

RAPID DETERMINATION OF DIETHYL ETHER LEVELS IN BLOOD

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BLOOD ether levels have been determined most frequently by direct chemical methods requiring from one to twelve hours for completion.¹⁻³ Recently a mass spectrographic technique has been utilized for this determination.⁴ Although this method has shortened the time of analysis, there is still considerable delay in obtaining the data. It would be desirable to develop a procedure which would give quantitative results in minutes. The technique of infrared spectroscopy of heteroatomic gases has been found suitable for this purpose.

METHODS

Calibration of Infrared Analyzer. The instrument used was the Beckman LB-1 infrared gas analyzer utilizing an ether detector unit equipped with a microcatheter cell. An Esterline-Angus Model AW graphic ammeter was placed in series with the amplifier for permanent recordings. The detector unit is capable of measuring ether concentrations up to 12 volumes per cent.

We have calibrated the analyzer so that one volume per cent will result in maximum meter deflection. A 1 volume per cent ether mixture was prepared by allowing liquid ether to attain room temperature (20 C.) and pressure (750 mm. Hg) in a glass tonometer. The volumes per cent of ether in the gas phase was then calculated from partial pressure data.⁵ A 485 ml.-glass tonometer was partially evacuated, and the calculated amount of ether mixture necessary to produce a final mixture of one volume per cent ether was bled into the tonometer. For example, at 20 C. and 750 mm. of mercury ambient temperature and pressure, the ether saturation is 60 volumes per cent. One milliliter of this mixture diluted to 60 ml., or 8.08 ml. diluted to 485 ml. would produce a one volume per cent ether mixture.

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Transfer of the gas was accomplished by means of glass syringes fitted with metal 3-way stopcocks. Approximately 30 ml. of gas were required to attain a peak reading from the analyzer at flow rates of 500 ml./minute (standard flow rate) through the microcatheter. Various dilutions of the one volume per cent mixture were made to obtain the straight line curve shown in figure 1. During this

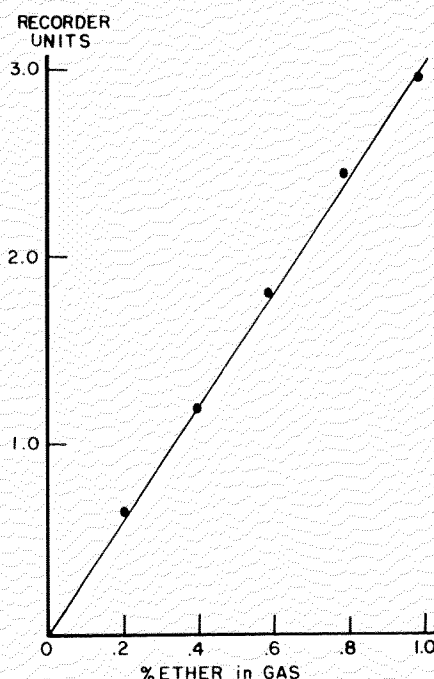


FIG. 1. Curve obtained by passing various dilutions of 1 per cent of ether in air through the infrared analyzer.

series of studies the curve was spot checked two or three times daily. This was most easily done by using the original 1 per cent ether mixture and observing the change, if any, from the original 1 per cent reading.

Blood Gas Extraction. Since ether is quite soluble in plasma, evacuation and/or heat would not release all of the dissolved ether. A "salting out" procedure was then devised to

obtain maximum extraction. Various salts were tried; potassium chloride, sodium chloride, potassium ferriyanide, sodium sulfate, and sodium hydrosulfite. Sodium hydrosulfite proved to be most efficient in releasing dissolved ether and the following procedure for extracting the ether was employed.

Materials: Sodium hydrosulfite ($\text{Na}_2\text{S}_2\text{O}_4$), mercury, glass tonometers of about 40 ml. capacity fitted with a stopcock at one end and a piece of rubber tubing at the other, and blood ether standards. The standards were prepared by diluting 1 ml. of ether to 100 ml. with blood in a volumetric flask. This gave a stock concentration of 714 mg. per cent. This stock solution was then serially diluted to give concentrations of 71.4, 57.1, 42.8, 28.6, and 14.3 mg. per cent.

Procedure:

1. Two milliliters of mercury were pipetted into a 42 ml. tonometer to facilitate subsequent mixing.
2. Two grams of sodium hydrosulfite powder were placed into the tonometer.
3. The tonometer was evacuated by attaching to a vacuum source and clamping the tubing with a hemostat.
4. Using a volumetric pipette 1 ml. of the blood to be extracted was transferred to the tonometer by inserting the pipette into the rubber tubing. The transfer was accomplished by placing the index finger over the pipette to maintain as much vacuum as possible and then opening the hemostat. Complete draining was allowed and the tubing resealed.
5. The mixture was shaken to produce a film over the inner surface of the tonometer.
6. The tonometer was placed in an 80–90 C. water bath for one minute and then cooled to room temperature by immersing the tonometer in a pan of water maintained at room temperature.
7. The gas mixture was brought to ambient pressure with room air and then transferred to a 30 ml. syringe by attaching the tonometer to a rubber tubing leading to a mercury-filled leveling bulb and allowing the gas to be displaced by the mercury.
8. The gas mixture was then evacuated from the syringe through the detector unit at a flow rate of 500 ml./minute.
9. The meter deflection was permanently recorded on the recording ammeter.
10. Blank blood samples were run for each separate blood to determine any inherent interference and the readings (range 0–0.2) obtained were subtracted from the sample readings.

