A Simplified Procedure for the Determination of Urinary δ -Aminolaevulinic Acid

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Urinary δ -aminolaevulinic acid (ALA) is commonly estimated by the method of Mauzerall and Granick (1956). In that method porphobilinogen (PBG) and ALA are successively separated from the urine by ion exchange resins.

A simplified procedure, omitting the initial separation of PBG, was investigated. Urine samples from 39 lead workers and controls were estimated for ALA by both the original procedure and by this simplified procedure. A correlation coefficient of 0.99 was obtained.

It was concluded that the simplified procedure may be used for screening lead workers so long as the possibility of false high readings is borne in mind.

Some sources of variation in standardizing the original procedure are also discussed.

The urinary excretion of δ -aminolaevulinic acid (ALA) is increased by lead absorption (Haeger, 1957, 1958; Haeger-Aronsen, 1960; de Kretzer and Waldron, 1963) and is recommended for the estimation of lead absorption (Haeger-Aronsen, 1960; Cramér and Selander, 1966) and of lead poisoning (Cramér and Selander, 1965). The method of Mauzerall and Granick (1956) in which porphobilinogen (PBG) and ALA are successively separated from urine by ion exchange resins is commonly used. Since PBG excretion is little affected by lead absorption (Griggs and Harris, 1958; Haeger-Aronsen, 1960; de Kretzer and Waldron, 1963), its determination is unnecessary when urinary ALA is used for screening lead workers. It therefore seemed possible that acceptable estimates of urinary ALA could be obtained more simply without first removing PBG. Such a procedure is described and evidence of its validity is presented.

Materials and Methods

Reagents and Resin Columns Reagents and resin columns were similar to those described by Mauzerall and Granick (1956), except that the resin columns were prepared using cotton wool

plugs (Haeger-Aronsen, 1960) and gave flow rates of about 10 drops per minute. δ -Aminolaevulinic acid hydrochloride (ALA.HCl) was obtained from Aldrich Chemical Co., Inc., Wisconsin, U.S.A.

Collection of Samples The pH of fresh urine was measured with pH paper and adjusted to 5-6 with glacial acetic acid as necessary. Samples were placed in 25-ml. screw-capped bottles and stored in the dark, at $-18^{\circ}\text{C}.$, until analysed.

Analytical Procedures

Modified Original Procedure The frozen urine was melted and shaken to suspend any sediment and 1 ml. was placed on a Dowex 2 column. After the urine had passed through, the column was washed twice with 2 ml. of water.

The combined eluate was transferred quantitatively to a Dowex 50 column. The urea was removed from the resin with water (10-20 ml.) until the eluate did not give a lemon colour on mixing with an equal volume of modified Ehrlich's reagent: 4.5 ml. and 5 ml. of 0.5M sodium acetate were then passed through the resin and all the eluate was collected in a 10 ml. measuring cylinder. The eluate was made up to 10 ml. with acetate buffer, pH 4.6.

Acetylacetone, 0.2 ml., was placed in a test tube

and 3 ml. acetate buffer, pH 4.6, was added. The mixture was shaken and the contents of the measuring cylinder were added. The tube, unstoppered, was held vertically in a boiling water-bath for 10 minutes and then cooled in a cold water-bath. A 2 ml. portion was added to 2 ml. of modified Ehrlich's reagent, and the mixture was shaken. The absorbance was read after not less than 15 minutes in a 1 cm. cuvette at 553 m μ in a Unicam SP 600 spectrophotometer. The blank consisted of 2 ml. of distilled water and 2 ml. of modified Ehrlich's reagent. When the whole procedure was performed using 1 ml. of distilled water instead of 1 ml. of urine, readings of zero were obtained.

The procedure was standardized by adding known amounts of ALA.HCl to stock urine and analysing the solutions obtained.

Simplified Procedure The frozen urine was melted and shaken to suspend any sediment. 1 ml. was placed on a Dowex 50 column, the Dowex 2 stage being omitted. Washing etc. was then continued as in the modified original procedure.

The procedure was standardized as described above, using this simplified method.

