Solubility of Nitrous Oxide in Water and in Canine Blood

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NEW "molecular" theories [2, 3, 15] of anesthesia necessitate for their confirmation precise measurement of the physico-chemical properties of the anesthetic agents. An extensive review of the literature reveals a wide variation in values for one of these properties, namely, solubility. Because of the disagreement concerning nitrous oxide solubility (table 1) and because solubility is important to an understanding of the mechanism of anesthesia, a study of the solubility of nitrous oxide in water and blood was undertaken. An attempt was made to specify and control those variables not taken into consideration in previous investigations.

Methods

The Van Slyke-Neill standard procedure for analysis of blood gases [19] was used with modifications. This method involves equilibration of a solvent with a known concentration of gas. The amount of gas in solution is subsequently determined by extraction of the dissolved gas in the Van Slyke apparatus.

For determination of the solubility of nitrous oxide in water, a 150-ml. glass tonometer with ground glass stopcocks at each end was suspended in a 37° C. constant temperature water bath and connected to a cylinder of the gas. The tubing through which the gas passed, prior to entering the tonometer, also was immersed in the bath to minimize the cooling effect during saturation. Approximately 50 ml. of glass-distilled water (*p*H 7) was placed in the tonometer. Nitrous oxide was bubbled through the water continuously for at least one hour while the tonometer was mechanically agitated. This procedure was judged sufficient to completely saturate the water with nitrous oxide to dispel any other dissolved gases.[5]

A drop in temperature of 1.3–2.0° C. occurred during saturation. After saturation, in order to permit the water in the tonometer to attain a temperature of 37° C., the tonometer was kept in the bath for 15 minutes with both stopcocks closed. At this time one stopcock was momentarily opened to expel the small amount of gas forced out of solution by the rise in temperature and to maintain the system at atmospheric pressure. At no time was a negative pressure observed, nor was room air allowed to come in contact with the water.

A pediatric anesthesia bag was flushed repeatedly with nitrous oxide and a quantity of gas allowed to remain in the bag at atmospheric pressure. This bag was connected to one stopcock of the tonometer. The time interval was not considered sufficient to allow a change in the concentration of nitrous oxide in the bag due to its solubility in rubber. A no. 16 French rubber catheter was connected to the other stopcock. With the tonometer still in the bath, both stopcocks were opened and the gas-saturated water allowed to flush the open catheter, thus avoiding contamination of the sample with room air. With the water still running, a 1-ml. Ostwald pipette with a ground glass stopcock was inserted into the catheter. The hydrostatic pressure of the water in the tonometer was usually sufficient to fill the pipette: if not, a minute pressure was exerted on the gas bag to aid filling of the pipette. Immediately upon completion of fill-

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TABLE 1. Solubilities of Nitrous Oxide in Blood and Water
A Review of the Literature

Reference	Water Alpha	Water Lambda	Temp. ° C.	Blood Alpha	Blood Lambda	Temp. ° C.
Siebeck[18]	—	0.435	38	—	0.486	38
Orcutt and Seevers[12]	0.549	0.599	25	0.416	0.472	37.5
Greene[4]	—	—	—	0.189-0.268	—	?37
Harris[6]	0.395	—	37	0.412	—	37
Nunn[10]	a) —	0.540	23	—	—	—
	b) 0.63	—	20	—	—	—
McIntosh[8]	0.68	—	20	—	—	—
Seidell[17]	0.545	—	25	—	—	—
Kety[7]	—	—	—	0.460	0.482	37
Orcutt and Seevers[13]	—	0.44	37.5	—	0.47	37.5

ing, the sample was transferred to the extraction chamber of the Van Slyke-Neill apparatus in the standard manner.[19] The mercury was then lowered to the 50 ml. mark and the extraction chamber shaken for three minutes. The level was then brought to the 2-ml. mark and the manometer reading recorded as P_1. Gas was then expelled and reextractions carried out. Gas was expelled each time. When no more gas could be obtained, the solution was brought to the 2-ml. mark and the manometric reading recorded as P_2. The difference, $P_1 - P_2$, was a measure of the amount of nitrous oxide extracted from the water. Further extractions were carried out until three consecutive, identical readings were obtained for P_2. Approximately 40 seconds[12] was used in raising the water to the 2-ml. mark with each determination.

The temperature at which extraction was carried out was noted on a thermometer placed in the water jacket surrounding the extraction chamber. This temperature was constant throughout any one experiment. Atmospheric pressure was read from a mercury barometer at each saturation period. It never varied significantly during the course of any experiment.

