Biophysical Notes

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Halothane Solubility in Blood and Solutions of Plasma Proteins:

Effects of Temperature, Protein Composition and Hemoglobin Concentration

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The solubility of halothane (2-bromo-2-chloro-1:1:1-trifluoroethane) in human blood is significantly changed by alterations in plasma protein and hemoglobin concentration. An albumin: globulin ratio of 3:1 in blood gives a λ value approximately 45 per cent higher than an albumin: globulin ratio of 1:2. Halothane is more soluble in protein solutions at lower temperatures, but increases in solubility are not linearly proportional to the solubilities of halothane in water. The authors' results indicate that solubility of halothane in blood and plasma depends partly upon the concentration and composition of the proteins in these body fluids. The variation of hematocrit in normal range has only a small effect upon the solubility of halothane in blood. (Key words: Halothane; Ostwald solubility coefficient; Temperature; Blood; Plasma proteins.)

The solubility of halothane in human blood and plasma varies from patient to patient.[2] This finding suggests that the solubility of halothane in blood may be influenced by the composition of blood and plasma. The purpose of this study was to evaluate the effects of plasma proteins and hemoglobin concentrations upon solubility of halothane in blood and plasma. In addition, the solubility coefficient of halothane (λ) for known concentrations of albumin, α-, β- and γ-globulins, fibrinogen and hemoglobin in Krebs-Henseleit solution were determined at 37, 30 and 25 C.

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Received from the Department of Anaesthesia, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts. Accepted for publication January 12, 1970. Supported by Grant 1 GM15904 from the National Institutes of Health. Portions of this work have been presented.[1]

Materials and Methods

Blood (Hb 13.7–14.9 g/100 ml) was obtained from ten healthy male subjects. Each 500-ml sample was heparinized by the addition of 0.5 ml heparin (20,000 units). Twenty-ml samples of blood, plasma, water, Krebs-Henseleit solution (table 1 of reference 3) and various protein solutions were equilibrated with equal volumes of halothane vapor in air (29.7 mg halothane/100 ml air) at 25, 30 and 37 C for four hours in a temperature-controlled water bath (±0.05 C). A capillary glass electrode G297/G2 and calomel electrode K497 were used to measure *p*H in blood, plasma and protein–Krebs Henseleit solutions prior to and after equilibration with the halothane. For readout the electrodes were attached to Radiometer PHA-927 and PHM-27 monitors. Calibrated, leakproof ground-glass syringes served as equilibration containers. The syringes were flushed four times with the halothane–air mixture and then filled with the mixture before determination of the halothane concentration in the syringe (initial concentration). The volume of gas was brought to 20 ml by expelling the excess gas, and the stopcock closed. The three-way stopcock was flushed with the liquid sample and 20 ml introduced into the syringe. After four hours of equilibration at 37, 30 or 25 C, the gaseous phase in the syringe was again analyzed for halothane (final concentration). The difference between initial and final concentrations was the amount of halothane absorbed by the liquid phase. From these values the solubility coefficient (λ) was calculated.[4] All analyses of halothane concentrations were done with a Hewlett-Packard Research Gas Chromato-

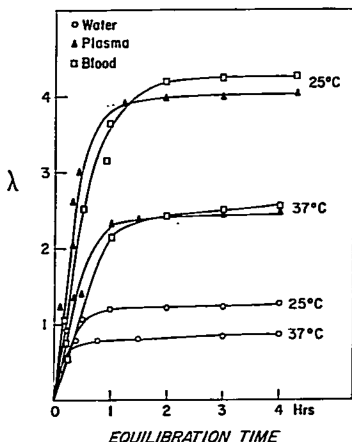

FIG. 1. Halothane equilibrates more slowly with blood than with plasma or water. Ostwald solubility coefficients (λ) of halothane are plotted against equilibration time.

graph, model 5750. The instrument was equipped with dual 6-foot columns, which were packed with 3.8 per cent (w/w) silicone fluid on Chromosorb W. A hydrogen flame ionization detector was used for quantitation of halothane. The temperatures of injection port, columns and detector were 102, 50 and 140 C, respectively, and were kept constant during the analysis. Reproducibility of the halothane determinations was ±2 per cent, and responses of the detector to halothane concentration were linear.

