Quantitative Analysis of Diethyl Ether in Blood

Blood/Gas Distribution Coefficient of Diethyl Ether

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A method for the determination of blood-ether concentration was developed, consisting of refluxing a sample of blood, condensing the ether in a liquid nitrogen trap and using gas chromatography for analysis. The method is applicable for other volatile anesthetics. A relative standard deviation of ±3 per cent was obtained. Recovery of ether added to blood was over 95 per cent. The blood/gas distribution coefficient for ether in human blood at 37° C. was calculated to be 12.

Gas chromatography is rapid and precise for the quantitative analysis of gases.[1, 2] Techniques have been developed to adapt gas chromatography for the analysis of volatile anesthetics in blood.[3-7] Each of these adaptations is unsatisfactory in some detail such as reproducibility of sampling, preparation of sample, or method of standardization. The present method eliminates these difficulties by measuring a volume of blood between two stopcocks, removing anesthetic from blood by refluxing and trapping in liquid nitrogen, for direct analysis in a gas chromatograph. The method is also applicable for gas samples; therefore standardization can be accomplished by analyzing an identical volume of gas of known concentration. Recovery data were obtained by equilibrating blood with a known gas mixture and analyzing both phases.

Apparatus

The complete system, figure 1, consists of two units: a standard gas chromatograph * with a hydrogen flame detector and an apparatus † for preparing the sample. Blood can be injected anaerobically into the glass pipette using a 15-gauge blunt-end needle inserted into Teflon tubing ⅛ inch, outside diameter, 1/16 inch, inside diameter, in turn inserted into port 5 of stopcock C. The connection between the outlet of the reflux apparatus and valve II is a *short* length of ⅛ inch, inside diameter, Teflon tube. For simplicity, the glass reflux apparatus is shown with an outer jacket condenser only. It also has an inner finger condenser.

Methods

Refluxing helium ‡ (50 ml./minute) is passed through ports 1, 4 and 6 of the glass pipette, the refluxing apparatus, the condensing coils of valve II and out the flow meter, figure 1. The condensing coils are immersed in a Thermos flask containing liquid nitrogen. When the coils reach −195° C. (the liquid nitrogen will become quiescent), stopcocks B and C are turned, connecting ports 5 and 3. The ¼ ml. glass pipette is then filled with well mixed blood, the excess passing out waste port 3.

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* Perkin-Elmer Corporation, Norwalk, Connecticut, model 154 D.G.

† Ace Glass, Inc., Vineland, New Jersey. Reflux condenser, quotation No. 07107A-10489.

‡ It is convenient to use two tanks of helium, each with separate pressure reducing regulators and valve controls: one, working pressure 0–150 cm. Hg for *carrier* helium for the gas chromatograph and the other, working pressure 0–1 cm. Hg for both *zero-gas* helium (25 ml./min. flow) and for *refluxing* helium (50 ml./min. flow). *Zero-gas* helium contains no anesthetic.

