A Simplified Method for the Measurement of Volatile Anesthetics in Blood by Gas Chromatography

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ALTHOUGH gas chromatography is a sensitive and rapid method of assaying the blood for volatile anesthetics, its use has been hampered by difficulties related to extraction of the anesthetic from blood. Various stratagems have been tried, including extraction of the anesthetic from the blood with N-heptane,[1,2,3] direct injection of blood on to the column,[4] injection into a heated antechamber,[5] distillation into a toluene trap,[6] distillation from a cold trap,[7] and simple equilibration of blood with air in a vial,[8,9] or in a syringe at 37 C.[10] This paper presents a further simplification of the latter method. The modification consists in performing the equilibrations at room temperature.

In brief, a blood sample containing the anesthetic is subjected to two consecutive equilibrations with air in a syringe, and a chromatographic peak is obtained from each of the gas phases. The two peaks allow calculation of the blood–air partition coefficient and the partial pressure of the anesthetic in the sample at room temperature. Correction to body temperature is achieved by means of a vapor-pressure curve.

Procedure

The equilibrations are performed in a 10-ml gastight syringe (Hamilton #1010, Luer-Lok nozzle). A plunger–adapter bearing two adjustable stops (fig. 1) allows introduction of volumes V_B of blood and V_A of air, as follows:

1. Blood from the collecting syringe is transferred via a 3-way metal stopcock to the gastight syringe, which is filled to stop 1, introducing volume V_B of blood.

2. The stopcock is removed and the plunger of the gastight syringe withdrawn to stop 2, introducing volume V_A of air. The syringe is then closed with a clean metal stopcock.

3. Equilibration of the blood and air phases is performed by attaching the syringe to a rotating drum for 15 minutes.

4. The syringe is removed from the drum and connected through its stopcock to the gas-sampling valve of the chromatograph. The inlet connection on the valve is a female Luer-Lok, sloping downward so as to make the blood in the syringe collect away from the inlet.

5. The valve is flushed with equilibrated gas from the syringe and peak P_1 recorded.

6. The syringe is disconnected and the residual gas (but not blood) cautiously expelled from it; the syringe is refilled with air to stop 2 and recapped with the stopcock. Steps 3 to 5 are now repeated, recording peak P_2.

The gas chromatograph used was a Varian Aerograph Model 1520 B; detector, hydrogen-flame ionization; column, 5-foot stainless steel $\frac{1}{8}$" packed with DMCS Chromosorb W 50–80 mesh coated with 5 per cent SE 30; gas-sampling loop, 0.5 ml; column temperature 85 C, detector temperature 90 C; carrier gas, helium, 25 ml/min; hydrogen flow, 20 ml/min. Each gas phase was analyzed in triplicate: the range did not exceed ±1.5 per cent of the mean. For halothane, the principal anesthetic used in this study, the retention time was about 1.5 minutes. Calibration was performed

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FIG. 1. Attachment to syringe for equilibrating fixed quantities of blood and air. The rod carrying the stops is hinged at the arrow.

before and after each blood analysis with a mixture of 0.31 per cent halothane in nitrogen stored in a cylinder. This mixture was itself calibrated with halothane–air mixtures prepared by evacuating a desiccator of known volume, introducing a weighed amount of liquid halothane and filling with air.

Calculations

(1) Calculation of the Anesthetic Partial Pressure in the Blood Sample

In the first equilibration the anesthetic vapor from volume of blood V_B redistributes with volume of air V_A. Equating the total mass of anesthetic before and after equilibration, we have:

$$P_0V_B\lambda_B = P_1V_B\lambda_B + P_1V_A\lambda_A \qquad (1)$$

P_0 = partial pressure of anesthetic in original blood sample at room temperature;
P_1 = partial pressure of anesthetic in the syringe after the first equilibration, measured by peak 1;
λ_B = solubility coefficient of anesthetic in blood at room temperature;
λ_A = solubility coefficient of anesthetic in air at room temperature, defined as unity.

During the second equilibration the remaining anesthetic in the blood redistributes with a volume of air V_A according to the formula:

$$P_1V_B\lambda_B = P_2V_B\lambda_B + P_2V_A\lambda_A \qquad (2)$$

P_2 = partial pressure of anesthetic in the syringe after the second equilibration, measured by peak 2.

