Emission spectrometric analysis of ^{15}N

By D. N. SALTER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

For many years the only feasible method of ^{15}N analysis was by means of the mass spectrometer, and for the highest precision this remains the method of choice. During the past 20 years improvements in the technique of emission spectroscopy have made this a useful alternative for the measurement of ^{15}N enrichments when high precision is less important. The earliest reports of the application of emission spectroscopy to ^{15}N analysis appeared in the German literature (Clusius & Becker, 1947; Hoch & Weisser, 1950) when a photographic method was used to measure the spectrum of enriched N. It was not until Broida & Chapman (1958) used a photoelectric method to measure ^{15}N abundance to a precision of a few percent that real progress was made and it became evident that emission spectroscopy could be useful for the isotopic analysis of micro samples of N. The prototype of the first commercial ^{15}N-analyser was developed by Meier & Müller (1965), whilst at the same time Faust (1965) and Münsche (1965) developed micro-methods for the preparation of emission tubes and analysis of 10 μg N based on the existing Rittenberg and Dumas methods. The use of rare gases to sustain the discharge facilitated the analysis of ^{15}N in as little as 0·5 μg N (Cook *et al.* 1967). Subsequently, details of an automatic ^{15}N-analyser were published in East Germany by Gerstenberger *et al.* (1974). This instrument was capable of carrying out eighteen ^{15}N analyses/h using the Rittenberg hypobromite method of preparing N, but required 120 μg N for each analysis. Soon after this Goulden & Salter (1975) described an automatic ^{15}N emission spectrometer with N preparation by the Dumas copper oxide method which required 10 μg N for each measurement. This was followed by the improved NIRD automatic ^{15}N analyser (Goulden & Salter, 1977) employing a catalytic method of N generation with platinum and rhodium which required only 1–10 μg N and was capable of measuring up to 60 samples/h.

The analysis of ^{15}N by the emission spectrometric technique depends on the property of N at relatively low pressure to emit light of characteristic wavelengths when energized by radio- or microwaves. Fig. 1 shows the emission spectrum of N which is composed of a succession of bands each of which corresponds to a transition between a particular pair of electronic states from a higher to a lower level in the diatomic molecule. The presence of an extra neutron in the ^{15}N nucleus causes shifts in the energy transitions of an ^{15}N-containing molecule which result in changes in the wavelengths of each of the bandheads. The 2–0 transition shows a pronounced shift resulting in a readily measurable displacement of the bandhead which normally appears in the ultraviolet region of 297·7 nm for the $^{14}N^{14}N$ molecule, to 298·3 nm for $^{14}N^{15}N$ and 298·8 nm for $^{15}N^{15}N$. The intensities of the

0029–6651/81/4033–5703 $01.00

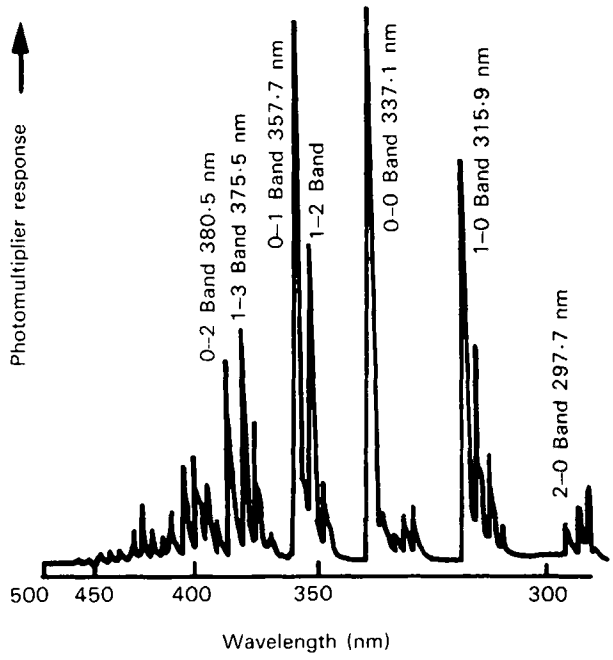

Fig. 1. Emission spectrum of natural nitrogen (0·37 atoms % ^{15}N).

bands are proportional to their nitrogen content (Fig. 2) so that in an equilibrium mixture of the three molecular forms of N, the proportion of ^{15}N can be calculated from the ratio of the intensities of the bands of mass 28 and 29 in a manner analogous to that used in mass spectrometry.