To obtain *in vivo* blood samples, which had been equilibrated with a known gas mixture, a healthy dog was anesthetized with sodium pentobarbital, the trachea intubated and the lungs mechanically ventilated with a mixture of 80 per cent nitrous oxide and 20 per cent oxygen delivered from a McKesson anesthesia machine. Since alveolar partial pressures were measured directly, the flow meter of the machine was not calibrated. The mechanical respirator was adjusted to maintain an end-expiratory P_{CO_2} of approximately 40 mm. of mercury (range from 39.5 to 40.5 mm. of mercury). End-expiratory gas samples obtained from a catheter in the endotracheal tube were taken as representative of alveolar concentrations.[1, 7] Oxygen concentration was measured with a Beckman (Model C) oxygen analyzer and $P_{A_{CO_2}}$ determined with a Beckman Spinco Model LB-1 medical gas analyzer. Readings were taken directly and continuously by means of a Texas Instruments' Rectiriter. After one hour of ventilation, nitrogen washout was assumed to be complete.[6, 7, 14]

When both oxygen and carbon dioxide values remained constant for 15 minutes, arterial puncture was performed and a polyethylene, 18-gauge catheter inserted. Ten milliliter samples of blood were collected in heparinized syringes after flushing the sampling catheter. The syringes were capped and the samples analyzed as soon as possible. Comparison of results indicated that storage in a refrigerator at about 10° C. did not alter the results if the syringes were sealed. The blood was delivered to Ostwald pipettes, utilizing an adequately flushed rubber catheter. The analyses were carried out in the Van Slyke apparatus.[19]

Arterial hematocrits were measured by the micro-hematocrit method at the time of sampling and repeat measurements were made at the time of analysis.[9] Body temperature was continuously monitored with a rectal thermometer.

Calculations

Pressure readings obtained were corrected according to the gas laws. In addition, when the Van Slyke apparatus is used, correction must be made for "unextractable" gas, *i.e.*, that gas which remains in solution at the end of an analysis due to the imperfect vacuum in the extraction chamber. When gases of low solubility, such as nitrous oxide, are being studied, the value for unextractable gas is a very small percentage of the amount originally in solution. However, when a more soluble gas is in the solvent, this unextractable portion may represent a considerable portion of the total gas content. Orcutt and Seevers attempted to calculate the values for unextractable gas.[12] In reviewing their original work, the derivation of their formula below was not clear:

$$V = \frac{(A - S)_p}{(B - W_S)S - S_P}$$

where:

- V = total volume of gas in solution, both extractable and unextractable.
- A = volume of the extraction chamber (50 ml.).
- S = size of sample in milliliters.
- p = observed pressure at the 50-ml. mark minus the blank manometer reading made on the liquid phase.
- B = barometric pressure.
- W_S = vapor pressure of liquid at the temperature of saturation.

The derivation is carried through several steps, involving the following expression:

$$V = v + \frac{SVv}{(A - S)}$$

where v = volume of unextractable gas.

Volumes "V" and "v" are related to the Ostwald solubility expression (lamba) for the gas studied. Lambda, however, is an unknown value and cannot be calculated or algebraically equated, but rather it must be meassured, or as pointed out below, reduced to a negligible value by appropriate methods. As Orcutt and Seevers point out,[12] "At the present time it is necessary to make this calculation [*i.e.*, lambda value] for each gas from the value of alpha [directly related to lambda] for water [as] found (in the literature). This procedure is open to two objections. The values found in the literature are not always in (close) agreement. Secondly, these constants are for pure water, whereas, the constants desired are for the acid and alkaline reagents containing blood. For gases with fairly high solubilities, such as the anesthetic gases, there may be considerable variation (between) the values for pure water and those for aqueous solutions."

In order to overcome problems of unextractable and reabsorbed gas, multiple extractions were used. When the amount of gas in the extraction chamber becomes very small due to multiple extractions, the amount of unextractable gas is minute.

The formula of Nunn [10] was used to calculate the quantity of gas in solution:

$$V = \frac{1}{\frac{(B - W_s)}{a \cdot i\,(P_1 - P_2)} - \frac{1}{50 - S}}$$

where:

- V = total amount of gas in solution.
- B = barometric pressure.
- W_s = vapor pressure of water under the experimental conditions.
- a = volume at which the pressure readings were obtained.
- i = correction factor for resorbed gas.
- $P_1 - P_2$ = observed pressure for nitrous oxide in the manometric apparatus.
- $\frac{1}{50 - S}$ = correction for the volume at which extraction took place. "S" equals sample size. (For water "S" equals one; for blood "S" equals 4.)

The steps involved in deriving this formula are outlined in Nunn's paper. They do not involve the use of alpha or lambda values from the literature as did the formulas of Orcutt and Seevers.[12] Because reabsorption of gas was not a significant factor as the final pressure readings were being obtained, the "*i*" factor, the correction factor for resorbed gas, no longer applies. Thus:

$$V = \frac{1}{\frac{(B - W_s)}{a \cdot (P_1 - P_2)} - \frac{1}{50 - S}}.$$

TABLE 2. Solubility of Nitrous Oxide in Water and in Canine Blood

Solvent	t_s °C.	t_e °C.	$B - W_s$ mm. Hg	$P_1 - P_2$ mm. Hg	Lambda
Water	37	26	699.8	122.9	0.367
Water	37	26	699.2	125.2	0.370
Water	37	28.5	699.2	123.7	0.367
Water	37	25	694.7	123.0	0.372
Water	37	25	694.9	121.0	0.365
Water	37	25.3	695.2	121.5	0.366
Water	37	25.5	695.5	123.1	0.369
Water	37	25.9	695.8	120.7	0.361
Water	37	26.0	695.8	123.3	0.367
				Mean	0.367
				S.D. ±	0.003