Dividing equation (1) by equation (2) yields:

$$\frac{P_0}{P_1} = \frac{P_1}{P_2} = r \qquad (3)$$

r is the ratio between the pressures of anesthetic in the blood before and after an equilibration, in particular, the ratio between measured peaks P_1 and P_2. From equation (3):

$$P_0 = rP_1 \qquad (4)$$

Note that V_A is identical in equations (1) and (2), and the same is true of V_B. Hence, V_A and V_B do not appear in equation (4) and P_0, the partial pressure of the anesthetic in the sample, can be calculated without knowing the exact volumes of blood and air introduced into the syringe. However, P_0 is the partial pressure at room temperature, and must be corrected to P, the partial pressure at body temperature. As shown in the appendix, the correction factor, F, consists simply of the ratio of the saturated vapor pressures of the anesthetic at body temperature and at room temperature. These pressures are obtained at once from inspection of the vapor pressure curve of the anesthetic. For example, in the case of halothane at body temperature, 37 C, and room temperature, 21 C, the vapor pressures of the anesthetic are, respectively, 63 and 33 per cent of a standard atmosphere. Then:

$$P = \frac{63}{33}P_0 = 1.9P_0$$

and, from equation (4),

$$P = 1.9P_1r$$

A more rapid, but approximate, estimate of the partial pressure of the anesthetic in the blood sample can be obtained with a single equilibration and the use of equation 1. In this case an arbitrary value must be assumed for λ_B, and V_A and V_B must be known:

$$P_0 = \frac{P_1V_B\lambda_B + V_A}{V_B\lambda_B} \qquad (5)$$

V_A and V_B are fixed by the stops and are readily determined by weighing. Since the ratio V_A/V_B is a constant, K, for any one

syringe, equation (5) reduces to:

$$P_0 = P_1 + \frac{P_1 K}{\lambda_B}$$

(2) Calculation of Solubility Coefficient

This requires knowledge of the volumes of the blood phase V_B and of the air phase V_A. From equation (2):

$$P_1 = \frac{P_2(V_B\lambda_B + V_A\lambda_A)}{V_B\lambda_B}$$

or

$$\frac{P_1}{P_2} = 1 + \frac{V_A\lambda_A}{V_B\lambda_B} = r$$

whence

$$\frac{V_A\lambda_A}{V_B\lambda_B} = r - 1$$

or

$$\frac{\lambda_B}{\lambda_A} = \frac{V_A}{V_B} \cdot \frac{1}{r-1}$$

and, since λ_A equals 1 by definition, finally

$$\lambda_B = K \cdot \frac{1}{r-1} \qquad (6)$$

where K is the syringe constant and r is the ratio between the first and second chromatograph peaks.

λ_B constitutes the solubility coefficient of the anesthetic in blood at room temperature. To obtain the solubility at body temperature, λ_B must be multiplied by 1/F, the reciprocal of the factor used to correct partial pressure. The reason for this is given in the appendix.

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Appendix

The objective is to calculate the partial pressure of anesthetic vapor in the blood at body temperature from a measurement at room temperature. The calculation depends on the relation between thermal changes in vapor pressure and thermal changes in vapor solubility. The following thermodynamic considerations show this relation to be a simple inverse one.

The Clapeyron-Clausius equation states the second law of thermodynamics in a form applicable to a two-phase equilibrium of a single substance, such as a liquid-vapor equilibrium:

$$\frac{dP}{dT} = \frac{\Delta H}{T\Delta V} \qquad (7)$$

P = vapor pressure of the liquid;
T = absolute temperature of the system.

