METHODS FOR THE ESTIMATION OF BLOOD HALOTHANE CONCENTRATIONS BY GAS CHROMATOGRAPHY

BY

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SUMMARY

Two methods using gas chromatography are described for the estimation of halothane levels in whole blood. The first technique based on the use of an electron capture detector requires the halothane to be extracted in heptane. The second utilizes a flame ionization detector with the direct injection of whole blood. Its rapidity makes it suitable for use with an on-line computer monitoring system.

The requirement to monitor blood halothane levels during anaesthesia demands a method of analysis which can be completed within a matter of minutes. This is particularly true when a digital computer monitoring system is in use on-line. For this purpose the method of choice is gas chromatography. During recent years the engineering of commercial chromatographs has improved significantly and they are available in many laboratories. Most of the papers dealing with the estimation of blood halothane levels by gas chromatography have been based upon the use of an extraction technique to remove the halothane from the blood sample, before the sample of the extracting agent plus halothane is loaded on to the chromatographic column. Although more time-consuming, this arrangement overcomes some of the difficulties which are encountered with the loading of whole blood into the chromatograph.

Butler and Hill (1961) used n-heptane as the extracting agent. Equal 1-ml volumes of whole blood and heptane were shaken up and left overnight. One microlitre of the heptane was then placed upon the column packed with silicone fluid type MS550 on Celite. The resulting halothane and heptane peaks were detected with a flame ionization detector. By leaving the samples overnight, an average recovery of 98.7 per cent was obtained (Butler, 1963). The flame detector responds well to the large heptane peak, and the time interval necessary to allow this peak to clear the detector reduced the number of samples that could be analyzed to four per hour. A similar approach was adopted by Wortley and associates (1968). These workers obtained an average 98.3 per cent recovery by shaking the blood and heptane vigorously for 3 minutes. The presence of the large heptane peak again meant that the interval between successive samples was 16 minutes. The need to load a precise volume of sample can be avoided by incorporating an internal marker substance into the sample. Cervenko (1968) in this laboratory used carbon tetrachloride as the extracting agent for halothane with diethyl ether as the marker. The calibration of the chromatograph is now based upon the ratio of peak areas or heights for halothane and ether and is independent of the sample volume. Cervenko used equal 1.5 ml volumes of blood and carbon tetrachloride, the mixture being spun in a centrifuge for 15 minutes at 2000 r.p.m. in order to separate the blood cells from the carbon tetrachloride. Successive samples can still only be injected at 10-12-minute intervals because of the time required for the carbon tetrachloride peak to pass. These considerations have led us to investigate the feasibility of other approaches.

METHODS

The electron capture detector.

Lovelock and Lipsky (1960) proposed the idea of the electron capture detector. The version used by us consists of a simple small concentric ionization chamber of approximately 3 ml volume. The...
central anode is 0.2 cm in diameter and 3.5 cm long. The outer cathode is made from a 6 x 1 cm tritiated zirconium foil having an activity of about 10 mc. A polarizing voltage of 8 V d.c. is placed across the chamber from a dry battery. The resulting electron current of about $10^{-9}$ A is measured with a vibrating reed electrometer or with an operational amplifier having a field effect transistor input stage. The detector is preceded by a 6 feet (180 cm) long ½ inch (3.1 mm) o.d. nylon column filled with 35 per cent by weight of silicone fluid type MS550 on 44-60 mesh Celite. The injection port for the column, the column and the detector are all maintained in an air oven at 44°C ± 0.1°C. The column carrier gas is oxygen-free nitrogen. When a halogenated compound is eluted from the column into the detector, the halogenated molecules capture some of the free electrons, thus producing a diminution in the chamber standing current. In practice, the standing current is backed-off by means of an auxiliary potentiometer and the chart recorder polarity is reversed so that the decrease in current now appears as a conventional positive peak on the chart recorder. The area under the peak of the chromatograph is automatically measured by means of a voltage-to-frequency type integrator which prints up a figure proportional to the area. This can be entered as data into the computer by means of the on-line teleprinter. A computer program has been prepared based on Lagrange's interpolation formula (Bull, 1966). This interpolates the integrator reading from a blood sample into the calibration curve for the chromatograph. It also checks that reproducibility of peaks obtained from aliquots of the same sample is within 5 per cent.

