Journal of Medical Genetics (1972). **9**, 57.

The Use of the AutoAnalyzer to Determine the Acetylator Phenotype

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It has been pointed out (Rao *et al*, 1970) that with the advent of intermittent treatment regimens the determination of the acetylator phenotype has become of practical importance. It has recently been shown that rapid acetylators have an inferior response to slow acetylators when their tuberculosis is treated with once weekly treatment regimens (Menon, 1968; Tuberculosis Chemotherapy Centre, Madras, 1970).

Sulfamethazine is polymorphically acetylated in man by the same N-acetyl transferase as isoniazid (Evans and White, 1964).

The stable storage properties, easy analysis, and speedy availability of results have led to sulfamethazine acetylation being developed as a phenotyping test in preference to isoniazid (Evans and White, 1964; Evans, 1969; Rao *et al*, 1970).

It has been shown that a single small dose of 40 mg sulfamethazine per kg body weight is sufficient (Evans, 1969); and either a single urine specimen (Rao *et al*, 1970), or alternatively one urine and one serum (or plasma) specimen (Evans, 1969) are required for analysis.

The purpose of the present communication is to show that a Technicon AutoAnalyzer can be used to perform the analyses required in the acetylator phenotyping procedure.

Materials

Sodium nitrite (British Drug Houses 'Analar' analytical reagent); ammonium sulphamate and N-1-naphthylethylene-diamine dihydrochloride (British Drug Houses Laboratory reagents).

The Technicon AutoAnalyzer flow diagram is shown in Fig. 1 and the details of the modules and the solutions are given in the caption.

Method

Estimation of sulfamethazine concentration in urine and serum samples was performed by a modification of the method of Falk and Kelly (1965), an automated adaptation of the Bratton-Marshall procedure for sulphonamides.

While serum samples generally require no dilution prior to analysis, some urine samples require dilution 1 in 10 with water, while others require no dilution.

The sampling module of the system was set to operate at a rate of 40 samples per hour, with a distilled water-wash between samples.

Free sulfamethazine was determined by first diluting the sample with O·4N hydrochloric acid. The acidification step before dialysis was incorporated in order to bring about the release of protein-bound sulfamethazine in serum or protein-containing urine samples (Falk and Kelly, 1965). The acidified sample was then dialysed into 0·3N hydrochloric acid and the dialysate properly mixed by passing the sample line through a double-length mixing coil before diazotization. Excess nitrite after diazotization was removed by reacting with ammonium sulphamate before coupling with naphthylethylene-diamine dihydrochloride.

The sample stream was then passed through a single mixing coil immersed in water at 37° C, mounted beneath the membrane dialysis module, and fed into a 15-minute delay coil to allow full colour development. The stream was finally read at 540 mμ and the absorbances recorded on a strip chart recorder.

For total sulfamethazine concentration, the mixed dialysate was passed into an 80 ft long coil in a heating bath at 95° C for the hydrolysis of acetyl-sulfamethazine. (The changes of circuit are effected very easily by slipping the plastic transmission tubing off and on to plastic nipples.) The stream was then passed through a double-length mixing coil immersed in water at 20°C to reduce the temperature of the sample stream before diazotization. Thereafter, the stream was treated as for free sulfamethazine estimation.

An array of standard aqueous solutions of sulfamethazine at 30, 60, 90, 120, 150, and 180 μg/ml concentration was run before and after each batch of unknowns.

Results

The results of phenotyping healthy students, doctors, nurses and laboratory staff with procedure II of Evans (1969) and performing the analyses on

Received 19 May 1971.

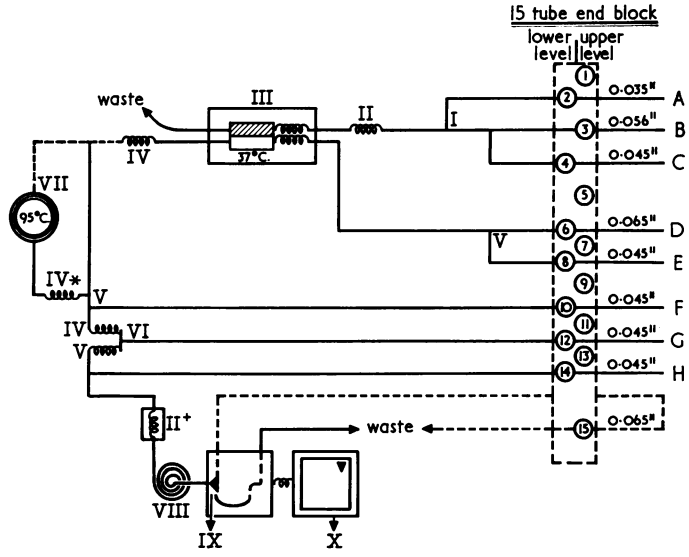

FIG. 1. Flow diagram for automated determination of free and total sulfamethazine in urine and serum (or plasma) using the Technichon AutoAnalyzer.

