Laboratory Method

Gas Chromatographic Analysis of Inhalation Anesthetics in Whole Blood by an Equilibration Method

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A simple gas chromatographic procedure for the quantitative analysis of the inhalation anesthetics in blood was devised. A blood sample was equilibrated in 10 ml. or 20 ml. glass vial. After equilibration 1 ml. of the gas in the vial was introduced into a gas chromatograph and the anesthetic concentration in blood was measured from that in gas phase by means of a calibration curve prepared from appropriate standards. A thermal conductivity detector was used for the analysis of nitrous oxide, cyclopropane and ether, while a flame ionization detector was used for halothane, methoxyflurane and low concentration of ether. Concentrations in blood of inhalation anesthetics now available can be measured accurately by this method.

WITH the recent development of gas chromatographic techniques, analysis of inhalation anesthetics in the gaseous phase has become more rapid and accurate than with other chemical or physical methods. Analysis of anesthetics in the liquid phase, however, is not easily performed by gas chromatography. While many methods have attempted to extract the gas from the liquid, they are tedious and time-consuming and no ideal method has been established. Thus, we have devised a new method which is simple and reliable for the quantitative analysis of inhalation anesthetics in blood.

Method

When an anesthetic in blood is in equilibrium with the overlying gas phase, the anesthetic concentration in blood can be calculated from the concentration in the gas phase if the blood-gas partition coefficient is known. The data on the blood-gas partition coefficients of the several anesthetics, however, differ widely from one investigator to another, so they can not be utilized for this calculation. Therefore, we have made several standard solutions containing anesthetics of known concentration in blood and a calibration curve was obtained from these standards. The blood sample of unknown anesthetic concentration was treated under the same conditions as the standard solutions and concentration measured by means of the calibration curve. With this method ether, halothane, methoxyflurane, nitrous oxide and cyclopropane in blood were analyzed.

Preparation of Standard Solutions

ETHER. One milliliter of blood from the patient, taken before anesthesia, was injected into each of six 20-ml glass vials through silicon rubber caps. One milliliter of aqueous solution containing 2.0, 1.8, 1.6, 1.4, 1.2 and 1.0 mg. of ether, respectively, was added to each vial. These standard solutions were made in quadruplicate.

HALOTHANE. Two milliliters of aqueous mixtures containing halothane ranging from 10 mg. to 20 mg. per 100 ml. (10, 12, 14, 16, 18, 20 mg. per cent) were prepared in quadruplicate in six 10-ml. vials with silicon rubber caps.

METHOXYFLURANE. Two milliliters of aqueous mixtures containing methoxyflurane ranging from 5 mg. to 10 mg. per 100 ml. were similarly prepared.

NITROUS OXIDE. Before anesthesia 1 ml. of the patient's blood was injected into each of five 10-ml. glass vials through silicon rubber caps and 1 ml. of gas mixtures of nitrous oxide and oxygen ranging from 10 to 50 volume per

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GAS CHROMATOGRAMS OF
ETHER, HALOTHANE AND METHOXYFLURANE (F.I.D.)

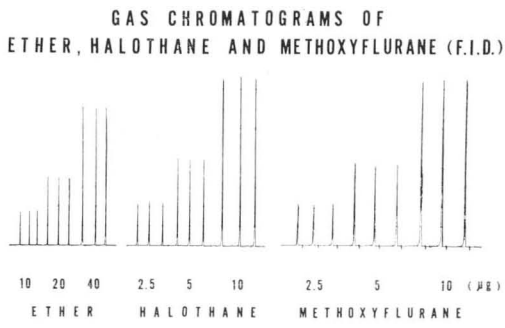| 10 | 20 | 40 | | 2.5 | 5 | 10 | | 2.5 | | 5 | | 10 | (μg) |
| ETHER | | | | HALOTHANE | | | | METHOXYFLURANE | | | | | |

FIG. 1. Linear relations observed between the amount of anesthetic and detector response.

cent was added. The concentration of nitrous oxide in the gas mixtures had been checked beforehand by gas chromatography. These were also prepared in quadruplicate.

CYCLOPROPANE. These were similar to those of nitrous oxide. However, 5 to 10 volumes per cent of cyclopropane in oxygen were used.