The hemoglobin concentration of heparinized blood obtained from one subject was changed seven times by subtraction of either plasma or cells after sedimentation. Samples of this blood were then used for determination of λ-halothane in blood at various hemoglobin concentrations. In addition, in order to evaluate the effect of hemoglobin concentration upon solubility of halothane in blood with different albumin:globulin (A:G) ratios, plasma samples with known protein concentrations (8 g/100 ml) and A:G ratios of 1:2 and 3:1 were prepared; hemoglobin concentration was adjusted to 7.5 and to 15.0 g/100 ml of blood by adding packed erythrocytes to the plasma. These erythrocytes were withdrawn from another sample of the same subject's blood after centrifugation of that blood. The hemoglobin concentrations of the resultant blood specimens were determined by the cyanmethemoglobin technique.[5]

The effects of serum albumin, α-, β- and γ-globulins, fibrinogen and hemoglobin upon solubility of halothane (λ) in plasma and Krebs-Henseleit solution were determined by adding plasma or Krebs-Henseleit solution to 4 g of each protein to give a volume of 100 ml. Purity of the proteins was evaluated by electrophoresis and the content of lipids (mg/g dry weight of protein) determined. Total serum protein contents of the various bloods were analyzed[6] and found to range between 6.5 and 7.9 g/100 ml serum; the relative compositions of serum proteins were determined

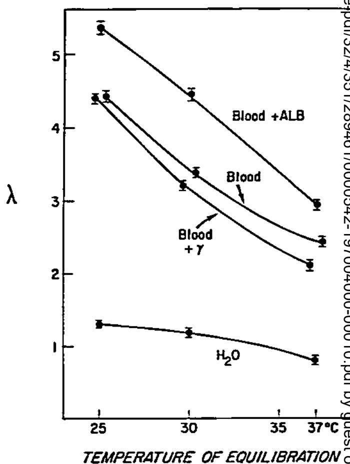

FIG. 2. The solubility of halothane in blood is increased by high serum albumin concentrations and decreased by γ-globulins. Solubility coefficients (λ) of halothane are shown for normal human blood (14.2 g Hb/100 ml) and for blood after a 4 g/100 ml increase in albumin concentration (top line) and an equal increase in γ-globulin concentration. There is no constant relationship* between solubilities of halothane in blood and in water.

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TABLE 1. Ostwald Solubility Coefficients λ ± SE for Water, Human Blood and Plasma at 25, 30 and 37 C

Sample	25 C (N)	30 C (N)	37 C (N)
Water	1.28 ± 0.01 (46)	1.16 ± 0.01 (35)	0.78 ± 0.01 (93)
a. Plasma Albumin = 4.45 ± 0.06; globulins, α_1 = 0.34 ± 0.03; α_2 = 0.45 ± 0.04 β = 0.99 ± 0.06; γ = 0.76 ± 0.03; fibrinogen = 0.30 ± 0.01 g/100 ml. Total lipids = 427 ± 13 mg/100 ml.	3.84 ± 0.05 (53)	3.27 ± 0.03 (56)	2.28 ± 0.02 (73)
b. Plasma + albumin 4 g albumin/100 ml added to *a*.	5.07 ± 0.09 (35)	4.28 ± 0.03 (30)	3.46 ± 0.04 (36)
c. Plasma + γ-globulin 4 g γ-globulins added to *a*.	3.81 ± 0.08 (26)	3.14 ± 0.02 (31)	2.16 ± 0.02 (31)
d. Blood 14.2 ± 0.05 g Hb/100 ml blood, plasma as *a*.	4.54 ± 0.04 (33)	3.38 ± 0.02 (38)	2.42 ± 0.02 (37)
e. Blood + albumin Plasma as *b*, 14.2 g Hb/100 ml blood.	5.40 ± 0.05 (39)	4.46 ± 0.02 (35)	2.91 ± 0.02 (27)
f. Blood + γ-globulin Plasma as *c*, 14.2 g Hb/100 ml blood.	4.41 ± 0.04 (21)	3.19 ± 0.04 (21)	2.11 ± 0.02 (32)

by electrophoresis using cellulose polyacetate strips and barbital buffer (*p*H 8.6 and $\mu = 0.03$). The lipid concentrations in plasma were determined by methanol-chloroform extraction.[7]