Various methods are available for preparing N for analysis in separate emission tubes by the manually operated emission spectrometer (Fig. 3). Biological samples are usually converted to ammonium chloride by a Kjeldahl digestion followed by steam distillation of ammonia from the alkali-treated digest into dilute hydrochloric acid. The ammonium chloride formed is converted under vacuum to N gas either with alkaline hypobromite (Rittenberg method) or by heating with copper oxide (Dumas method). Calcium oxide is included to absorb water which interferes with the emission spectrum. On average an operator can process and measure some seventy samples/week. The process is tedious since reagents and emission tubes require repeated degassing and the advantages of an automatic method are obvious.

The main features of the NIRD automatic ^{15}N-analyser (Goulden & Salter, 1979) are illustrated in Fig. 4. Samples of ammonium chloride containing ^{15}N prepared from biological materials are injected into a stream of purified helium, converted by reaction with heated soda lime to NH_3 which is decomposed to N and hydrogen gases over a platinum–rhodium catalyst. N is separated from H and

https://doi.org/10.1079/PNS19810049 Published online by Cambridge University Press

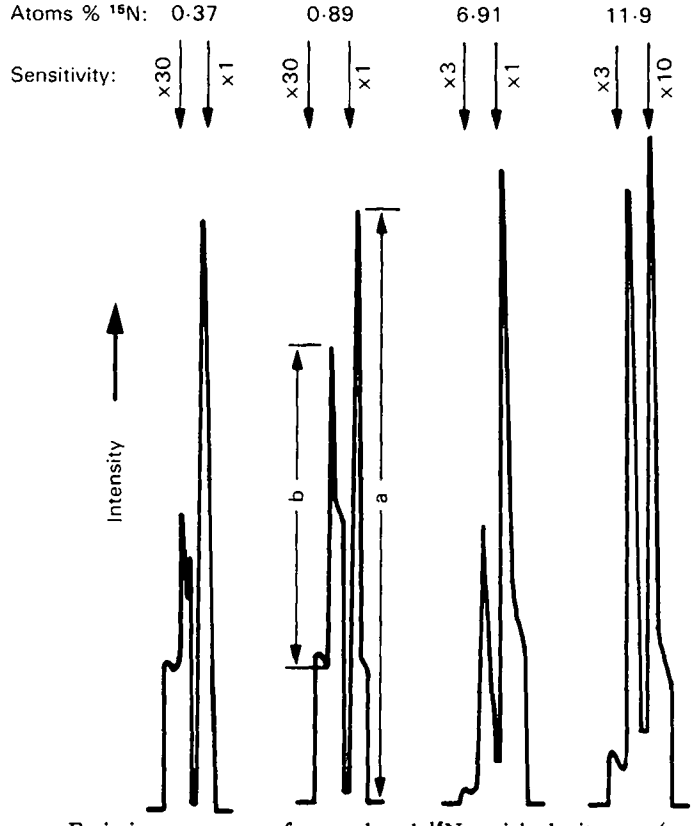

Fig. 2. Emission spectrum of natural and ^{15}N-enriched nitrogen (2–0 transition).