Calibratoin Curve. Vials containing the standard solutions were manually shaken, then placed in a constant temperature chamber at 25° C. for one to two hours, since at this or lower temperatures no untoward effect of the water vapor was observed. When complete equilibrium of the anesthetic between gas and liquid phase was obtained, 1 ml. of the gas from the upper portion of each vial was withdrawn into a syringe of convenient size and injected into a gas chromatograph. The calibration curve was obtained by plotting the

peak height against calculated mg. of anesthetic per 100 ml. of solution. The gas chromatographs used in this experiment were an Okura flame ionization-type gas chromatograph and GC-2B-type Shimazu gas chromatograph. Operating conditions are shown in table 1. The range of linear response of a flame ionization detector is largely influenced by the structure of the detector itself and the type of amplifier.[1-3] As shown in figure 1, we obtained a highly reproducible and linear response over the range necessary for our method of analysis.

Measurement of Anesthetic Concentration in Unknown Blood Sample. In order to analyze nitrous oxide or cyclopropane, 1 ml. of the blood containing an unknown amount of anesthetic was injected into a glass vial of the same size as that of the standard. In the case of volatile anesthetics, 1 ml. of distilled water was injected beforehand into a vial, then 1 ml. of blood sample was added. After equilibration, 1 ml. of gas sample from the upper portion of the vial was withdrawn into a syringe and introduced into the gas chromatograph. The anesthetic concentration in the blood sample was determined by comparing peak height with that of the calibration curve obtained from corresponding standards. The accuracy of these results was confirmed by comparing the above values with those obtained by the "trap" method reported by Yamamura and his co-workers,[4] using the same blood samples (table 2).

TABLE 1. Gas Chromatographs Used and Operating Conditions

Anesthetics	G C	Columns		Temp.	Flow Rate of Carrier Gas
Nitrous oxide		Activated charcoal	1 m.	35° C.	100 ml./min.
Cyclopropane	Shimazu GC·2B (Det. curr. 300 mA.)	25% DOP on celite	3 m.	22° C.	50 ml./min.
Ether		25% PEG on celite	2 m.	60° C.	60 ml./min.
Cyclopropane			2 m.	24° C.	
Ether	Ohkura flame ioniz. G C	25% PEG on celite	1.5 m.	40° C.	N₂ 50 ml./min. H₂ 50 ml./min. Air 0.4 l./min.
Halothane			1.5 m.	60° C.	
Methoxyflurane			1 m.	80° C.	

Results

As shown in figure 2, calibration curves were rectilinear over the range of clinical anesthetic concentrations and a high degree of correlation was observed between the anesthetic concentration in blood and that in the equilibrated gas phase. Table 2 shows a comparison of the analytical results obtained by this method and the "trap" method on the same blood sample. The results of two methods are almost identical.

Technical Considerations

Accuracy of the Amount of Anesthetic Injected Into the Vial. After injection of the liquid anesthetic from a syringe into a vial, weight gain of the vial and weight loss of the syringe were compared. The differences were less than 2 per cent of the total amount injected, even in the case of ether, the most volatile anesthetic. From these results we determined the amount of anesthetic injected into a vial from the loss of weight of the syringe. For the gaseous anesthetics, a 1,475-ml. glass bottle was filled with an anesthetic gas and oxygen mixture. After gas chromatographic determination of the anesthetic concentration, 1 ml. of the mixture was introduced into a 10 ml. empty glass vial, and the product of the anesthetic concentration and gas volume in the vial was noted to approximate closely the total amount of the anesthetic injected. We also noted that the reproducibility of the volume of gas injected with a syringe was quite satisfactory.

Homogeneity of the Anesthetic Solution. In preparing the standard solution, the ether-water solution was found to be homogenized after stirring with a magnetic stirrer for one hour. In the case of halothane and methoxyflurane, a blood-water mixture was better than water as a solvent because the former produced a more homogeneous anesthetic solution.

