ANALYSIS OF GASES IN BLOOD WITH THE MASS SPECTROMETER. III. A METHOD FOR THE DETERMINATION OF NITROUS OXIDE IN BLOOD *†

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As for the determination of diethyl ether in blood (1), the problem concerning use of the mass spectrometer for analysis for nitrous oxide in blood divides itself into two basic steps: 1. A representative sample of the gas contained in the blood must be obtained. 2. This sample of gas must be analyzed for its nitrous oxide content.

THE QUANTITATIVE DETERMINATION OF NITROUS OXIDE IN A MIXTURE OF GASES

Inspection of the fragmentation pattern represented by the mass spectrum of nitrous oxide shown in figure 1 revealed that mass 30 existed in ample abundance for analysis.¶ Further, inspection of the spectra of other gases likely to be present (water vapor, nitrogen, oxygen and carbon dioxide) (see figure 2 of part II of this study [1]), revealed no significant contribution to mass peak 30 by these other gases. Furthermore, no significant contribution appeared to be made by the nitrous oxide spectrum to mass 40. Therefore, it seemed plausible to follow the same analytic procedure as for ether except that mass 30 was used to determine the nitrous oxide present instead of mass 31.

For the analysis of nitrous oxide in a gas mixture then,

$$\frac{M_{N_2O}}{Ma} = k \frac{V_{N_2O}}{Va} \qquad (1)$$

when M_{N_2O} = mass of nitrous oxide in the sample
Ma = mass of argon
V_{N_2O} = output voltage at peak 30
Va = output voltage at peak 40
k = a calibration constant.

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¶ Analysis on the unfragmented mass peak 44 would be complicated because carbon dioxide, present in our samples of gas obtained from blood, has the same unfragmented peak.

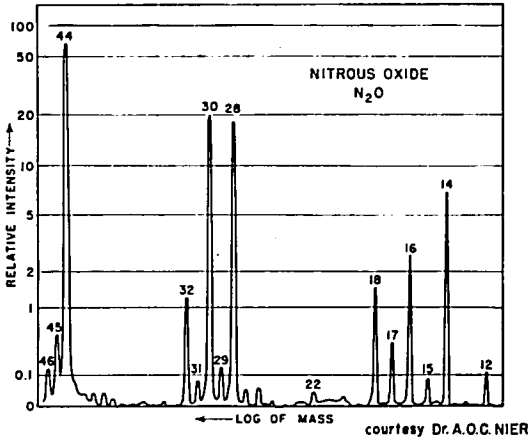

FIG. 1. Mass spectrum of nitrous oxide. Mass peak 30 is used in these studies as representing nitrous oxide.

The Calibration Constant k.—In order to determine k in equation 1, several mixtures containing varying amounts of nitrous oxide and argon were prepared. Ratios of the amount of nitrous oxide to that of argon varied from 0.13 to 5.7. All the mixtures were prepared on a vacuum gas manifold which was equipped with an accurate burette for measuring small volumes of gases. A manometer was available for measuring the pressure of each of the two gases when a flask containing several liters of the mixture was to be filled. The calibration constant for nitrous oxide was determined by recording the output voltage for peak 30 and peak 40 of these known mixtures. By substituting these known values in equation 1, k could be determined. It was noted that this constant varied at times as much as 15 per cent during the course of one day. If a number of samples were being analyzed it became necessary to calibrate the instrument after analysis of approximately every four samples of unknown.

Calibrated 5-liter flasks, containing known mixtures of nitrous oxide and argon in approximately the same ranges of concentration as those ordinarily dealt with in this work, were prepared in the following manner: The flask was completely evacuated and then filled with nitrous oxide to a certain manometric pressure. Argon was then added and the pressure was increased by a certain value. This mixture was diluted with dry air in order to obtain mixtures with concentrations comparable to those seen in samples of blood being analyzed. By

knowing the partial pressure and densities of these gases, the mass ratio could be calculated. This mixture was utilized for recalibration of the instrument at frequent intervals.

A standard procedure for analysis of the samples of gas for nitrous oxide in the mass spectrometer was followed. The unknown sample to which a known mass of argon had been added was admitted to the analyzer tube at a pressure of approximately 50 mm. of mercury in the inlet manifold. (This pressure is not critical.) After several minutes the output voltages of the peaks (nitrous oxide at 30 and argon at 40) became stabilized and were read alternately several times and averaged. Since the composition of the samples in which we were interested was close to air, dry air was admitted to the analyzer tube at the same manifold pressure before each sample of gas to determine the residual background for peak 30. This background was subtracted from the output voltage before the ratio was calculated. Some of the nitrogen and oxygen in the air apparently combine to contribute to peak 30 (NO^+) and give a higher background than would be obtained if these gases were not present. The 30 background from the air in the sample limits the accuracy of the determination of low concentrations of nitrous oxide. A pump-out interval between samples of about five minutes reduced background voltages to satisfactory values. After the voltage ratio was determined on the spectrometer, the data were applied in equation 1:

$$M_{N_2O} = k\,Ma\left(\frac{V_{N_2O}}{Va}\right).$$

If the volume, V, of the original gas sample is expressed in milliliters and Ma in milligrams, the concentration of nitrous oxide per 100 ml. of the original sample of gas is

$$\frac{100\,k\,Ma}{V}\left(\frac{V_{N_2O}}{Va}\right). \qquad (2)$$

CORRECTION FOR THE PRESENCE OF ETHER

When ether and nitrous oxide are present simultaneously in the sample, a correction of the voltage read at peak 30 is required because of the contribution to this peak by ether. The correction is made by determining the amount of ether present as described in study II of this series basing the determination on the relative abundance of peak 31. Since the ratio of peak 31 to peak 30 for ether is constant, it is a matter of simple calculation to determine the contribution of ether to peak 30. When this contribution is known and is subtracted from the total abundance of the fragment mass 30, the remainder represents the contribution of nitrous oxide.

ANALYSIS OF SAMPLES OF BLOOD FOR NITROUS OXIDE

The partial extraction method described in the previous paper (1) was used for the purpose of deriving a representative sample of nitrous oxide from the blood to be analyzed. The gas is partially released from the blood by equilibrating it with a known amount of air. Air was utilized in this procedure since it provided a convenient source of gases containing a known percentage of argon, the gas used as an internal standard. The concentration of argon in air is constant at 0.93 per cent. The amount of nitrous oxide released depends solely on the solubility of the gas in the blood at the temperature of equilibration. It is essential, then, that in determining the amount of nitrous oxide in any sample of blood with this method, the distribution ratio of that gas in blood be known.

Distribution Ratio.—The method for determining the distribution ratio was based on that described for ether in the previous paper (1). It involved equilibration of a known volume of nitrous oxide-argon mixture with a known volume of blood, and measurement of the nitrous oxide remaining in the gas phase on the mass spectrometer. The amount of nitrous oxide dissolved in the blood was then calculated and the distribution ratio determined.

The same equation used in developing the analytic method for ether is applied. Here nitrous oxide is substituted for ether:

$$DR_{N_2O} = \frac{Vg}{Vb}\left[\frac{Rs}{Rg}\left(1 + DRa\,\frac{Vb}{Vg}\right) - 1\right] \qquad (3)$$

when: Vg = volume of final gas phase
Vb = volume of final blood phase
Rs = spectrometric nitrous oxide-argon voltage ratio before equilibration
Rg = spectrometric nitrous oxide-argon voltage ratio after equilibration
DRa = distribution ratio of argon in blood or 0.034.

The technic of the determination of the distribution ratio of nitrous oxide in blood involved the admission of approximately 5 ml. of a known nitrous oxide-argon mixture to a calibrated 20 ml. syringe containing approximately 10 ml. of blood. The blood and known mixture of gas contained in the syringe was then equilibrated for forty-five minutes in a constant temperature water bath. This time was demonstrated to be ample for completion of the process. After equilibration, a small sample of the gas phase was admitted to an evacuated gas sampling bulb on a vacuum gas manifold system. This sample of the gas was then analyzed on the mass spectrometer for the nitrous oxide-argon ratio. After appropriate substitutions of these values in equation 3, the distribution ratio was determined.

The distribution ratio for argon in blood was not determined, but it is assumed that it is very similar to that for water or 0.034. Considerable error in this assumption would result in only minimal error in the percentage of nitrous oxide determined.

Blood Content.—The determination of nitrous oxide content of blood is somewhat the reverse of the determination of the distribution ratio; blood containing nitrous oxide is exposed to the gas phase containing a known amount of argon and then equilibrated. When the distribution ratio of nitrous oxide has been determined, the amount of nitrous oxide originally contained in the blood may be calculated.

As demonstrated in the previous paper (1), the following equation may be derived for this determination. Again, nitrous oxide is substituted for ether.

$$N_2O \text{ in milligrams per 100 ml.} = \frac{100\ Ma\ k\ R_{N_2O}}{Vb}\left[\frac{1 + DR_{N_2O}\frac{Vb}{Vg}}{1 + DR_a\frac{Vb}{Vg}}\right] \quad (4)$$

when: Ma = mass of argon present in gas phase
k = mass spectrometer calibration constant
R_{N_2O} = spectrometric voltage ratio after equilibration
Vb = volume of blood
DR = distribution ratio (a for argon; N_2O for nitrous oxide)
Vg = volume of gas.

