Section of Anæsthetics

President A C Forrester FFA RCS

Meeting December 1 1967

Gas Chromatography

Wing Commander W T Fothergill
(Research Department of Anæsthetics, Royal College of Surgeons of England, London)

Technique

Gas chromatography is a method of separating the components of mixtures of gases and vapours; it is widely applied in analytical chemistry and is finding an increasingly useful place in physiological laboratories. The method is more versatile, more accurate, simpler to operate and less dependent upon technical skill than many of the old-established ways of identifying and measuring gases contained in biological fluids. Thus a chromatographic system suitably designed will identify and measure oxygen, carbon dioxide and nitrous oxide in less than fifteen minutes and just as readily, gas chromatography can be employed to measure levels of volatile anæsthetics and alcohol in blood and tissues.

The simplest concept of a chromatograph is to consider it as a molecular filter which selectively retards the passage of molecules according to certain physical characteristics such as shape, size, weight and boiling point. A mixture of gases, in the course of its passage through the chromatograph, will divide into its components and emerge from the distal end of the column in regular order, to be measured by a detector whose output may be recorded graphically or electronically.

In order to carry a sample through the chromatograph, a carrier gas is required. The choice of carrier gas is dictated both by the nature of the sample and by the type of detector to be used. The use of a thermal conductivity detector demands that the thermal conductivity of the carrier gas is widely different from that of the samples. Hydrogen and helium with relatively high thermal conductivities are used when the separation of physiological gases is required; helium is less reactive than hydrogen but its cost limits its use. Nitrogen may be used as a carrier gas when flame ionization detectors or gas density balances are used, if nitrogen detection is not required. Operating pressures for the carrier gas vary between 10 and 60 lb/in^2 (0·7–4·2 kg/cm^2) and accurate needle valves are required to control its flow.

Immediately proximal to the chromatograph column is the injection apparatus. This may take the form of a simple diaphragm, a gas sample loop or a blood/gas extraction device. A simple diaphragm may be used for the injection of liquid or gas samples from a syringe: the injection port must be heated to allow liquid samples to be instantaneously vaporized upon injection; slow vaporization of samples will give rise to unsatisfactory peak characteristics. Blood may be injected by this route if suitable cleaning methods (e.g. disposable glass liners) can be devised (Laasberg & Etsten 1965). Gas sample loops enable known fixed quantities of gas samples to be injected from either a static reservoir or a stream of sample gas (Theye 1964). Blood/gas extraction devices provide a method whereby dissolved and combined gases may be extracted from blood and then injected on to the column; to date, these have been either adaptions of Van Slyke chambers (Ramsey 1959, Lukas & Ayres 1961, Summers & Adriani 1961, Wilson, Jay, Doty *et al.* 1961, Wilson, Jay & Holland 1961, Johns & Thompson 1963, Yee 1965, Hill 1966) or gas bubble equilibration chambers (Bowes 1964). The gas liberated from the blood is swept on to the column by diverting the carrier gas stream through the reaction chamber or by any alternative method which will ensure rapid and complete injection.

The analytical process takes place in the chromatograph column, which is merely a length of tube (glass, stainless steel or copper, depending upon the reactivity of the sample components)

containing a packing material, known as the stationary phase. The stationary phase may be liquid (as in gas/liquid chromatography, GLC) or solid (as in gas/solid chromatography, GSC). Liquid absorbents may be dispersed on the wall of the column if capillary tubing is used or may be held upon the surface of an inert supporting material such as firebrick, Celite or Chromosorb if wider bore tubing is to be utilized.

The length of the column depends on the retention time required – the longer the column, the longer the retention time. Other factors concerned in retention time are oven temperatures and carrier gas flow rate. It is possible to calculate in advance, on a theoretical basis, the performance of any given column and thus to predetermine the length required (Kaiser 1963, Szymanski 1964) but in practice, especially when parallel columns are used and sample splitting is required, a process of trial and error is more satisfactory.

The sensing column may consist of one or more units each containing a different stationary phase, arranged in series with a detector between each unit or in parallel with one detector shared by both units. In the latter situation the length of each unit is adjusted to avoid overlapping of peaks.

