DETERMINATION OF HALOTHANE IN GAS, BLOOD, AND TISSUES
BY CHEMICAL EXTRACTION AND GAS CHROMATOGRAPHY

BY

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SUMMARY

The method is an adaptation of those of Wolfson, Ciccarelli and Siker (1966) and of Cervenko (1968). Halothane is extracted into Analar carbon tetrachloride which contains chloroform as a trace impurity. This chloroform is used as an internal standard. Detailed tests were made of the stability and reproducibility of gas and liquid standards and of the extraction efficiency and its reproducibility (mean efficiency within 1–2 per cent of 100 per cent for blood, brain, heart, kidney, liver and muscle, with 95 per cent confidence limits for a single extraction with duplicate analyses of ±2.3 per cent). As an overall test of performance a mean 0.9 per cent NaCl/gas partition coefficient of 0.75 at 37.5°C was obtained which agrees with, or is slightly greater than, previous determinations.

In an investigation of tissue/gas partition coefficients for halothane it was found necessary to measure the concentration in gas, blood and tissues. We based our methods on the extraction techniques of Baker and King (1963), Wolfson, Ciccarelli and Siker (1966) and Cervenko (1968), but made a number of modifications designed to improve the accuracy and submitted the modified technique to rigorous tests.

METHOD

Extraction of halothane from blood and tissues.

This procedure was basically the same as that of Cervenko (1968): about 0.5–2 ml of tissue was added to an 8-ml glass phial containing 2 ml of carbon tetrachloride; the phial was then sealed with a nylon-lined screw cap, shaken for 6 min in a mechanical shaker and centrifuged for 15 min at 1250 g. Our technique differed from that of Cervenko in that all quantities were measured gravimetrically and that various pre-treatments were applied to the tissues (see below).

Following the example of Wolfson, Ciccarelli and Siker (1966) and Cervenko (1968) it was decided to add an internal standard to the carbon tetrachloride extractant; thus the halothane concentration in an injected sample could be determined from the ratio of the halothane and internal-standard peaks, instead of depending on the absolute value of the halothane peak. On analysis, however, it was found that BDH Analar carbon tetrachloride contained a convenient trace impurity which gave a suitably sized, sharp peak and was eluted after halothane but before carbon tetrachloride. Circumstantial evidence suggested that the impurity was chloroform and this was confirmed by mass spectrometry of the peak, carried out by the Physico-Chemical Measurements Unit (PCMU), Harwell, Didcot, Berks. The concentration was normally about 250 mg/l., but one batch of carbon tetrachloride contained too much chloroform to be used in this way.

Chromatographic details.

A Pye series 104 chromatograph with a flame ionization detector was used in conjunction with a potentiometric recorder. The column was glass, 5 ft × ½ inch o.d. (1.5 m × 6 mm), packed with 3 per cent SE30 on 100–120 mesh diatomite CQ, with both the column and support silanized with dimethyl-dichlorosilane. The carrier gas was nitrogen flowing at 40 ml/min whilst hydrogen at 40 ml/min and air at 600 ml/min were employed for the flame.

Liquid samples (0.8 μl) were injected from a 1-microlitre syringe and analyzed using an injection port temperature of 100°C and a column...

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temperature of 50°C. Gas samples (0.5 ml) were introduced by means of a Pye, manually operated, gas sampling valve and were analyzed using a column temperature of 100°C.

The minimum time interval between injections was 7 min for liquid samples and 1 min for gas samples. Since sharp peaks were obtained with little tailing (fig. 1), measurements were based on peak heights.

Preparation of standards.
Following the technique of Jones, Molloy and Rosen (1971) gas standards were prepared by vaporizing accurately weighed amounts of halothane in 10-l. aspirator bottles of accurately measured volume. The apertures were closed by epoxy-resin-coated rubber bungs pierced by nylon stopcocks. The inclusion of a few pieces of aluminium foil ensured thorough mixing after shaking for 10–15 min. The concentration of halothane within the bottle was then calculated with correction for the volume of the vaporized halothane and for subsequent changes in room temperature and barometric pressure. The samples of gas to be analyzed were taken by way of a Luer stopcock using a 2-ml, unlubricated, glass syringe. Flushing the bottle with air for 30 minutes removed all traces of halothane preparatory to making a fresh standard.

For liquid standards known concentrations of halothane in carbon tetrachloride were prepared by a double dilution technique. The solutes, 100 µl halothane and then 2 ml concentrated halothane in carbon tetrachloride were handled in syringes which were weighed to 0.1 mg when full and then when empty. The solutions were made up to 50 ± 0.1 ml in volumetric flasks.