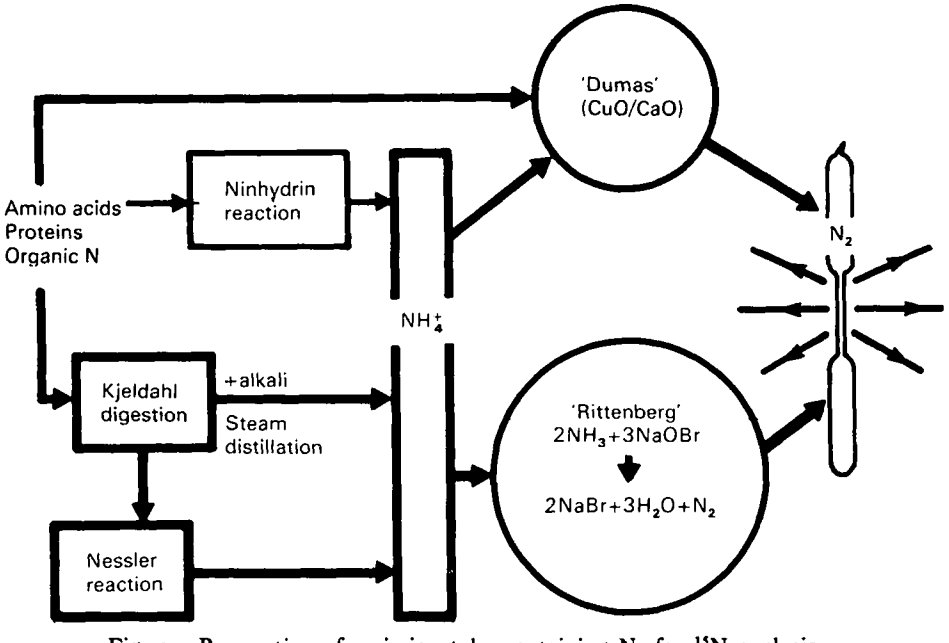

Fig. 3. Preparation of emission tube containing N_2 for ^{15}N analysis.

https://doi.org/10.1079/PNS19810049 Published online by Cambridge University Press

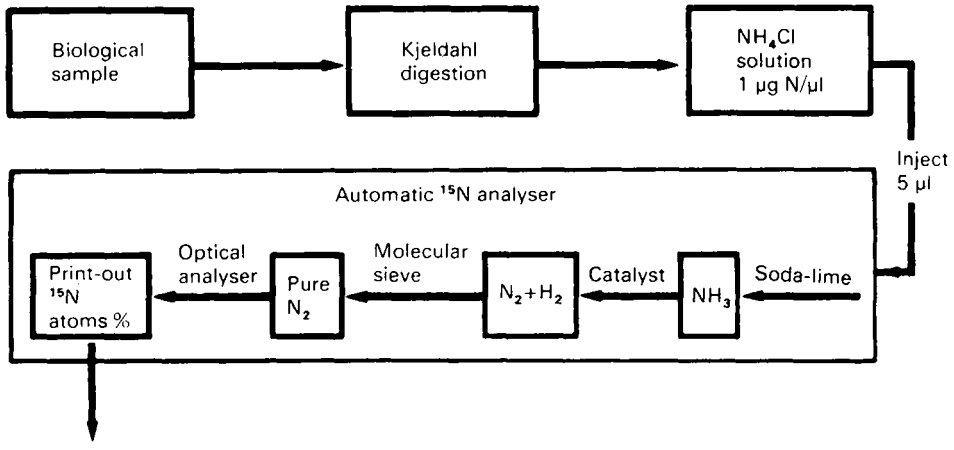

Fig. 4. Method of processing samples and measurement of ^{15}N in the NIRD automatic ^{15}N analyser.

water on a molecular sieve column and passes through a quartz tube held in a microwave cavity where a continuous He emission is sustained. Light emitted from the N gas as it passes through the discharge tube is analysed in a dual wavelength monochromator in which the $^{14}N^{14}N$ and $^{15}N^{15}N$ hands are monitored simultaneously by a pair of photomultipliers. The signals from the photomultiplier tubes are converted electronically to atoms % ^{15}N and printed out.

