

LETTERS TO THE EDITORS

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Assay of Isotopic Nitrogen by Mass-Spectrometer

In a recent communication, Capindale and Tomlin¹ have pointed out some of the difficulties in measuring accurately the abundance of isotopic nitrogen (nitrogen-15), a measurement which is generally required when this isotope is used as a tracer in biological experiments.

One of the chief difficulties in estimating the abundance of isotopic nitrogen in small samples (20–300 $\mu\text{gm.}$) is the correction for contamination by air. Two methods are commonly used, each of them suffering from some drawbacks. One is based upon a measurement of the oxygen peak at mass number 32 and, by means of a predetermined nitrogen/oxygen ratio (28/32) for air (≈ 5.5), correcting for contamination by atmospheric nitrogen. This method is accurate provided that all the oxygen in the sample comes from air, but is invalidated if any oxygen is formed by decomposition of hypobromite, when this is used for oxidizing the ammonia to gaseous nitrogen. The other method of correction is more specific in that it depends on measurement of the nitrogen/argon ratio (28/40); but it has the disadvantage that argon is present only in traces and experimental errors are magnified by the high nitrogen/argon ratio (≈ 73) in air.

Both methods have been tested in these laboratories during the course of work carried out by Yemm and Willis² and by Cocking³. More recently, it has been possible to apply corrections, based on measurements of the oxygen peak, with greater confidence by virtually eliminating the release of oxygen from hypobromite. As shown by Fleury⁴, the decomposition, $2\text{NaBrO} \rightarrow 2\text{NaBr} + \text{O}_2$, is catalysed by traces of copper (Cu^{++}), but the decomposition is prevented by adding potassium iodide, which presumably forms a complex with the catalyst. Many tests have shown that no detectable release of oxygen occurs when 0.1 per cent potassium iodide is added to the hypobromite reagent. Moreover, by immersing the reaction vessel in liquid air after completion of the oxidation, it is possible to freeze out other by-products, such as nitrous oxide and carbon dioxide, which may interfere with measurements of nitrogen at mass numbers 28, 29, 30.

For routine analyses Rittenberg tubes⁵, which can be fitted directly to the gas inlet system of a mass spectrometer, are convenient. By evacuating the tubes in two stages, first to about 10^{-2} mm. mercury by a mechanical pump (warming the solution to dispel dissolved gases) and then to about 5×10^{-3} mm. mercury by a backed mercury diffusion pump, nearly all the air can be removed prior to oxidation of ammonia by hypobromite. After the oxidation, the Rittenberg tube is attached to the mass spectrometer and immersed in liquid air to freeze out impurities. The gas is then expanded into a highly evacuated vessel of suitable volume (usually 50 ml.) for measurement of the peaks at mass numbers 28, 29, 30 and 32. In this way it is possible to obtain consistent measurements in samples containing as little as 30 $\mu\text{gm.}$ nitrogen with very small or negligible corrections for

Table 1. NITROGEN-15 ABUNDANCE IN AMINO-ACIDS

Amino-acids	Nitrogen ($\mu\text{gm.}$)	Relative peak heights				Isotope abundance (atom per cent nitrog n-15)	
		Mass 28	Mass 29	Mass 30	Mass 32	28 : 29	28 : 29 : 30
Aspartic acid	92	280	28.25	0.700	0.600	4.86	4.85
Threonine	49	100	17.75	0.825	0.215	8.24	8.25
Serine	29	150	19.0	0.630	0.125	5.98	6.00
Glutamine	91	347.5	66.6	3.26	0.340	8.76	8.78
Glycine	30	189	23.6	0.775	0.125	5.93	5.96

contamination by air. Some typical examples of the estimates of nitrogen-15 abundance in amino-acids, separated chromatographically during the course of tracer experiments on yeast, are given in Table 1.

In samples of gas containing about 5 atom per cent nitrogen-15 or above, it is possible to calculate the abundance either from the ratio of mass numbers 28 : 29 or from the measurements of the three peaks 28, 29, 30 (Rittenberg⁶). Close agreement between the two estimates is obtained, thus indicating the validity of the small correction for contamination by air, based on the measurement of the peak for oxygen at mass 32.

With the precautions outlined above, measurements have been made over a period of several months on replicate samples containing 30 $\mu\text{gm.}$ nitrogen of a standard solution of an enriched ammonium salt; fifteen measurements had a mean of 31.67 atom per cent nitrogen-15 with a standard deviation of ± 0.17 .

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¹ Capindale, J. B., and Tomlin, D. H., *Nature*, **180**, 701 (1957).

² Yemm, E. W., and Willis, A. J., *New Phytol.*, **55**, 229 (1956).

³ Cocking, E. C., Ph.D. thesis, University of Bristol (1956).

⁴ Fleury, P., *C.R. Acad. Sci., Paris*, **171**, 957 (1920).

⁵ Sprinson, D. B., and Rittenberg, D., *J. Biol. Chem.*, **180**, 707 (1949).

⁶ Rittenberg, D., "Preparation and Measurement of Isotopic Tracers" (Edwards, Ann Arbor, Michigan, 1946).

Mass-spectrometric Observations of Ions in Flames

As an extension of our studies of ionization in flames, previously based largely on conductivity measurements¹, an apparatus has been constructed for direct mass-spectrometric analysis of ions drawn from a flame. Whereas previously information about the nature and relative amounts of positive ions could only be obtained by inference from the electron concentration, it is now in principle possible to obtain directly the concentration of ions of any given mass number. It is the purpose of this communication to give preliminary qualitative results obtained with this apparatus, which prove to be of considerable interest.

In brief, the flame, burning at atmospheric pressure, is directed against a thin (0.002 in.) window in which