Technical methods

Quantitative estimation of formiminoglutamic acid in urine

J. KOHN *From Queen Mary's Hospital, Roehampton, London*

The need for a technique for quantitative determination of formiminoglutamic acid (FIGLU) in urine became apparent soon after the publication of the paper 'Conventional voltage electrophoresis for formiminoglutamic-acid determination in folic acid deficiency' (Kohn, Mollin, and Rosenbach, 1961). The following method has been used for the past two years in this laboratory under routine conditions and has given satisfactory and reproducible results.

PRINCIPLE

The ninhydrin-staining spots corresponding to the FIGLU position both on the ammoniated and non-ammoniated parts of the strip, prepared as described by Kohn *et al.* (1961), are cut out and dissolved or eluted. The solutions or eluates from the test urine and the standard urine are then compared in a suitable colorimeter using the solutions or eluate from the non-ammoniated spot as the blank. This eliminates any possible interference by urinary amino-acids having an electrophoretic mobility similar to that of FIGLU.

PROCEDURE

The preparation of the patient, collection of the specimen, and electrophoresis of the urine sample are performed as previously reported (Kohn *et al.*, 1961). After the strips have been developed with ninhydrin and dried, the acidic amino-acid spots corresponding to the FIGLU position, on both the ammoniated and the non-ammoniated part of the strips, are cut out. The spots from the non-ammoniated part serve as a blank. This applies to test urines as well as to the standard urine. The cut-out spots are crushed into small fragments, placed in small labelled glass tubes, and 1 ml. of a solvent is added to each tube. The simplest and most convenient consists of 9 vol. chloroform and 1 vol. ethanol (Kohn, 1957a and b). The tubes are stoppered and shaken for about 15 minutes (the shaking is not essential but it accelerates the solution) until the cellulose acetate is completely dissolved. The resultant coloured solutions obtained are transferred to 1 ml. cuvettes and the optical densities measured at 525 m μ using the solutions from the corresponding non-ammoniated spots as a blank.

Alternatively, the cut-out spots can be fragmented and placed in tubes to which 1 ml. of eluting fluid (38 vol. 75% ethanol and 2 vol. 0.1% copper sulphate in H₂O) is added. The tubes are stoppered and vigorously shaken for 15 to 30 minutes and then centrifuged hard. The supernatant eluate is measured as above at 540 m μ .

The calculation is as follows:

$$\frac{(\text{NH}_3 \text{ test urine}) - (\text{test urine blank})}{(\text{NH}_3 \text{ standard}) - (\text{standard blank})} \times \frac{\text{concentration of standard urine}}{\text{concentration of standard}} = \text{FIGLU concentration in test urine}$$

FIGLU¹ for the preparation of marker urine is expensive but only very small quantities are needed and it has only to be obtained once, as each laboratory can thereafter prepare its own standards from positive urine. It has to be borne in mind that the commercially available FIGLU preparation is a barium salt. In order to obtain the figures for free FIGLU a conversion factor of 0.67, i.e., 100 μ g. of barium FIGLU = 67 μ g. of pure FIGLU must be used.

As FIGLU is expensive and rather difficult to obtain an alternative standard substance was sought and glutamic acid was the obvious choice. It has been found that 0.001 M glutamic acid (chromatographic standard: in 1% acetic acid) corresponds approximately to a concentration of 400 μ g./ml. FIGLU (as barium salt) or 270 μ g./ml. of free FIGLU. If glutamic acid is used as the standard for quantitation, any FIGLU-positive urine will serve as a check on the method itself. The following pattern is proposed Fig. (1).

• 0.001 M Glutamic acid	
• Patient's urine	
• Patient's urine	} NH ₃
• Any FIGLU positive urine	

FIG. 1. Pattern of application of samples when glutamic acid is used as a standard marker.

If glutamic acid is used as a standard the calculation is as follows:

$$\frac{(\text{NH}_3 \text{ test urine}) - (\text{test urine blank})}{(\text{Glutamic acid (0.001M) spot})} \times 400 = \text{FIGLU } \mu\text{g./ml.}$$

In order to check the accuracy of the technique a series of increasing concentrations of FIGLU was prepared and these were used as samples for FIGLU determination. The optical densities obtained from the eluates of the

FIG. 2. Increasing concentrations of FIGLU and glutamic acid plotted against optical densities of eluates from FIGLU and glutamic acid spots.

