Chromatographic Analysis of Multiple Tracer Inert Gases in the Presence of Anesthetic Gases

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A gas chromatographic method for simultaneous analysis of multiple tracer inert gases in blood and expired gas samples is described. The method enables determination of the distribution of ventilation-perfusion ratios in the lungs during anesthesia with nitrous oxide and halothane. In addition, simultaneous analysis of anesthetic gas concentration in blood permits calculation of the amount of uptake or elimination of anesthetic gases from the Fick principle. (Key words: Measurement techniques; chromatography. Pharmacokinetics: uptake; solubility. Lung: ventilation-perfusion.)

General anesthesia is frequently associated with abnormalities of gas exchange that necessitate an increased inspired oxygen concentration. Understanding of the causes of these alterations in pulmonary function would be improved if the patterns and severity of alterations in the distribution of ventilation-perfusion (V,/Q) ratios produced by anesthesia could be determined. Though suitable for this purpose in the absence of inhaled anesthetics, the multiple-tracer inert gas-elimination technique described by Wagner et al.5-6 utilizes a chromatographic technique in which the retention times and the concentrations of commonly used anesthetic gases (nitrous oxide, halothane, enflurane) in the expired gas and blood samples interfere with analysis of the tracer gases. Alternative methods described earlier for analysis of multiple anesthetics in blood are also not suitable for simultaneous multiple-tracer inert gas analysis.6,7

We have modified the chromatographic analysis of six tracer inert gases in the presence of anesthetic concentrations of nitrous oxide and halothane. It is the purpose of this paper to demonstrate that with these modifications we are able to obtain tracer inert gas elimination data comparable to those obtained with the original method. In addition, this modified chromatographic technique enables simultaneous determination of blood concentrations of nitrous oxide and halothane.

Methods

The original technique of multiple-inert gas analysis for determination of V,/Q distribution involved analysis of tracer concentrations of sulfur hexafluoride (SF6), ethane, cyclopropane, ether, acetone, and halothane in expired gas and in arterial and venous blood samples. Sulfur hexafluoride analysis was performed with an electron-capture detector (ECD), and the remaining five gases with a flame ionization detector (FID) gas chromatograph.

In the present study, halothane (blood:gas partition coefficient \( \lambda = 2.3 \)) was replaced with enflurane (\( \lambda = 1.8 \)) as a tracer gas, allowing us to use six tracer gases in the presence of nitrous oxide and halothane in anesthetic concentration. In order to provide adequate separation of both tracer and anesthetic gases, we used two separate detector and column systems in two separate ovens. Additional use of temperature programming enabled greater resolution of compounds with short retention times and increased the peak heights of compounds with relatively long retention times. A byproduct of this configuration was the ability to measure simultaneously arterial and mixed venous blood concentrations of nitrous oxide and halothane in the same samples used to measure tracer inert gas concentrations.

Instrumentation

The analytic procedure was performed with a Hewlett-Packard model 5711 FID and model 5713 ECD gas chromatograph. This particular ECD utilizes a \( ^{60} \)Ni electron source. A 0.5-ml constant-volume gas-sampling valve was used for introducing the sample onto the columns of each chromatograph. The inlet loops were adequately flushed and filled with 5-ml volumes of sample.

Two stainless steel columns (12 feet long, \( \frac{1}{8} \) inch diameter) filled with Porapak-Q, 80/100 mesh (Applied Science Laboratories), were placed in the dual-column oven of the model 5711 FID. Nitrogen was used as the carrier gas at a flow rate of 22–24 ml/min. Upon introduction of the gas sample onto the FID

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column, the temperature of the column oven was immediately programmed to increase at a rate of 4 °C/min from 140 to 170°C and held at 170°C for 8 to 16 min, depending on the amount of halothane present in a given sample. The FID detector was held at a constant temperature of 250°C. An example of a chromatogram obtained with the FID, containing five tracer gases and two anesthetic gases, is shown in figure 1a. Total FID analysis times for individual samples ranged from 16 to 24 min.

A single stainless steel column (6 feet long, ½ inch diameter) packed with molecular sieve 5-A, 80/100 mesh (Applied Science Laboratories) was placed in the model 5713 ECD oven. A 5 per cent methane in argon carrier gas was used at a flow rate of 20 ml/min, at a constant column oven temperature of 65°C. The detector temperature was held constant at 150°C. A typical ECD chromatogram is shown in figure 1b. Oxygen and nitrogen in the sample were adequately separated from sulfur hexafluoride, and nitrous oxide and halothane did not appear in the 4.5 min required for sulfur hexafluoride concentration analysis. In fact, nitrous oxide retention time was 6.5 hours at 65°C, (halothane retention > 24 hours), after which time both nitrous oxide and halothane accumulated on the column could be cleared by increasing the column temperature to 225°C and the detector temperature to 250°C for 45 min. Following this clearance, the usual operating conditions could be resumed.