Since the apparatus is comparatively inexpensive, the optical method has been used for a wide range of applications in agriculture, animal science and medicine, but its main advantage which has not been fully exploited is in measuring ^{15}N in samples containing very limited amounts of N. A comparison of the performance of the average mass spectrometer with various kinds of emission spectrometer is made in Table 1. The modern automatic mass spectrometer, with a precision in measuring ^{15}N to $\pm 0{\cdot}00002$ atoms %, is clearly capable of much greater accuracy than the emission spectrometer, measuring to $\pm 0{\cdot}01$ atoms % for samples in the range from natural abundance to $3{\cdot}0$ atoms % ^{15}N. However it is doubtful whether this degree of accuracy in the mass spectrometer can be achieved with samples of less than 1 mg total N. For emission spectrometry much smaller amounts of N are required (1–10 μg) for optimum working and the rate of analysis with the automated analyser is much more rapid. The high sensitivity of the emission spectrometer is particularly important in relation to the amounts of nitrogen available for ^{15}N measurement in blood or tissue free amino acids.

The concentrations of amino acid N in the blood plasma of man, steer and rat for five of the amino acids most commonly used as tracers in studies of protein turnover are shown in Table 2. Sufficient blood to measure the ^{15}N enrichments of these amino acids by the optical method can be relatively easily obtained and repeated sampling is possible. Direct measurement by mass spectrometry is less practical, on account of the excessive volumes of blood required and the difficulties

https://doi.org/10.1079/PNS19810049 Published online by Cambridge University Press

Table 1. *Performance of emission spectrometer compared with mass spectrometer*

(Samples presented as NH_4Cl)

	μg N/single determination		Rate of analysis†		Claimed accuracy
	Optimum	Minimum	Samples/h	Samples/wk	(atoms % ^{15}N)
Mass spectrometer:					
Manual	5,000	200 (?)	6	120	±0·0001
Automatic	5,000	200 (?)	~12	250	±0·00002
Emission spectrometer:					
Manual, standard tube	10	5	*	80	±0·01
Manual, micro-tube	1–2	0·1	*	60	±0·02
Automatic, commercial	40	—	18	500	±0·01
Automatic, NIRD	5	1	60	1500	±0·01

*2–3 d for a batch of 40 tubes.
†Average, single operator, 40 h week.

Table 2. *Amino acid-N concentrations in plasma* (μg *N/ml*)

	Man*	Steer†	Rat*
Glycine	2·5	5·3	2·2
Leucine	1·7	1·7	2·8
Lysine	4·8	4·6	18·4
Phenylalanine	0·7	0·9	1·0
Tyrosine	0·8	0·8	1·8

*Calculated from data of Waterlow *et al.* (1978).
†Calculated from data of Williams *et al.* (1980).

of isolating amino acids from them. Measurement by addition of carrier unlabelled N can introduce uncertainties, including the problem of measuring the ratio of carrier to isolated amino acid N. For the optical method there should be little difficulty in achieving the relatively high ^{15}N excess of twice natural abundance that is necessary to measure enrichment to a precision of ±2%, because of the small size of the amino acid pool.

Some modification to the method of preparing N for ^{15}N analysis is necessary when dealing with the small quantities of amino acids isolated by ion exchange chromatography from blood samples. The Nessler method of Lloyd-Jones *et al.* (1977) has been adapted for use with a Conway microdiffusion technique (J. S. Blake, unpublished results, Fig. 5) to prepare ammonium chloride from plasma amino acid samples containing as little as 2 μg N and found to give satisfactory results. Working with such small quantities of total N necessarily imposes stringent precautions to avoid the introduction of unlabelled N as a contaminant in reagents or apparatus.