The electron capture detector is so sensitive to halothane that the blood samples can be diluted by a factor of 1000 with n-heptane. Extraction is complete within 2 minutes of vigorous shaking. The detector is correspondingly insensitive to heptane, so that the heptane peak is only of the order 1 cm high, whilst the halothane peaks are several cm high. Halothane is eluted after 35 seconds and heptane after 88 seconds. The small heptane peak means that successive samples can be injected at 3-minute intervals. Using a 10 µl syringe fitted with a Chaney adaptor, 10 µl of blood is added to 10 ml of heptane in a volumetric flask, 2 µl of the heptane solution then being placed on to the column.

The calibration curve for the electron capture detector is not quite linear, so that three standards of blood in heptane are used to determine the curve at the start of the day, and one standard subsequently. The standards are prepared in 25-ml flasks by adding known volumes of halothane and heptane and weighing to a known weight of heptane. The density of halothane at 20°C is 1.87 g/ml and that of n-heptane is 0.68 g/ml. Each standard is then diluted by adding 5 µl of it to 5 ml of heptane. The standards should be prepared daily as there is evidence that the heptane evaporates more rapidly than the halothane, giving rise to an apparent increase in the strength of the standard with time.

Experiments were also carried out with a pulsed polarizing voltage applied to the detector. The pulse repetition rate was 10,000/sec, the pulse width 5 m.sec and the pulse amplitude 20 V. By suitably adjusting the pulse parameters it was found possible to linearize the calibration curve (fig. 1). However, no increase in sensitivity over the simple d.c. system could be obtained and the method was not pursued. With halothane and the d.c. system no evidence was found of the build-up of a contact potential on the cathode as described by Lovelock (1963). The sensitivity with argon as the carrier gas was less than that obtained using nitrogen.

**The direct injection system.**

The maximum number of blood samples can be handled in a given time if it is possible to dispense with the heptane extraction and simply inject a sample of whole blood containing halothane into a heated injection port where the halothane will be vaporized. This approach was described by Lowe (1964), but no details were given of its efficiency, and it does not appear to have been widely adopted. The sensitivity of the electron capture detector is too great for it to be used with blood directly, so that the flame ionization detector is employed. The flame detector responds to water vapour (Hill and Newell, 1965), so that it is arranged to saturate the carrier gas with water vapour to prevent effects arising from the water vapour liberated from the blood sample. The column used is 18 inches long by ½ inch
Calibration curves for the electron capture with pulsed or d.c. operation.

(3.1 mm) o.d. nylon. It is filled with plain 60–85 mesh Chromosorb P. At the start of each day 0.5 ml of distilled water is injected through the sample port and a period of about 1 hour allowed for this to diffuse through the column. At the end of this time the sensitivity of the chromatograph has increased to a maximum, constant, value. This injection of water will last for 4–5 hours. The column operates at room temperature with the detector in an oven at 130°C and the injection port maintained at 50°C. The injection port contains a detachable glass liner packed with glass wool. This is changed after every three samples to remove dried residues. Sample injections are made using a 1 μl syringe, the plunger of which is arranged to protrude from the needle tip to remove any dried blood from the tip.

The needle is rotated during injection with a circular wiping motion to deposit the blood evenly throughout the port as an aid to evaporation. The halothane peak emerges after only 35 seconds so that this is a very rapid system.

Calibration is accomplished by means of halothane standards prepared in distilled water. Two to five μl of liquid halothane are added to 20 ml of water in a syringe containing mercury. The syringe is shaken vigorously after capping and left for 20 minutes for mixing to be complete.

RESULTS
Based on the results obtained from twenty-five blood samples in the range 9.0–21.5 mg halothane per 100 ml of blood, the electron capture method gave a mean recovery of 94.3 per cent. This compares with the 98.7 per cent of Butler (1963), 94.5 per cent of Cervenko (1968) and 98.3 per cent of Wortley and associates (1968), using flame ionization techniques. For the electron capture
TABLE I
The measurement by electron capture gas chromatography of known concentrations of halothane in whole blood.