FIGLU spots were plotted against the concentration. The graph obtained (Fig. 2) shows a straight line except for very low concentration near the limit of sensitivity. Glutamic acid dilutions gave a similar curve.

My thanks are due to Mr. P. H. Motivala for his technical assistance.

REFERENCES

- Kohn, J. (1957a). *Biochem J.*, **65**, 9p.
 — (1957b). *Clin. chim. Acta*, **2**, 297.
 —, Mollin, D. J., and Rosenbach, L. M. (1961). *J. clin. Path.*, **14**, 345.

CORRECTIONS

On page 222 of the paper 'Histology of the aortic media in dissecting aneurysm' by Gerald Manley (*J. clin. Path.*, **17**, 220-224) the two parts of Fig. 2 have been interchanged.

Figure 1 on page 244 of the paper by M. Patricia Jevons and M. T. Parker, 'The evolution of new hospital strains of *Staphylococcus aureus*' (*J. clin. Path.*, **17**, 243-250) has been printed upside down.

On page 301 of the paper 'A shortened automated procedure for the determination of alkaline phosphatase' by Joyce L. Bell and M. Collier (*J. clin. Path.*, **17**, 301-303) under Reagents '1-aminoantipyrine' please read 'Dissolve 1 g. 4-aminoantipyrine in 1 l. distilled water'

A new system for rapid haemoglobin estimations and leucocyte counts

R. E. DAVIS and D. J. NICOL *From the Department of Haematology, Royal Perth Hospital, Western Australia*

Blood samples are usually diluted 1 in 200 for the estimation of haemoglobin and 1 in 500 when counting leucocytes in the Coulter counter. These procedures are performed separately and require two tubes and two diluting pipettes. The purpose of this paper is to describe a system which enables the haemoglobin estimation and leucocyte count to be made from the one diluted sample. The system dispenses with the need for pipettes and diluting tubes and enables both examinations to be completed in less than 40 seconds.

PRINCIPLE

To enable both the haemoglobin estimation and leucocyte count to be made from the one dilution it was necessary to increase the dilution for haemoglobinometry to 1 in 500. Compensation for this was made by increasing the light path in the haemoglobinometer from 10 to 25 mm. The sample of blood is first diluted with N/150 ammonia directly into the haemoglobinometer tube using an automatic dispenser. This lyses the red cells and also causes some swelling of the leucocytes. Without delay the sample is further diluted by adding a saline solution of sufficient strength to make the whole mixture isotonic. This saline diluent also contains saponin to ensure adequate stromatolysis. The leucocytes quickly return to their former size and remain stable. The haemoglobin concentration is read in a photoelectric colorimeter as oxyhaemoglobin and the leucocytes are counted in a Coulter automatic cell counter.

APPARATUS

A standard type of rotary specimen mixer is followed by a Trimatic¹ dispenser fitted with pumps suitable for removing a 40 c.mm. sample of blood and delivering 10 ml. of diluent. Next to the dispenser is a 10 ml. semi-automatic pipette² fitted with a small plastic tube in place of the needle thus reducing frothing. It is set to deliver 10 ml. A Spectronic 20 spectrophotometer, fitted with an adaptor to give a 25 mm. light path, is used to measure haemoglobin. This adaptor is a standard accessory. Leucocytes are counted in a Coulter cell counter model A.

REAGENTS

The diluent used in the Trimatic dispenser is N/150 ammonia and in the semi-automatic pipette is 1.7%

¹Manufactured by Research Specialities Corp. U.S.A. Model No. 2642.

²Manufactured by Becton, Dickinson & Co., Cat. No. 1271.

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practical help to pathologists called upon to unravel the intricacies of these often puzzling anomalies.

T. CRAWFORD

OCCLUSION OF THE SUPERIOR MESENTERIC ARTERY By Benjamin B. Jackson. (Pp. xiv + 141; 41 figures. \$7.50.) Springfield, Illinois: Charles C. Thomas. 1963.

