

Laboratory Note

Determination of the Solubility of Halothane in Canine Blood and Cerebral Tissue at Hypothermia, Using a Tonometer for Constant-gas-flow Equilibration

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The solubilities of halothane in distilled water and canine whole blood and cerebral tissue were measured at four temperatures for evaluation of the effects of temperature on halothane solubility. Equilibration was performed in a bubble tonometer which included a humidifying chamber and an equilibration chamber. The whole system was circulated by a stirring pump in a temperature-controlled water bath. Test samples were subjected to continuous flows of anesthetic gas for equilibration. Halothane concentrations in both liquid and gas phases were analyzed by gas chromatography. Ostwald solubility coefficients obtained were distilled water, 0.76, 0.88, 1.06, and 1.12 at 37, 33, 29 and 25 C, respectively; blood, 2.78, 3.35, 4.03, and 4.58 at 37, 33, 29 and 25 C; cerebral tissue, 8.28, 9.00, 10.54, and 11.86 at 37, 33, 29 and 25 C. Equilibration time in 10 ml distilled water was 30 minutes at every temperature tested. Using this method, the technique is easy to follow, and the same sample may be used at different temperatures, thus eliminating variation among samples. (Key words: Ostwald solubility coefficient; Hypothermia; Halothane; Blood; Brain tissue.)

ONE OF THE PROBLEMS in the measurement of anesthetic solubility is the difficulty of equilibrating anesthetics with the test materials in a short time. Equilibration methods thus far available have proved time-consuming and have entailed complicated pretreatment of

samples before measurement of anesthetic concentration. With some methods, it is not possible to prevent leakage of anesthetic gas during equilibration. Furthermore, most equilibration procedures do not allow for measurement at different temperatures. This report describes a simple tonometric method for determination of the solubility of inhalation anesthetics and its application in estimating the effects of temperature on the solubility of halothane in canine blood and cerebral tissue.

Materials and Methods

The equilibration system consists of a bubble tonometer, a constant-temperature bath with a circulating pump, and a conventional anesthetic machine equipped with a vaporizer. The bubble tonometer is essentially the same as that used by Adams and Morgan-Hughes for determination of the blood-gas factor of the oxygen electrode.¹ The tonometer includes a humidifying chamber and an equilibration chamber. The whole chamber is circulated at the desired temperature by a stirring pump in a temperature-controlled water bath.

For this purpose, we used the constant-temperature bath equipped with an IL-Meter (Model 113, Bath Model 102-T, Instrumentation Laboratory Inc., Lexington, Mass.). With modification, the temperature of the bath can be varied from 5 to 55 C.

A continuous anesthetic gas flow from an anesthetic machine is passed through a humidifying chamber with a capacity of 10 ml. It is then led to the equilibration chamber, which is filled with 10 ml of the test media (distilled water, blood, or cerebral tissue ho-

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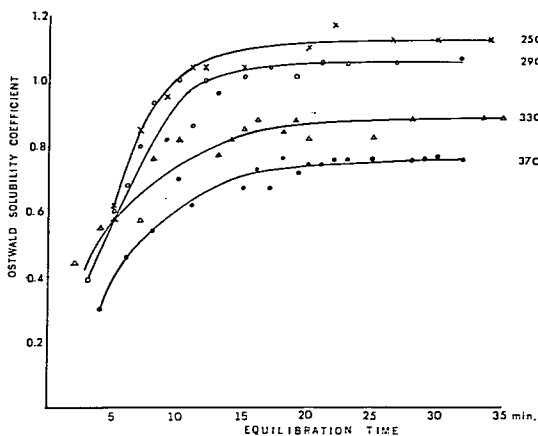


FIG. 1. The Ostwald solubility coefficients of halothane plotted against equilibration temperatures. Curves are the lines of best fit to the data. A 10-ml sample of distilled water was equilibrated with a 500-ml/min constant flow of 1 per cent halothane in oxygen at four temperatures: 25, 29, 33, and 37 C. Distilled water was equilibrated with halothane for 30 minutes at each temperature.

mogenates). The humidifying chamber brings the temperature of the equilibrating gas to that of the tonometer and also saturates the gas with water vapor, thus preventing loss of water from test samples during equilibration. The design of this system did not permit evaluation of saturation of inflowing gas with water vapor in the humidifying chamber. However, since the level of the test material in the equilibration chamber did not change during equilibration, it does not seem unreasonable to assume that there was no significant loss of water from test materials during equilibration, and that the humidifying chamber saturated the equilibrating gas with water vapor. The flow of the anesthetic gas mixture is adjusted to 500 ml/min. Gas leaving the equilibration chamber flows through an outlet to the atmosphere, ensuring maintenance of the contents at atmospheric pressure. This escaping gas is used for measurement of the concentration of the test anesthetic in a gas phase. An equilibrated liquid sample is drawn into a microsyringe through a small outlet from the equilibration chamber. Withdrawal of an equilibrated sample does not necessitate stopping the gas flow through the chamber. All analyses of anesthetic concentrations in samples, in both gas and liquid phases, were done with a gas chromatograph equipped with

a hydrogen-flame ionization detector. Dual 75-cm columns (0.3-cm ID) were packed with Molecular Sieve 5A coated with 0.3 per cent diethylene glycol succinate. Temperature settings were 100, 120, and 160 C for the sample space, column and detector, respectively. A sample-vaporizing apparatus was attached to the gas chromatograph at the entrance to the column.² By the use of this vaporizer, gas and liquid samples were measured in the same manner. Each sample was analyzed at least three times.

