The Solubility of Halothane in Blood and Tissue Homogenates

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THE dynamic distribution of any drug in the body is determined in large part by its solubility in the body parts. Knowledge of these solubilities is a prerequisite for understanding drug uptake and distribution, and hence the rational use of that drug. To date, little information is available concerning the absolute or relative solubility of halothane in the body. Duncan and Raventós [1, 2] have reported the blood-air partition coefficient for halothane at 37° C. to be 3.6, the oil-water partition coefficient to be 330, with a water solubility of 0.345 parts per 100 parts water. This paper will present the results of more extensive determinations of the solubility of halothane in various body fluids, tissues and tissue components. The term "partition coefficient" and the symbol λ will be used, the latter being numerically equal to the Ostwald solubility coefficient when a gas phase exists.

Method

TECHNIQUE OF ANALYSIS FOR HALOTHANE

Five 2,000 ml. rubber stoppered Erlenmeyer flasks containing blood or tissue samples were flushed with oxygen and evacuated to about two thirds of an atmosphere. From a calibrated micropipette, 0.1 ml. ($\pm$ 0.001 ml.) liquid halothane * was aspirated by the flask vacuum through a stopcock and needle into each of four flasks. A fifth control flask was identical to the others except that halothane was not added. Equilibration of halothane was accomplished by periodic manual agitation at 37° C. for two to four hours. Oxygen was then admitted to bring the pressure within the flasks to ambient.

The concentration of halothane in the gas phase was determined with a Beckman infrared halothane analyzer, connected by a nylon catheter to the one-way stopcock in the stopper. About 10 ml. of oxygen was injected with a syringe through the analyzer head into the flask so that a slight positive pressure existed within the flask. With release of the syringe barrel, the pressure forced flask gas containing halothane back through the analyzer. The flask was then shaken vigorously and the gas phase reanalyzed, the procedure being repeated until three consecutive identical readings were obtained. All samples had attained equilibration within two to four hours, as evidenced by no further change in concentration of the gas phase up to six hours. Human fat and lecithin were arbitrarily equilibrated for 18 to 24 hours prior to analysis because their heavy consistency prevented adequate shaking and more rapid equilibration.

Rate of loss of halothane from stoppered flasks with time was determined and found to be insignificant in four hours, with about 3 per cent loss in 24 hours.[3] Corrections for halothane loss with time were included in the calculations of solubility coefficient for human fat and animal lecithin.

PREPARATION OF SAMPLES

Human blood from the blood bank or from the cardiac surgery pump oxygenator was pooled according to the type of anticoagulant used and quadruplicate determinations run on each pool. The pooled blood hematocrit ranged from 42 to 45. Three independent pools of fresh, heparinized beef blood were also obtained, the hematocrit ranging from 40

* Supplied by Ayerst Laboratories.

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to 45. Partition coefficients were also determined in human and beef blood diluted 50 to 67 per cent with 0.9 per cent saline.

Hemoglobin solutions were prepared from packed, heparinized human red cells by washing twice with two volumes of 0.9 per cent saline and then hemolyzing with two volumes of distilled water. Red cell ghosts were removed by ultracentrifuging at 35,000 g's for 15 minutes.[4]

Human tissues from autopsy material and corresponding beef tissues were stripped of capsular and vascular connective tissue and the quantity (ml.) determined by saline displacement. The tissues were then homogenized with known volumes of 0.9 per cent saline. Partition coefficients were obtained for both gray and white cortex as well as for intact human brain. Separation of the brain into these two components was done by careful dissection, but because of the undulating cortical convolutions, there was undoubtedly a small amount of tissue overlap in these homogenates.

The total volume of blood or tissue homogenate added to each flask ranges from 300 to 500 ml. Silicone antifoam (GE-10), 0.1 to 1.0 ml., was added to prevent foaming during shaking or homogenization. Flasks containing antifoam alone did not take up a measurable amount of halothane.

