Technical methods

Assessment of automated nitrogen analysis of biological fluids with reference to the Kjeldahl method

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By incorporating a digestor module in the Auto-Analyzer it is possible to automate the estimation of nitrogen, using an adaptation of the classical Kjeldahl technique. To evaluate the reliability of this method the reproducibility and accuracy have been determined and a comparison made with the manual technique for urine, faeces, and food.

METHODS AND MATERIALS

The established Kjeldahl technique for estimating nitrogen (King and Wooton, 1956) was employed using a Markham still and standardized sulphuric acid and sodium hydroxide with phenolpthalein as indicator. Urinary nitrogen was estimated on 1 ml aliquots. For faeces and food 10-20 g aliquots of homogenate previously prepared with a Silverson mixer were weighed and digested on the macro scale. The digests were then diluted to 100 ml and 10 ml aliquots used for distillation.

All estimations were carried out at least in duplicate.

The AutoAnalyzer method used was that of Marten (1964) (flow diagram Fig. 1) with a digestion mixture consisting of 90% sulphuric acid, 2% perchloric acid, 0·5% mercuric sulphate, and 0·02% selenium dioxide. The digestion current was set at 4 amps and 7 amps and the sampling rate 60/hr with a water wash between each sample. These conditions were adhered to for all materials. A suggestion that foodstuffs would require more catalyst and a higher temperature proved invalid; increasing the perchloric acid concentration to 10% did not affect the results and the equipment fused whenever the current was raised to 5 amps and 8 amps.

All assays incorporated two series of standards using dilutions of nicotinamide and of a urine of known nitrogen content, determined earlier by replicate manual Kjeldahl estimations. These standards curves were found to coincide for nitrogen values up to approximately 1,200 μg.N/ml. Assays were performed in duplicate on urine and in quadruplicate on faeces and diets.

Estimations were carried out on pools of urine and of faeces and on samples of patients' daily diets. Faeces and diets were obtained as homogenates. Because a Solid Prep. Sampler was not available sampling of these homogenates and blockage of the manifold proved a persistent problem, so all nipples and fine sampling tubing were eliminated from the introduction manifold
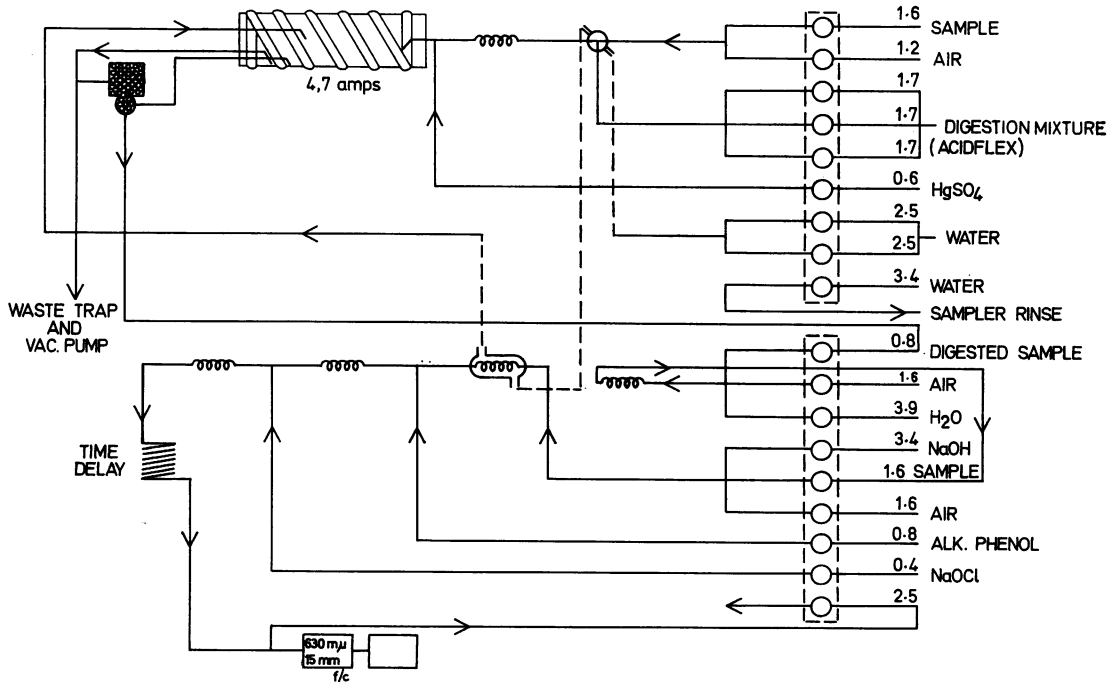

FIG. 1. *Nitrogen flow diagram.*

and weighed aliquots of the semisolid were suspended in alkaline Polycell (10-20 g homogenate diluted to 100 ml with 0·25% Polycell in 1% NaOH). These alkaline mixtures were found to be quite stable; the nitrogen content did not vary after several weeks at room temperature. Aliquots of the mixtures undergoing continuous vibratory stirring were then analysed on the AutoAnalyzer. Dietary homogenates provided most trouble with severe blockage but with greater dilution reproducible results could be obtained. These results agreed well with those obtained by the manual method and also with those calculated from dietary tables, provided all meat was minced before being homogenized.

