GAS CHROMATOGRAPHY AS A METHOD FOR ESTIMATING CONCENTRATIONS OF VOLATILE ANAESTHETICS IN BLOOD

BY

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SUMMARY

A method of estimating any volatile anaesthetic in blood by means of gas chromatography is described. It requires only a small sample which may be conveniently stored and from which many readings may be taken. Possible uses of the method are indicated.

Gas chromatography offers a simple, cheap and reliable method of estimating any of the volatile anaesthetics in blood or tissues and is extremely sensitive. Measurement of clinical concentrations does not require such extreme sensitivity, but other features of the method present certain advantages. Thus sample size is small, and many estimations can be performed on each sample; storage is convenient and may be prolonged; other volatile substances in the blood, including other anaesthetics, acetones and alcohol, do not interfere with the analysis and may be estimated simultaneously if desired; repeatability is good and does not depend on a high standard of technical skill.

METHOD

Gas chromatography is a physical method of separating materials from one another by distributing them between two phases, one of which is a stationary, liquid phase and the other is a mobile, carrier gas phase. The stationary phase liquid is normally a heavy, non-volatile fluid which is chosen to have different "affinities" for the materials to be separated. The liquid must present a large surface area to the carrier gas and for this reason it is usually absorbed on to fine particles of diatomaceous earth or ground firebrick, or it may be deposited as a thin layer on the internal surface of a fine capillary tube.

The homogeneous mixture is separated into its components due to their varying affinities for the stationary phase liquid. Figure 1 shows this process in diagrammatic form, where the more volatile components are emerging before the less volatile. On emergence from the column the separated components are, in this instance, detected by a flame ionization detector (Harley, Nel and Pretorius, 1958). In this form of detector the increase in electrical conductivity of a small hydrogen flame is measured by an electrometer amplifier and displayed on a potentiometric recorder as a "peak".

The method previously described (Butler and Hill, 1961) employed columns made from copper refrigeration tubing, 1/2 in. (6.4 mm) internal diameter, packed with 80–100 B.S. mesh Celite which was impregnated with 10 per cent by weight of silicone fluid M.S.550. The column was maintained at 30°C in a hot air oven. A flame ionization detector was used with hydrogen as the carrier gas at a flow rate of 45 ml per minute. The apparatus is shown in block form in figure 2.

It is now apparent that the original flame ionization detector is modified into a thermionic detector by virtue of the relatively large hydrogen flow producing a larger flame (Lovelock, 1961, personal communication). This form of detection is related to the "hot wire" detectors used for halogenated gases and offers more sensitivity than the true flame ionization detector for halogenated hydrocarbons.

With this arrangement, separation of halothane from the solvent took 12 minutes, with a sensitivity of better than 10 parts per million.

Clinical work requires greater speed and less
AN ALIQUOT OF ABOUT 0.2-1 µL OF LIQUID OR 0.2-2 µL OF VAPOUR OF THE MIXTURE IS INJECTED INTO THE COLUMN.

A column consists of a tube which is maintained at a constant temperature. In packed columns the solvent phase is distributed on an inert supporting medium such as Kieselguhr or firebrick. In capillary columns the solvent phase is coated on to the walls of the tube. Typical solvent phases are glycerol, squalane oil, di-nonyl phthalate, silicones. Packed columns may vary in length from 12 in. (30 cm) to 20 ft. (600 cm); capillary columns from 10 ft. (300 cm) to 100 ft. (3000 cm).

**Fig. 1**

**Fig. 2**

Gas chromatographic apparatus.
sensitivity. The original method has been modified to these requirements by increasing the oven temperature to 80°C and calibrating the chromatograph on peak heights rather than peak areas when known standard solutions are compared to the samples.

The amount of solution or mixture necessary for analysis on the gas chromatograph is small, of the order of 1 microlitre (1 μl), and consequently the volume of blood sample that has to be taken need not be larger than 0.1 ml. In practice, however, a sample of 2 ml is more convenient to handle.