Known amounts of animal lecithin (90 per cent) † dissolved in chloroform were added to each of the flasks. The chloroform was slowly evaporated in a 60 to 80° C. waterbath, leaving the waxy lecithin coating the inner surface of the flasks. Each flask was stoppered for 24 hours or more and the gas phase analyzed for any residual chloroform vapor, with the halothane analyzer, which is sensitive to chloroform. When none was present, halothane was added to each flask and the partition coefficient determined as previously described.

Calibration of Instruments

The infrared halothane analyzer was calibrated with an H cylinder containing 2.5 per cent halothane in oxygen, prepared by adding 70 ml. liquid halothane to an empty cylinder and pressurizing it with oxygen to 175 pounds per square inch. Halothane concentration in the tank was determined by measuring the oxygen concentration difference from 100 per cent with a paramagnetic oxygen analyzer.‡ Using 2.54 per cent halothane for full scale deflection and pure oxygen for zero, intermediate points were obtained by volumetric dilution of the reference sample with oxygen. These points were joined for a calibration curve.

The halothane analyzer head was filled with 16 per cent CO_2 to minimize response to CO_2 liberated from the tissues within the stoppered flasks. Since zero deflection was not entirely eliminated by this maneuver, a control flask, containing the same substance without halothane was analyzed in each set and the appropriate zero correction determined.

Calculations

The Ostwald solubility coefficient (λ) is the volume of gas dissolved per unit volume of solvent measured at equilibration temperature when the partial pressure of the gas equals one atmosphere.[5] It is numerically equal to the partition coefficient, which is the ratio of the concentration of gas in the two phases. Under the conditions of this experiment:

$$\lambda = \frac{C_L}{C_G} \qquad (1)$$

$$C_L = \frac{V_I - C_G(V_F - V_L)}{V_L}$$

Therefore: $$\lambda = \frac{V_I - C_G(V_F - V_L)}{V_L C_G}$$

Where:

C_L = concentration of halothane in liquid phase

* C_G = concentration of halothane in gas phase

* V_I = volume of halothane vapor injected

V_F = volume of flask

V_L = volume of test material

* Corrected to 760 mm. of mercury.

For blood or tissue homogenates diluted with normal saline, prior determination of the par-

† This material is the commercially prepared acetone insoluble, alcohol soluble phospholipid fraction from bovine brain and spinal cord.

‡ Beckman-Model E_2.

tition coefficient of halothane in saline permitted calculation of the partition coefficient for the test material by modification of formula 1 to:

$$\lambda = \frac{V_I - C_G[V_S\lambda_S + V_E - (V_L + V_S)]}{V_L C_G} \quad (2)$$

Where:

V_S = volume of saline

λ_S = solubility coefficient of halothane in saline (0.70)

To obtain V_I, we vaporized 0.100 ml. liquid halothane in oxygen in each of the stoppered flasks at 37° C. and analyzed the halothane concentration of the resultant gas mixture. In 64 determinations, 0.100 ml. liquid halothane gave an average of 23.99 ml. (± 0.3 ml.) halothane vapor. § This agrees with the predicted volume of 23.96 ml. halothane vapor per 0.1 ml. liquid obtained from mole volume calculations.

V_L for saline or water was obtained by weighing the fluid and multiplying by the appropriate conversion factor to volume.[6,7] V_L for blood, plasma and tissue homogenates was determined by measuring the volume of water needed for displacement of the gas volume after the analysis was complete. The volume (± 1 ml.) of each stoppered flask was obtained by filling the flask with water and converting the differential weight to volume with a conversion factor.[7] V_L for olive oil, human fat and lecithin was obtained by differential weight, with independent determinations of the density of these substances.

Results

The partition coefficient of halothane for water, 0.9 per cent saline, olive oil, human blood and tissue homogenates at 37° C. are shown in table 1. Corresponding values for bovine blood and tissue homogenates are included for comparison. Table 2 shows the relationship of the partition coefficients of halothane in blood, oil and tissues to those of other commonly used anesthestic gases. These data were compiled from many sources in which varied techniques and methods were used. Where conflicting values were obtained, only the one considered most reliable was included in this table. Solubility coefficients have traditionally been expressed with gas volumes corrected to 0° C. (Bunsen coefficient). We have corrected such Bunsen coefficients at 37° C. to partition coefficients at 37° C. by multiplying by 310/273.[8]

§ Corrected to 760 mm. of mercury.