Change of Anesthetic Concentration During Shaking. In order to measure the amount of anesthetic absorbed in the silicon rubber cap or adsorbed to the wall of a glass bottle during shaking, a fixed amount of solution was extracted and the anesthetic concentration determined every 30 minutes up to 3 hours. No change of anesthetic concentration was
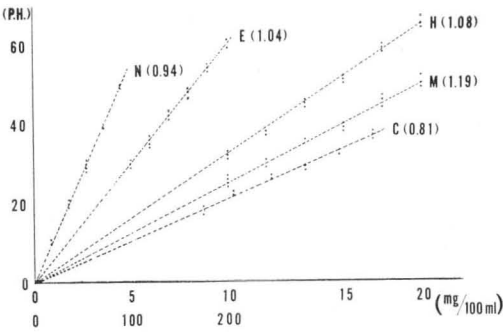

FIG. 2. Calibration curves for ether, halothane, methoxyflurane, nitrous oxide and cyclopropane. The upper figure along the horizontal axis represents concentration (mg./100 ml.) for halothane, methoxyflurane and cyclopropane, the lower figures for nitrous oxide and ether. Figures in brackets show the standard error of the means. N = Nitrous oxide, E = Ether, H = Halothane, M = Methoxyflurane, C = Cyclopropane, P.H. = Peak Height.

found with the time. As shown in table 3, ether content in the gas and liquid phases were measured after complete equilibrium was attained and the sum was found to coincide closely with the amount of ether injected into the vial.

Change of Anesthetic Concentration During Equilibration. As shown in figure 3, the ether concentration in the gas phase reached a maximum about 30 minutes after injection of the blood sample into the vial and a constant value was observed for five hours thereafter. With halothane and methoxyflurane some decrease in anesthetic concentration in the gas phase was observed after a maximum level was once attained; but a relatively constant value was obtained with halothane from

TABLE 2. Comparison of Values Obtained by Equilibration Method and "Trap" Method, on the Same Blood Samples (mg./100 ml.)

No.	N₂O		Cyclo.		Ether	
	Equil.	Trap	Equil.	Trap	Equil.	Trap
1	33	37	8	9	80	90
2	41	40	10	10	118	110
3	51	53	13	12	135	130
4			15	15	142	149
5			16	16	178	171
6			18	18	185	191

TABLE 3. Comparison of Amounts of Ether
Before and After Equilibration
(Expressed in Milligrams)

Before	After		
Inj.	Gas	Liq.	Total
2.00	0.54	1.55	2.09
2.40	0.63	1.74	2.37
2.80	0.73	2.03	2.76
3.20	0.83	2.29	3.12
3.60	0.92	2.85	3.77
4.00	1.01	3.03	4.04

Inj. = amount injected into the vials. Gas = amount of ether in the gas phase. Liq. = amount of ether in the liquid phase.
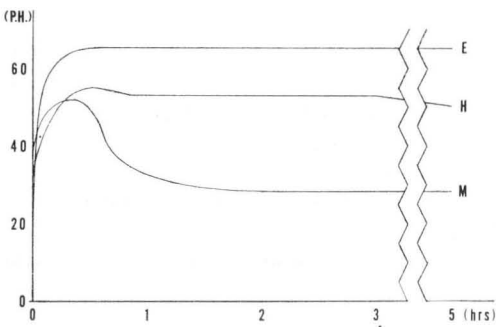

FIG. 3. Change of anesthetic concentration in the gas phase plotted against time after the blood samples were injected. E = Ether, H = Halothane, M = Methoxyflurane.

one to three hours after injection and with methoxyflurane from two to five hours. The observed decrease in anesthetic concentration in the gas phase during equilibration may be due to adsorption of anesthetic vapor to the glass or absorption into the rubber cap.[5, 6] So long as the blood sample of unknown concentration is treated in the same manner as the standard solution, no gross error can be expected from the equilibration technique. In the case of nitrous oxide or cyclopropane no change of anesthetic concentration in the gas phase was observed from ten minutes to three hours (fig. 4). Table 4 shows a comparison of the total amount of nitrous oxide and cyclopropane, before and after the equilibration, respectively. Anesthetic concentration in the liquid phase was determined by the "trap" method.

Effect of Pressure Change Within the Vial. The pressure in the vial increased after injection of the blood sample causing leakage of anesthetic from the syringe at the time of gas sampling, thereby resulting in an underestimation of the actual anesthetic concentration in the blood sample. On the other hand, in order to keep the pressure in the vial constant, the use of a manometric apparatus is cumbersome and unpractical. We then compared the concentration of anesthetic in the gas phase in the vials which had the same amount of anesthetic, under various pressures.