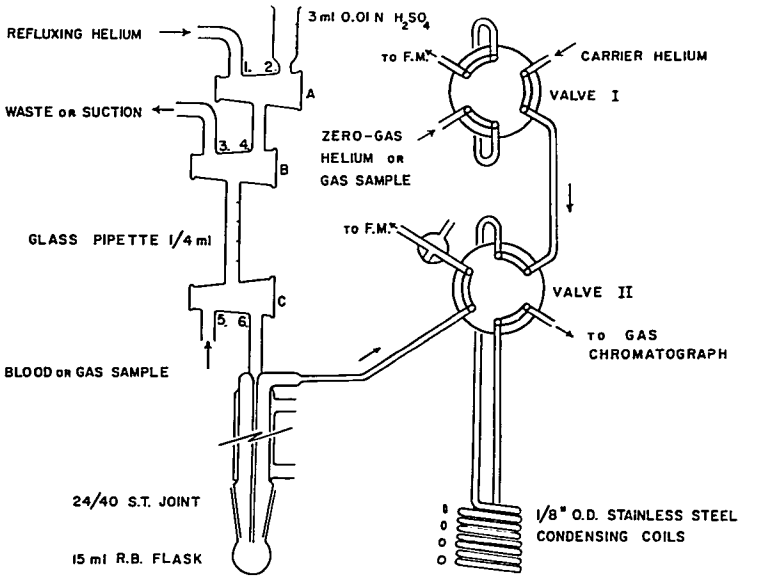

FIG. 1. Glass pipette and refluxing apparatus connected to Valve II. Valve II has stainless steel coils for condensing in place of a standard flow-through loop used for measuring a volume of a gas sample. Valve I has a standard flow-through loop of ¼ ml. volume. Valve I (control knob pulled out, not indicated in figure) is shown with the ¼ ml. loop connected to the sample inlet port and to the outlet port to a flow-meter. The stream of *carrier* helium is shunted through the valve. Valve II (control knob pushed in, not indicated in figure) is shown with *carrier* helium passing through the condensing coils for analyzing a sample trapped in the coils, while *refluxing* helium from the glass apparatus is shunted through the valve and out to flow-meter, F.M.

Stopcocks C, B and A, in that order, are quickly turned, connecting ports 2, 4 and 6, and allowing 3 ml. of 0.01 *N* H_2SO_4 previously added to the funnel to wash the blood into the round-bottom flask. As soon as the last of the 3 ml. of 0.01 *N* H_2SO_4 passes stopcock A, it is turned to permit *refluxing* helium to flow through the apparatus and a timer is set for 4 minutes. A gas flame about ¼ inch § high is swung under the round-bottom flask. Boiling begins in about one minute. Foaming of the acidified blood does not occur with this low flame. After 4 minutes, the control knob of valve II is turned into the gas chromatograph and the Thermos of liquid nitrogen is immediately replaced with a container of boiling water. After 1 minute, the control knob is turned back again to the original position. The glass pipette is cleaned by aspirating water at port 5. The apparatus is dried with air suction by opening the stopcock between valve II and flowmeter, F.M., for 2 minutes, with the condensing coils still immersed in hot water.

Standardization is accomplished by analyzing a *gas* mixture of exactly known concentra-

§ Made with a blunt 18-gauge needle, mounted in a cork for fastening. The gas flame is made without prior mixing with air or oxygen.

tion ¶ (*i.e.*, 1 per cent ether in nitrogen, obtained from a tank) in the ½ ml. blood sample pipette (inlet at port 5, outlet at port 3) and proceeding with a "dry, *frozen*" analysis (the 3 ml. of 0.01 *N* H_2SO_4 and the boiling are omitted).‡ The 4-minute period of *refluxing* helium is repeated at the same flow of 50 ml./minute. The condensed diethyl ether represents a known concentration in identical volume as taken for blood. The concentration of standard gas should be of the same order of magnitude as the unknown sample, since linearity cannot be expected over the entire range of sample mass which the hydrogen flame can handle. Peak areas (integrals) show better reproducibility than peaks heights and are used throughout.

Calculations

$$\frac{\text{Known concentration of standard gas}}{\text{Integral of standard gas, measured}} = \frac{\text{Unknown concentration of blood}}{\text{Integral of blood, measured}}$$

Checking Standards of Performance

(a) Leaks in the sampling pipette, refluxing apparatus and condensing coil unit are determined by admitting helium into the entire system to a pressure of 30 mm. of mercury, closing inlet and outlet, and noting the rate of fall in pressure. Maximum rate of fall should not exceed 1 mm. of mercury per minute.**

(b) Baseline stability of the gas chromatograph is usually present if the entire instrument, including column oven and flame detector is kept turned on, day and night. Baseline drift should not be detectable at the working attenuation of the instrument.