Results

The effects of equilibration time upon solubility of halothane in blood, plasma and water at 25 and 37 C are shown in figure 1. Approximately two hours were required for equilibration of gaseous and liquid phases to obtain reasonably reproducible solubility coefficients of halothane for blood and plasma. The mean values (±SEM) of Ostwald solubility coefficients (λ) for water, normal blood and plasma specimens at 25, 30 and 37 C are given in table 1. When the concentration of albumin in plasma was increased by 4 g/100 ml the λ-halothane values for plasma changed from 2.28 to 3.46 at 37 C, from 3.27 to 4.28 at 30 C, and from 3.84 to 5.07 at 25 C. Equal increases of γ-globulin concentrations in plasma caused small but significant ($P < 0.01$) depressions of the solubility coefficients (λ) at 30 and 37 C; at 25 C there was no noticeable effect (table 1).

At 37 C an increase of albumin concentration (4 g/100 ml plasma) raised the solubility coefficients of halothane in blood from 2.42 to 2.91; in contrast, an equal elevation of γ-globulins decreased the λ value from 2.42 to 2.11 ($P < 0.01$) (fig. 2). Similarly, at 30 and 25 C the solubility of halothane in blood was depressed when concentration of γ-globulins was increased (table 1, fig. 2).

For blood samples the *p*H values averaged 7.8 (range 7.9–7.7) before, and 7.3 (range 7.0–7.4) after, equilibration with halothane. Corresponding average values for plasma were 7.7 and 7.6.

The effects of different albumin:globulin (A:G) ratios upon solubility of halothane in blood with hemoglobin concentrations of 7.5 and 15 g/100 ml are shown in table 2. When the A:G ratio was increased from 1:2 to 3:1 the λ value for plasma at 37 C was raised from 2.02 to 2.78; similar increases in halo-

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TABLE 2. Solubility Coefficients (λ) for Halothane in Bloods with Various Concentrations of Hemoglobin and Albumin-Globulin Ratios (A:G) at 37 C*

Hb (g/100 ml)	A:G Ratio	$\lambda \pm$ SE (N)
0	1:2†	2.02 ± 0.02 (15)
7.5	1:2	1.97 ± 0.02 (11)
15.0	1:2	2.15 ± 0.02 (14)
0	3:1‡	2.78 ± 0.03 (15)
7.5	3:1	2.82 ± 0.03 (15)
15.0	3:1	3.11 ± 0.03 (16)

* Total serum protein concentration of every sample was 7.7 g/100 ml.
† A:G = 1:2: albumin = 2.65; α_1 = 0.52; α_2 = 0.70; β = 0.98; γ = 2.85; fibrinogen = 0.30.
‡ A:G = 3:1: albumin = 5.96; α_1 = 0.28; α_2 = 0.38; β = 0.49; γ = 0.64; fibrinogen = 0.25.

thane solubility were obtained in blood with hemoglobin concentrations of 7.5 and 15 g/100 ml. An elevated A:G ratio had a greater effect upon the solubility of halothane than an increase of the hemoglobin concentration. Thus, a change in hemoglobin concentration from 7.5 to 15 g/100 ml of blood increased λ-halothane from 1.97 to 2.15 at 37 C.

In figure 3 the solubility coefficients of halothane at 37 C are shown for blood with hemoglobin concentrations from 0 to 27.8 g/100 ml of blood and normal plasma protein composition. In anemic blood the solubility of halothane is lower than in normal blood, and in blood with very high hematocrits (Hb > 20 g/100 ml) the solubility of the anesthetic is significantly ($P < 0.001$) lower than in normal blood (Hb 15 g/100 ml).