In group A in table 5, 1 ml. of air in the vial had been withdrawn before injecting the blood sample. In group B, the gas in the upper portion of the vial was allowed to escape momentarily through a small bore needle to keep the pressure at atmosphere, just after in-

TABLE 4. Comparison of Amount of Cyclopropane and Nitrous Oxide, Before and After Equilibration (Expressed in Milligrams)

Sample No.	Cyclopropane				Nitrous Oxide			
	Before	After			Before	After		
	Inj.	Gas	Liq.	Total	Inj.	Gas	Liq.	Total
1	0.175	0.165	0.008	0.173	1.00	1.00	0.07	1.07
2	0.158	0.150	0.007	0.157	0.90	0.86	0.06	0.92
3	0.140	0.135	0.006	0.141	0.80	0.70	0.06	0.76
4	0.123	0.120	0.006	0.126	0.70	0.60	0.04	0.64
5	0.105	0.105	0.005	0.110	0.60	0.52	0.04	0.56
6	0.088	0.080	0.004	0.084	0.50	0.46	0.03	0.49

Inj. = amount injected into the vials. Gas = amount of anesthetics in the gas phase. Liq. = amount of anesthetics in the liquid phase.

TABLE 5.   Comparison of Anesthetic Concentrations in the Gas Phase of the Vials With the Same Amount of Anesthetics at Various Pressures (Expressed in Peak Heights)

	Ether	Halothane	Methoxy-flurane	Nitrous Oxide		Cyclopropane	
	Blood Sample	Blood Sample	Blood Sample	Blood Sample	Gas Sample	Blood Sample	Gas Sample
A	39	52	32	25	21	28	33
B	38	52	31				
C	38	53	33				
D	38						
E	36	49	31	25	21	28	31

Each value is the mean of four determinations.

jecting the blood sample. In group C, the gas from the upper portion of the vial was allowed to escape to the atmosphere after equilibrium was once attained, and then re-equilibrated. In group D, by removing the gas as the pressure rose, the pressure was maintained at atmosphere throughout equilibration. In group E, no pressure regulation was attempted; thus, equilibration was performed under pressure.

With any of the volatile anesthetics examined, no significant differences were observed among the peak heights of group A, B, C and D, while a slight decrease in peak height was observed in group E. From these results we may conclude that the most simple and practical method is to allow the gas from the upper portion of vial to escape momentarily just after the injection of the blood sample, then to equilibrate for one to two hours at 25° C. Although some of the anesthetic vapor may escape with this procedure, it is not enough to affect the analytical results. In the case of the gaseous anesthetics, pressure change is not as important in obtaining an accurate result.

The Effect of Change in the Blood Components. Since the principle of the equilibration method is based on the blood-gas partition coefficient of the anesthetic, the result of analysis will be affected if the components of a blood sample are changed and this causes a fluctuation in the value for the blood-gas partition coefficient.[7-13] We have examined the effects of hematocrit on anesthetic concentration in the gas phase and found no uniform fluctuation in peak height, as shown in table 6.

Discussion

Among the extraction procedures for a gas dissolved in blood, solvent extraction, distillation, vacuum extraction, bubbling and equilibration methods have been employed. Although these methods have been applied to the gas chromatographic analysis of the anesthetics in blood, they are tedious and time-
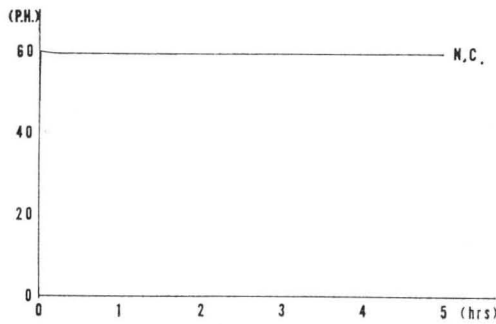

FIG. 4. Change of anesthetic concentration in the gas phase plotted against time after the gas samples were injected. N = Nitrous oxide, C = Cyclopropane.

