Anesthesiology

THE JOURNAL OF THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, INC.

VOL. 22 JULY-AUGUST 1961 NO. 4

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/22/4/519/613808/0000542-196107000-00001.pdf by guest on 01 February 2023

ETHYL ETHER CONTENT IN BLOOD AS DETERMINED BY GAS CHROMATOGRAPHY

Theodore H. Noehren, M.D., and John W. Cudmore

Recent developments in gas chromatography have made available methods of quantitative and qualitative determination of great rapidity and accuracy.[1, 2, 3] The introduction of this principle to the determination of gas concentration in biologic fluid has been a significant adaptation of this technique. This paper will describe such a method, ethyl ether being the inert gas and blood the biologic fluid.

Previous methods for determination of ether in blood have involved a more lengthy procedure and, in our hands a significant degree of error.[4-5] The technique under consideration is that of gas extraction in a tonometer with analysis of the sample by a gas chromatograph. With this method, results for each sample are available within 15 minutes. Accuracy is good, as compared with previous methods.

Apparatus

The gas chromatograph used in this study was a Model 154-C Vapor Fractometer * with a gas sampling valve accessory, and an L. & N. Model G. Recorder.† Helium was employed as the carrier gas. The column is described as "Column F 154-0013 Fx" and is constructed of stainless steel tubing, two meters in length. The separating agent (fixed phase) is tetraethyleneglycol dimethyl ether. The packing or supporting agent is diatomaceous earth.

The Thomas-Van Slyke Manometric apparatus was employed in this series. Blood samples to be analyzed were admitted by means of Ostwald-Van Slyke pipettes.

Method

The chromatograph is calibrated with prepared mixtures of diethyl ether in nitrogen, at 1 and 2 per cent concentrations.‡ The carrier gas flow, helium, is set at 30 psi, and the temperature at 60 C. A gas sampling valve is employed with a sample loop which admits a constant volume of 25 ml. to the instrument. A steady baseline is obtained on the recorder. The sample loop is flushed with the standard mixture before the sample is admitted. Care must be taken to maintain the gas at ambient pressure. Each gas is recorded at several ranges of sensitivity, as close as possible to the sensitivity setting at which eventual determinations will be made. A baseline must be re-established before each new recording.

Blood is drawn anaerobically, with a standard needle and syringe of convenient size. The sample is transferred to an Ostwald-Van Slyke pipette (2 or 3 ml.) by inserting the

* Perkin-Elmer Corporation, Norwalk, Connecticut.

† Leeds & Northrup Company, Philadelphia, Pennsylvania.

‡ Supplied by The Matheson Company, East Rutherford, New Jersey.

Received from the Department of Medicine, University of Buffalo School of Medicine, and accepted February 23, 1961. Dr. Noehren is a Henry C. and Bertha Buswell Fellow, and Assistant Professor of Internal Medicine.

syringe into the bottom end of the pipette. A rubber washer is employed between the head of the needle and the tip of the pipette to ensure an airtight seal.

The sample is pipetted into the mercury-filled Van Slyke chamber. The liquid is then equilibrated with room air to a total combined volume of 50 ml. by lowering the mercury. Care is exercised that only a one-way inward flow of air is allowed. Complete equilibration between the liquid and gas phases is achieved by stirring with the magnetic rod for five minutes at approximately 90 rpm. More aggressive stirring may cause red cell hemolysis which can seriously alter the analysis, since the solubility of ether is higher in the erythrocyte than in plasma. Caprylic alcohol is not used, for it too may alter the solubility coefficient of blood.

The tonometer is attached by glass tubing to the 25 ml. sample loop on the chromatograph which is then flushed with the equilibrated gas. The last 25 ml. are introduced to the instrument for analysis. Recording is done at a suitable sensitivity which will produce tall, sharp peaks (fig. 1). The air peak appears promptly and is constant so long as the same sample loop is used. The ether peak, which appears later, is recorded at a suitable sensitivity range as determined by the concentration of the sample, the apparatus, and the age of the column. The exact time of the peak appearance will vary with these individual characteristics but identification is not difficult since there are only three peaks, air, ether, and water, the last with considerable delay in elution time. Samples in a series should be so timed that peak recordings do not superimpose on a peak of a previous sample.

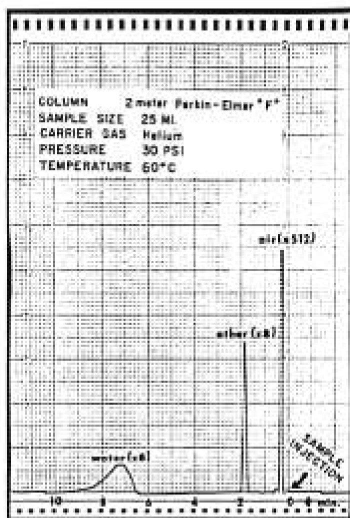

Fig. 1. Sample chromatogram for ethyl ether in blood. The analysis is recorded from right to left as indicated by the time in minutes. The sensitivity at which each peak is recorded is indicated in parentheses for each gas.