The solubility coefficients of halothane for various protein solutions at 25, 30 and 37 C are given in table 3. Solubility of halothane in Krebs-Henseleit solution was practically the same as in water at all temperatures (tables 1 and 3). Of the various plasma protein solutions, albumin had the highest λ-halothane values: 1.72, 2.38, and 3.29, at 37, 30, and 25 C, respectively. The presence of 4 g of either fibrinogen or γ-globulin in Krebs-Henseleit solution had an insignificant ($P < 0.2$) effect upon the solubility of halothane at 37 C; these proteins decreased the solubility of halothane at 30 C in Krebs-Henseleit solution. However, at 25 C γ-globulins raised the solubility coefficient of halothane from 1.27 to 1.37 ($P < 0.001$), and a concentration of 4 g fibrinogen/100 ml Krebs-Henseleit solution depressed λ-halothane from 1.27 to 1.18 ($P < 0.001$). The solubility coefficients of halothane for α-globulin solutions were 1.55, 2.04, and 2.52, at 37, 30, and 25 C, respectively. The corresponding λ-halothane values for β-globulin solutions were 1.48, 2.15, and 2.47. The presence of hemoglobin (4 g/100 ml) in Krebs-Henseleit solution always increased the solubility of halothane as compared with λ-halothane values for the solvent. Krebs-Hense-

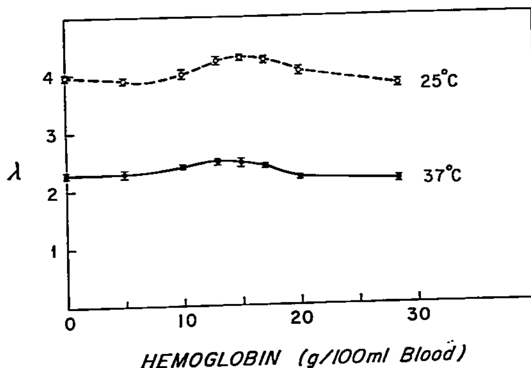

FIG. 3. The solubility coefficients (λ) of halothane in human whole blood at 25 and 37 C are shown for blood samples with different hemoglobin concentrations. The solubility of halothane in anemic blood (Hb 10 g/100 ml) is lower than that in normal blood (Hb 15 g/100 ml). Thus, λ-halothane for blood is not proportional to λ plasma and λ erythrocytes. Concentrations of plasma proteins are 4.18 g albumin, 2.12 g globulins ($\alpha_1 = 0.35$; $\alpha_2 = 0.63$; $\beta = 0.67$; $\gamma = 0.47$) and 0.28 g fibrinogen in 100 ml. Concentration of total lipids is 410 mg/100 ml plasma.

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TABLE 3. Ostwald Solubility Coefficients (λ) ± SE for Proteins (4 g/100 ml) in Krebs Henseleit Solution at Various Temperatures*

Sample	25 C (N)	30 C (N)	37 C (N)
Krebs-Henseleit solution (KH)	1.27 ± 0.01 (65)	1.16 ± 0.01 (37)	0.76 ± 0.01 (37)
KH + albumin†	3.29 ± 0.03 (31)	2.38 ± 0.02 (41)	1.72 ± 0.02 (32)
KH + α-globulins†	2.52 ± 0.02 (50)	2.04 ± 0.02 (39)	1.55 ± 0.02 (22)
KH + β-globulins‡	2.47 ± 0.03 (22)	2.15 ± 0.02 (12)	1.48 ± 0.02 (26)
KH + γ-globulins†	1.37 ± 0.02 (35)	1.11 ± 0.01 (37)	0.79 ± 0.04 (18)
KH + fibrinogen§	1.18 ± 0.01 (31)	1.13 ± 0.01 (29)	0.80 ± 0.03 (18)
KH + hemoglobin†	2.18 ± 0.02 (29)	1.68 ± 0.02 (36)	0.99 ± 0.04 (21)

* Purity of human plasma protein fractions by electrophoresis: albumin 100 per cent; α-globulins 92 per cent; β-globulins 90 per cent; γ-globulins 97 per cent; fibrinogen 73 per cent; hemoglobin 100 per cent. The lipid content in albumin, γ-globulin, fibrinogen, hemoglobin was less than 1 mg/1 g of dry protein. The extractable lipids in α-globulins and β-globulins averaged 99 mg and 36 mg/g of dry protein, respectively.
† Mann Research Lab., Inc., New York, New York.
‡ Pentex, Inc., Kankakee, Illinois.
§ E. R. Squibb & Sons, New York, New York.

leit solution was at pH 7.4 both before and after equilibration with halothane vapor. For protein solutions, pH was adjusted to 7.4 prior to equilibration (range 7.44–7.39), and was practically unchanged after equilibration (range 7.42–7.37).