The Ostwald solubility coefficient was calculated from the ratio of peak height in the liquid phase to peak height in the gas phase recorded on the gas chromatograph.

Fresh blood from dogs was obtained in a heparinized syringe. The hematocrit was adjusted to 42 per cent by adding a serum or cell fraction from the same sample, simply because the average hematocrit obtained from seven dogs in our laboratory was 42 per cent. Blood samples were centrifuged after each measurement. When hemolysis and its resultant decrease in hematocrit occurred, the samples were discarded.

Using pentobarbital anesthesia, cerebral tissue was obtained from the dogs, which had had no previous exposure to any inhalation anesthetic. An attempt was made to separate the

TABLE 1. Ostwald Solubility Coefficients of Halothane for Distilled Water, Blood, and Cerebral Tissue at 25, 29, 33 and 37 C*

	25 C	29 C	33 C	37 C
Distilled water	1.12 ± 0.02 (10)	1.06 ± 0.04 (10)	0.88 ± 0.05 (10)	0.76 ± 0.03 (10)
Blood	4.58 ± 0.11 (12)	4.03 ± 0.15 (11)	3.35 ± 0.13 (12)	2.78 ± 0.16 (13)
Cerebral tissue	11.86 ± 0.57 (13)	10.54 ± 0.72 (11)	9.00 ± 0.60 (11)	8.28 ± 0.62 (10)

* Solubility expressed as mean ± SD. Numerals in parentheses are numbers of determinations.

tissue into white and gray matter, but because of the technical difficulty in completely separating the brain tissue into two components, whole cerebral tissue was used in this study. Segments of connective tissue were removed. The volume of the tissue was determined by measuring the displacement of distilled water. The tissue was then homogenized with nine times its volume of distilled water by an ultrasonic probe (Biosonik III Ultrasonic System, Bronwill Scientific, Rochester, N. Y.). The prepared samples were used within two hours of homogenization and liquefaction.

Results

The times necessary to equilibrate 1 per cent halothane in oxygen with 10 ml of distilled water at four temperatures, 25, 29, 33, and 37 C, are shown in figure 1. Approximately 30 minutes were needed for equilibration of 10 ml of distilled water with 500 ml/min continuous gas flow at 25 C. Distilled water was equilibrated with halothane for 30 minutes at each temperature.

The times necessary for equilibration of blood and cerebral tissue homogenates were determined by measuring halothane concentrations in both inflow and outflow gas in the tonometer. Blood and cerebral tissue homogenates were equilibrated for 30 and 40 minutes, respectively, prior to analysis at any temperature. Mean values and standard deviations of the Ostwald solubility coefficients determined by this method are shown in table 1. Coefficients of variation, determined by the peak heights, were ±3.5 per cent for distilled water, ±3.8 per cent for blood, and ±3.8 per cent for cerebral tissue.

Solubilities vary inversely with temperature (fig. 2), but the relationship was not necessarily linear, nor logarithmic as has been pro-

posed.³ Brain-blood partition coefficients calculated from the values obtained in this study were 2.59, 2.62, 2.69, and 2.98 at 25, 29, 33, and 37 C, respectively, tending to increase with increasing temperature.

Discussion

The Ostwald solubility coefficient for halothane in distilled water at 37 C has been reported to be 0.74 (Laasberg *et al.*)⁴ and 0.78 (Larson *et al.*)⁵ These findings are in agreement with our value. However, the present value at 25 C differs considerably from the 1.28 reported by Laasberg *et al.*⁴

The solubility coefficient for human blood at 37 C was previously reported to be 2.42,⁴ 2.56,⁶ and 2.3.^{5,7} A previously reported value at 25 C was 4.54.⁴ Larson *et al.*⁵ reported the same value for human and bovine blood. However, Lowe *et al.*⁶ have reported different values for human and calf blood (2.56 and 2.87, respectively). Our value for canine blood at 37 C is identical to that for calf blood, but it is 9 to 21 per cent higher than values for human whole blood. Our value at 25 C is not different from the reported value. Slight qualitative variations in hematocrit, hemoglobin, and other blood components are seen among these different samples.^{8,9} However, these variations are not consistent either with previous reports or with the present findings of halothane solubility in whole blood. Studies of quantitative variations in solubility coefficients among different species have thus far not been reported. These contradictory results suggest that solubility discrepancies are due neither to species differences nor to variations of technique. The reasons for the discrepancies remain obscure.