<table>
<thead>
<tr>
<th>Blood halothane concentration (produced by weighing) (mg/100ml)</th>
<th>Peak area from integrator</th>
<th>Concentration found on two aliquots</th>
<th>Average</th>
<th>Per cent recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7</td>
<td>104</td>
<td>10.7</td>
<td>10.6</td>
<td>90.6</td>
</tr>
<tr>
<td>16.3</td>
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<td>14.98</td>
<td>15.0</td>
<td>92.0</td>
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<tr>
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<td>19.1</td>
<td>19.6</td>
<td>85.2</td>
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</tr>
<tr>
<td>25.8</td>
<td>247</td>
<td>23.9</td>
<td>26.0</td>
<td>100.8</td>
</tr>
</tbody>
</table>

method, ten injections of the same diluted sample (22.6 mg halothane per 100 ml of blood diluted 1000 times in heptane) gave a standard deviation of 1.8 per cent. The injection of nine separate dilutions of the 22.6 mg/100 ml of blood gave a standard deviation of 1.9 per cent, thus showing that the dilution was reproducible. The direct injection method gave a mean recovery of 92.9 per cent on six samples. The details of the direct injection results are given in table I.

DISCUSSION
The electron capture detector is by far the most sensitive of the detectors currently available for the gas chromatographic analysis of halothane. Because of this it has an obvious application to wash-out studies. It is easily constructed and requires only one carrier gas, oxygen-free nitrogen. Hence the equipment can be used in the operating room close to the patient if desired. The detector is sensitive to temperature variations, so that an efficient thermostat is essential. The use of a high dilution ratio requires care, but is feasible in practice. Chloroform and trichloroethylene also exhibit marked electron-capturing properties. The sensitivity for methoxyfluorane is about 1000 times less than that for halothane and is commensurate with that obtained using a flame ionization detector.

The direct injection approach using a flame ionization detector is invaluable when results have to be obtained in the shortest possible time. Care must be taken to master the technique of injection and to change the sample port lining at regular intervals. The water loading of the column must also be maintained. We have found the method to be invaluable in a study aimed at using the digital computer to analyze electroencephalograms obtained from dogs in which anaesthesia is maintained at known halothane blood levels.

ACKNOWLEDGEMENTS
It is a pleasure to acknowledge the co-operation of Drs Ling and Denison-Davies in the use of the electron capture detector system, and for Professor H. J. Lowe's guidance on the direct injection approach. This study was supported financially by the Liverpool Children's Fund, and by the Science Research Council. The digital computer was generously provided by the Medical Research Council.

REFERENCES
ESTIMATION OF BLOOD HALOTHANE CONCENTRATIONS


BOOK REVIEW


When the frequency, extent and importance of disturbances in fluid balance are considered it is remarkable just how much ignorance of this subject still prevails among clinicians. This book will do much to dispel that ignorance and for the examination candidate it should prove invaluable. The author has resisted the temptation to extend his text to the complicated fields of renal dialysis and cardiopulmonary bypass techniques, with the result that he is able to present a compact, concise and lucid account of the principles and management of fluid balance disturbance in less than 300 pages.

The text is divided into twelve chapters to which are added three appendices vital for the proper understanding of the text. It is unfortunate, therefore, that the content of these appendices is not designated on the title page. Approximately one-third of the material is devoted to a presentation of normal physiology and the remainder to a discussion of pathological disturbances and their treatment. In particular the chapter on shock should be compulsory reading for all clinicians involved in fluid therapy, despite a rather superficial and somewhat inaccurate account of the influence of anaesthesia on the shocked state. The chapters on plasma substitutes and disturbances during infancy and childhood reflect not only the author's research interests but also his long clinical experience in these fields.

When this book comes to be reprinted it would perhaps be wise to adopt the SI units of measurement throughout in place of the imperial units. Similarly the substitution of BP names for drugs instead of proprietary names would be an improvement. Again, except perhaps in the case of infants, it is surely a relic of a bygone age to advocate that whenever possible an infusion should be administered through a needle inserted into an accessible superficial vein (p. 242); certainly there can be few anaesthetists who do not prefer a short polyethylene cannula to a rigid needle.

Finally, although the bibliography is comprehensive the absence of titles in the reference list is an irritating inconvenience which could be remedied.

These, however, are minor criticisms of an excellent book which can be recommended with confidence. It is also nicely produced; the illustrations are clear and for the most part simple, and the print is easy to read.

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