Tubes to receive these samples are prepared as follows: into glass-stoppered test tubes (100 × 12 mm) are placed 2 ml of n-heptane (L. Light & Co.), a suitable anti-coagulant (e.g. oxalate) and four glass balls (3–4 mm). If possible the prepared, stoppered tube is weighed. Blood samples are taken by syringe and the volume adjusted to 2 ml. The blood is then injected as a fine spray under the surface of the n-heptane in the sample tube. The difficulties which may occur in obtaining and measuring exactly 2 ml of blood during clinical anaesthesia can be largely compensated for by weighing the sample tubes; no sample need then be wasted since an altered extraction ratio may be calculated.

Samples may be stored at this stage in a domestic refrigerator (2–7°C) allowing estimations to be performed later. Samples have shown no significant loss of their volatile anaesthetic over a period of two months storage.

Blood samples have their volatile anaesthetic content extracted into the solvent by gentle shaking; the glass balls assist the process and extractions in excess of 95 per cent may be expected. Too vigorous or prolonged shaking will lead to the formation of an emulsion of n-heptane in blood; in most cases storage over night results in sufficient separation to allow estimations to be performed, but on rare occasions centrifuging may be necessary.

Standard solutions for calibration are prepared of the relevant volatile anaesthetic in n-heptane. The usual range of values expected during surgical anaesthesia is of the order of 8–12 mg of halothane per 100 ml of blood (Duncan and Raventós, 1959; Bull, Ozinsky and Harrison, 1960) and 40–120 mg of di-ethyl ether per 100 ml of blood (Hill et al., 1952; Criscuolo et al., 1959). For comparison with 2 ml of blood in 2 ml of n-heptane, a standard solution, in 10 ml of n-heptane, would contain 8 μl of di-ethyl ether to be equivalent to 57 mg/100 ml of blood, or 0.8 μl of halothane to be equivalent to 15 mg/100 ml of blood. Suitable standard solutions are kept in glass-stoppered test tubes which also contain three or four glass balls (3–4 mm) to aid mixing.

Calibration is performed by injecting into the chromatograph a set volume of a standard solution and measuring the response. This response, which appears as a peak on the record (fig. 3), may be measured most accurately as the area under the peak or more conveniently as its height. A calibration graph is then constructed from the response to several standard solutions. The height, but not the area, of a peak for a standard solution may vary if temperature and gas flows alter during a run of estimations, and for this reason a calibration on peak height should be checked at frequent intervals.

The same set volume of n-heptane plus anaesthetic must be used both for calibrating solutions and for the unknown solutions. This set volume (about 0.5 μl) is injected into the chromatograph either by a modification of the capillary pipette described by Tenney and Harris (1957) or by means of a micro-syringe, such as a Hamilton 701N, which may also be used for preparing the standard solutions.

RESULTS

Using the less accurate system of measuring peak heights rather than areas, a coefficient of variation of between 0.8 and 2 per cent was obtained for forty-eight estimations of a single calibrating solution on various occasions. By bracketing an unknown solution C with two standard solutions A and B, as in figure 3, mean values and standard deviations may be obtained. If this degree of accuracy is not required, three or four readings of each unknown will suffice and many more samples may be dealt with per hour.

Arterial concentrations of halothane and ether found in a series of experimental animals are shown in table I. The constancy of the inspired anaesthetic tension was verified by continuous monitoring with an infra-red gas analyzer, pre-
GAS CHROMATOGRAPHY FOR ESTIMATING ANAESTHETICS IN BLOOD

TABLE I

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Duration of anaesthetic (min)</th>
<th>Number of observations</th>
<th>Inspired concentration (per cent)</th>
<th>Mean arterial concentration (mg/100 ml)</th>
<th>S.D. of mean</th>
<th>Range (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>5-6</td>
<td>36</td>
<td>1.5</td>
<td>9.9</td>
<td>1.9</td>
<td>6.8-13.7</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>23</td>
<td>1.5</td>
<td>11.5</td>
<td>2.1</td>
<td>8.2-14.5</td>
</tr>
<tr>
<td>Di-ethyl ether</td>
<td>5-6</td>
<td>24</td>
<td>7.0</td>
<td>93.0</td>
<td>13.0</td>
<td>67-113</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>18</td>
<td>7.0</td>
<td>92.0</td>
<td>11.0</td>
<td>74-115</td>
</tr>
</tbody>
</table>

FIG. 3

A chromatogram showing peaks recorded to measure an arterial blood halothane concentration. Chart speed 1 in./min. Solvent peaks were not recorded. Sample concentrations:

- Standard A 14.9 mg/100 ml
- Standard B 7.5 mg/100 ml
- Unknown C 11.8 mg/100 ml

Previously calibrated on vapour/gas mixtures prepared by pressure dilution (Hill, 1961).