RESULTS AND DISCUSSION

The statistical techniques used in the analysis of the results were all taken from Davies (1957).

Reproducibility of chromatographic analyses.
For a single measurement of a gas sample the 95 per cent confidence limits varied over a period of 21 months from ± 0.5 to ± 1.5 per cent. For a single measurement of a liquid sample the limits were initially ± 3.7 per cent with a simple, silicone-rubber septum at the injection port. When Hamilton “sandwich” septa were used the limits fell to ± 1.7 per cent but then gradually rose to ± 3.4 per cent over 12 months. All the changes were significant and the changes with time may indicate a gradual deterioration of the column.

Stability and reproducibility of standards.
In an initial study two gas standards were made in separate aspirator bottles and compared by analyzing ten samples from each. During the following week one of the standards was remade on five different days and similar comparisons made with the one original standard. Regression analyses of the results showed that loss of halothane from the gas standard was significant but small: 0.34 ± 0.14%* per day. Neglecting random

* Throughout this paper, the form “a ± b” is used to indicate an estimate of some parameter (mean or, as here, regression coefficient) ± the 95 per cent confidence limits of the estimate.
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variation of this loss, the 95 per cent confidence limits for the deviation of a single gas standard from the regression line were ±0.5 per cent.

During routine use, every gas standard made was compared with some other standard, made a day or two before or after. The occasional “rogue” could thereby be detected and remade. Under these working conditions 20-40 samples were drawn per day instead of only 10. Neglecting the increased variation in loss that this would probably cause, the 95 per cent confidence limits for the deviation of a single gas standard from the mean increased to ±2.4 per cent of the mean.

Similar comparisons were attempted with the liquid standards but, because the response of the chromatograph to liquid samples was more variable and much slower than to gas samples, no very precise information could be obtained in a reasonable time. However, the following conclusions could be drawn. No significant change in peak height ratio was found in the two-week period for which any liquid standard was used. The 95 per cent confidence limits for the deviation of the mean peak height ratio for a single liquid standard from the grand mean for several standards ranged from ±1.3 per cent in preliminary tests to ±2.4 per cent under working conditions, but the whole of this variation could be attributed to experimental error in the analyses.

Linearity tests.
A series of five liquid standards was made up containing 70-1000 mg of halothane per litre. The peak height ratio was found to be independent of sample size between 0.5 and 1 µl and accurately linearly related to halothane concentration: there was no significant curvature and the 95 per cent confidence limits of the slope of the regression line were only ±1 per cent.

A less exacting test of linearity for gas samples showed that this was certainly adequate for the narrow range of concentrations of interest and probably at least as good as the linearity for liquid samples.

Efficiencies of the extraction procedure.
Preliminary tests with 2-ml samples of distilled water containing known amounts of halothane (about 100 mg/l.) gave an extraction efficiency of 99.7 ± 1.6 per cent.

In a further series of tests extraction phials, containing halothane already in the carbon tetrachloride (about 200 mg/l.), had 2-ml halothane-free samples of saline, of blood and of homogenates of brain, heart, kidney, liver and muscle added to them. After the normal shaking and centrifuging procedure, samples of the extractant were analyzed after half an hour and again after 1, 2, 3 and 6 days. Two phials were used for each sample: in one the screw cap was nylon lined, in the other it was aluminium-foil lined. There was no significant difference between the two sets of phials.

Regression analysis showed a small, initial, apparent gain in the amount of halothane present in all samples followed by a continuous apparent loss from samples of brain, kidney, liver and heart. The initial apparent gain did not differ significantly between tissues and had a mean value of 1.1 ± 0.8 per cent of the original concentration. The continuous apparent loss was in fact due to a rise in the chloroform peak height with no significant trend in the halothane peak height. The rate of “loss” was small for heart and brain (0.9 and 1.8% per day respectively) but quite large for kidney and liver (initially 0.7 and 0.44% per hour respectively). A “blank” phial, containing only extractant plus halothane, showed a non-significant loss of 0.1% per day.

The rise in the chloroform peak height was indeed due to an increase in chloroform (PCMU analysis). It seems likely that this can be attributed to a conversion of carbon tetrachloride to chloroform, either metabolically (Fowler, 1969) or catalytically (Finar, 1967). The initial apparent gain is in agreement with the results of Wolfson, Ciccarelli and Siker (1966) who obtained a mean efficiency for the extraction of halothane from blood of 100.8 ± 1.2 per cent. The gain may be attributable to the carbon tetrachloride/tissue partition coefficient being slightly greater for chloroform than for halothane.