The commonly used detectors are katharometers (which are thermal conductivity cells wired together to form a Wheatstone bridge) and flame ionization detectors. The latter are more sensitive but are unsuitable for the detection of oxygen, carbon dioxide and nitrous oxide. Argon ionization detectors have been recommended by Hill (1962) as being suitable for portable apparatus and gas density balances are suggested by Kaiser (1963). Electron capture detectors are also

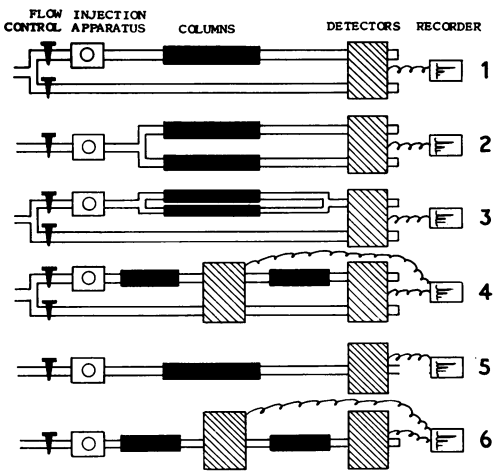

Fig 1 *Schematic diagram illustrating several possible methods of assembling the components of a gas chromatography system*

available in suitable form. When a katharometer is used it is important to ensure that carrier gas flow is equal across both the detector and reference arms of the bridge, in order to eliminate a common cause of baseline drift. Fig 1 demonstrates some of the possible variations of chromatograph assembly.

Quantification of sample components is more accurately achieved by measurement of peak areas which do not vary much with fluctuations of temperatures and carrier gas flow and are not dependent upon injection technique. However, some authors (Hamilton 1962, Theye 1964, Hill 1966) note that measurement of peak height is accurate if the peaks are tall and narrowly based.

The retention time of sample components and the peak characteristics will remain constant if variables such as carrier gas flow and pressure, injection technique, oven temperature and detector sensitivity are not altered.

Blood Gas Analysis

Ramsey (1959) appears to be the first to have used a chromatographic process to measure the gas content of biological fluids. He described a Van Slyke extraction process for use with plasma and reported very good reproducibility and accuracy, plus high degrees of sensitivity. Lukas & Ayres (1961) followed with a development of this work and succeeded in extracting gases from whole blood. More recently Hill (1966) has described the use of the Beckman blood/gas accessory (Johns & Thompson 1963) in association with silica gel, charcoal and Molecular Sieve sensing columns.

With the improvement of electrode determination of oxygen and carbon dioxide tensions in blood, little interest has been shown in measuring blood gas content; in particular very little of the work done on nitrous oxide anæsthesia has been related to blood gas levels, probably because of a lack of a reliable, simple method of blood gas analysis. It was decided, therefore, to attempt to design a chromatographic system which could be used routinely to determine blood concentrations of oxygen, carbon dioxide and nitrous oxide, with an analysis time of less than ten minutes. Accordingly, after prolonged trial and error, a gas chromatograph was assembled which was capable of splitting the gases in question. The instrument used was an F & M 700 gas chromatograph fitted with a gas sampling loop and a thermal conductivity detector. Helium was used as the carrier gas. A pressure of 30 lb/in^2 (2·1 kg/cm^2) was required to produce a flow rate of 80 ml per minute. The sensing column, constructed from 0·25 in (6 mm) copper tubing, consisted of a 7 ft 11 in (2·4 m) type Q Poropak limb in parallel with a 9 ft (2·7 m) Linde type 5A Molecular Sieve. The layout was as in Fig 1 (3). Poropak is a preparation of cross-

bonded polymer beads; it is inert and stable below 250°C. It will split carbon dioxide and nitrous oxide at room temperature, oxygen and nitrogen at −75°C. Unfortunately this latter temperature is too close to the boiling point of nitrous oxide and carbon dioxide to utilize Poropak as a single column for all four gases. Molecular Sieve is a trade name applied to preparations of alumino-silicates; No. 5A is crystalline alumino-silicate and separates oxygen and nitrogen at room temperatures. Argon is contained in the oxygen fraction but accounts for less than 0·05% (Lukas & Ayres 1961). Carbon dioxide and nitrous oxide are permanently absorbed by Molecular Sieve which is activated by heating, preferably in a stream of carrier gas, in order to drive off water vapour. The column must subsequently be protected from contamination with water vapour and, to this end, the Molecular Sieve column was fitted with a forecolumn containing powdered firebrick. In order to balance gas flow, both through the limbs of the sensing column and across both arms of the detector bridge, forecolumns of powdered firebrick were fitted to the Poropak limb and to the connecting column carrying gas to the reference arm of the bridge.