RESULTS

ACCURACY   To assess the accuracy of the method recovery experiments were carried out adding known quantities of nicotinamide to each sample aliquot (Table I).

TABLE I

RECOVERY OF NICOTINAMIDE NITROGEN FROM URINE, FAECES, AND DIET

Material	No. of Recoveries	Amount Added (μN)	Mean result ($\pm$ S.D.)
Urine	30	250-1500 μg N	100·4 $\pm$ 5·0%
Faeces	30	200- 600 μg N	98·1 $\pm$ 3·2%
Diet	15	100- 600 μg N	100·1 $\pm$ 5·7%

REPRODUCIBILITY   Reproducibility was determined by comparing the results of duplicate determinations in a series of assays. The estimated standard deviation was calculated by the method of Snedecor (1952) from the formula $s = \sqrt{\dfrac{\Sigma d^2}{2N}}$ where d is the difference between duplicates and N the number of duplicate assays in the series (Table II). As the ranges given are rather wide the figures can be considered as an approximate indication of the results which can be obtained.

TABLE II

REPRODUCIBILITY EXPRESSED AS THE ESTIMATES OF S OF RESULTS OF DUPLICATE DETERMINATIONS

Material	No. of Pairs	Range Covered (g/24 hr)	s
Urine	30	4·0-15·0	0·13
Faeces	22	0·6- 6·0	0·06
Diet	15	6·0-17·0	0·21

COMPARISON WITH KJELDAHL METHOD   The nitrogen content of two series of samples, 59 urines and 25 faeces and diets, was estimated by both methods and scatter graphs plotted of the results (Figs. 2 and 3). Statistical analysis showed the regression coefficients, r, to be respectively 0·981 and 0·998. Calculation of both F and t tests proved there was no significant difference from 1 in either case.

CONCLUSION

From the foregoing it appears that the AutoAnalyzer provides an accurate and reproducible alternative to the
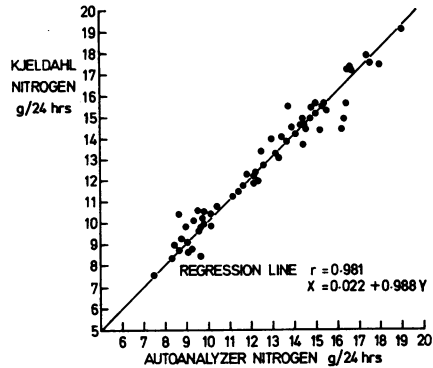

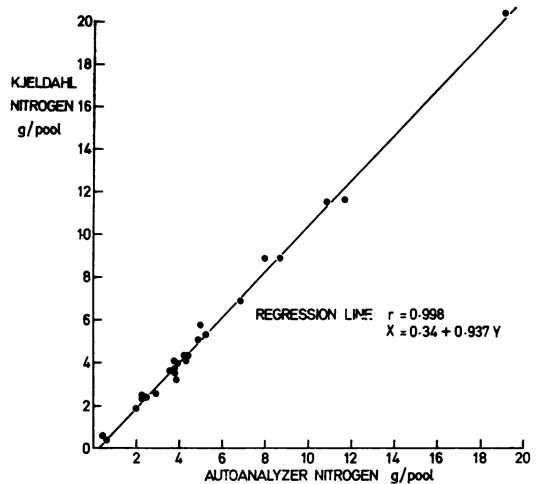FIG. 2. *Comparison of micro Kjeldahl and AutoAnalyzer urine nitrogen.*

FIG. 3. *Comparison of macro Kjeldahl and AutoAnalyzer faecal and dietary nitrogen.*

manual Kjeldahl technique for determining nitrogen in urine and in faecal and dietary homogenates. The Auto-Analyzer can supply results for nitrogen at some seven times the manual rate. Any one result can be obtained within 20 minutes instead of several hours. The Auto-Analyzer involves very much greater ease of manipulation with correspondingly less chance of error than does the manual method. For all these reasons, therefore, this is obviously the method of choice where large numbers of samples must be analysed under comparable conditions. We have found this to be of particular advantage in metabolic balance studies.

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