Calculations

An example of the calculations necessary to this method of blood-ether determination is as follows (table 1):

Since there are 0.71 mg. of diethyl ether in 25 ml. of 1 volume per cent gas (STPD) and 1.43 mg. of ether in 25 ml. of 2 volumes per cent (STPD), we may calculate in this case that 1 mg. of ether would give a recorded peak of 27.8/0.71 or 39.2 chart units when recorded at a sensitivity of × 32. Similarly, using the 2 volumes per cent recording, 1.0 mg. of diethyl ether would produce a peak of 55.9/1.43 or 39.1 chart units in height when recorded at an attenuation of × 32. The average calibration factor at × 32 is then 39.2.

If it is not possible to standardize at a sensitivity applicable to the unknown sample, as in this case, the factor from the closest sensitivity, extrapolated accordingly, should be used.

As an example the following determinations were made:

Blood aliquot—0.2 ml.

Tonometer gas volume (50 ml.–2 ml. blood)—48 ml.

Extraction temperature—22.0 C. (solubility coefficient—31.6)

Recorded peak height (× 8) 33.7 units

Calibration factor (× 8) = 157.0

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/22/4/519/613808/0000542-196107000-00001.pdf by guest on 01 February 2023

TABLE 1

CALIBRATION

Recorder Sensitivity	1 vol. % Standard Gas	2 vol. % Standard Gas	Units/mg. 1 vol. %	Units/mg. 2 vol. %	Calibration Factor
×16	55.7 units	—	78.5	—	78.5
×32	27.8 units	55.9 units	39.2	39.1	39.2
×64	13.9 units	28.1 units	19.6	19.7	19.7

Gas sample concentration—0.84 mg. per cent

Tonometer gas content—0.40 mg./48 ml.

The peak height (33.7 at × 8) is divided by the previously extrapolated calibration factor for that sensitivity (157.0) to give the total ether (0.21) in the 25 ml. sample of tonometer gas. This multiplied by 4 will determine diethyl ether concentration in the gas sample in mg. per cent. From this, mg./ml. is determined and the content of the 48 ml. of tonometer gas calculated (0.40) mg.

The ether content of the original blood sample may be determined from the following formula:

$$Q_{gas} = \frac{E \times V_{gas}}{V_{gas} + SV_{liquid}}$$

where Q_{gas} is the amount of ether in the gas phase after equilibrium has been reached in the tonometer; E is the total amount of ether in the pre-equilibrated sample of blood; V_{gas} is the volume of gas admitted to the tonometer; S is the solubility coefficient of ether in blood at the temperature of the tonometer (See fig. 2); V_{liquid} is the blood sample volume

$$\therefore \quad E = Q_{gas} \left[\frac{V_{gas} + SV_{liquid}}{V_{gas}} \right]$$

$$E = 0.40 \left[\frac{48 + 31.60(2)}{48} \right]$$

$$E = 0.93 \text{ mg./2 cc. aliquot}$$

$$E = 46.5 \text{ mg. per cent.}$$

EVALUATION OF METHOD

Aliquots of ether-blood standards were analyzed. These standards were established from a saturated ether-saline solution, prepared as follows:

One liter of physiologic saline solution was sealed in a chemically clean bottle with a three-holed stopper. A centigrade thermometer, an air relief glass tube extending within 1 inch of the bottom of the bottle, and a syringe adapter fitted with a B-D Luer-Lock stopcock, were inserted into the stopper openings. Fifty milliliters of ethyl ether were added to the saline and shaken for 20 minutes. Release of excess pressure was frequently necessary during this period. An additional 50 ml. of ether was then added and shaking continued for another 10 minutes.

At the end of this mixing period the bottle was inverted, effectively sealing it. A significant layer of ether was present on the surface of the saline-ether mixture, indicating saturation. A slight rise in temperature was generally noted by this time. When the solution had cooled to the temperature of the environment, the mixture was again shaken thoroughly and allowed to layer completely in the inverted position. This was repeated whenever the temperature of the solution changed.

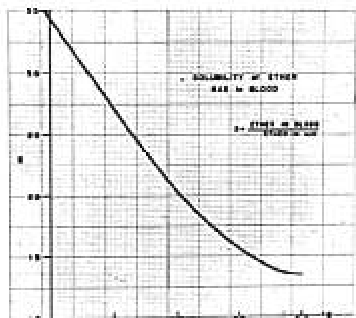

FIG. 2. The solubility coefficient of ethyl ether in blood as determined by Shaffer and Ronzoni.[6]

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/22/4/519/613808/0000542-196107000-00001.pdf by guest on 01 February 2023

If this is not done true saturation may not be assured.