These results indicate that, for tissues as well as for blood, extraction efficiencies within 1-2 per cent of 100 per cent can be expected, provided that the extractant is analyzed soon enough, and that the exchange of halothane between sample and extractant is complete.

The experiments with homogenates suggest that “soon enough” means within about 3 hours for...
kidney and liver and 30 hours for the other tissues. However, later experiments have revealed substantial increases in chloroform peak height with time for heart samples taken directly from an anaesthetized rabbit. Therefore it seems advisable to analyze all tissues within 2-3 hours.

The experiments with homogenates show that, with the routine shaking procedure, exchange between sample and extractant is complete within 30 minutes. By contrast, with 2-3 ml lumps of tissue, it was found that exchange was only about 90 per cent complete in 24 hours, and that the addition of glass beads to the phials was not effective in pulverizing skeletal and cardiac muscle pieces. Therefore it has become our practice to immerse lumps of tissue in liquid nitrogen and crush them before adding them to the extraction phials.

Reproducibility of the extraction procedure.
Duplicate extractions from 22 samples of rabbit blood equilibrated with 1 per cent halothane showed that the 95 per cent confidence limits for a single extraction, with duplicate analyses, were ±2.3 per cent.

Overall performance.
Determination of the saline/gas partition coefficient for halothane involves all aspects of the technique (gas and liquid analyses and standards, and extraction efficiency) and therefore comparison of the result with published values provides a useful test of the overall performance of the techniques.

On three separate occasions, using different standards, a total of eight tonometries were performed at 37.5°C. From a hierarchical analysis of variance of the results it was calculated that the mean coefficient for 0.9 per cent saline was 0.75 ± 0.03 at 37.5°C. This was increased by 2.3 per cent (Eger, Saidman and Brandstater, 1965) for comparison with values published for 37°C (table I).

Our results are clearly higher than those of Larson, Eger and Severinghaus (1962) and perhaps a little higher than those of Laasberg and Hedley-Whye (1970). We can see no obvious cause of systematic error in any of the three techniques. However, although both the other methods have the advantage of depending on only a single (gas) standard they have the disadvantage of depending on the difference between two, not very dissimilar, gas concentrations. Therefore an error of 1 per cent in final gas concentration gives a greater error in calculated partition coefficient: 3 per cent with the proportions of liquid and gas used by Laasberg and Hedley-Whye and 6-10 per cent with those used by Larson, Eger and Severinghaus. The corresponding disadvantage of our method is that the result depends on two separate standards (gas and liquid) but our final figure is derived from three separate sets of experiments using three different pairs of standards. The confidence limits given are based entirely on data from the saline experiments but are wide enough to include the independently-estimated variation between individual standards.

### CONCLUSIONS

There is a variety of techniques for the determination of halothane in blood (see Douglas, Hill and Wood, 1970; Bracbet-Liermain, Ferrus and Caroff, 1971) but the only ones for determination in tissue are that of Larson, Eger and Severinghaus (1962), which requires large samples, and those of Cervenko (1968) and of Lowe (1969) for which only limited information on reliability is available. Therefore the chief value of this report is in the results of the extensive testing which we have undertaken. We believe these results demonstrate that, given certain precautions, our method is reliable and reproducible in

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<td>Liquid/gas partition coefficients for halothane at 37°C (mean ± 95 per cent confidence limits).</td>
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<td>Larson, Eger and Severinghaus (1962)</td>
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<td>Laasberg and Hedley-Whye (1970)</td>
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determining the halothane content of tissues as well as of blood.

Thus, even taking account of 95 per cent confidence limits instead of standard deviation or standard error, the extraction efficiency is effectively within 1–2 per cent of 100 per cent, reproducibility of a single extraction with duplicate analyses is within ±2.3 per cent, and standards are reliable to within 1–2 per cent even under working conditions. The overall accuracy of the technique is confirmed by the degree of agreement with other determinations of the saline/gas partition coefficient.

An important condition for obtaining the stated performance is observance of the 3-hour time limit on the storage of at least liver, kidney and heart in the extractant, due to the formation of chloroform. However, it is possible that the carbon tetrachloride could suppress the breakdown of halothane, a process which might place a similar limitation on other extraction methods.

ACKNOWLEDGEMENTS

We are grateful to Professor William W. Mushin, Head of this Department, for his encouragement and advice and to ICI Ltd, for generous supplies of halothane.

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REFERENCES


Ciccarelli et Siker (1966) and de Cervenko (1968). Halothane is extracted from the tetrachloroform of Analar, which contains chloroform as a trace.

