ml acetylacetone (BDH laboratory grade) in acetate buffer and make up to 1 litre with more buffer. Store in a dark bottle at room temperature.

Stock standard ALA, 240 μg ALA/ml

Dissolve 31 mg delta-aminolaevulinic acid hydrochloride (Sigma Chemical Co.) in water and make up to 100 ml. Refrigerate. This solution is stable for several months.

Working ALA standard, 8 μg /ml

Dilute the stock standard 1 in 30 with water. Prepare fresh.

Modified Ehrlich's reagent

Dissolve 1 g *p*-dimethylaminobenzaldehyde in about 30 ml glacial acetic acid and 8 ml 70% perchloric acid and make up to 50 ml with more acetic acid. This reagent is prepared freshly before use.

Procedure

Set up five tubes as follows: A, 4.0 ml acetate buffer; B, 4.0 ml acetate buffer + 100 μl urine; C, 4.0 ml acetate/acetylacetone buffer; D, 4.0 ml acetate/acetylacetone buffer + 100 μl urine; E, 4.0 ml acetate/acetylacetone buffer + 100 μl urine + 100 μl standard.

Place the unstoppered tubes vertically in a boiling water bath for 10 minutes. Cool and add 4.0 ml of the modified Ehrlich's reagent to each tube. Mix and allow to stand for 15 min after which the solutions are read at 553 nm on the Unicam SP500 series II spectrophotometer using 4 cm light path glass cells. Solution B is read with solution A set to zero absorbance, while solutions D and E are read against solution C. Solutions A and C will serve as reagent blanks for any number of urines, it being necessary to set up tubes B, D, and E for every additional urine.

Calculation

$$\text{Urine ALA (mg/l)} = \frac{\text{Absorbance (D - B)}}{\text{Absorbance (E - D)}} \times 8$$

Results

The varying absorbance slopes due to the added ALA exhibited by different urines are shown in

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Fig 1 Linear absorbance with different ALA concentrations. A, aqueous standard curve; B, C, and D, internal standard curves of three different urines.

figures 1 and 2. Linearity of colour formation was obtained for both low and high ALA concentrations. With different normal urines the absorbance of the internal ALA standard (8 μ g/ml) ranged from 0.098 to 0.147 while the same ALA standard without urine had absorbance around 0.155. For this reason the use of a pooled urine is not recommended and it is necessary for each urine to have its own internal standard. Absorbance up to 1.0 representing added ALA concentration equivalent to 60 mg/l was measured. For a more intense colour the absorbance should be read using cuvettes with a 1 cm light path. This gives similar results as when the proposed method was repeated using a smaller urine volume.

By the proposed method the ALA output in 24-hr urine samples taken from 20 adults gave a range of 2.5 mg to 5.0 mg (mean 3.8 mg). Several groups of workers have given the normal daily output of ALA for adults as 1.3-7.0 mg (cited from Fernandez and Jacobs, 1970).

The expression of urinary ALA as a concentration

Fig 2 Linear absorbance with different ALA concentrations. K, aqueous standard curve; L, M, and N, internal standard curves of three different urines.

from random urine specimens is employed by several workers, but results generally show a wide range. To overcome the problem of a dilute or concentrated urine which gives this scatter of results, we have also collected a working day (8 hr) urine specimen from 20 normal subjects and found the urinary ALA excretion to have a range 0.9-6.7 mg/l (mean 2.8 mg/l).

Analyses of normal urines with a range 1.5-6.1 mg ALA/l (mean 3.0 mg/l) gave a between-batch coefficient of variation of 3.47% from 16 duplicate analyses. The precision (95% limit) for our method is ± 0.2 mg ALA/l.

Lead factory workers were also screened for raised ALA excretion using the eight-hr urine collection. The range obtained from these workers was 5.6-88.4 mg/l, of which 80% of them were

found to show varying degrees of elevated urinary ALA levels.

Comment

The direct measurement of ALA in urine as proposed by Grabecki *et al* (1967) and Basecqz *et al* (1971) is not accurate for values within and bordering the normal range and these methods can at best serve as screening procedures for high ALA levels. Low ALA concentrations determined by these methods are also in error as there is considerable suppression of the Ehrlich colour complex by various urinary constituents. These workers have not reported the normal values for urinary ALA by their methods. We have shown by using a smaller urine volume (100 μ l) that this colour suppression can be minimized, and the accuracy of the estimation assured through the use of an internal standard. However, the low intensity of the Ehrlich colour resulting from the low urine volume is compensated for by the use of a 4 cm light path cuvette.

The endogenous Ehrlich-positive substances in urine were measured in solution B when read against the first reagent blank (solution A). We have found that acetylacetone reacts with the Ehrlich's reagent. To correct for this, we set up a second reagent blank (solution C) against which solutions D and E are read.

When several urine samples are processed, the addition of the Ehrlich's reagent is staggered to allow for the 15-min reaction time. The cell holder of the spectrophotometer which accepts four cells was used to facilitate readings. It is important that cuvettes are matched, otherwise this might cause substantial error at low ALA concentrations.

References

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A simple method for the estimation of plasma ammonia using an ion specific electrode

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Recently a new specific electrode has been introduced for the measurement of ammonia. Until now this electrode appears to have been used only for industrial purposes (Midgley and Torrance, 1972) but this instrument can be used for the determination of ammonia in blood, and the method is sufficiently sensitive and simple for use in a routine clinical laboratory. A preliminary report on the use of a similar electrode system has been made in the United States (Coleman, 1972).

The method depends on the microdiffusion of ammonia vapour through a plastic membrane. This allows a great increase in the speed and sensitivity of the analysis in comparison with the microdiffusion technique of Conway (1939).

Samples of plasma are mixed with an alkaline buffer solution and the mixture is then brought immediately into contact with the undersurface of the water-repellent plastic membrane. Ammonia vapour from the sample diffuses selectively through the membrane into a thin film of dilute ammonium chloride solution which lies in contact with the lower end of the glass electrode. Changes in the potential from the glass electrode, which is coupled to a silver/silver chloride reference electrode within the probe, are a reflection of changes of the pH within the film, and are hence proportional to the ammonia concentration in the plasma sample.

In practice, it is difficult to obtain stable reproducible readings from the electrode when used for ammonia estimations on single samples because of the tendency of the instrument to suffer from electronic drift. When, however, the electrode is incorporated as part of an automated continuous flow system, it becomes much easier to standardize and this is the recommended mode of operation which is described below.

Apparatus and Materials

Ammonia probe, model 8002-2 with flow-through

¹This report forms part of a thesis to be submitted by Mrs N. Park to the Institute of Medical Laboratory Technology.