Comparison of the Modified Original and Simplified Procedures Samples of urine were collected at mid-day from 39 workers in an electric accumulator factory, 29 of whom were on work involving contact with lead. The samples were stored as described previously and analysed by both the modified original procedure and the simplified procedure.

Results

Standardization of Simplified Procedure

The results of quadruplicate analyses of urine standards containing 0, 2, 4, and 6 mg. of added ALA.HCl/100 ml., when corrected for the original ALA in the stock urine, showed the absorbance was directly proportional to the concentration of ALA.HCl. Assuming 10 mg. ALA.HCl correspond to 7.28 mg. ALA, the slope of the calibration curve was 0.136 per mg. ALA/100 ml. The mean coefficient of variation of the quadruplicate estimations was 2.9%.

Comparison of the Modified Original and the Simplified Procedures The Figure shows a scatter diagram made by plotting the coordinate

FIGURE Comparison of the original and simplified procedures for the determination of urinary ALA. The corresponding absorbances determined by the original procedure (y) and the simplified procedure (x) are plotted for 39 urine specimens. The regression lines, the correlation coefficient (r), and a 1:1 line through the origin are also shown.

points of the two absorbences obtained for each urine sample, together with the regression lines. A correlation coefficient of 0.99 was obtained. It can be seen that the regression line lies close to the 1:1 line through the origin; that no sample diverged greatly from the regression; and that the samples at the lower (more normal) end of the range gave slightly higher absorbences with the simplified than with the modified original procedure.

Discussion

Modified Original Procedure The modified original procedure described differs slightly from previous methods.

Elution of ALA Mauzerall and Granick (1956) and Haeger-Aronsen (1960) discarded the first portion of sodium acetate passed through the Dowex 50 column. But Rimington (personal communication) found that this discarded portion, due to resin batch differences, might sometimes contain ALA. He therefore used a total of 9.5 ml. of sodium acetate and, like de Matteis and Prior (1962), retained all the eluate. Loss of ALA occurred with the resin used in the present instance, so Rimington's modification was adopted.

Condensation Reaction Mauzerall and Granick (1956) and Haeger-Aronsen (1960) used 0.2 ml. of acetylacetone; Rimington, having retained all the eluate, used 0.3 ml. Whilst standardizing Rimington's method, it was found that a deeper colour developed if 0.2 ml. of acetylacetone were used instead of 0.3 ml. Similarly, a deeper colour developed if unstoppered tubes were used during boiling; and colour development was more consistent if the unstoppered tubes were held vertically in a boiling bath than if they were placed non-vertically in a beaker containing boiling water. The quenching effect of excess acetylacetone and the variation with different boiling techniques has also been noticed by Rainsford (personal communication). The reasons are not known, but the method was modified accordingly.

Standardization Mauzerall and Granick (1956) gave a factor to convert the absorbence into the concentration of ALA in urine. Since small variations in technique produce relatively large changes in the absorbence (see above) and since ALA.HCl is now commercially available, we have preferred to run standards with each batch of urines analysed.

Recovery of ALA Mauzerall and Granick (1956) showed the absolute percentage recovery of ALA from urine was about 90%. They used techniques beyond the scope of the present inquiry so it was decided to obtain a standardization curve using ALA.HCl added to normal urine and to use this to estimate ALA in lead workers' and non-lead workers' urine. It was assumed that ALA.HCl behaved like ALA and that the percentage recovery in lead workers and non-lead workers was similar, as Haeger-Aronsen (1960) showed.

Simplified Procedure The results of the comparison of the modified original and simplified procedures suggest that PBG, and other possible interfering substances, were not retained by Dowex 50 in sufficient quantity to interfere greatly with the determination of ALA and that the Dowex 2 stage was unnecessary for screening these 39 workmen. However, if this simplified procedure were used in other subjects, false high readings from large amounts of PBG, as found in porphyria, might occur. Further investigation is desirable to reveal the probability of this event.

Summary and Conclusion

When urinary ALA is used for screening lead workers, the Dowex 2 stage of the analytical procedure of Mauzerall and Granick may be omitted. However, this may result in lower specificity, and falsely high values may then occur in pathological conditions.

Since small variations in technique produce relatively large variations in the absorbence obtained, controls were run with every batch of samples analysed.

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