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

- Basecqz, J. M., Lauwerys, R., and Buchet, J. P. (1971). Étude comparative de divers tests biologiques d'exposition au plomb. *Arch. Mal. prof.*, **32**, 453-463.
- Fernandez, A. A., and Jacobs, S. L. (1970). Porphyrins, Porphobilinogen, and aminolaevulinic acid in urine. In *Standard Methods of clinical Chemistry*, edited by R. P. MacDonald, Vol. 6, pp. 57-71. Academic Press, New York.
- Grabecki, J., Haduch, T., and Urbanowicz, H. (1967). Die einfachen Bestimmungsmethoden der δ -Aminolävulinsäure im Harn. *Int. Archiv für Gewerbepathologie und Gewerbehygiene*, **23**, 226-240.
- Mauzerall, D., and Granick, S. (1956). The occurrence and determination of δ -aminolevulinic acid and porphobilinogen in urine. *J. biol. Chem.*, **219**, 435-446.

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.

Technical methods

cap (Messrs E.I.L. Chertsey), pH meter, and amplifier, model 7030.

Servoscribe pen recorder.

Technicon AutoAnalyzer, mark II sampler and mark I peristaltic pump.

Heparinized blood collecting tubes and stock standard solutions of ammonia were prepared as described by Fenton and Williams (1968).

Ammonia-free distilled water was prepared by allowing distilled water to percolate through a 6×15 cm column of ion exchange resin Amberlite IR 120 (analytical grade) in the sodium cycle.

Ammonium chloride solution, 0.1 M, is used as the internal filling solution for the ammonia electrode.

Potassium chloride solution, 0.1 M, is best prepared by dilution from a stock 1.0 M potassium chloride solution which has been rendered ammonia free by vigorous boiling after the addition of 1 ml of N sodium hydroxide solution.

Potassium carbonate solution, 0.1 M, is rendered ammonia free by boiling.

All solutions are stored and used at room temperature.

Method

The flow diagram for the automated method is illustrated in figure 1. When required for use, the inlet of the electrode flow cap is connected to the manifold, the sampler change is set for continuous aspiration of the wash solution, and reagents are pumped through the manifold until the chart recorder reaches a stable baseline. A period of approximately 30 minutes is required for the electrode to achieve a steady response. Standard

solutions containing 50, 100, 150, 200, and 250 μg ammonia nitrogen per 100 ml are used for calibration with a sampling rate of 20 per hour, using a 2:1 cam. Up to 10 samples of plasma may be analysed before re-calibration is required.

As the electrode response is proportional to the logarithm of ammonia concentration, a plot of peak height on semilog paper gives a straight line plot for the standard values.

When work has been completed, the manifold is disconnected from the electrode and the flow compartment is syringed through with 0.1 M ammonium chloride solution. The electrode is stored by leaving the flow compartment filled with this solution.

Results

The validity of the electrode method was tested by comparison with the ion-exchange method of

Fig 2 Results of plasma ammonia analysis by the electrode compared with an ion exchange method.

Fenton and Williams (1968). Eighteen samples of fresh plasma obtained from normal individuals and from patients with chronic liver disease were analysed in duplicate by both methods. The results are shown in figure 2. The correlation coefficient was found to be 0.979 (P is less than 0.001) and the regression line, y , equal to $0.939x + 5.205$.

Recovery experiments were carried out on a pooled sample of plasma. The ammonia content of the plasma pool was reduced to a low initial level by passage through a column of cation exchange

Fig 1 Flow diagram for automated ammonia analysis

resin in the sodium cycle. Triplicate experiments were carried out with the addition of concentrations of ammonia equivalent to between 25 and 300 μg of ammonia nitrogen per 100 ml. Recoveries were found in all cases to vary between 100 and 102%.

The total carryover for this system was determined by analysing alternating groups of three plasma samples according to the scheme suggested by Broughton, Buttolph, Gowenlock, Neill, and Skentelbery (1969). Carryover was found to be negligible and the K factor was 0.009.

Comment

The estimation of plasma ammonia has long been considered a difficult analysis, the volume of literature on the subject bearing witness to the truth of this statement (Lorentz and Ossenberg, 1967). Most of the methods currently available give reliable results only when used regularly in the laboratory, and for this reason, since the estimation is of clinical value only at relatively infrequent intervals, it is omitted from the repertoire of most hospital laboratories.

Only two difficulties have been encountered in the routine use of the electrode over six months. Although the electrode is moderately sensitive to changes in temperature, it has been found to function without drift provided that both the reagents and the equipment are allowed to equilibrate to ambient temperature. Major changes in the osmolarity of the flow stream have been found to cause instability

of the electrode through fluctuations in the concentration of the ammonium chloride film behind the membrane. However, if 0.1 Molar solutions are used both for the wash fluid between specimens and the diluent, this problem is overcome.

The electrode would thus seem to show advantages in both simplicity and reliability, which render it preferable to the methods at present in use for the estimation of plasma ammonia, either on an occasional diagnostic or on a routine research basis.

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References

- Broughton, P. M. G., Buttolph, M.A., Gowenlock, A. H., Neill, D. W., and Skentelbery, R. G. (1969). Recommended scheme for the evaluation of instruments for automatic analysis in the clinical biochemistry laboratory. *J. clin. Path.*, **22**, 278-284.
- Conway, E. J. (1939). *Micro-diffusion Analysis and Volumetric Error* 1st ed. Crosby, Lockwood, London.
- Coleman, R. L. (1972). Practical aspects of automating an ammonia specific electrode. (Letter to the Editor.) *Clin. Chem.*, **18**, 862.
- Fenton, J. C. B., and Williams, A. H. (1968). Improved method for the estimation of plasma ammonia by ion exchange. *J. clin. Path.*, **21**, 14-18.
- Lorentz, K., and Ossenberg, F. W. (1967). Untersuchung zur Isolierung von ammoniak aus dem Blut. *Med. Lab.*, **20**, 77-88.
- Midgley, D., and Torrance, K. (1972). The determination of ammonia in condensed steam and boiler feed with a potentiometric ammonia probe. *Analyst*, **97**, 626-633.