Calculation:

The percentage of ether in the gas phase was obtained by the use of the curve in figure 1. By multiplying the per cent ether by the known gas volume of the tonometer (39 ml.) the total ml. of ether vapor present could be determined. To convert ml. of ether vapor to mg. of ether, the

Avogadro principle was used, with corrections for temperature and pressure.

The calculation then became:

$$22.4 \text{ (gram-mole volume)} \\ \times \frac{760}{750} \times \frac{293}{273} = 24.361 \text{ liters}$$

One gram-mole of ether (74.12 gm.) now occupies 24.361 liters or $74.12/24.361$ equals 3.043 mg. of ether per milliliter of gas at these conditions. Then finally: (gas volume) (per cent ether/100) \div (3.043) = mg. of ether/ml. blood. As an example of the complete calculation, with a meter reading of 1.66 minus a blank reading of 0.2 which was equivalent to 0.50 per cent ether (from fig. 1); the calculation became:

$$(39) (.5/100) (3.043) = .5936 \text{ mg./ml.} \\ \text{or } 59.36 \text{ mg. per cent.}$$

Ten samples of each known concentration were analyzed with the results shown in table 1 and figure 2.

TABLE 1
RECOVERY OF ETHER ADDED TO BLOOD

Mg. % Ether Added	Mg. % Ether* Recovered	Per Cent Recovery
71.4	59.2 \pm 1.86	82.9
57.1	47.3 \pm .75	82.8
42.8	35.8 \pm .81	83.6
28.6	23.9 \pm .63	83.6
14.3	12.1 \pm .63	84.6
Mean and S.D.		83.5 \pm .68

* Mean of 10 determinations plus standard deviation.

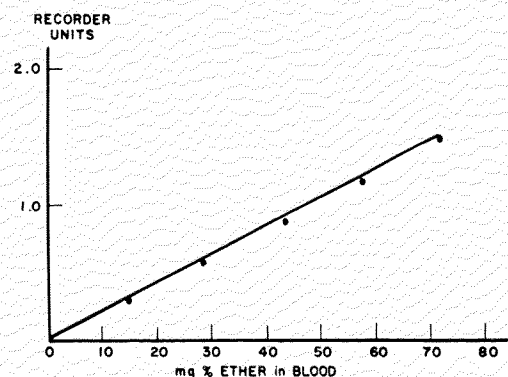


FIG. 2. Curve obtained by plotting known concentrations of ether in blood against recorder units.

Clinical Application. Since the percentage of recovery was consistent at all concentrations, the method was reduced to the extraction of ether from the blood sample, as previously

described, subtracting its blank value and calculating the unknown sample concentrations from a curve derived from a set of three or more known standards. The procedure then consisted of the following four steps: (1) Extract and plot the meter readings (minus their blank) of three or more known standards. (2) Extract the unknown blood samples and record meter readings. (3) Extract the unknown blood blank samples. (4) Subtract the blank blood reading from the sample reading and determine the ether concentration from the standard curve.

Nine unknown samples were obtained from patients anesthetized with ether. The samples were collected in heparinized syringes and kept on ice until analyzed. These samples were also used to determine the accuracy of the method as compared to the chemical procedure of Price and Price.³ The values obtained by the infrared method and the chemical method are shown in table 2 and figure 3.

RESULTS

In the extraction of ether from blood by the "salting out" technique, 5 known standards at various concentrations below 100 mg. per cent gave a consistent percentage of recovery; 82.9, 82.8, 83.6, 83.5, and 84.6 (table 1). The milligrams recovered, when plotted against recorder readings, resulted in a straight line curve (fig. 2). The 9 unknown samples used in the clinical procedure gave 98.0 ± 1.0 per cent recovery when compared to the chemical

TABLE 2
PER CENT OF BLOOD ETHER IN NINE PATIENTS

Sample	Recorder Units		Infrared Analysis (mg. %)	Chemical Analysis (mg. %)	Per Cent Recovered
	Sample	Blank			
1	2.02	.15	106.8	109.1	97.9
2	1.08	.15	57.1	58.3	98.0
3	2.67	.15	141.2	143.1	98.7
4	1.90	.10	100.5	103.1	97.4
5	2.13	.15	112.6	113.2	99.5
6	1.59	.15	84.1	84.7	99.3
7	0.25	.15	13.2	13.6	97.1
8	0.85	.15	45.0	46.9	95.8
9	1.60	.15	84.6	86.3	98.0
Mean			82.8	84.3	98.0 ± 1.0 *
Standards					
142.8 mg. %	2.80	.20			
71.4 mg. %	1.40	.20			
35.7 mg. %	.65	.20			

* Standard deviation.