(c) Constant response of the gas chromatograph is determined by reproducibility of analyses of standard gas, *unfrozen*, using valve I. Blood analysis should not begin until these gas analyses show a relative standard deviation †† of ±1 per cent. Continued stability is monitored with standard gas before or after each blood analysis. Standard gas should be passed through valve I only as long as required to fill the measuring loop of the valve, because the surfaces of the valve contain a trace of lubricant which slowly absorbs gas. During measurement, this absorbed gas will be eluted to *carrier* helium and added to the analysis. In order to eliminate this potential source of error, the lubricant is kept gas-free by continually passing zero-gas helium (*i.e.*, containing no anesthetic) through the sample ports of valve I (25 ml./minute) while *carrier* helium flows through the gas chromatograph ports. For analysis of standard gas (*i.e.*, 1 per cent diethyl ether in nitrogen v/v, from a tank), *zero-gas* helium is interrupted and the 1 per cent diethyl ether passed through for 15 seconds, at 50 ml./minute. The valve control knob is sharply turned into the gas chromatograph, allowing *carrier* helium to sweep out the sample for analysis. After 15 seconds, the control knob is turned back to the original position and *zero-gas* helium reinstituted until the next gas sample. The lubricant does not absorb or elute detectable amounts of gas in this period.

(d) Condensing coils of valve II. The trapping efficiency of the condensing coils was tested in two ways: (1) One per cent diethyl ether in nitrogen was sampled in valve I. The coils of valve II were kept in the *carrier* helium stream. Alternate analyses were made: freezing the diethyl ether with liquid nitrogen, and not freezing. The frozen analyses averaged 98 per cent of the unfrozen analyses. (2) One hundred per cent ethylene was analyzed using two sets of condensing coils in series, in valve II. The first coil consistently trapped 98 per cent and the second coil 2 per cent of the total sample. Similar results were obtained with 100 per cent cyclopropane.

(e) Figure 2 shows the results of varying reflux times. The sample was diethyl ether in

¶ An H tank of 1 per cent diethyl ether in nitrogen, v/v, 700 psi, is a convenient source of standard gas sample. We have calibrated such standards using a Beckman gas density balance, model 3A.

‡ Washing in the standard gas sample with 3 ml. of 0.01 *N* H_2SO_4 and boiling for 4 minutes gives the same result as the dry analysis.

** SNOOP, a non-residue liquid leak tester applied to joints will indicate leaks by bubbling. Nuclear Products Company, 15635 Saranac Road, Cleveland 10, Ohio.

†† Recommended term and defined in Analytical Chemistry, Vol. 36, Dec. 1964, as the standard deviation of a series of test results expressed as the percentage of the mean of that series.

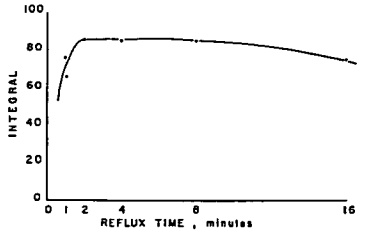

FIG. 2. Effect of varying reflux time.

water, 40 m*M* per liter. The points are the average of duplicate analyses which agreed within 2 per cent except for the 1 minute pair which are not averaged. Complete refluxing requires more than 1 minute; loss due to sublimation is measurable at 16 minutes. A refluxing time of 4 minutes was chosen.

TABLE I. Recovery Data of Ether Added to Blood at 37° C. Calculations for Blood/Gas Distribution Coefficient

Sample: fresh, heparinized blood: hematocrit	43.5%.
Tonometer volume	105.5 ml.
Blood volume	20.0 ml.
Initial gas volume, 25° C., dry	75.5 ml.
Balance, mercury volume	10.0 ml.
Equilibrated gas volume, 37° C., wet	82.4 ml.

