THE UPTAKE AND ELIMINATION OF HALOTHANE IN DOGS:
A TWO- OR MULTICOMPARTMENT SYSTEM?

I: Gas chromatographic determination of halothane in blood and in inspired and end-tidal gases

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SUMMARY

The gas chromatographic determination of volatile organic anaesthetic agents in blood and in respiratory gases is described. The analysis in blood is based on a head space technique. Sampling of inspired and end-tidal gases is carried out by means of a system including a gas sampling valve.

In recent years there has been a growing interest in methods for measurement of volatile organic inhalation anaesthetics. Determinations of the concentrations in respiratory gases, blood and body tissues are necessary in many experiments, such as investigation of the pharmacokinetics of new anaesthetic agents, while monitoring of the expired concentration may contribute to better techniques of anaesthesia.

Until now several different techniques have been used (Hill, 1973). Recently, however, most analyses have been performed using gas chromatography (GC); by this method an accurate analysis can be made in a short time. The sample size for GC is relatively small and the technique has the advantage that several anaesthetics and respiratory gases can be analysed simultaneously. A disadvantage is the fact that continuous measurement of the concentrations is impossible.

The methods described here were applied to the in vivo investigation of the uptake and elimination of halothane in dogs (Beneken Kolmer et al., 1975).

APPARATUS

The conditions for the analyses are such that, besides halothane, other anaesthetic agents must be determined also. The measurements are carried out using a Perkin Elmer 900 gas chromatograph. This instrument is equipped with a flame ionization detector, which is used for the detection of the organic anaesthetics, and a katharometer which can be used for the detection of nitrous oxide and respiratory gases.

Figure 1 shows a chromatogram obtained after injection of a mixture of air and some common anaesthetic agents. The separation of the components took place on a 20-cm stainless steel column (i.d. 22 mm), packed with Porasil S and Carbowax 400 (80–100 mesh).

The analysis conditions were as follows:

<table>
<thead>
<tr>
<th>Carrier gas</th>
<th>nitrogen, flow 55 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection port temp.</td>
<td>100°C</td>
</tr>
<tr>
<td>Column temp.</td>
<td>60°C</td>
</tr>
<tr>
<td>Detector temp.</td>
<td>150°C</td>
</tr>
<tr>
<td>Injection</td>
<td>200 μl litre of gas</td>
</tr>
</tbody>
</table>

FIG. 1. Gas chromatogram of some anaesthetics and acetone.

The injected sample contained mixture components in concentrations varying from 0.1 to 0.7 vol%. Acetone was included in the analysis because it may be present in expired gases and in blood in disease states. Concentrations of anaesthetics in gas samples of unknown composition can be derived from measurements of the peak height only. The coefficient of variation of the peak height measurement in a series of 25 injections from a Hamilton gas-tight precision syringe is less than 2%. The relationship between concentration and peak height is determined daily with standard samples.

ANALYSIS OF ORGANIC ANAESTHETIC AGENTS IN BLOOD
(HEAD SPACE TECHNIQUE)

Several methods for determining volatile anaesthetic agents in blood have been described. The most obvious would be the direct introduction of a small blood sample into the gas chromatograph, having the advantage of a short total analysis time (Lowe, 1964; Cousins and Mazze, 1972; Yokota et al., 1967).

For several reasons, however, the reproducibility of the quantitative analysis is poor; at low temperatures of the injection port the organic anaesthetic agent is released slowly, resulting in broad and asymmetrical peaks. At high inlet temperatures, clogging of the microsyringe occurs frequently, furthermore the gas chromatograph system is contaminated easily with protein material and "ghost" peaks may appear in the chromatogram.

Better results are obtained by methods in which the anaesthetic is released from the blood sample by extraction (Douglas, Hill and Wood, 1970; Brachet-Liermain, Ferrus and Caroff, 1971; Atallah and Geddes, 1972; Jones, Molloy and Rosen, 1972), distillation (Dyferman and Sjövall, 1962; Gadsden, Risinger and Bagwell, 1965) or equilibration techniques (Yamamura et al., 1966; Fink and Morikawa, 1970). A common disadvantage of these techniques is the additional time required for the preparation of the sample.

The method selected in our laboratories is a head space technique. The sample preparation is as follows: one hundred microlitre of blood is introduced into a 25-ml volumetric flask, shortened at the graduation mark and closed with a silicone rubber serum cap (fig. 2c). The flask is placed in a shaker and agitated for 10 min. During this time the anaesthetic is equilibrated between the gas phase and the blood. Then 200 µlitre of the gas phase is introduced into the gas chromatograph.

Calculation of the concentration of anaesthetic agent in blood

A precisely known volume $V_{\text{blood}}$ containing $\varphi$ g of anaesthetic is introduced into the volumetric flask. After equilibration, the anaesthetic is divided between the gas and the blood phase and the following relation exists:

$$\varphi = C_{\text{blood}}\frac{V_{\text{blood}}}{C_{\text{gas}}V_{\text{gas}}}$$  \hspace{1cm} (eqn. 1)

$V_{\text{gas}}$ being the gas volume of the volumetric flask (ml) after introducing the blood sample. $C_{\text{blood}}$ and $C_{\text{gas}}$ are the concentrations of anaesthetic agent in blood and gas respectively (g/ml). At equilibrium:

$$\lambda = \frac{C_{\text{blood}}}{C_{\text{gas}}}$$  \hspace{1cm} (eqn. 2)

where $\lambda$ is the blood-gas partition coefficient.