There are clear species differences in the values for cerebral tissue components.⁹ For

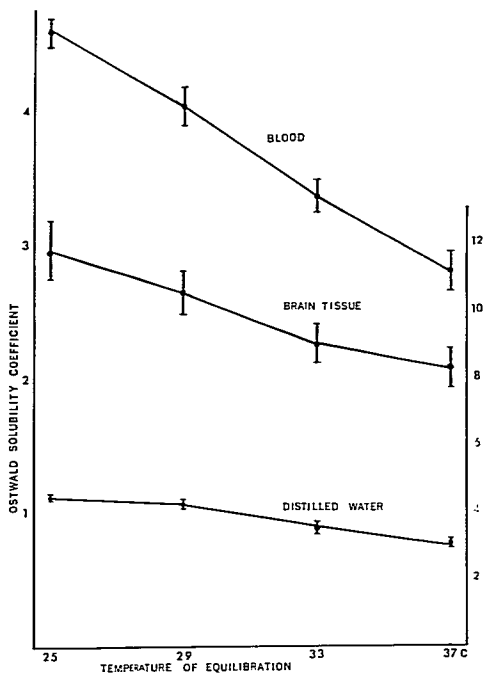


FIG. 2. Effects of temperature on Ostwald solubility coefficients in distilled water, blood, and cerebral tissue. Solubility coefficients for distilled water and blood are plotted on the left ordinate, that for cerebral tissue on the right ordinate. Standard deviations are indicated by vertical bars.

example, canine whole-brain tissue contains 5 to 20 per cent more total lipid than human whole-brain tissue, 48 to 55 per cent more than human gray matter, and 38 to 63 per cent less than human white matter. Canine whole-brain tissue contains more total protein than human brain tissue, irrespective of the components; 8 to 17 per cent more than whole-brain tissue, 23 to 36 per cent more than white matter, and 32 to 39 per cent more than gray matter. Solubility increases with the increasing amounts of lipid and protein. It would appear that the differences in solubilities for brain tissues are due to differences in species and samples compositions. Our results are inconsistent with those reported by Han and Helrich,⁷ who found no differences in solubility for human gray matter at 28, 33,

and 43 C. Their report provides insufficient data to permit the evaluation of their conclusions and comparison with the present result.

Using this method of equilibration, any concentration of gas can be used so long as it is constant during equilibration. It is not necessary to know either the concentration of gas or the volume of liquid anesthetic as a standard for calculation of solubility. Agitation is not needed in a bubble tonometer during equilibration. Speed of equilibration in a tonometer depends on the area of liquid exposed to the gas, the volume and the nature of the liquid, the rate of flow of the gas, and its solubility in the liquid at each temperature.¹ Considering that a continuous flow of 500 ml/min passes through 10 ml of distilled water, it is no wonder that equilibration can

be attained within 30 minutes at any temperature above 25 C. One of the disadvantages of bubble tonometer is that it is difficult to prevent foaming at high gas flows without using antifoam which, we confirmed, produced hemolysis. A thin film of silicone grease applied inside the equilibration chamber will prevent foaming. It is crucial to adjust the optimal flow rate (in this case 500 ml/min) even if silicone grease is used to prevent foaming and hemolysis.

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Drugs and Their Actions

BRAIN AMINE CONCENTRATIONS Analyses of 5-hydroxytryptamine, norepinephrine, and dopamine in the brain stem, hypothalamus, and caudate nucleus were made postmortem. Subjects were 11 patients who had received isocarboxazid; 13 who had received clorgyline; nine who had received tranlycypromine; and 11 controls. Increases in all values were found in brains of patients who had received the drugs, especially dopamine in the hypothalamus and caudate nucleus following tranlycypromine. The administration of chlorpromazine, 100 mg daily, did not appear to have antagonized these effects; hence, it appears not to be contraindicated as an adjunct to therapy with monoamine oxidase inhibitors. The differences in dopamine concentration following tranlycypromine may reflect differences in monoamine oxidases present, as described by others. For example, the isoenzyme which oxidized dopamine was found by other authors to be inhibited by tranlycypromine but not by isocarboxazid or dopamine. Four patients with parkinsonism, one in each of the above-mentioned groups, had no abnormal increase in 5-hydroxytryptamine and norepinephrine concentrations in the brain stem and hypothalamus; indeed, they had markedly low levels of dopamine in the caudate nucleus. The findings reflect the clinical ineffectiveness of these drugs in Parkinson's disease. This is unlike the effect of L-dopa, which increases dopamine levels in the caudate nucleus. (*Bevan Jones, A. B., and others: Brain Amine Concentrations after Monoamine Oxidase Inhibitor Administration, Br. Med. J. 1: 17-19, 1972.*)