Ether Concentration, v/v, %

Initial Gas Phase Dry, 25° C	Equilibrated Gas Phase Wet, 25° C	Equilibrated Blood
1.12	0.278	3.14
1.16	0.269	3.12
1.16	0.272	3.10
1.15	0.269	
1.17		
1.16		
1.15 % average	0.272 % average	3.12 % average

Temperature Correction, equilibrated air phase, from 25° C. to 37° C.:
0.272% × 298/310 = 0.261 %
Blood/Gas distribution coefficient at 37° C.:
3.12/0.261 = 12.0
Initial ether content, air phase: 75.5 ml. × 1.15% = 0.868 ml.
Equilibrated ether content, air phase: 82.4 ml. ×0.261% = 0.215 ml.
Equilibrated ether content, blood: 20.0 ml. ×3.12% = 0.624 ml.
Recovery: 0.839/0.868 × 100 = 96.6%

Results

Gas Samples. The ratio of 2 series of analyses of standard gas, one measured in valve I, *unfrozen*, the other in the glass pipette and condensed in the coils of valve II, *frozen*, was 1.14. The ratio of 2 similar series, both *unfrozen*, was 1.10. The difference in the ratios indicates a 3.5 per cent loss in the *frozen* series. The relative standard deviation of all 4 series was less than ±1 per cent.

Water and Blood Samples. A series of 10 consecutive analyses, in water and blood, each of approximately 10 m*M* diethyl ether per-liter, had a relative standard deviation of less than ±1 per cent for water and less than ±2 per cent for blood. Gas samples using valve I monitored the chromatograph for each *frozen* analysis, and had a relative standard deviation of less than ±1 per cent.

Recovery of Diethyl Ether. The average recovery of diethyl ether equilibrated with water in 3 experiments was over 98 per cent; in 5 experiments with blood, over 95 per cent. Table 1 shows the data sheet for an experiment on blood. The average distribution coefficient for water at 25° C. was 23 and for blood at 37° C., 12.

Discussion

Several methods have been used for introducing volatile anesthetics present in blood into a gas chromatograph. The simplest is the injection of blood directly into the helium stream of the chromatograph.[4, 7] We have been unable to obtain precision with this method. Another disadvantage of direct injection is that it requires a drying column to remove excess water in blood; the $CaCl_2$, $CaSO_4$ or P_2O_5 of the drying column also removes certan volatile anesthetics, notably diethyl ether.

A common method of avoiding direct injection of blood is to extract the anesthetic from blood by an anhydrous solvent;[5, 6] the dry solvent, containing the anesthetic is then injected into the chromatograph. A potentially serious disadvantage is that the solvent peak is many thousand times the size of the anes-

thetic peak. Even slight carry-over of part of the solvent peak from the same or previous analysis to the location of the anesthetic peak may lead to considerable error.

In our method, measuring the volume of blood between two stopcocks is automatic and accurate. Refluxing removes most of the water, all of the protein, fat and other substances which may decompose and interfere with the analysis. Because gas samples can also be analyzed, these are used for precise and convenient standardization. Jones *et al.*[8] have commented on the standardization of their method for blood diethyl ether, using the mass spectrometer. They attributed loss of approximately 16 per cent to the difficulty of standardization and to the nonhomogeneity of blood. Using a tank of diethyl ether in nitrogen as a gas standard, requires only one calibration for the tank, after which it can be used for thousands of blood analyses. This is in contrast to the classical method of using an analytical balance to weigh sealed ampules for each standardization.