An amount, dependent upon the final dilution desired (1: 10, 1: 25, 1: 50, etc.) was removed by syringe from the saturated stock bottle solution and transferred to an Ostwald-Van Slyke pipette. The solution temperature was carefully noted at this time, for the exact ether content is dependent upon the solubility coefficient at that temperature (table 2). A Van Slyke extraction chamber was employed as a volumetric flask. Delivery of the sample was made under mercury into the mercury-filled extraction chamber. A syringe, fitted with a rubber-tipped adapter, added a measured amount of saline, bringing the total volume in the chamber to 50 ml. Thorough mixing was ensured by gently raising and lowering the mercury reservoir bulb, producing a flushing movement between delivery syringe and the Van Slyke extraction chamber. Before doing so, however, the saline remaining in the adapter tip had to be replaced with mercury to prevent this additional saline dilution. Care had to be exercised that air be excluded from the system.

The blood-ether standard was prepared as follows, always in a 1: 10 dilution, from the ether-saline solution in the extraction chamber. Five milliliters of this solution were added, under mercury, to a second Van Slyke tonometer, also filled with mercury. Forty-five milliliters of freshly drawn blood were added and mixed as in the previous step with the ether-saline dilution. Great care was necessary to prevent foaming of the blood without anti-foaming agents.

Samples of ether-blood solution were analyzed in a consecutive series. Multiple aliquots were 2 and 3 ml. in size. The ether content, determined from the known solubility coefficient of ether in the above ether-saline solution, ranged from 25.4 to 168 mg. per cent. The average results are seen in table 3.

TABLE 2

SOLUBILITY OF ETHER IN PHYSIOLOGIC SALT SOLUTION*

Temperature of Solution, Degrees C.	Grams of Ether 100 ml. of Solution
0	13.0
5	11.2
10	9.5
15	8.1
20	6.9
25	6.0
30	5.3

TABLE 3

RESULTS OF CHROMATOGRAPHIC ANALYSIS OF ETHER-BLOOD SAMPLES

Number of Samples	Sample Size (ml.)	Std. Ether in Blood (mg. %)	Recovered Ether (aver. mg. %)	Diff. from Std. Ether (aver. mg. %)
4	2	25.4	23.79	−1.61
4	3		24.89	−0.51
3	2	26.8	26.97	+0.17
3	3		26.60	−0.20
4	2	35.7	35.35	−0.35
4	3		35.34	−0.36
6	2	36.0	35.71	−0.29
6	3		36.47	+0.47
4	2	49.5	46.70	−2.80
4	3		45.98	−3.52
4	2	52.4	52.36	−0.04
4	3		52.52	+0.12
4	2	54.56	50.71	−3.85
4	3		51.67	−2.89
5	2	57.0	56.92	−0.08
4	3		54.73	−2.27
4	2	114.0	114.27	+0.27
4	3		113.23	−0.77
4	2	150.0	149.66	−0.34
4	3		150.53	+0.53
4	2	168.0	166.97	−1.03
4	3		168.56	+0.56

DISCUSSION

Statistical analysis of the 91 individual determinations included in table 3 indicate that the accuracy for any single value is ± 3.05 mg. (± 5.9 per cent) at the 95 per cent confidence limit, and ± 4.57 mg. at the 99 per cent confidence limit. However, multiple determinations of any single blood sample were found to be more reproducible than these confidence limits would suggest. There was a more consistent deviation from the predicted ether content than between

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/22/4/519/613808/0000542-196107000-00001.pdf by guest on 01 February 2023

duplicate determinations and, as such, suggests that some of the scatter in results is attributable to the technique in preparation of the standards. It is our belief that the chromatographic determination of ether in blood is even more accurate than these results indicate. Careful analysis of the data also indicates greater accuracy with blood ether levels over 100 mg. per cent.

The method of preparing standards was used for the speed and simplicity it afforded. It is useful for anyone planning to become practiced in the adaptation of gas chromatography to the analysis of ether in biologic fluids. The standard ether solution, however, must be prepared daily as we have found significant deterioration in the ether-saline mixture, even when stored in air-sealed containers.

Readily reproducible results may be obtained with this procedure if careful consideration is given to other details. It is essential to allow the chromatograph to warm up for not less than 45 minutes prior to use. The carrier gas pressure, the column temperature, and the detector current must be kept constant. It is also necessary to calibrate the chromatograph at the start of each day for we have found that a column of this type is subject to "wear" as evidenced by progressive shortening of the elution time, and increasing peak heights on successive calibrations. The active life of the column in the series reported was over 300 hours. Upon eventual replacement of the column, or source of power supply such as new batteries, it is well to allow the apparatus to run constantly for several days to achieve equilibrium. We have avoided this problem by replacing the batteries with the accessory Zener-regulated constant power supply.