The mechanics of this procedure involved the admission of approximately 10 ml. of air to a syringe containing 5 ml. of blood in which was dissolved an unknown amount of nitrous oxide. The system was then subjected to equilibration by continuous rotation in a water bath at 37 C. for forty-five minutes. After equilibration, a representative sample of gas was delivered to the mass spectrometer in the manner described. After V_{N_2O} and Va were determined, the mass of nitrous oxide present per 100 ml. of the original sample of blood was determined by substitution in equation 4.

Validation of the Method

Blood, containing known amounts of nitrous oxide, was prepared by first saturating nitrous oxide or known mixtures of nitrous oxide and oxygen with water vapor in a water bath at a constant temperature of 37 C. This gas was then led through the blood at the same temperature until equilibrium was established. The process of equilibration was found to require about three hours. The amount of nitrous oxide actually contained in the blood was calculated from the distribution ratio of nitrous oxide in blood and the density of the gas.* Several

* Density (STPD) $\times \frac{273}{T} \times \frac{\text{P-water vapor pressure}}{760} \times$ distribution ratio $\times$ 100 = calculated blood level when P = pressure of N_2O in mm. of mercury.

TABLE 1

MASS SPECTROMETRIC ANALYSIS OF BLOOD SATURATED WITH NITROUS OXIDE OR NITROUS OXIDE AND OXYGEN

Sample	Nitrous Oxide, Mg. per 100 Cc. in Blood Saturated with: 100 Per Cent N_2O	50 Per Cent N_2O and 50 Per Cent O_2
1	71.3	37.3
2	70.6	35.4
3	68.9	36.4
4	71.6	36.7
5	68.4	37.2
Mean	70.2	36.6
Standard deviation	±1.45	±0.75
Per cent standard deviation	±2.1	±2.0
Calculated value	70.6	37.0
Error of method, per cent	0.6	1.1

samples of blood were analyzed for nitrous oxide by the mass spectrometric method. Mixtures of two gases in single cylinders were utilized in this proof of the method—100 per cent nitrous oxide and a mixture of approximately 50 per cent nitrous oxide and 50 per cent oxygen. These mixtures were analyzed for oxygen content with a Beckman Oxygen Analyzer, Model C. The residual gas was considered to be nitrous oxide since the gas mixtures in the cylinders were specially prepared to be free from impurities. The results of this process are presented in table 1. The results of 5 mass spectrometric analyses of blood equilibrated with 100 per cent nitrous oxide were found to have a standard deviation of ± 2.1 per cent, and a like number of analyses of blood equilibrated with 50 per cent nitrous oxide revealed a standard deviation of ± 2 per cent. The per cent error was 0.6 per cent and 1.1 per cent respectively.

Simultaneous distribution ratios of nitrous oxide and ether in blood were determined by the same method. A gas mixture containing known tensions of ether and nitrous oxide was prepared in a calibrated flask. The ether was carefully weighed in a specially prepared ampule and broken inside the sealed flask after it has been filled with a gas mixture prepared by using pressure manometers on a gas manifold. The results of these determinations are found in table 2.

The mean distribution ratio for ether in 4 simultaneous determinations was found to be 11.2 which is very near the over-all figure found when distribution ratios of ether in the absence of nitrous oxide were

TABLE 2

SIMULTANEOUS DISTRIBUTION RATIOS OF NITROUS OXIDE AND ETHER IN BLOOD

Sample	N_2O	Ether
1	0.453	11.4
2	0.451	10.8
3	0.454	11.3
4	0.460	11.4
Mean	0.455	11.2

determined. The mean distribution ratio for nitrous oxide in the same determination was 0.455 in comparison to the over-all mean of 0.457 found in the series when nitrous oxide distribution ratios were determined.

SUMMARY

A method adapting the mass spectrometer to the analysis of the nitrous oxide content of blood is described. The method involves extraction of a sample of gas from the sample of blood by equilibration at a constant temperature with a gas containing a known amount of argon (in this case, air). The resultant mixture of gases may then be analyzed on the mass spectrometer and the original nitrous oxide content of the blood calculated. This process may be carried out in the presence of varying amounts of the usual respiratory gases and ether vapor. It is shown that simultaneous determinations of the distribution ratio of nitrous oxide and ether in the same sample of blood yield results in close agreement with determinations for these two agents made separately.

Accuracy of analysis for nitrous oxide in blood containing a known and substantial amount is shown to be to the order of ± 2 per cent.

REFERENCE

1. Jones, C. S.; Saari, J. M.; Devloo, R. A.; Faulconer, Albert, Jr., and Bahles, E. J.: **Analysis of Gases in Blood with Mass Spectrometer. Method for Determination of Diethyl Ether in Blood,** Anesthesiology **14:** 490–497 (Sept.) 1953.