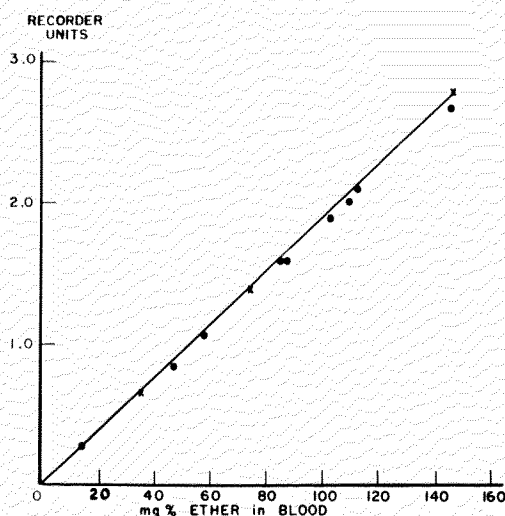


FIG. 3. (x) represents known concentrations of ether plotted against recorder units. (●) represents chemical determinations of nine unknown samples plotted against recorder units.

procedure of Price and Price (table 2 and fig. 3).

DISCUSSION

Although 100 per cent recovery is not obtained by this extraction procedure, the consistency of recovery lends itself to quantitative analysis. This is further substantiated by the fact that a straight line results from plotting the mg. of ether found against the recorder readings. When consistent incomplete extraction is offset by the use of standards which have this same technical disadvantage, an apparent 98 per cent recovery is obtained. This is demonstrated by the nine determinations used in the clinical procedure described.

We have found that daily plotting of the standard curve is advisable since meter readings are not absolutely reproducible from day to day. After a standard curve is produced for any particular group of blood samples and a straight line obtained, occasional checking at one point of the curve will assure constancy of the curve. In our experience with the procedure, standards will remain stable for one week if kept under refrigeration or on ice while in use.

In performing a recovery study, such as is described in Blood Gas Extraction above, particular attention must be taken of ambient

temperature and pressure. The clinical procedure eliminates, to a great extent, the influence of these two factors, since the unknown and the standards are analyzed within a short time of each other.

The inherent "background" interference of the blanks, from 0-0.20 recorder unit, is probably due to the release of carbon monoxide from the blood. The absorption peak of carbon monoxide is close enough to that of ether to cause cross-interference.⁷ This is the result of commonly shared absorption bands between the specific gas being measured and the diluent gas or gases. Before utilizing infrared spectroscopy in analysis of any heteroatomic molecule, precautionary measures should be taken to rule out cross-interference. This may be done by passing the diluent gases through the specific detector unit and noting the deflection, if any. If the diluent gases cause a deflection, then a method must be devised for separating the interfering gases from the specific gas to be measured.

Nitrous oxide, carbon dioxide, and carbon monoxide were the heteroatomic gases present in the series of analysis performed on patients receiving ether anesthesia. The first two gases mentioned, nitrous oxide and carbon dioxide, were passed through the detector unit and no cross-interference were noted. The cross-interference of carbon monoxide was measured by the use of the blank blood samples. The close agreement of the chemical determinations and the infrared spectroscopy values substantiates the lack of cross-interference. The phenomena of collision broadening must also be considered since there are diluent gases present with the specific gas being analyzed.⁷ Infrared spectroscopy measures the absorption of infrared radiation by atoms vibrating in the frequency range of the infrared portion of the electromagnetic spectrum. The vibration of these atoms is a function of several physical phenomena, one of which is collision between molecules of dissimilar gases. Collision broadening causes an increase in the total amount of infrared absorption by the heteroatomic molecule being analyzed, thus resulting in a falsely high value. The concentrations at which ether is found in the blood during anesthesia are below the concentrations at

which collision broadening phenomena exhibits a significant effect. This is apparent by the fact that the values obtained by the infrared analysis of known blood ether samples never exceeded the known concentrations present.

The determination of blood ether levels by means of extraction and infrared spectroscopy is simple and sufficiently accurate for clinical and research problems. At low concentrations (*i.e.*, during induction or recovery) an increase in accuracy may be obtained by increasing the quantity of blood sample to be analyzed.

SUMMARY

Ether dissolved in blood may be determined quickly and accurately by employing sodium hydrosulfite as a "salting out" agent. The resulting ether mixture can then be measured in an infrared analyzer and quantitated. Nine infrared determinations of unknown concentrations resulted in a 98 per cent recovery when compared to the chemical method of Price and Price.

This paper represents the personal viewpoint of the authors and is not to be construed as a statement of official Air Force policy.

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