TABLE 1. Ostwald Solubility or Partition Coefficient of Halothane for Water, Saline, Oil and Human and Beef Blood and Tissue Homogenates at 37° C. and 760 mm. Hg

Substance	Number of Determinations	Partition Coefficient	1 Standard Deviation or Range
Distilled water	11	0.74	0.05
0.9 per cent saline	12	0.70	0.05
Whole blood, human	16	2.3	0.1
Whole blood, bovine	11	2.3	0.1
Whole blood, human (saline dilution method)	4	2.4	0.2
Whole blood, bovine (saline dilution method)	12	2.3	0.1
Hemoglobin	4	6.7	0.5
Whole brain, human	4	6.0	0.3
Whole brain, bovine	2	4.8	4.75 to 4.91
Cerebral cortex, human white matter	7	8.3	0.8
Cerebral cortex, human gray matter	4	5.4	0.1
Liver, human	4	6.0	0.3
Liver, bovine	2	4.2	4.14 to 4.22
Kidney, human	3	3.6	3.32 to 3.76
Kidney, bovine	4	3.5	0.04
Muscle, human psoas	4	8.0	0.03
Muscle, bovine	4	7.0	0.2
Fat, human perirenal	4	138	7
Lecithin	3	126	122 to 130
Olive oil	12	224	15

Discussion

The partition coefficient for both human and bovine whole blood was 2.3. Essentially the same values were obtained for saline diluted specimens of these bloods. Since the partition coefficient for saline was 0.70, the protein and lipid elements in blood must constitute the chief source of halothane uptake. The partition coefficients for hemoglobin (6.7), saline (0.70) and phospholipid (126) were used to predict a solubility coefficient for whole blood from assumed concentrations of protein 19.5 per cent, lipid 0.5 per cent and saline 80 per cent.[9] This calculated value of 2.5 agrees well with the observed value of 2.3, but both values differ considerably from the 3.6 reported by Duncan and Raventós. Later data by Duncan and Raventós [2] indicate that a halothane blood-gas partition coefficient of 3.6 is too high. Assuming near equilibration of halothane between inspired gas and arterial blood in six hours,[10] and knowing the inspired anesthetic concentration (1.5 per cent v/v less 6 per cent

TABLE 2. Partition Coefficients of Common Anesthetic Gases at 37° C. ± 0.5° C.

Anesthetic Gas	Water / Gas	Blood / Gas	Oil / Gas	Tissue / Blood	Clearance Rate in One Passage Through Lungs (Per Cent)
Ethylene	0.081* [12]	0.140* [20]	1.28 [21]	1.0 (Heart) [22] 1.2 (Brain) [22]	84
Cyclopropane	0.204 [23]	0.415 [24]	11.2* [25]	0.92 (Muscle)† [26] 1.34 (Liver)† [26]	66
Nitrous oxide	0.435* [27]	0.468* [28]	1.40 [29]	1.13 (Heart) [30] 1.06 (Brain) [28] 1.0 (Lung) [31]	63
Halothane	0.71	2.3	224	2.6 (Brain) 2.6 (Liver) 1.6 (Kidney) 3.5 (Muscle) 60 (Fat)	26
Trichloroethylene	1.55 [35]	9.15 [35]			9
Chloroform	3.8 [31]	10.3 [31]	265 [29]		7
Diethyl ether	15.61 [32]	15.2 [32]	50.2 [29]	1.14 (Brain) [33] 1.0 (Lung) [31]	5

* Bunsen coefficient corrected to 37° C.
† Calculated from existing data.
Superior figures are references to the literature.

for water vapor or 1.41 per cent v/v), the average concentration of halothane in rat blood at equilibrium (21.5 mg. per 100 g. blood after six hours anesthesia) and the specific gravity of rat blood (1.055),[11] the calculated blood-gas partition coefficient at 37° is:

$$\lambda = \frac{C_L}{C_G} = \frac{21.5 \times 22.4 \times 310 \times 1.055}{197.39 \times 273} \quad (3)$$

$$\lambda = 2.1$$

Presuming the halothane blood-gas partition coefficient to be 3.6, would mean that after six hours of anesthesia, the alveolar halothane concentration would be only 52 per cent of the inspired concentration, a finding not consistent with known data.[10]

Small changes in fat or protein content will affect the partition coefficient for halothane in blood. These variations will be greater for halothane than for ethylene,[12] nitrous oxide [13] or cyclopropane [14] because of halothane's greater fat solubility. Use of pooled blood samples eliminated such variations among the flasks.