DISCUSSION

Gas chromatography offers a versatile system for the analysis of a wide variety of compounds in a large range of concentrations, both in a gas or vapour phase (Hill, 1960; Hill, Hook and Mable, 1962) and in blood or other tissues. There is a large number of ways in which this method may be applied. In this department it has been used in studies on the uptake, distribution and elimination of volatile anaesthetics; in monitoring new anaesthetic agents; in determining relative activity coefficients for volatile anaesthetics; and for forensic purposes in determining in blood and tissues the content of anaesthetics and other volatile compounds. In conjunction with cardiac output studies, the rate of uptake of anaesthetic may be calculated by determination of the difference in content of anaesthetic in the arterial and the mixed venous blood.

ADDENDUM

Dr. A. S. Curry (1962, personal communication) of the Home Office Forensic Science Laboratory (N.E. Area) has found that, for clinical concentrations, a modified extraction method is very satisfactory. One ml of blood is injected through a silicone diaphragm into a tube containing one gram of potassium carbonate. After shaking, the gas above the liquid is sampled and injected into the chromatograph. The chromatograph is calibrated by known vapour concentrations, or by extracting blood samples to which weights of anaesthetic have been added.

ACKNOWLEDGMENTS

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REFERENCES


**BOOK REVIEW**


Reviews must take into consideration not only the merits of a new book, but also its value in relation to other available books on the same subject. Apart from Adriani's books, there are no comprehensive reviews of the pharmacology of drugs used by anaesthetists, except for chapters in the standard textbooks and a few special articles in some of the journals. Wood-Smith and Stewart have produced a volume which fills this deficit and deals not only with the drugs used in the actual management of cases during anaesthesia, but also with such topics as bronchoconstrictors and bronchodilators, cardiovascular drugs, diuretics, uterine stimulants, histamine and antihistamines, enzymes, chemical transmitters, hormones, anticoagulants, and electrolyte and infusion fluids. Apart from the antibiotics they have truly dealt with all drugs likely to be used by the anaesthetist. In addition to this they have included a useful eight-page appendix of "normals" for many biochemical tests.

Many readers find the picturesque approach of Adriani an unsatisfactory method of presentation of data on drugs, and the volume under consideration has adopted a completely different approach which is somewhat similar to that used by the British Pharmacopoeia. There is a general discussion at the beginning of most chapters which is followed by a review of the actions of individual drugs. This gives the chemical, official and proprietary names for all drugs and it is a great help to readers to find the latter included in the index. There is a short but useful bibliography after most drugs, and the brevity of this can be excused in view of the large number of drugs reviewed.

One could easily criticize this book on the grounds of inadequate information in some sections, and also on the paucity of structural formulae. However, as stated above, the authors have attempted to give the essential data on every drug likely to be used by the anaesthetist, and yet keep the book within a reasonable size, and it is obvious that there had to be some omissions. Readers will have to consult publications of authors with a specialized knowledge of various subjects to get a complete picture of the actions of all drugs. As an example, it is obviously quite impossible to expect a complete review of muscle relaxants in twenty-three pages. Perhaps more information should have been given on thiopentone (eight pages), trichloroethylene (four pages), and nitrous oxide (four pages).

It will be obvious that the reviewer feels that, although this book is not a complete survey of all actions of all drugs in anaesthetic practice, it fills a very great need in our current anaesthetic literature. Provided readers are aware of its limitations it can be recommended to those preparing for higher examinations. It is well produced, but perhaps a bit inadequately illustrated. The price may prove a deterrent to a few, but it should be a standard reference book in all libraries.

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