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Laboratory Note

A Rapid Direct-injection Method for Measuring Volatile Anesthetics in Whole Blood

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A direct-injection gas chromatographic method for the measurement of volatile anesthetics in samples of whole blood is described. The method is simple, uses small samples, has a coefficient of variation of less than 2 per cent, and can be used for on-line monitoring of blood anesthetic levels in the operating room and laboratory.

GASES SAMPLED from patients receiving inhalation anesthetics can be rapidly analyzed with ease and accuracy by gas chromatography. However, an equally satisfactory method for the analysis of volatile anesthetics in whole blood is not as readily available.[1, 2] This paper presents a direct-injection method which is as rapid and simple as gas analysis and produces reproducible symmetrical peaks.

Methods

A gas chromatograph † equipped with a flame ionization detector was used with a 1-millivolt recorder and disc integrator. The injection port was modified to accept pyrex glass liners. Temperatures of injection port, column, and detector were 100, 40, and 120 C, respectively.

Columns made of ⅛-inch stainless steel were packed with 100–120-mesh Chromosorb-W solid support and O.V. 101 liquid phase. For methoxyflurane analysis, a 6-foot column with 10 per cent O.V. 101 was used, and for halothane a 10-foot column with 5 per cent O.V. 101. The latter column was also used for analyses of diethyl ether, fluroxene, and trichloroethylene.

Carrier gas flow rates were 30 ml/min of nitrogen for methoxyflurane analysis and 25 ml/min of nitrogen for halothane. Hydrogen and air flow rates were 30 and 300 ml/min, respectively.

ANALYSIS TECHNIQUE

Samples were placed in heparinized glass syringes and sealed with diaphragms which allowed repeated sampling without loss of agent.[3] Samples from small animals were collected in heparinized capillary tubes. A push-button Hamilton syringe (CR-700-20),‡ adjusted to deliver 1 μl, was used for injection. As soon as the needle point penetrated the septum, the syringe was triggered and rapidly withdrawn. It was then rinsed several times with distilled water.

CALIBRATION

One, three and five μl of liquid anesthetic were added to sealed syringes[3] each containing 20 ml of distilled water to make 14.3, 21.3, and 35.5 mg/100 ml water standards, respectively. Blood standards were prepared similarly, resulting in a calibration curve identical to that obtained with water standards. A calibration curve was drawn from both water and gas standards, and its linearity was taken as an indication of their accuracy (fig. 1). This curve was redrawn after each 25 blood analyses.

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† Varian Aerograph, model 1445, Varian Aerograph, Walnut Creek, California.

‡ Hamilton Company Inc., Whittier, California.

Results

Precision

Consistently linear calibration curves were obtained for methoxyflurane in whole blood over the concentration range of 1 to 40 mg/100 ml, and for halothane over the range of 6 to 50 mg/100 ml. Analyses of ten blood samples to which 0.355 mg/ml of methoxyflurane had been added gave a mean area of 903 ± 4.73 (SE), with a coefficient of variation of ± 1.7 per cent, a figure comparable to that found by Butler[1] and Fink[4] using equilibration techniques. Figure 2 shows a chromatogram of duplicate injections of methoxyflurane blood standard. Similar sharp peaks and precision were obtained with halothane; the water peak followed rather than preceded that of the anesthetic agent in this analysis (fig. 3).

Accuracy

The addition of known amounts of methoxyflurane to blood produced mean peak areas which fell on the calibration curve drawn from gas standards (fig. 1). Similar results were obtained with halothane, fluroxene, diethyl ether, and trichloroethylene. This indicated that close to 100 per cent of the anesthetic agent was recovered from the blood.

Speed of Determination

Retention time for methoxyflurane was 4 minutes; thus, 15 samples per hour can be analyzed. Analysis time for halothane, diethyl ether, fluroxene, and trichloroethylene was 3 minutes.

Discussion

During the past ten years, methods for the analysis of volatile anesthetics in blood have

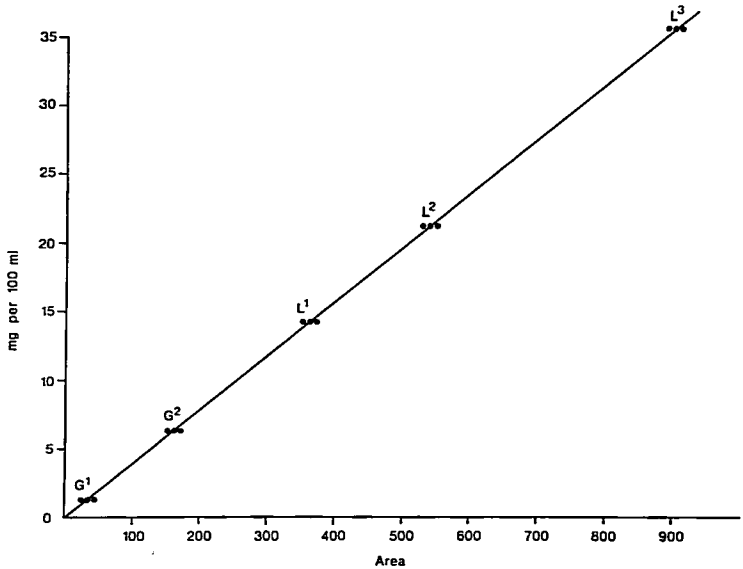

Fig. 1. Calibration curve drawn from both gas and water methoxyflurane standards. $G^1 = 0.22$ vol per cent (1.41 mg/100 ml); $G^2 = 1.0$ vol per cent (6.4 mg/100 ml); $L^1 = 14.3$ mg/100 ml; $L^2 = 21.3$ mg/100 ml; $L^3 = 35.5$ mg/100 ml.