The human perirenal fat partition coefficient was 138 or about 60 per cent that for olive oil, indicating that the adipose tissue contained about 40 per cent water. The halothane partition coefficient for 90 per cent bovine lecithin was 126. Here again a partition coefficient less than that for the same volume of olive oil might be predicted since lecithin contains phosphoric acid and choline in addition to glycerol and fatty acid radicals.[15]

Halothane partition coefficients in tissue homogenates would be expected to vary with the fat content of those tissues. In general, we found this to be true. Bovine kidney, whose total lipid content is about 4 per cent of the fresh weight, had a lower partition coefficient than bovine liver, which is about 6 per cent lipid or bovine muscle, which may contain as much as 10 to 14 per cent lipid.[9] Human cortical white matter, which is about 17 per cent lipid, had a higher partition coeffi-

cient than gray matter, which is about 5 per cent lipid.[9] Furthermore, by comparing halothane solubility in oil and lecithin, it is evident that tissues whose fat compartment is chiefly in the form of neutral fat (muscle)[9] may have a higher tissue-gas partition coefficient than will tissues whose total fat content is greater but which exists principally as phospholipid (brain).[9]

It must be emphasized that the partition coefficients for various body tissues, as for blood, are only approximate values. Lipid and protein content of any tissue or organ may vary as a result of several factors, including nutritional status of the organism and relationship of the study to the time of feeding. Again pooled specimens were used to minimize such fluctuations.

The tissue homogenate studies indicate that halothane is about 1½ to 3½ times as soluble in tissue compartments as in whole blood. This finding is at variance with that for most of the common anesthetic gases (table 2), which have a tissue-blood partition coefficient of about 1. The explanation for this difference is probably the result of halothane's high lipid solubility compared to the lipid solubility of other commonly used anesthetic gases (table 2). Confirmation of a halothane tissue-blood partition coefficient greater than 1 can be obtained from Duncan and Raventós, who reported the concentration of halothane in various rat tissues during anesthesia.[2] Using their data at the end of six hours of anesthesia, we calculate the rat brain-blood partition coefficient to be 2.1 and the rat liver-blood partition coefficient to be 2.2, both of which approach the human brain-blood and liver-blood partition coefficients of 2.6 obtained by us (table 2). Their tissue-blood values are slightly lower probably because complete tissue-blood equilibration had not occurred in six hours as shown by the still rising tissue concentrations of halothane.

Chloroform would also be expected to have a tissue-blood partition coefficient of greater than 1, based on a calculated oil-gas coefficient of 265. Nicloux[16] reported tissue-blood partition coefficients for chloroform in brain and liver to be about 1. However, one may object that dogs breathing a constant chloroform mixture could not reach tissue-blood equilibration during the period of inhalation of chloroform (110 minutes), since the arterial chloroform concentration was rising at the time of supposed equilibration. Both Storm van Leeuwen[17] and Hansen[18] reported tissue-blood partition coefficients for chloroform of about 1, but sufficient time was not allowed for equilibration in these *in vivo* experiments.

Knowing the partition coefficient of halothane in bloody and body tissues, one can make certain predictions about the rate of rise of halothane concentration in the human body during the course of general anesthesia. The high blood solubility of halothane relative to air results in a slower rise in the alveolar and arterial halothane tensions toward the inspired tension than would occur with less soluble anesthetics such as nitrous oxide, ethylene or cyclopropane. Blood returning to the lungs from the tissues initially has a low halothane tension because of the loss of halothane to tissue compartments. This serves to maintain a high alveolar gas-arterial blood concentration gradient and impedes the rise in halothane concentration in the alveoli beyond that occurring with other agents, whose tissue-blood partition coefficients approach 1.