Key to components: **I**—Cactus special with 0·023 I.D. plat. (Cat. no. H3). **II**—Mixing coil, standard size (Cat. no. MSC-1). **II⁺**—Mixing coil, standard size (Cat. no. MSC-1) immersed in water with temperature controlled at 37° C (one of the additional coils mounted in the 37° C-bath beneath the membrane dialysis module of III). **III**—Dialyzer, temperature controlled at 37° C (Cat. no. 105—A00–01). **IV**—Mixing coil, double length (Cat. no. MSC-II). **IV***—Mixing coil, double length (Cat. no. MSC-II) immersed in water at 20° C. **V**—h Junction (Cat. no. D1). **VI**—T junction, standard (Cat. no. A0). **VII**—Heating bath (95° C), double coil (1·6 mm I.D. × 80 ft) (Cat. no. 105—A100–01). **VIII**—Time delay coil (see text). **IX**—Flow cell, tubular 15 mm (N. method) (Cat. no. 112—728–15). **X**—Recorder, single pen. (Cat. no. 011—A00–01). **A**—Sample line. **B**—0·4N hydrochloric acid. **C**—Air. **D**—0·3N hydrochloric acid. **E**—Air. **F**—0·1% sodium nitrite. **G**—0·5% ammonium sulphamate. **H**—0·1% N-(1-naphthyl) ethylene diamine dihydrochloride.

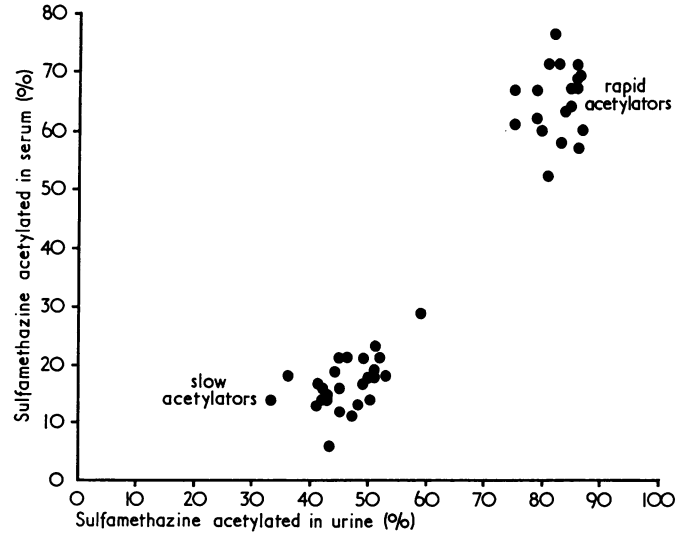

FIG. 2. Results of acetylator phenotype determinations performed using the apparatus shown in Fig. 1.

the AutoAnalyzer, are shown in Fig. 2. The phenotypes are easily identified. When a scattergram was constructed from the results of analysing the same specimens manually, it looked closely similar to Fig. 2. Plasma samples give very similar results to serum samples by this AutoAnalyzer technique.

Conclusion

Technicon AutoAnalyzer apparatus can be used to determine 'free' (ie, unconjugated) and 'total' (ie, free plus acetylated) sulfamethazine concentrations in urine and serum (or plasma). Large numbers of subjects can be accurately acetylator-phenotyped by this technique, which may, therefore, be a contribution to the institution of more rational and successful therapy with 'intermittent' (eg, once weekly) dosage regimens for tuberculosis.

Summary

Technicon AutoAnalyzer modules can be utilized to estimate 'free' and 'total' sulfamethazine concentrations in urine and serum (or plasma). The laboratory procedures required for acetylator phenotyping of populations (for such purposes as monitoring of 'intermittent' tuberculosis chemotherapy or gene frequency determinations) can thus be automated.

The authors would like to thank the Medical Research Committee of United Liverpool Hospitals (Chairman, Professor C. A. Clarke, FRS) and the Nuffield Foundation (via Professor C. A. Clarke, FRS), also Mr Hasse Schröder of Uppsala, Sweden, who aided in obtaining all the serum samples.

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