Repeated changing of the columns predisposed to leaking joints and polytetrafluoroethylene sealing tape was found to prevent this irksome complication.

Prior packing and conditioning of a variety of lengths already fitted with couplings, greatly reduced the time taken to adjust the column length. Packing of the columns was achieved with the aid of vibration. Oven temperature was 40°C; detector temperature was 50°C. A filament current of 250 milliamps was used. Peaks were recorded on a Hook and Tucker self-balancing potentiometric pen recorder. Peak heights were measured by means of a Solatron Digital voltmeter using the peak holding setting.

Gas samples were obtained from Wosthoff gas mixing pumps and were injected through the gas sample loop. Calibration curves for the apparatus were virtually straight lines; the curves for oxygen and nitrous oxide were almost identical, that for carbon dioxide a little steeper. Multiple readings were taken to determine each point through which the curves were drawn.

A Beckman blood/gas accessory was introduced in series with the sample injection apparatus in the carrier gas stream; the flow of carrier gas must be carefully balanced in the various valve positions in order to avoid flow fluctuations consequent upon valve operation. This apparatus is basically a reaction chamber which may be isolated to allow reaction between blood and reagent and then vented on to the columns or to the atmosphere. Fig 2 illustrates the reproducibility obtained when four separate analyses were carried out on a venous blood sample from a patient under nitrous oxide-oxygen anæsthesia. It has not been possible to eliminate an injection peak and this may well invalidate the use of peak height for quantification of samples.

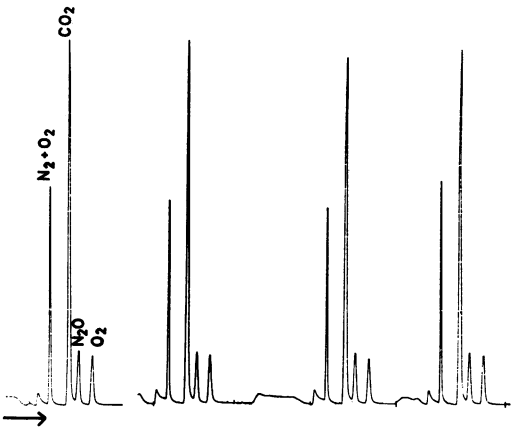

Fig 2 *Chromatograms obtained from identical venous blood samples using Poropak Q and Molecular Sieve columns in parallel. The initial small peak is caused by the injection of the sample*

Calibration of the accessory is proceeding, using potassium carbonate/hydrochloric acid mixtures for carbon dioxide calibration and ceric sulphate/hydrogen peroxide mixtures for oxygen calibration (Yee 1965) but, so far, results have been less consistent than with gas mixtures.

There is in anæsthetic practice a need for simple, inexpensive apparatus for the determination of blood-gas and anæsthetic content. Gas chromatography partially satisfies this need but the problem of extraction of combined gases from the blood is incompletely solved, possibly due to a lack of interest in the problem.

Acknowledgments: I wish to thank the Director General of Medical Services, Royal Air Force, and Air Commodore R L Soper, Consultant Adviser in Anæsthetics, Royal Air Force, for the opportunity to do this work; Professor J P Payne, Director of the Research Department of Anæsthetics, Royal College of Surgeons, and his staff for the generous facilities and advice afforded to me; and Dr D D Davies for his assistance in the latter part of the work.

REFERENCES

Bowes J B (1964) *Anæsthesia* **19**, 40
Hamilton L H (1962) *Ann. N. Y. Acad. Sci.* **102**, 15
Hill D W
(1962) PhD Thesis, London
(1966) In: Oxygen Measurements in Blood and Tissues. Ed. J P Payne & D W Hill. London; p 63
Johns T & Thompson B (1963) *Analyzer* April, p 13