Combining equations 1 and 2 yields:

$$\varphi = C_{\text{gas}}\left[V_{\text{gas}} + \left(C_{\text{blood}}/C_{\text{gas}}\right)V_{\text{blood}}\right] = C_{\text{gas}}\left(V_{\text{gas}} + \lambda V_{\text{blood}}\right)$$  \hspace{1cm} (eqn. 3)

Since $V_{\text{gas}}$ (24.9 ml) and $V_{\text{blood}}$ (0.1 ml) are known, the amount $\varphi$ of the anaesthetic originally present in 100 µlitre of blood can be calculated if $C_{\text{gas}}$ and $\lambda$ are determined.

It should be noted that, because of the large ratio $V_{\text{gas}}/V_{\text{blood}}$ (approximately 249:1), small errors in the determination of $\lambda$ do not influence appreciably the calculation of $\varphi$ using equation 3. This is especially so because of the small value of $\lambda$ as demonstrated in table I.
TABLE I. Human blood-gas partition coefficients at 37°C.

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>λ* (measured)</th>
<th>λ† (proposed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>enflurane</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>halothane</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>diethylether</td>
<td>10.5‡</td>
<td>12</td>
</tr>
</tbody>
</table>

*Graven and Jongen, 1974; †Steward et al., 1973; ‡bovine blood.

Determination of \( C_{\text{gas}} \) in the volumetric flask

\( C_{\text{gas}} \) of unknown samples is derived from the peak height measurements. The relationship between the concentration and the peak height is determined daily from standard samples.

These standards are prepared by evaporation of a precisely known amount of anaesthetic in an air-tight closed bottle of known volume (approximately 1 litre (fig. 2B)). The flask is provided with a serum cap. The glass stopper is sealed with a small amount of grease.

The peak height \( v \) concentration curve is linear for concentrations between about 1 p.p.m. (vol/vol) and 5 vol%. In spite of the grease sealing and the rubber serum cap, no loss of halothane is demonstrated within a 3-hr period.

Determination of the blood-gas partition coefficient \( \lambda \)

From equation 3 it follows that \( \lambda \) can be determined if \( \varphi, V_{\text{gas}}, V_{\text{blood}} \) and \( C_{\text{gas}} \) are known.

A round bottom flask of 500 ml is closed at the top and provided with a serum cap (Cowles, Borgstedt and Gillies, 1971) (fig. 2A). The volume of the flask has to be determined precisely.

A known volume of blood \( V_{\text{blood}} \) (say 20 ml) is introduced into the round bottom flask by means of a precision syringe. An exactly known amount of anaesthetic \( \varphi \) (say 100 mg) is now introduced with a syringe via the serum cap. The flask is rotated. It should be noted that the partition coefficient \( \lambda \) is influenced strongly by temperature. Therefore the rotating flask is placed in a water bath kept at the temperature at which the 25-ml flasks are shaken. At the end of each 2-min period the concentration of the anaesthetic in the gas phase (\( C_{\text{gas}} \)) is determined as described.

After approximately 6 min \( C_{\text{gas}} \) will become constant. Since \( V_{\text{gas}} = V_F - V_{\text{blood}}, \lambda \) can be calculated according to equation 3.

ANALYSIS OF ORGANIC ANAESTHETIC AGENTS IN INSPIRATORY AND END-TIDAL GASES

Organic anaesthetics in respiratory gases are measured by introducing a 0.5-ml sample into the gas chromatograph via a gas sampling valve.

The respiratory gases are transported continuously from the endotracheal tube via the sample loop to a vacuum system (pressure 0.37 atm). The transport line (fig. 3) from the patient to the gas sampling valve consists of a stainless steel capillary tube of 5 m length and 0.25 mm i.d.

The dimensions of the transport tube are such that...

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**Fig. 3.** Schematic representation of a gas chromatograph method for the analysis of inspired and end-tidal gas.
The concentrations of the anaesthetic agent in the respiratory gases are determined by comparison with a calibration curve. The calibration curve is constructed by inserting one end of the capillary connection tube through a serum cap into a flask containing a known concentration of anaesthetic. The pressure in the buffer vessel and therefore the pressure in the sample loop must be the same during analysis and calibration.

The system ensures very accurate analysis. The coefficient of variation of the peak height measurements is less than 2%.

REFERENCES


LA FIXATION ET L'ELIMINATION DE L'HALOTHANE CHEZ LES CHIENS: SYSTEME A DEUX OU A PLUSIEURS COMPARTIMENTS?

1: Estimation par la chromatographie en phase gazeuse de l'halothane contenu dans le sang et dans les gaz à l'inspiration et en fin de flux

RESUME
Les auteurs décrivent le moyen de déterminer à l'aide de la chromatographie en phase gazeuse les agents volatils organiques anesthésiants contenus dans le sang et dans les gaz du système respiratoire. L'analyse de la quantité contenue dans le sang est fondée sur la technique de l'espace de tête. On effectue un échantillonnage des gaz à l'inspiration et en fin de flux en adoptant un système qui comporte une soupape d'échantillonnage des gaz.

AUFNAHME UND ELIMINIERUNG VON HALOTHAN BEI HUNDEN: EINE ZWEI-ODER VIELTEILIGES SYSTEM?

1: Gaschromatografische bestimmung von halothan im blut und in respiratorischen gasen

ZUSAMMENFASSUNG

LA ABSORCCION Y LA ELIMINACION DEL HALOTANA EN PERROS: ¿UN SISTEMA DOBLE O PLURICOMPARTIMENTADO?

1: Determinacion cromatografica gaseosa del halotana en la sangre y en los gases inspiratorios y periodicos

SUMARIO
Se describe la influencia cromatografica gaseosa de los agentes anestéticos organicos volatiles en la sangre y en los gases respiratorios. El análisis en la sangre se basa en una técnica de espacio muerto. Las muestras de gases inspiratorios y periódicos residuales se lleva a cabo por medio de un sistema que comprende una válvula de muestra de gas.