Discussion

The solubility of halothane in blood and its major proteins has been determined. An increase in albumin concentration in blood or plasma increased λ-halothane, whereas a high concentration of γ-globulins either decreased it or had no significant effect. The α- and β-globulins contained considerable amounts of extractable lipids (table 3). Purification of these globulins is impossible without denaturation of the proteins. Therefore, we did not evaluate the effects of different concentrations of α- and β-globulins upon solubility of halothane in blood and plasma. Variations of hemoglobin concentration (12 to 17 g/100 ml) in blood samples with normal plasma protein patterns did not cause any considerable changes in solubility of halothane in blood. However, when the concentration of hemoglobin in blood was increased to 27.8 g/100 ml, the solubility coefficient of the anesthetic was significantly ($P < 0.001$) reduced. The decrease occurred despite a relatively high solubility of halothane in solutions of pure hemoglobin. Lower-than-expected solubility coefficients of halothane for blood with high hemoglobin concentrations (fig. 3) may be due partly to protein-protein interaction and to interaction of other intracellular constituents with hydrophobic sites on hemoglobin molecules in intact erythrocytes. Possibly, crystallized hemoglobin dissolved in Krebs-Henseleit solution might have more hydrophobic sites free and be more able to interact with halothane than the intracellular hemoglobin of the blood we used. Depression of λ-halothane in blood with high hematocrit may also be related partly to reduced plasma volume and, possibly, to aggregation of erythrocytes.

Differences in solubility of halothane related to the presence of albumin, γ-globulins and fibrinogen in Krebs-Henseleit solution are probably caused by the primary structures and conformations of these proteins. Thus, human serum albumin has a higher content of amino acids with non-polar side-chains than γ-globulin and fibrinogen. In addition, albumin contains only 13.8 g of hydroxyamino acids (serine, threonine, proline) per 100 g of protein, whereas γ-globulin has 28.6, and fibrinogen 18.8 g, of those amino acids per 100 g. The hydroxy groups in serine, threonine and hydroxyproline are able to form hydrogen bonds with either water or other amino-acid residues. This may partly account for the marked differences in solubility of halothane in albumin, γ-globulin, and fibrinogen solutions. Secondary and tertiary structures, and number of intramolecular hydrophobic bonds between amino-acid residues in a protein

molecule, may all be important in halothane–protein interaction; charge distribution and protein–water interaction may be other significant factors which affect λ-halothane for protein solutions. The effect of variation of *p*H was not evaluated in this study, although we recently measured the effects of *p*H change on λ-halothane for polypeptides and their monomers in 0.2 N sodium chloride solution.[10]

The mean value of λ-halothane for normal bloods at 37 C is 2.42, which is in agreement with other standard values.[2,4,11] An approximation of the proportion of halothane in blood that is associated with proteins can be made. Assume mean values of 0.4 g lipids (including free fatty acids[12]) and 21 g protein per 100 ml blood (the rest is water); a λ-halothane for water of 0.78 and a λ-halothane of 138[4] for lipids. Then, by calculation, approximately half the uptake of halothane by blood results from the presence of proteins. The data we have given, and knowledge of the concentrations of total plasma proteins, lipids, hemoglobin, and the A:G ratio, enable an estimation of λ-halothane for blood.

Recent work from our department has explored the relationships between the solubility coefficients (λ or α) of gases and volatile anesthetics in blood and in water. The solubility coefficients of an aqueous solution in which the solute is inert to the gas will always be a constant times the solubility coefficient of the gas in water. For example, consider oxygen in blood: the solubility coefficient of oxygen in blood (α_B) varies according to temperature but is always a constant times α-water at the same temperature.[13] This suggests that O_2 does not change the configuration of blood proteins (hemoglobin was inactivated in order to determine α_B). Similarly, the solubility of nitrous oxide in blood is a constant times its solubility in water at the same temperature.[14] Halothane, nitrogen and helium do not have constant relationships between their solubilities in water and in blood at various temperatures.[14] It will be interesting to see the correlation between changes in blood protein structure on exposure to gases and anesthetics and solubility data for these interactions.

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