**Preparation of Samples**

Expired gas samples were collected in dry matched-barrel glass syringes with 1.0-ml graduated markings. This enabled flushing of the FID gas sampling valve with an adequate volume of sample, while at the same time retaining a suitable volume in the syringe for reproducible dilutions prior to introducing the sample into the ECD gas-sampling valve.

Measurement of gases present in blood samples was performed as described earlier. Nitrogen was added in a volume similar to that of the blood sample in a 30-ml matched-barrel glass syringe (1-ml graduations). Equilibration of the gas and liquid phases was performed at the desired temperature (±0.1°C) in a shaking water bath (Precision Scientific, model W3382-1) for a minimum of 40 min. Volume of the gas and liquid phases in the equilibrated samples was again determined by subtracting blood volume (weight of the blood + density) from the total volume of gas and liquid at the equilibrated temperature.

Solubility of the gases in blood was also measured as described earlier, except for a minor modification for gases with solubility less than 1 ml/100 ml/mm Hg.
That is, for determining the solubility of sulfur hexafluoride, ethane, cyclopropane and enfurane, gas samples of approximately $10^{-4}$ ml/ml and $10^{-6}$ ml/ml (enfurane) concentration were added in approximately equal volumes to 10-ml volumes of blood for equilibration and re-extractions. This *in-vitro* method provided a higher signal-to-noise ratio for gas concentrations remaining in the re-extraction samples.

**Equilibration Time**

The time required for equilibration in the extraction procedure was tested for the less soluble gases, to assure that the large fluxes of nitrous oxide and halothane (anesthetic concentration) did not prolong equilibration time (ether and acetone were shown earlier to reach equilibrium in 1–2 min). This was determined by measuring the solubilities of sulfur hexafluoride, ethane, cyclopropane, and enfurane in six 10-ml samples of dog blood after 5, 10, 15, 30, 40, and 60 min of equilibration (fig. 2). However, instead of an initial complete equilibration with an appropriate concentration of the test gases, as described previously, the initial equilibration time was equal to the re-equilibration time. This resulted in low values for solubility at the shorter equilibration time rather than high values as described in the earlier publication. The time required for equilibration, however, remained the same.

**Performance Characteristics of the Gas Chromatographs**

The linearity of the HP model 5711 FID was tested with gas concentrations of ethane, cyclopropane, ether, acetone, and enfurane ranging from $10^{-9}$ to $10^{-6}$ ml/ml concentrations. The diluent gases used for the test were: 1) N₂; 2) N₂ in N₂O, 70 per cent; 3) a mixture of N₂ in N₂O, 70 per cent, halothane 1.0 per cent. Linearity was determined for nitrous oxide over the range 100 per cent to 0.1 per cent and for halothane 2.0 per cent to 0.1 per cent to determine feasibility of measuring their concentrations in the same blood samples used for the tracer gas measurements. The linearity of the HP model 5713 ECD for sulfur hexafluoride concentration was tested with concentrations ranging from $10^{-9}$ to $10^{-12}$ ml/ml.

The concentration of gases (ml/ml) at which baseline noise level reached 5 per cent of the peak height was used to determine the sensitivities of the FID and ECD for each of the tracer and anesthetic gases.

Reproductibilities of both detectors were determined by successively analyzing eight samples of a

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**Fig. 2.** Calculated solubilities *in vitro* vs. time of the four least soluble tracer gases as a function of the period of equilibration of blood sample with the original gas sample and the same re-extraction (with N₂) period. Note that the equilibration is complete for even the least soluble gas (SF₆) in less than 30 min.
gas mixture contained within one syringe. Reproducibility of the combined techniques of equilibration and chromatographic analysis was further determined by measuring the concentrations of the six tracer gases and nitrous oxide and halothane in ten samples of 10 ml each of dog blood.

Results

Linearity

Linearity was consistent with all diluent gases tested (nitrogen, nitrogen + nitrous oxide (3:7), nitrogen + nitrous oxide + halothane (fig. 3)). In addition, the FID also demonstrated a linear response to halothane in the concentration range 2.0 to 0.1 per cent, as expected. Nitrous oxide peaks, however, were non-linear with respect to concentration (fig. 4a). This was also true for sulfur hexafluoride concentration as measured by the ECD (fig. 4b). Therefore, calibration curves had to be constructed for determining nitrous oxide concentration by FID, as well as for sulfur hexafluoride concentration by ECD.

Since sulfur hexafluoride concentration need only be measured in relative terms for $V_r/Q$ analysis (e.g., arterial:venous or expired:venous concentration ratio), the calibration curve for sulfur hexafluoride has been expressed in terms of percentage concentration (highest peak) with respect to peak height. On the other hand, the nitrous oxide calibration curve must be expressed in terms of percentage concentration with respect to atmospheric pressure, to allow measurement of absolute nitrous oxide concentrations in gas and blood samples. Similarly, a known standard was necessary to calibrate halothane peak height with respect to concentration, although a dilution curve was not needed (since the FID response was linear).