Kety[19] has published human anesthestic uptake curves for common anesthetic gases based on solubility of these gases in blood. Superimposing a halothane uptake curve on these predicted curves, the "knee" of the curve would lie somewhere between that for chloroform and cyclopropane. The "tail" of the curve would rise only very gradually, ultimately being crossed by the curve for ether, which has a greater blood solubility but not so great a fat solubility.

In their study on the pharmacokinetics of halothane anesthesia, Duncan and Raventós[2] estimated it would take about 20 hours of anesthesia in the mouse to attain equilibration of the tissues with the inspired halothane concentration (1.5 per cent v/v). Because of the extreme solubility of halothane in fat and the relatively poor blood flow to fat compartments, we calculate it would take about 140 hours to attain 95 per cent tissue-blood equilibration in an adult man of 15 liters fat volume, assuming a constant anesthetic tension in the arterial blood and constant blood flow to fat (equation 4).

$$T = \frac{\lambda t V_t}{Q_t} \cdot \log_e \frac{1}{0.05} \qquad (4)$$

Where:

T = time to 95 per cent tissue-blood equilibration (in minutes)

λt = tissue-blood partition coefficient (60 for fat)

V_t = volume of tissue (15 l. fat)

Q_t = blood flow to tissue compartment (0.3 l./minute)

Duncan and Raventós [2] reported that the clearance of halothane from the mouse occurs in an exponential fashion with time. They further stated that venous blood is almost completely cleared during its passage through the lungs, with only small amounts of halothane being carried over to arterial blood. Using a 2.3 blood-gas partition coefficient and assuming a constant alveolar ventilation of 4 l./minute, constant pulmonary capillary blood flow of 5 l./minute and normal ventilation-pulmonary blood flow relationships, we have calculated the clearance rate of halothane from venous blood during a single passage through the lungs (equation 5):

$$\text{Per cent clearance} = \frac{\dot{V}_A \times 100}{\dot{V}_A + \lambda \dot{Q}_c} \qquad (5)$$

Where:

$\dot{V}_A$ = alveolar ventilation per minute

$\dot{Q}_c$ = pulmonary capillary blood flow per minute

Approximately 26 per cent of the halothane in the mixed venous blood will be removed by the alveolar ventilation during a single passage of blood through the lungs. The remaining 74 per cent of the halothane will be recirculated in the arterial blood, to be removed on subsequent passages through the lungs. The greater the clearance rate of an anesthetic by the lungs, the less anesthetic will be recirculated to the brain and hence the shorter the recovery period. On this basis, patients will awaken more rapidly from ethylene, nitrous oxide or cyclopropane anesthesia than from halothane, but will awaken more slowly from either chloroform or ether. This prediction, however, ignores the effects of the tissue-blood coefficients, which, if elevated, would tend to maintain venous anesthetic concentration and thereby prolong the awakening time. Halothane being the only anesthetic known to date with an elevated tissue-blood partition coefficient, is the only anesthetic in which this would be a significant factor.

Summary

The Ostwald solubility coefficients (37° and 760 mm. of mercury), λ or partition coefficients of halothane in blood and body tissue homogenates of man and cattle were determined by equilibrating these substances with a known volume of halothane in closed flasks and analyzing the halothane concentration of the overlying gas phase with an infrared halothane analyzer. For human or bovine whole blood, the partition coefficient was 2.3 and in tissue homogenates ranged from 3.6 for human kidney to 8.3 for human cerebral white matter.

The finding of a tissue-blood partition coefficient greater than 1 is at variance with previously reported data for other anesthetics, the explanation probably being halothane's extreme fat solubility ($\lambda = 138$ in perirenal fat and 224 in oil). With this information, predictions can be made concerning the rate of rise of halothane in the human body during general anesthesia. Furthermore, we calculate that it would take about 140 hours to attain 95 per cent tissue-blood equilibration in an adult man of about 15 liters fat volume. Additional calculations indicate that approximately 26 per cent of the halothane in mixed venous blood is cleared in one passage through the lungs.

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