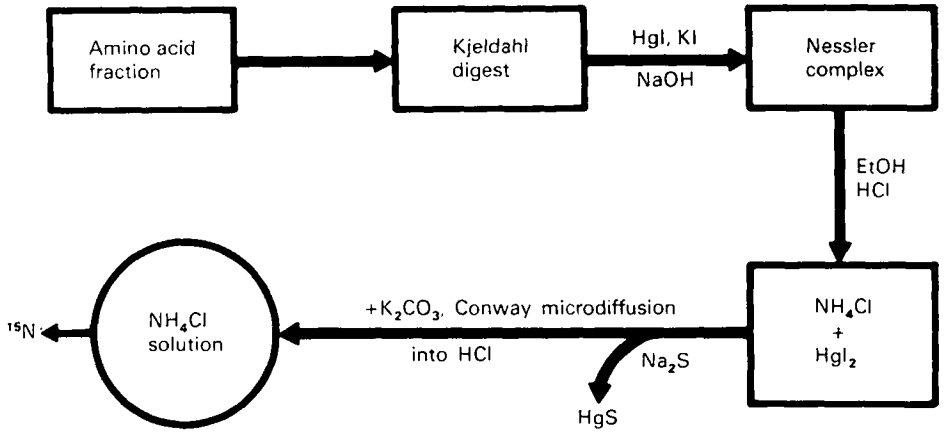

Fig. 5. Method of preparing small quantities of N_2 from amino acid fractions for optical ^{15}N analysis (adapted from Lloyd-Jones *et al.* 1977).

Flux values for some of the amino acids that have been used in studies of protein turnover by end-product or plasma analysis are given in Table 3. The magnitude of the flux determines the rate at which an ^{15}N-labelled amino acid must be infused to achieve an accurately measurable ^{15}N excess since for a steady state infusion ^{15}N atoms % excess $= \frac{i}{Q}$, where i is the infusion rate and Q is the flux (Golden & Waterlow, 1977). For example, to attain 1 atom % excess in plasma lysine would require a constant infusion of 1·67 mg ^{15}N/h, assuming a lysine flux of about 4 g N/d for a 70 kg man. This represents only 1% of the lysine flux and is therefore not an excessive load.

Few studies of N metabolism in man using optical emission to measure ^{15}N have yet been reported, although it has been extensively used in animal studies. Stein *et al.* (1975; 1976) have obtained satisfactory results by the optical method in human studies. When [^{15}N]glycine was infused into a 70 kg man at a rate of 35 mg ^{15}N/h for 8 h, enrichments in urinary amino acids reached 0·6 atoms % excess. Similarly when [^{15}N]glycine was administered orally at a rate of 19·8 mg ^{15}N/h, plateau ^{15}N values for total plasma amino N reached 0·5 atoms % excess. This rate of infusion represented about 3% of the glycine flux, but the enrichment of ^{15}N in the purified amino acids would be considerably higher and could be achieved with much lower infusion rates. It has also been found (Salter and Smith, unpublished results) that

Table 3. *Amino acid flux values (man)*

	Amino acid-N	
	mg/kg per d	g/70 kg per d
Glycine	110–190	7·7–13·3
Leucine	34–46	2·4– 3·2
Alanine	124	8·7
Lysine	38–72	2·7– 5·0
Tyrosine	13	0·9

https://doi.org/10.1079/PNS19810049 Published online by Cambridge University Press