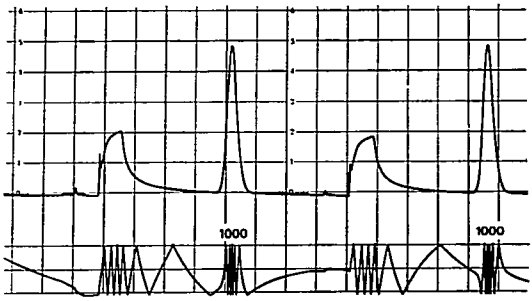

FIG. 2. Chromatogram of duplicate injections of methoxyflurane blood standard.

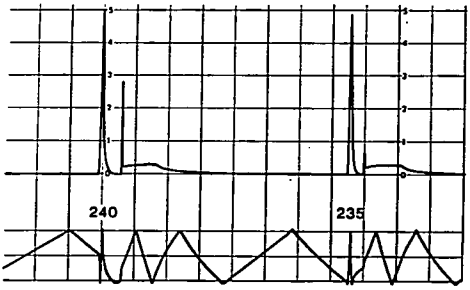

FIG. 3. Chromatogram of duplicate injections of halothane blood standard.

appeared almost yearly, attesting to the difficulty experienced with this analysis. Indirect methods, which require pretreatment of the blood sample, include: extraction techniques[5-7]; distillation[8,9]; use of a pre-column sample-vaporizing space[10]; and equilibration of blood with air.[1,2,4,11] Direct-injection techniques have also been developed.[12,13] Indirect methods cannot be used for small samples and involve potential loss of agent in the course of pretreatment. They also take more time and labor than direct-injection methods.

Direct-injection analysis of whole blood poses three main problems: interference from elution of water; production of multiple peaks at high temperatures; and contamination of columns with protein material. Water is eluted quickly on ethylvinylbenzene columns (Porapak Q); however, high injection-port and column temperatures may be necessary. High injection-port temperatures clog syringes and produce a forest of peaks from a sample of whole blood. A low-temperature direct-injection technique presently available requires periodic addition of water to the column, necessitating a relatively cool (50 C) injection port.[13] These temperatures may result in a broad peak, with tailing due to sequential release of anesthetic agent from serum, proteins, and erythrocytes.

The technique described in this report eliminates the problems mentioned above. Duplicate determinations can be performed with total samples as small as 50 μl. Since pretreatment is not necessary, a potential source of error due to handling is eliminated, as is the delay in obtaining results necessary for on-line adjustment of anesthetic requirements. The recently developed O.V. 101 (liquid methyl silicone) is a liquid at room temperature and

may be used as a liquid phase on one of the diatomaceous silica solid phases such as Chromosorb-W. At 40 C water is eluted rapidly from this column and can be made either to precede or to follow the volatile agent, depending upon column length and percentage of liquid phase. An injection-port temperature of 100 C is high enough to produce rapid elution of anesthetic agents when the sample is quickly spread over a large surface area, as can be done with the CR-700-20 syringe. In addition, a temperature of 100 C does not cause multiple blood peaks, nor does it clog syringes if injections are made rapidly. Baseline drift due to slow release of volatile blood components is negligible if liners are changed after every 25 injections.

The precision of the technique depends on achievement of a uniform injection of samples. The fixed setting of the push-button syringe provides a more reproducible sample size,§ and also allows more rapid injection than can be obtained with standard 1-μl syringes. The use of glass liners minimizes reaction of blood components with the surface of the injection port. The small piece of glass wool at the end of the liner prevents blood debris from reaching the column. Liners are reusable after cleaning.

This technique has now been used for more than 500 measurements of concentrations of volatile anesthetic agents in whole blood for on-line monitoring in the operating room and laboratory. In the hands of an operator with a basic knowledge of gas chromatography, the method can be quickly set up, and reproducibility is rapidly achieved.

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Addendum

The analysis time for methoxyflurane has been shortened to 2 minutes by substituting the synthetic solid support Chromosorb-G for Chromosorb-W. A precise and rapid injection may also be achieved by attaching the Hamilton 7101N 1-μl syringe to the Reproject apparatus (Shandon, Sewickley, Pa. 15143).

§ The teflon barrel plungers eventually wear and are replaced when injections of standards show a diminution of peak area.

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