

some gross errors in a simple graph-reading exercise in which the readers were aware that the results were to be analysed for correctness.

On the whole the gain in precision in going from the non-linear, hand-drawn chart technique (where the main error is probably in absolute accuracy) to the semi-log computed standard curve is fairly small, but the importance of precision was shown to us in our routine laboratory serum calcium estimations, in which transcription is carried out by the Wang technique. Comparison of recovery of a quality control serum for runs over a period of 3 months in which transcription was by manually drawn graphs with those from 3 months of the Wang technique showed a halving of the coefficient of variation from about 7% to 3.5%.

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#### REFERENCES

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### **Determination of nitrogen in trichloroacetic acid filtrates of milk using the Technicon AutoAnalyzer**

By E. J. HINDLE and J. V. WHEELOK, *School of Biological Sciences, The University, Bradford, Yorks*

We have successfully adapted an automated procedure for the estimation of nitrogen in trichloroacetic acid filtrates of milk. These milk filtrates contain numerous small peptides, free amino acids, urea, creatinine and, with low concentrations of trichloroacetic acid, some whey proteins. The N content varies from 200 mg/l in a 12% (w/v) trichloroacetic acid filtrate to 1000 mg/l in a 2% (w/v) filtrate. As in the Kjeldahl procedure, the organic N must first be converted into ammonium sulphate. The ammonia released is then estimated colorimetrically. To obtain a satisfactory method for our samples, it was necessary to make a number of modifications in the recommended Technicon procedure for total N.

For digestion, the amount of selenium dioxide-sulphuric acid-perchloric acid mixture and the temperature are critical. Use of two or three acidflex tubes, as recommended, results in a large excess of digestion mixture being introduced so that when the sodium hydroxide is added for neutralization, excess sodium sulphate forms and blocks the tubes. We have found that one Acidflex tube (size 0.081 in) is sufficient. If the temperature of digestion is too high some of the ammonium sulphate is decomposed and also some catalyst precipitates when water is added to dilute the sample at the end of digestion. If the temperature is reduced too much there is again incomplete digestion. These problems can be overcome by using a

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 digester: (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.

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### Nitrogen balance by Technicon AutoAnalyzer

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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  $\mu\text{g N/ml}$ . 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  $\mu\text{g N/ml}$  over a series including urine, food and faeces with a N concentration range of 150–830  $\mu\text{g N/ml}$ . 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