For the particular case of a liquid-vapor equilibrium ΔH is equal to L_e, the molar heat of evaporation, and the volume change ΔV is $V_V - V_L$, where V_V and V_L are the molar volumes of vapor and liquid, respectively. Hence, for the variation of vapor pressure with absolute temperature we may write (7) as follows:

$$\frac{dP}{dT} = \frac{L_e}{T(V_V - V_L)} \qquad (8)$$

But the volume of the liquid may be considered negligible compared with the volume of vapor to which it gives rise, so that equation (8) reduces to:

$$\frac{dP}{dT} = \frac{L_e}{TV_V} \qquad (9)$$

TABLE 1. Solubility of Halothane in Blood–Saline Mixtures

Blood Sample	Partial Pressure of Halothane in Mixture (atm × 0.01)	Solubility Coefficient (corrected to 37 C)
1	7.73	2.28
	2.83	2.28
2	42.50	2.03
	13.70	2.03
3	17.90	2.08
	5.57	2.37
4	12.20	1.66
	3.31	1.84
5	2.54	2.16
	0.77	2.48
6	2.06	2.55
	0.68	2.51
7	1.12	2.39
	0.28	2.42
8	1.30	1.85
	0.40	2.13
9	1.00	1.85
	0.40	2.30
10	3.06	2.28
	1.34	2.07
11	2.11	2.22
	0.59	2.19

Assuming that the vapor behaves as an ideal gas, for one mole we have:

$$PV_V = RT$$

or,

$$V_V = \frac{RT}{P} \tag{10}$$

Substituting from (10) into (9) gives

$$\frac{dP}{dT} = \frac{L_e P}{RT^2}$$

and by rearrangement:

$$\frac{dP}{P} = \frac{L_e}{R} \cdot \frac{dT}{T^2} \tag{11}$$

If L_e is regarded as constant over the range of temperature under consideration (for two-carbon hydrocarbons the value at 37 C is about 98 per cent of the value at 21 C) equation (11) can be integrated. Integrating between the temperature limits T_1 and T_2, and the corresponding vapor pressures P_1 and P_2, one obtains:

$$\ln \frac{P_2}{P_1} = -\frac{L_e}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{12}$$

Equation (12) describes the effect of a temperature change on the vapor pressure of a liquid, and a graph of this equation constitutes the ideal vapor-pressure curve of the liquid. Still needed is an equation for the effect of temperature on the solubility of the vapor in a second liquid. When gases dissolve in water there is generally a liberation of heat. For an ideal gas, and at constant pressure, say one atmosphere, it is possible by thermodynamic methods to deduce[11] the relation

$$\ln \frac{C_2}{C_1} = -\frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{13}$$

where C_1 and C_2 are the concentrations of the gas (in moles per liter) in the liquid phase at absolute temperatures T_1 and T_2, respectively, and ΔH, the differential heat of solution of one mole of gas in a saturated solution, is assumed to be independent of temperature.[11]

The number of moles of the gas, of course, is proportional to the volume of the gas at S.T.P., so that C_1 and C_2 can also be expressed as volumes of gas per unit volume of liquid, in other words, as the Bunsen adsorption coefficient (at a pressure of one atmosphere) or as the Ostwald solubility coefficient (at other pressures). Hence, equation (13) may be rewritten

$$\ln \frac{\lambda_2}{\lambda_1} = -\frac{\Delta H}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{14}$$

Equations (12) and (14) are obviously similar in form, and since the factors L_e and ΔH in these equations can both be regarded as constants, it would appear that with a change in temperature, the logarithm of the vapor pressure changes proportionally to the logarithm of the solubility coefficient. However, the signs attached to L_e and to ΔH must be taken into account. L_e, the latent heat of vaporization, signifies an addition of heat to the system, whereas ΔH, the differential heat of solution of the gas, signifies a loss of heat from the system. In other words, L_e and ΔH have opposite signs, consequently the sign of $\ln \lambda_2/\lambda_1$ is opposite to the sign of $\ln P_2/P_1$, which amounts to saying that $\ln \lambda_2/\lambda_1$ is proportional to $\ln P_1/P_2$ or that λ varies inversely with P.