A heat-evaporated solution of 15 grams of potassium oxalate and 5 grams of lithium oxalate in 100 ml. of distilled water (0.1 ml. per 10 ml. of blood sample) was found suitable to prevent clotting. A dry anticoagulant is essential, since any liquid added to the sample will alter the solubility coefficient. A preliminary sample of blood must be analyzed to determine any possible pre-existing ether concentration. It is also advisable to process a saline blank prior to a series of determinations for the detection of extraneous ether in the tonometer or in the column of mercury. This also emphasizes the necessity of thoroughly cleaning the apparatus with several washings of distilled water after each determination.

Although recorded peaks of water vapor are inconvenient, we have been unable to dry the air samples without also removing ether. It has been suggested that superimposed peak heights can be used for some analyses as the recordings appear to be additive. However, interpretation of such combined peaks is frequently inaccurate and best to be avoided. It is necessary to space sample analysis sufficiently to prevent the occurrence of simultaneous peaks.

Accuracy in the tonometer temperature determinations of the extraction chamber is most important since any error will significantly influence the solubility coefficient factor in calculating the results. It is also essential that air admitted to the tonometer for equilibration be of ambient pressure and only sufficient to bring the total chamber volume (liquid and gas) to 50 ml.

The standard gas mixtures provide a rapid, accurate, and convenient means of calibration. It has been suggested that the gas chromatograph can be calibrated with liquid ether samples. We found this to be impractical. Calibration becomes non-linear for liquid ether samples or for blood ether mixtures of excessive concentration.

While the accuracy of this method has been demonstrated with aliquots of 2 ml. and 3 ml. samples, spot checks with larger aliquots (5 ml., etc.) suggest similar results. However, it has been found that a sample of 1 ml. is too small for use in an extraction chamber of this type. Recovery of ether is incomplete with this size sample.

Summary

A method for the analysis of ethyl ether content in blood by gas chromatography is presented. The gas is extracted in the tonometer of a Van Slyke apparatus and, after column separation, is analyzed by thermal conductivity.

Results are obtainable within 15 minutes. The accuracy of the method, within the range

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/22/4/519/613808/0000542-196107000-00001.pdf by guest on 01 February 2023

of 25 to 175 mg. per cent, was found to be ± 3.05 mg. at the 95 per cent confidence limit, and ± 4.57 mg. at the 99 per cent confidence limit. A discussion of the method and its requirements is presented.

This study was supported in part by Research Grant H-2225 from the National Institutes of Health.

REFERENCES

1. Jay, B. E., and Wilson, R. H.: Adaptation of gas absorption chromatographic technique for use in respiratory physiology, J. Appl. Physiol. 15: 298, 1960.
2. Dressler, D. P., Mastio, G. J., and Allbritten, F. F.: Clinical application of gas chromatography to analysis of respiratory gases, J. Lab. Clin. Med. 55: 144, 1960.
3. Summers, F. W., and Adriani, J.: Gas Chromatography: analytical method for anesthesiology research, ANESTHESIOLOGY 22: 100, 1961.
4. Price, H. L., and Price, M. L.: Determination of diethyl ether in blood, ANESTHESIOLOGY 17: 293, 1956.
5. Criscuolo, D., Lee, W. L., Tarrow, A. B., and Ward, R. J.: Rapid determination of diethyl ether levels in blood, ANESTHESIOLOGY 20: 593, 1959.
6. Shaffer, P. A., and Ronzoni, E.: Determination of ethyl ether in air and in blood, and its distribution ratio between blood and air, J. Biol. Chem. 57: 741, 1923.
7. Jones, C. S., Baldes, E. J., and Faulconer, A., Jr.: Ether concentration in gas and blood samples obtained during anesthesia in man and analyzed by mass spectrometry, Fed. Proc. 9: 68, 1950.
8. Haggard, H. W.: Accurate method of determining small amounts of ethyl ether in air, blood and other fluids, together with determination of coefficient of distribution of ether between air and blood at various temperatures. J. Biol. Chem. 55: 131, 1923.
9. Adams, R. C.: Intravenous Anesthesia. New York, Paul B. Hoeber, Inc., 1944, p. 56.

OUR NEW ADDRESS

EDITORIAL AND BUSINESS OFFICES

ANESTHESIOLOGY

J. B. Lippincott Co.
East Washington Square
Philadelphia 5, Pa.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/22/4/519/613808/0000542-196107000-00001.pdf by guest on 01 February 2023