Sensitivity

In spite of the smaller sample size (0.5 ml), all five tracer gases, as well as halothane, as determined with the FID, were measurable at levels as low as one part per million (ml/ml) with a noise level of no more than 5 per cent of the signal. Nitrous oxide was measurable to as low as 0.1 per cent concentration with a noise level of no more than 5 per cent of the signal.

Reproducibility

Reproducibility of the HP 5711 FID, using temperature programming, was determined after correction of each subsequent sample analysis for diffusion loss (25 min interval). The correction for diffusion loss was applied from the coefficients of regression.
lines obtained from the respective peak heights of each of the gases measured repeatedly from a mixture of these gases in a matched-barrel glass syringe over a five-hour period. The standard deviation of peak height measurements with this correction was less than 0.4 per cent of the mean peak height for all five tracer gases. This slightly higher standard deviation for FID measurements (compared with 0.2 per cent reported by Wagner et al.) is probably in part due to the smaller sample size, 0.5 ml, compared with 2.0 ml, and in part due to minor variations in starting times of the temperature programming with respect to timing of gas sampling. Reproducibility of sulfur hexafluoride measured by the HP 5713 ECD (SD 0.4 per cent, with 5-min injection intervals) was essentially equal to that reported earlier (SD 0.5 per cent), however, in spite of the smaller sample size (0.5 ml) and the presence of nitrous oxide and halothane in the sample. This may be related to the more linear response of the HP 5713 ECD to sulfur hexafluoride, in comparison with the Beckman model GC 72-5 ECD used previously.4

Reproducibility of all tracer gas measurements in blood samples was comparable to that described ear-

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**Table 1. Solubilities of the Six Tracer Gases in Human and Dog Blood**

<table>
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<tr>
<th></th>
<th>Human Blood, 37 C</th>
<th>Dog Blood, 50 C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wagner et al. (N = 9)</td>
<td>Present Study (N = 9)</td>
</tr>
<tr>
<td>SF6</td>
<td>0.00084 ± 0.00013</td>
<td>0.00065 ± 0.00010</td>
</tr>
<tr>
<td>Ethane</td>
<td>0.0133 ± 0.0011</td>
<td>0.01028 ± 0.00116</td>
</tr>
<tr>
<td>Cyclopropane</td>
<td>0.0702 ± 0.0056</td>
<td>0.06703 ± 0.00705</td>
</tr>
<tr>
<td>Enthranne</td>
<td>0.2249 ± 0.025</td>
<td>0.22649 ± 0.0279</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.641 ± 0.124</td>
<td>1.583 ± 0.108</td>
</tr>
<tr>
<td>Ether</td>
<td>44.9 ± 3.2</td>
<td>39.8 ± 6.1</td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
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Values of solubility in ml gas/100 ml blood/torr in human and dog blood, comparing the findings with those of Wagner et al.,7 using Porapak T columns for both FID and ECD.
lier. The standard deviation of percentage difference from the mean for each gas concentration in eight aliquots of blood was 2.8 per cent for SF₆, 3.5 per cent for ethane, 2.8 per cent for cyclopropane, 1.4 per cent for enflurane, 0.8 per cent for ether, and 2.6 per cent for acetone.

Equilibration (fig. 2) was complete for all gases within 30 min in the shaking water bath.

A small but consistent difference is seen in mean solubilities for tracer gases in dog blood and in human blood (table 1). This difference may be related to differences in hemoglobin, plasma protein, and plasma lipid levels. In any event, these differences are well within the range of differences for measured blood-gas partition coefficients reported by other investigators, as can be seen by multiplying our solubility values by the factor 713/100.

Discussion

A modified method for multiple-inert gas analysis by gas chromatography enables measurement of six tracer inert gases in samples of gas or blood in the presence of anesthetic concentrations of nitrous oxide and halothane. The levels of sensitivity and reproducibility of this method were essentially equivalent to those described earlier by Wagner et al., and thus the method is suitable for estimation of ventilation-perfusion ratio distributions in subjects anesthetized with these inhalation agents.

Furthermore, this method is suitable for simultaneous analysis of both multiple tracer inert gas concentrations and nitrous oxide and halothane concentrations. Concentrations of both nitrous oxide and halothane in mixed venous and arterial blood samples may be determined with the use of chromatography calibration factors from known gas samples, and the formula described earlier by Noehren and Cudmore. This enables calculation of nitrous oxide and halothane uptake or elimination in ml/min by the Fick principle, without the requirement for further blood sampling and without additional blood handling.

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References