Kaiser R (1963) Gas Phase Chromatography, Vol 1. London
Laasberg L H & Etsten B E
(1965) *Anesthesiology* **26**, 216
Lukas D S & Ayres S M
(1961) *J. appl. Physiol.* **16**, 371
Ramsey L H (1959) *Science* **129**, 900
Summers F W & Adriani J
(1961) *Anesthesiology* **22**, 100
Szymanski H A (1964) Biomedical Applications of Gas Chromatography. New York
Theye R A (1964) *Anesthesiology* **25**, 75
Wilson R H, Jay B, Doty V, Pingree H & Higgins E
(1961) *J. appl. Physiol.* **16**, 374
Wilson R H, Jay B & Holland R H
(1961) *J. thorac. Surg.* **42**, 575
Yee H Y (1965) *Analyt. Chem.* **37**, 924

Dr F W Cervenko[1]
(Research Department of Anæsthetics, Royal College of Surgeons of England, London)

Halothane in Blood and Tissues

The determination of concentration of inhalation anæsthetics in blood and tissue has now been made practicable by the use of gas chromatography. When the detector is of the flame ionization type a high degree of sensitivity is achieved. The present investigation concerns the development of a technique of estimating halothane in the blood of man and dog and in various tissues of the dog by gas chromatography. In the dog the results were used to calculate the uptake of halothane from breath to blood and from blood to tissue.

Methods

The chromatograph and hydrogen flame detector were constructed in the Research Department of Anæsthetics, Royal College of Surgeons of England, and were used in conjunction with a vibrating reed electrometer with head amplifier and a 1 mV recorder. A column 1·22 m long 0·40 cm internal diameter packed with silicone oil M.S. 550 35% w/w on Celite 44–60 mesh maintained at 85°C was used. The carrier gas was nitrogen flowing at 60 ml/min; hydrogen at 55 ml/minute and air as an oxygen source were needed for the flame. Samples, 3 to 5µl in size, were introduced into the chromatograph with a 5 µl Guild syringe. Diethyl ether was used as an internal standard to calculate halothane concentration on the basis of halothane-ether ratio. Halothane was extracted from whole blood and tissue using carbon tetrachloride (Wolfson *et al.* 1966). The three components were eluted from the column in order of their boiling points within seven minutes. Since sharp separated peaks were produced, measurement was based on the peak heights.

Preparation of halothane standards: Six µl of diethyl ether was added to 100 ml of carbon tetrachloride in a volumetric flask, glass-stoppered and mixed. It was found that this quantity of ether produced a near full-scale deflection on the recorder. To make a standard of known halothane concentration, 25 ml of the carbon tetrachloride-ether mixture was added to a volumetric flask, sealed with a rubber stopper and weighed. A quantity of halothane, usually 2–3 µl, was injected through the rubber stopper and the flask reweighed. A polyethylene stopper was substituted for the rubber and the flask was agitated. The concentration of halothane in the standard was then calculated.

Extraction of halothane from blood: A rubber stopper was placed on the 100 ml flask containing the same carbon tetrachloride-ether mixture used to make the standard and with a calibrated adaptor and glass syringe with needle, 2·85 ml was drawn up and injected into an 8 ml glass phial which was half filled with glass beads to facilitate mixing and to decrease phial air space. The phials were rapidly sealed with a sheet of nylon and a bakelite screw cap. Blood samples, approximately 3·5 ml in size, were collected anærobically into heparinized disposable polyethylene syringes and sealed with a polyethylene cap. The cap of the phial was removed and, using a calibrated adaptor and a needle fitted on to the syringe, 2·58 ml of blood was injected under the carbon tetrachloride-ether mixture; the phial was resealed and its contents were mixed on a mechanical shaker for ten minutes to complete extraction, then centrifuged at 3,000 rev/min for ten minutes; a sample of the clear extractant layer was injected into the chromatograph. Since 2·85 ml of carbon tetrachloride was used to extract 2·58 ml of blood a ratio correction factor of 1·10 was used.

Extraction of halothane from tissues: Halothane standards were made as described above. Glass beads were added to half fill an 8 ml glass phial, which was stoppered with a sheet of nylon and a bakelite screw cap and weighed. A biopsy specimen of tissue weighing approximately 1·5 g was blotted free of blood and added to the phial which was restoppered and weighed; the weight of the tissue was calculated; 2·58 ml of the carbon tetrachloride-ether mixture used to prepare the standard was added; the phial was resealed, mixed and centrifuged as described for blood. The clear extract layer was then sampled and injected into the chromatograph. The total amount of halothane in 2·58 ml of carbon tetrachloride was cal-

[1]Present address: Department of Anaesthesiology, Kingston General Hospital, Kingston, Ontario, Canada