This method is an adaptation of Wolfson's method (1966) and that of Cervenko (1968). The halothane is extracted from tetrachloroform with a small amount of chloroform contained within it as an internal standard.

A detailed study was carried out to establish the reliability and reproducibility of the gas-liquid standards and the extraction process, with limits of confidence of ±2.3% for halothane in blood, brain, heart, kidney, liver and muscle, with limits of confidence of ±5% for a single extraction with analyses duplicates of ±2.3%.

A method was developed using a test general performance which showed the efficiency of a coefficient mean of partition 0.9% NaCl/gas of 0.75 to 37.5°C, which corresponds to a lower limit of ±2.3%.

On a obtenu comme résultat d'un test général de performance un coefficient moyen de répartition 0.9% NaCl/gaz de 0.75 à 37.5°C, ce qui correspond à un coefficient moyen de répartition de ±2.3%.

DIE BESTIMMUNG VON HALOTHAN IN GASEN, BLUT UND GEWEBEN DURCH CHEMISCHE EXTRAKTION UND GASCHROMATOGRAPHIE

ZUSAMMENFASSUNG


Es wurden detaillierte Untersuchungen durchgeführt, um die Stabilität und Reproduzierbarkeit der Gas- und Flüssigkeitsstandards und der Extraktionswirkung und ihrer Reproduzierbarkeit (mittlere Wirksamkeit in Grenzen von 1 oder 2% von 100% für Blut, Hirn, Herz, Niere, Leber und Muskulatur bei 95% Vertrauensgrenzen pro Extraktion mit Doppelanalysen von ±2.3%).

Die Gesamt durchführung des Tests ergab einen durchschnittlichen 0.9% NaCl/Gas-Verteilungskoeffizienten von 0.75 bei 37.5°C, was mit früheren Bestimmungen gut übereinstimmt, bei geringem Druck liegt.
DETERMINACION DEL HALOTANO EN GAS, SANGRE Y TEJIDOS MEDIANTE EXTRACCION QUIMICA Y CROMATOGRAFIA GASEOSA

RESUMEN
Este método es una adaptación de los de Wolfson, Ciccarelli y Siker (1966) y de Cervenko (1968). El halotano es extraído dentro de tetracloruro de carbono Analar que contiene cloroformo como vestigio de impureza. Este cloroformo es utilizado como standard interno. Fueron efectuadas pruebas detalladas de estabilidad y reproductibilidad de los standards de gas y líquido y de la eficacia de extracción y de su reproductibilidad (eficacia media dentro del 1 ó 2% del 100% para sangre, cerebro, corazón, riñones, hígado y músculo, con límites de confianza del 95% para una sola extracción con análisis duplicados de ±2,3%). Como prueba global de actuación fue obtenido un coeficiente de partición medio de NaCl al 0,9%/gas de 0,75 a 37,5°C que está de acuerdo con las determinaciones anteriores o es ligeramente superior a las mismas.

BOOK REVIEW


This book contains fifty-six papers dealing with various aspects of respiratory care, all of which have been published previously in a wide range of scientific journals. The selection of papers has been made by the editor and is primarily intended for inhalation therapists and inhalation therapy technicians. It is obvious that the selection has been governed by a number of factors, not the least being the willingness of the original publishers to allow reproduction and the need to keep the volume within reasonable proportions.

The book is divided into six sections with a brief introduction at the beginning of each section. These are entitled "Inhalation therapy—organisation, administration and philosophical considerations", "Physiologic, pathologic, diagnostic and prognostic considerations of respiratory diseases", "The management of respiratory diseases", "Problems of respiratory care equipment", "Complications of respiratory care" and "Miscellanea".

Most of the selected papers have appeared within the last four years and all are from transatlantic journals. The collection of papers contains some review articles and others that are more concerned with specific aspects of respiratory care, so that there is a wide spread of interest and information. The editor is particularly concerned with the need for respiratory care in modern American medicine, which he views as shortly to become one of the most significant public health issues. If inhalation therapy training programmes have reached the stage at which their participants can digest the articles in this book, then the editor need have no concern since many of the articles were originally intended for more highly qualified and trained medical readers. Whilst all these articles are available in a well-stocked medical library, it is convenient and practical to have these articles reproduced in a single collection. The papers have been published in their original form so that there is considerable variation in format and the type has lost some clarity in reproduction.

Because of the different approach to respiratory care in the United Kingdom, this volume will not arouse great interest amongst anaesthetists. Physiotherapists, however, will find in these pages a good deal of important information that may not otherwise be readily available to them. In those countries possessing inhalation therapy departments, this volume will be of value in stimulating reading and giving added breath to training.

Gordon H. Bush