TABLE 6. The Relation Between the Hematocrit Values and Anesthetic Concentration in the Gas Phase (Expressed in Peak Heights)

PH \ Ht	N	C	E	H	M
70	23	59	32	42	30
50	23	58	31	41	31
30	25	60	31	41	34
Water	24	59	25	45	44

Each value is the mean of four determinations. N = Nitrous oxide, C = Cyclopropane, E = Ether, H = Halothane, M = Methoxyflurane.

consuming. Besides, some are not as suitable for the extraction of the volatile as for the gaseous anesthetics.

Recently with the development of flame ionization detectors, a direct injection method has been attempted without the previous extraction procedure.[14-16] But many problems must be settled before this method can be accepted. These include the error of the injection volume owing to handling of small samples, treatment of water and contamination of the column. Of the extraction procedures, the equilibration method is considered to be the most readily adaptable for the gas chromatographic analysis. Various equilibration methods have been reported,[17-20] but none have achieved wide acceptance for a variety of reasons.

Many blood samples can be analyzed in a few hours by our method employing several common analytical tools including glass vials, glass bottles and syringes. By selecting vials of proper size, the influence of the change in blood components of a sample and in the pressure in the vial can be minimized, resulting in accurate determinations. In this study we employed five inhalation anesthetics. They cover a wide range of physical characteristics, such as boiling point, solubility in blood and oil, so that it is likely that our equilibration technique can be applied to other anesthetic agents as well.

Summary and Conclusions

A simple gas chromatographic procedure for the quantitative analysis of inhalation anesthetics in blood has been devised. A blood sample was equilibrated in a 10 ml. or 20 ml. glass vial. After equilibration, 1 ml. of the gas from the upper portion of vial was introduced into a gas chromatograph and the anesthetic concentration in blood determined from that in the gas phase, using a calibration curve.

A thermal conductivity detector was used for the analysis of nitrous oxide, cyclopropane and ether, while a flame ionization detector was used for halothane, methoxyflurane and low concentration of ether.

A close relation was observed between our results and that obtained by a "trap" method on the same blood samples of ether, nitrous oxide and cyclopropane. With ether, nitrous oxide and cyclopropane, no significant loss of anesthetic was observed during equilibration. With halothane and methoxyflurane, although anesthetic concentration in the gas phase decreased with lapse of time during equilibration, fairly accurate determinations can be expected if the unknown blood sample is treated in the same manner as the standard solutions.

The inhalation anesthetics now available can be simply and accurately analyzed by this method.

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P_{CO_2} and P_{O_2} in whole blood, J. Appl. Physiol. 11: 245, 1957.

TOXIC HEPATITIS Fatal hepatic necrosis following halothane anesthesia in 5 patients is reported. In addition, four instances of nonfatal postoperative jaundice after halothane anesthesia were observed. In 3 of the 5 patients who died, the anesthetic was administered for a second time in intervals ranging from three weeks to nine months. The earliest appearance of hepatic damage in the fatal instances occurred in four days and the longest interval between exposure and symptoms was eight days. The gross and microscopic findings were remarkably similar in all the fatal instances. The syndrome of unexplained postoperative malaise, fever and jaundice accompanied by significant serum glutamic-oxaloacetic transaminase elevation, occurring early in the postoperative period in all these patients, is presumptive if not diagnostic evidence of hepatocellular damage. While specific conclusions cannot be drawn as to a causal relation, the sequence of events is suggestive of such an association. (*Morgenstern, L., Sacks, H. J., and Marmer, M. J.: Postoperative Jaundice Associated with Halothane Anesthesia, Surg. Gynec. Obstet. 121: 728 (Oct.) 1965.*)

TOXIC HEPATOSIS Five incidents of toxic hepatosis following halothane anesthesia occurred within the last two years. Two patients died in hepatic failure and 3 died of other causes but had microscopic evidence of liver damage including focal necrosis. The failure may be explained by postulating a special "affinity" of halothane to the liver or on the basis of specific sensitivity of a given individual. The term "toxic hepatosis" represents a separate entity and can be differentiated from liver damage of other origin. Despite the vague clinical symptomatology of liver damage attributable to halothane, the morphological appearance, when correlated with the patient's history, seems to be typical. Histological evaluation by liver biopsy is usually possible. On the other hand, the morphological differential diagnosis later in the course of a liver disease is difficult. (*Klinge, O.: Toxic Hepatosis Associated with Halothane Anesthesia, Klin. Wschr. 43: 1042 (Oct.) 1965.*)