The author, a surgeon, has based this monograph on his own clinical and experimental work and on some 1,500 cases of acute and chronic mesenteric artery insufficiency which have, so far, been reported in the literature. The various aspects of mesenteric artery occlusion are considered and the resulting account is well balanced. While the book will probably be of most interest to surgeons, pathologists and others will find much of value in it.

P. D. STEWART

THE PATHOGENESIS OF LEPROSY Ciba Foundation Study Group No. 15. Edited by G. E. W. Wolstenholme and Maevae O'Connor. (Pp. ix + 101; 19 figures. 15s.) London: J. & A. Churchill Ltd. 1963.

This is a record of papers read at a special study group in 1963 and of the discussions which followed. Anyone interested in leprosy or Mycobacteria generally will find, in this small volume, much valuable information concerning recent experimental and research work into the pathogenesis of leprosy.

P. D. STEWART

PROGRESS IN MEDICAL LABORATORY TECHNIQUE 2 Edited by F. J. Baker. (Pp. vii + 191; illustrated. 35s.) London: Butterworth & Co. Ltd. 1963.

This is the second in a series of articles on new laboratory techniques written by those well versed in them. In comparison with the first it is disappointing. The methods are well described but highly specialized and on the whole are suitable for those working in reference or research laboratories. Those finding this book useful in one subject will have little practical use for the methods described in the others. There are 10 articles all by experienced technicians: two are histological, one chemical, two about cardiovascular bypass, three on haematology, one on bacteriology, and one on virology.

Some of the articles need more drastic editing. For example, advice on Mantoux tests and chest radiographs for laboratory staff is out of place as it is not laboratory technique and has been standard practice for many years. In the same chapter hypochlorite is recommended for pipette disinfection but no strength is given. A method for pyrazinamide sensitivity would have been valuable and also advice on weighing tubercle bacilli safely. Both are mentioned but no details are given.

The book is well set out, clearly printed, and for the most part easy to read. The price is reasonable.

JOAN STOKES

AUTOMATION IN CLINICAL CHEMISTRY By Walton W. Marsh. (Pp. xiv + 132; 38 figs. \$6.00.) Springfield, Illinois: Charles C. Thomas. 1963.

This little book is a valuable quick reference to automatic techniques which can be applied in clinical chemical laboratories. Its greatest value will be for those who are contemplating the introduction of machines such as the Technicon AutoAnalyzer into their departments. The early chapters on the principles of the various types of apparatus are the most helpful and the author discusses many practical points not mentioned elsewhere. This aspect could well be expanded in a later edition. The description of individual methods inevitably leads to a discussion of dated techniques though there is much of interest in the less familiar methods using such apparatus as the Benchtop Robot Biochemist.

J. RIDEOUT and M. G. RINSLER

CORRECTION

The author of 'Quantitative estimation of formimino-glutamic acid in urine' (*J. clin. Path.*, 17, 466) wishes to make the following amendments. The eighth sentence of the Procedure should read 'The resultant coloured solutions are transferred to small cuvettes of 1 cm. light-path and the optical densities measured, without delay, at 540 m μ using the solutions from the corresponding non-ammoniated spots as a blank'. The eleventh sentence should read 'The tubes are stoppered, the stained spots eluted for about 5-10 minutes with occasional shaking and centrifuged if the eluate shows any turbidity. The eluate is measured at 525 m μ '.

CLINICAL CHEMICAL PATHOLOGY, 3rd ed. By C. H. Gray. (Pp. viii + 196; 32 figures. 16s.) London: Edward Arnold. 1963.

This is the third edition of a valuable little book. It contains the chemical pathology that an undergraduate medical student must learn set out in very readable and comprehensible form. The whole book can be read in an evening: it should be in the possession of every medical student.

ARTHUR JORDAN

BOOK RECEIVED

(Review in a later issue is not precluded by notice here of books recently received.)

ENDOCRINOLOGY By A. Stuart Mason. (Pp. 136. 36s.) London: Staples Press. 1963.