	t_s °C.	t_e °C.	E_p mm. Hg	$P_1 - P_2$ mm. Hg	Lambda
Blood	37.5	28.1	499.1	96.8	0.400
Blood	37.5	28.2	499.1	97.2	0.395
Blood	37.5	28.1	499.1	97.4	0.396
Blood	37.5	28.1	499.1	96.9	0.392
Blood	37.5	28.2	499.1	97.2	0.393
Blood	37.5	28.1	499.1	97.4	0.395
				Mean	0.395
				S.D. ±	0.002

t_s = temperature of saturation.
t_e = temperature of extraction.
$B - W_s$ = barometric pressure minus vapor pressure of water.
E_p = equilibration pressure of nitrous oxide ($B - W_s - P_{CO_2} - P_{O_2}$).
$P_1 - P_2$ = observed manometric pressure of nitrous oxide.
Lambda = Ostwald solubility coefficient.

Results

The solubility of nitrous oxide in water at 37° C. was found to be 36.7 ml./100 ml. ± 0.003 (table 2). In heparinized dog blood at 37.4° C., the solubility was 39.5 ml./100 ml. (hematocrit = 43 per cent; standard deviation = ± 0.002) (table 2).

Discussion

Many of the values quoted (table 1) for the solubility of nitrous oxide were obtained under non-physiologic conditions. Variations from the physiologic state can be ascribed to three major categories: (1) temperature, (2) equilibration pressures, and (3) the solvent.

The solubility of a gas in a liquid varies with the temperature. Reference to table 1 indicates the different temperatures at which the solubility of nitrous oxide in water has been determined. In the present report *in vitro* experiments were controlled at 37° C., and the *in vivo* work performed at 37.4° C. At the end of equilibration the temperature in the tonometer was below 37° C. due to evaporation of water during equilibration. At the lower temperature, the quantity of nitrous oxide in solution was increased. Failure to control these changes is a source of error in earlier work.

Secondly, although Henry's law defines the pressure: solubility relationship as a linear one for ideal gases and solvents, Nunn's work on chloroform in oil [11] and pilot studies in this laboratory indicate that deviations from the ideal curve may be significant with anesthetics under clinical conditions. Heretofore, solubilities have been measured at pressures far removed from those effective clinically. Usually the pure agent has been equilibrated at ambient pressure assuming that extrapolation to clinical conditions was only a matter of calculation. A nitrous oxide solubility over the full range of pressures has not yet been determined. Variations from Henry's law when applied to "real" gases are probably due to van der Waals' forces.

To study Henry's law *in vivo*, the partial pressure of the anesthetic agent in the alveoli must be known. It is that pressure with which the pulmonary venous blood is equilibrated. Measurement of alveolar partial pressure of nitrous oxide is based on the work of Greene [4] and Comroe.[1] In these experiments, alveolar partial pressure of nitrous oxide is less than pressure of nitrous oxide in the inspired air because carbon dioxide and water vapor are added to alveolar gas. After one hour of denitrogenation, partial pressures of carbon dioxide and oxygen were constant. This method offers advantages. First, it is applicable to studies in man. Secondly, *in vivo* studies overcome the problems encountered when attempts are made to control *p*H, red cell viability, and hematocrit *in vitro*. Thirdly, this method can be used to measure equilibration pressures of two or more gases simultaneously. Nitrogen pressure does not affect equilibration pressure after a total body nitrogen

"wash out" period has been effected.[6, 7] A pressure differential exists across the alveolar membrane for gases such as oxygen and carbon dioxide. A similar pressure differential probably exists for nitrous oxide also. Our study indicates that nitrous oxide is more soluble in blood than in water. It follows that the air : blood solubility ratio is larger than the air : water solubility ratio. This may in part be due to a pressure differential at equilibrium for nitrous oxide across the alveolar membrane.

The third factor altering solubility is the nature of the solvent. Previous workers have given little attention to the components of non-homogeneous solutions and to the manner in which they may affect solubility. Such considerations are of little importance when "pure"solvents, such as water, are being used. In blood, many components influence the solubility value. Possati has shown that the solubility of cyclopropane in blood varies directly with the hematocrit.[16] The solubility of cyclopropane[14] and of nitrous oxide[10] vary inversely with the *p*H of the solution. Recently, Featherstone *et al.*[2] have shown the solubility of gases to vary with different concentrations of protein in the solvent. The concentrations of ions and of lipids are examples of other important variables.

Summary

Technical and mathematical problems relating to the solubility of nitrous oxide in blood and water are reviewed. Under controlled conditions the solubility of nitrous oxide in water at 37° C., using 100 per cent nitrous oxide at atmospheric pressure, is 36.7 ml./100 ml. The value obtained for dog blood *in vivo* at 37.4° C. using an inspired 80 per cent–20 per cent nitrous oxide-oxygen mixture is 39.5 ml./100 ml.

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