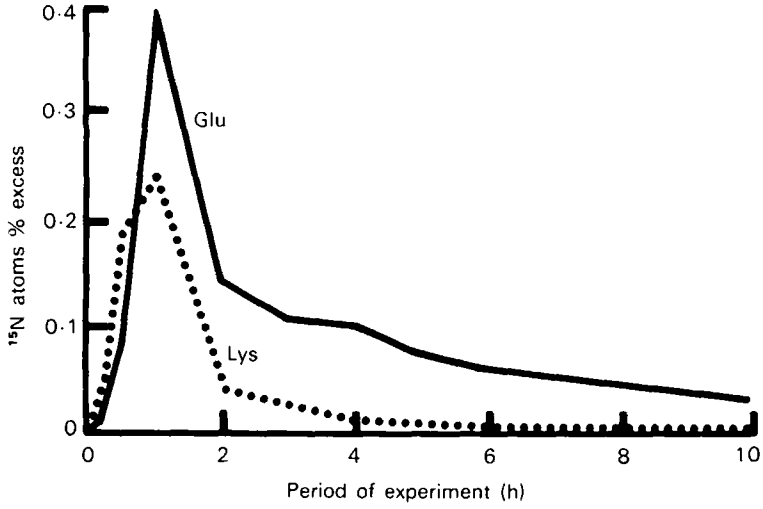

Fig. 6. ^{15}N concentration in non-protein fraction of portal blood plasma during absorption of ^{15}N-labelled amino acids from the small intestine of the steer; 1 g of each amino acid (95 atoms % ^{15}N) was infused into the duodenum.

the enrichment of ^{15}N in portal blood amino acids in the steer may be readily measured during absorption of ^{15}N labelled amino acids. Fig. 6 shows the course of absorption of 1 g each of [^{15}N]lysine or [^{15}N]glutamine (95 atoms %) infused into the duodenum of a 100 kg steer during the digestion of a meal. Despite a portal blood flow rate of 3·5 l/min the enrichment reached a maximum of 0·28 and 0·4 atoms % excess in total non protein N for lysine and glutamine respectively. Since lysine and glutamine each comprise only about 1% of total non protein N, the enrichment of ^{15}N in the separated amino acids would be correspondingly greater and enrichments accurately measurable by emission spectroscopy could be achieved with much smaller inputs of amino acid ^{15}N. Results of this type are an indication of the potential of emission spectrometry in protein turnover studied by blood ^{15}N amino acid analysis.

REFERENCES

Broida, H. P. & Chapman, M. W. (1958). *Analyt. Chem.* **30**, 2049.
Clusius, K. & Becker, E. W. (1947). *Z. Naturforsch.* **2a**, 154.
Cook, G. B., Goleb, J. A. & Middelboe, V. (1967). *Nature, Lond.* **216**, 475.
Gerstenberger, H., Meier, G., Mauersberger, K., Müller, G., Pabst, D., Riedel, S., Rose, W. & Schmidt, G. (1974). *Glas. Instrument Technik*, pp. 452, 547, 612.
Golden, M. H. N. & Waterlow, J. C. (1977). *Clin. Sci. Mol. Med.* **53**, 277.
Goulden, J. D. S. & Salter, D. N. (1975). *U.V. Spectrometry Group Bulletin*, **3**, 74.
Goulden, J. D. S. & Salter, D. N. (1977). *Proc. Nutr. Soc.* **36**, 132A.
Goulden, J. D. S. & Salter, D. N. (1979). *Analyst, Lond.* **104**, 756.
Hoch, M. & Weisser, H. R. (1950). *Helv. Chem. Acta* **33**, 2128.
Lloyd-Jones, C. P., Adam, J. S., Hudd, G. A. & Hill-Cottingham, D. G. (1977). *Analyst, Lond.* **102**, 473.
Meier, G. & Müller, G. (1965). *Isotopenpraxis* **1**, 53.

Münsche, D. (1965). *Isotopenpraxis* **1**, 32.
Stein, T. P., Leskiw, M. J., Liquori, E. M., Brooks, H. B., Wallace, H. W. & Blakemore, W. S. (1975). *Analyt. Biochem.* **66**, 481.
Stein, T. P., Leskiw, M. J. & Wallace, H. W. (1976). *Am. J. Physiol.* **230**, 1326.
Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978). In *Protein Turnover in Mammalian Tissues and in the Whole Body*, p. 119. Oxford: North Holland Publishing Co.
Williams, A. P., Hewitt, D., Cockburn, J. E., Harris, D. A., Moore, R. A. & Davies, M. G. (1980). *J. Sci. Fd Agric.* **31**, 474.

Printed in Great Britain