It follows that the vapor-pressure change with temperature, observed on a vapor-pressure curve, is the reciprocal of the vapor's solubility change with temperature. Hence, a solubility measured at one temperature can be readily converted to the solubility at any other temperature on the vapor-pressure curve. To do so, the vapor pressures at the two temperatures are read on the vapor-pressure curve: the reciprocal of the ratio between the two pressures constitutes the ratio of the corresponding solubilities. It is true that this rule has been derived for conditions where the vapor forms an *ideal* solution in the liquid but, within limits to be specified, the rule may be extended to the solubility in blood, notwithstanding that some of the anesthetic may be bound to macromolecules. When small molecules combine reversibly with macromolecules the usual laws of equilibrium govern the reaction. The number of combining sites on a macromolecule may be large, so that a large number of equilibrium constants may be needed, but no new principle is thereby introduced. The equilibirum at low concentrations of the smaller molecule may be defined in terms of a series of equilibrium con-

stants $k_1, k_2 \cdots k_n$:

$$k_1 = \frac{(PA_1)}{(P)(H)}$$

$$k_2 = \frac{(PA_2)}{(PA_1)(H)}$$

$$k_n = \frac{(PA_n)}{(PA_2)(H)}$$

where A_1, A_2, A_n represent adsorption sites of the anesthetic on macromolecule P, and (H) represents the concentration of the smaller molecule. Hence,

$$(H) = \frac{(PA_1)}{k_1(P)} = \frac{(PA_2)}{k_2(PA_1)} \cdots = \frac{(PA_n)}{k_n(PA_2)}$$

The above relations merely say that the ratio of occupied-to-unoccupied sites on the macromolecule is proportional to (H), provided no site is saturated. Since the measured solubility includes the adsorbed molecules as well as those in solution, if the number of small molecules adsorbed is proportional to the pressure, the sum of small molecules added per unit pressure will be constant. In other words, so long as no site is saturated with the anesthetic the solubility of the vapor in the system will appear constant.

The matter is easily put to a test. Applying the present technique, it is necessary only to measure the solubility of the anesthetic in blood at two differential partial pressures, within or above the clinical range. If the two measured solubilities are the same, the correction is validated. Table 1 shows the results of a series of such tests carried out with halothane. Different quantities of halothane or of a solution of halothane in isotonic sodium chloride were added to two aliquots of blood. The partial pressure and solubility of the halothane in the two mixtures were measured using equilibrations (4) and (6). Results with 11 different bloods are shown in the table, after correcting for the volume of saline solution ($\lambda = 0.73$) mixed with each blood. It is clear that, over the clinical range, the solubility of halothane in the mixtures is independent of the partial pressure of halothane.

In conclusion, it may be pointed out that the correction factors F and 1/F described in this article are necessary even when the equilibration with air is carried out at 37 C,[8-10] if the temperature of the subject differs significantly from the temperature of the equilibration.

Obstetrics and Pediatrics

RESPIRATORY DISTRESS IN INFANTS Respiratory symptoms in acyanotic infants who have congenital heart disease may result from several factors, including cardiac failure and bronchial obstruction. Significant bronchial obstruction can be caused by hypertensive pulmonary arteries or an enlarged left atrium. The left main bronchus, left upper and right middle bronchus are most commonly involved. Infantile lobar emphysema is associated with acyanotic congenital cardiac disease and may represent another sequela of bronchial compression by distended pulmonary arteries and the enlarged left atrium. The deformity of the bronchus may remain for some time after the cause of the deformity is removed, and may be responsible for respiratory complications in infants following cardiac surgical procedures. *(Stanger, P., Lucas, R. V., Jr., and Edwards, J. E.: Anatomic Factors Causing Respiratory Distress in Acyanotic Congenital Cardiac Disease, Pediatrics 43: 760 (May) 1969.)*

OMPHALOCELE The care of seven newborns with omphalocele is described. If immediate replacement into the abdomen is attempted, 50 per cent mortality results from pressure on the vena cava and the diaphragm. The method recommended is to cover the omphalocele with skin, or to apply mild antiseptics repeatedly. The result is an enormous ventral hernia. A plastic catheter is inserted into the peritoneal cavity. Air is injected daily to distend the peritoneal cavity. Increasing amounts of air generally stretch the abdominal wall until it is ready to accommodate the viscera. The amount of air injected each day is 150 to 250 ml. Excess air is indicated by development of grunting respiration. Use of the indwelling plastic catheter is safer than repeated needle punctures to introduce air. There have been no complications in seven cases treated with this technique. The longer the pneumoperitoneum is maintained, the easier it is to replace the viscera and repair the hernia. *(Ravitch, M.: Omphalocele: Secondary Repair with the Aid of Pneumoperitoneum, Arch. Surg. 99: 166 (Aug.) 1969.)*