The standard gas also serves to monitor the entire method since errors (mainly leaks) would immediately be detected by a change in the expected ratio of integrals of gas analyses in valve I, *unfrozen*, to integrals of gas analyses in valve II, *frozen*. Theoretically, blood could be standardized using only gas analyses in valve I *unfrozen*. In this case, a loss of anesthetic of 3.5 per cent must be corrected for; this is the difference in ratio of 1.10 and 1.14 (seen in Results of gas samples). Thus this method is not recommended. The ratio itself provides important information, that is, it acts as a monitor for the method. In addition, if standard gas is analyzed in the same way as the unknown blood sample, no correction will be needed. Part of the 3.5 per cent loss is the result of a 2 per cent loss in trapping within the stainless steel coils shown by all samples—both blood and standard gas. The remainder of the loss is due to the method of testing the sampling performance of the glass pipette. In this test, the volume between stopcocks B and C replaces the condensing coils of valve II. A very small leak, difficult to control, may develop at port 3 and 5 of the glass pipette, at a connection made with Teflon tubing (¼ inch × ⅛ inch). This temporary connection is not present in the standard refluxing procedure and is difficult to render leak-proof since it is subject to *carrier* helium pressure (up to 150 cm. of mercury). In the standard procedure, the pressure of *refluxing* helium is only 0.4 cm. of mercury.

Our technique of using gas samples for standardization was extended to serve as the basis for recovery experiments. The data could also be used to calculate blood/gas distribution coefficients of diethyl ether. Although the exact coefficient is still in question, it is an important physical constant which should be known as accurately as possible. In a review of previous determinations of this ratio, it was found that reports did not include recovery. Haggard [1] obtained a ratio of 15.2 on defibrinated dog blood at 37° C., analyzing both the blood and the air phases. Jones *et al.*[8] using a mass spectrometer, reported a ratio of 37° C. of 12.2 and 12.5 in two samples of human blood. Only the air phase was analyzed. Hattox *et al.*[10] using the same technique obtained a ratio of 11.2 for ether in the presence of N_2O. Again, only the gas phase was analyzed. Eger,[11] with a nonspecific infrared detector obtained results similar to those of Jones *et al.* and of Hattox *et al.* The blood phase was not analyzed and recovery could not be determined. Lowe [7] using gas chromatography, found a ratio of 15.6 but did not report any details.

Our determinations of the blood/gas distribution coefficient for diethyl ether at 37° C. also confirm the ratio reported by Jones *et al.* Since the determinations were done on both blood and gas phases, and in addition, since recovery of over 95 per cent was obtained, the value of 12 appears reasonably correct.

The distribution coefficient of any highly soluble substance such as ether is difficult to measure with accuracy. Even if the total loss in a determination is small, if most occurs in the denominator phase (gas), it appears as a falsely high ratio. Measurement of the gas phase is therefore important. However, measurement of this phase only may result in considerable error since only 1 of every 13 ether molecules would then be accounted for (as-

suming a ratio of 12/1 and that equal volumes of blood and gas were used).

The present method utilizes the full potential of gas chromatography as an analytical tool. Several anesthetics in the same blood sample can be determined. An unusually wide range of concentrations of anesthetic can be analyzed, from 100 per cent, to less than 0.01 per cent, v/v. The hydrogen detector unfortunately is not sensitive to inorganic molecules such as N_2O and CO_2 and O_2 which are of interest to anesthesiologists, but it is sensitive to all organic anesthetics. The insensitivity to inorganic molecules is, in one respect, a decided advantage, since small amounts of water do not interfere with the analysis.

Summary

A method for the quantitative analysis of diethyl ether in blood was developed. It is applicable to other volatile anesthetics. Standardization and monitoring of the method are easily and precisely done by gas analysis. The blood/gas distribution coefficient of ether in human blood at 37° C. was calculated, analyzing the blood and the gas phases, with recovery data.

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HYPERBARIC OXYGEN Guinea pigs with paired surface wounds infected with *Pseudomonas pyocyanea* or *Staphylococcus aureus* were placed in a chamber under two atmospheres of air pressure for 72 hours. Each wound was covered and was exposed either to flowing humidified air or oxygen during the period of pressurization. During treatment with oxygen, significant growth inhibition of both microorganisms was observed but after the treatment was stopped, infection increased to levels comparable with those in control wounds. (*Irvin, T. T., and others: Hyperbaric Oxygen in the Treatment of Infections by Aerobic Microorganisms, Lancet 1: 392 (Feb.) 1966.*)