high temperature during the initial stage of digestion and much lower temperatures in the latter stages. We used control settings of (1) 89 and (2) 37, which gave the following temperatures in the three segments of the digestor: (1) 320°, (2) 150° and (3) 120°.

As we were using much less acid for digestion than is recommended, it was also necessary to reduce the amount of sodium hydroxide added for neutralization. High concentrations of sodium hydroxide may result in loss of ammonia and so we reduced the concentration from 35% (w/v) to 10% (w/v).

Ammonium sulphate was used as a standard. There was a satisfactory correlation between the values obtained by this method and those obtained by the micro-Kjeldahl method.

We are grateful to Professor J. A. F. Rook of the University of Leeds for allowing us to use the Technicon AutoAnalyzer. E. J. H. thanks the University of Bradford for a Research Studentship.

Nitrogen balance by Technicon AutoAnalyzer

By SHEILA C. JACOBS and CHRISTIAN G. THIN, *Metabolic Unit, Western General Hospital, Edinburgh*

By the incorporation of a digester module in the AutoAnalyzer it is possible to automate the estimation of nitrogen, using an adaptation of the classical Kjeldahl technique. The method employed, the flow diagram and an evaluation of the reliability of the method have already been published (Jacobs, 1968). This method has been used in the Metabolic Unit for over 3 years without any new problems arising. All assays incorporated a series of standards using dilutions of nicotinamide containing 250–2000 μg N/ml. Recovery of added known quantities of nicotinamide to urine, food and faeces samples was invariably close to 100%. Reproducibility of the method was determined by comparing the results of duplicate determinations in a series of assays. The estimated standard deviation s (Snedecor, 1952) was 6·5 μg N/ml over a series including urine, food and faeces with a N concentration range of 150–830 μg N/ml. No significant difference has been found between results obtained by the manual Kjeldahl technique and duplicate samples estimated by the AutoAnalyzer (regression coefficient 0·99).

The estimation of N in urine samples was uncomplicated but when the method was applied to the estimation of N in samples of food and faeces difficulties arose. Flakes or particles in these heterogeneous samples persistently blocked the manifold causing breakage at various pressure points (Acidflex–glass joints). All nipples and fine sample tubing were eliminated from the introduction manifold and Acidflex–glass joints protected with a length of sleeving tubing. In order to soften or disintegrate the particles weighed samples of homogenates of food and faeces are suspended

in an alkaline Polycell solution for at least 24 h before analysis. When these steps are taken blockage of the manifold occurs infrequently.

N excretion in man is predominantly urinary; about 10% of the intake is excreted in the faeces. The main source of error in a N balance study is in the estimation of dietary N. Because of the mixed nature of the diet it is difficult to obtain a truly homogeneous sample and to draw from it representative portions. This difficulty can be overcome to a great extent by mincing all meat and vegetables, by using an efficient homogenizer, and sampling by siphon while mixing.

Values for N balance have been obtained for many patients in the Metabolic Unit. Large numbers of samples are required and these must be analysed under comparable conditions. We have found that the Technicon AutoAnalyzer provides an accurate and reproducible alternative to the manual Kjeldahl technique.

REFERENCES

Jacobs, S. C. (1968). *J. clin. Path.* **21**, 218.
Snedecor, G. W. (1952). *Biometrics* **8**, 85.

Problems associated with the automated analysis of mixtures of amino acids

By J. DAVIDSON and W. R. HEPBURN, *Rowett Research Institute, Bucksburn, Aberdeen*

Many nutritional investigations now in progress require a knowledge of amino acid concentrations in the diet and in tissues so that the requirements of the animal under study for each amino acid may be estimated, and adequacy of dicts assessed. Analysis for amino acids was difficult and slow before the advent some two decades ago of methods using ion exchange resins for separating all the amino acids in a mixture by column chromatography. The original method of Moore & Stein (1951) taking about 1 week per analysis was developed (Moore & Stein, 1954*a*,*b*; Moore, Spackman & Stein, 1958) and then automated (Spackman, Stein & Moore, 1958) until one complete analysis could be achieved each day.

More rapid analysis has been possible following (1) the introduction of a spherical form of resin which can be graded accurately for size according to methods such as that of Hamilton (1958) thus allowing more uniform packing of columns and (2) the development of pumps reliable at high pressures (up to 1000 lb/in^2) and low volume deliveries (3–120 ml/h).

At the Rowett Institute the advent of rapid and more precise chromatographic methods gave rise to an increasing demand for amino acid analysis and the single column system purchased from the Technicon Instruments Company, Chertsey, Surrey, allowing one analysis/d was soon unable to satisfy the demand for analyses. Increased output could be achieved either by replicating the whole or part of the system or by decreasing the time of each analysis from 20 h to about 5 or 6 h and arranging to have an automatic procedure for the necessary loading, development and regeneration during periods when the column was unattended