THE SOLUBILITY OF NITROUS OXIDE IN BLOOD AND BRAIN*

BY SEYMOUR S. KETY, MEREL H. HARMEL,† HANNAH T. BROOMEILL, AND CLARA BELLE RHODE

(From the Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia)

(Received for publication, December 29, 1947)

Since the formulation of the lipide theory of anesthesia (1) there has been some interest in the solubilities of various volatile anesthetics in the brain (2–6). Unfortunately the results obtained in vivo have been equivocal and inexact because of the technical difficulties of obtaining and analyzing without loss samples of brain containing volatile gas. Even the studies on solubility in vitro, on which the theory was largely based, were made on peanut, olive, or similar oils rather than on brain lipides. Our attention was drawn to the problem of the solubility of gases in brain in the course of the development of a method for measurement of cerebral blood flow by means of the blood-brain exchange of an inert gas, nitrous oxide (7). It can be shown that cerebral blood flow may be calculated from the arterial \( A \) and cerebral venous \( V \) blood nitrous oxide concentrations over a time interval \( u \) measured from the onset of inhalation of a comparatively low tension of nitrous oxide. The interval \( u \) must be of sufficient length to insure practically complete equilibrium between brain and blood draining the brain with respect to mean nitrous oxide tension. If the results are to be expressed in flow per unit weight of brain rather than in terms of flow per unit of nitrous oxide capacity, a factor \( S \) must be introduced, representing the brain-blood partition coefficient of nitrous oxide or the ratio of nitrous oxide dissolved per gm. of brain to that dissolved per cc. of blood at a constant nitrous oxide tension and at 37°. The formula for calculation of cerebral blood flow, which is derived elsewhere may be expressed as follows:

\[
\text{Cerebral blood flow} = \frac{V_u S}{\int_0^u (A - V) \, dt}
\]

* The expenses of this investigation were defrayed in part from funds granted by the Committee on Research in Dementia Precox, founded by the Supreme Council, 33° Scottish Rite, Northern Masonic Jurisdiction, United States of America, and in part by the Life Insurance Medical Research Fund.

† National Research Council Fellow in Anesthesiology.
In order to make possible the calculation of absolute values it was necessary to evaluate the partition coefficient ($S$) and the minimum time ($u$) for equilibrium between the brain and its venous blood. This was attempted by means of both *in vitro* and *in vivo* techniques.

**Methods**

_Determination of Nitrous Oxide Solubilities in Blood and Brain in Vitro_—Freshly shed, heparinized whole blood is used as such. The brain, however, is too viscid for convenient handling; a representative sample of about 10 gm. is accurately weighed and homogenized with exactly 5 cc. of distilled water in a Potter glass homogenizer (8). The homogenate is pressed through coarse gauze to remove small shreds of connective tissue and transferred to a 50 cc. glass syringe in which equilibration with nitrous oxide is to occur. All air is expelled from the syringe which is then filled with pure nitrous oxide after several flushings with the same gas. The tip of the syringe is immediately closed by means of a sealed needle hub and the syringe containing the nitrous oxide and blood or brain homogenate is rotated slowly in a water bath maintained at 37°. It is important to make sure frequently that the plunger of the syringe is free to move in the barrel to compensate for temperature-induced volume changes and that the syringe is not more than a cm. below the surface of the water of the bath. At intervals of 15 minutes the syringe is removed from the bath, the gas is expelled, and fresh nitrous oxide added for three such flushings. Equilibration is then allowed to proceed for another hour. At the end of that time, with the syringe held vertically in the bath, the gas is entirely expelled and the cap replaced. The syringe may now be removed from the bath and part of its contents analyzed for nitrous oxide as follows, after which the remainder may be reequilibrated: 5 drops of caprylic alcohol plus 3 cc. of distilled water are extracted for 3 minutes in the 50 cc. chamber of a Van Slyke-Neill manometric apparatus, then expelled as completely as possible, 1 cc. of mercury in addition being permitted to rise into the cup. The water and caprylic alcohol are removed from the surface of the mercury in the cup. A stout capillary tube of about 2 mm. bore and a cm. longer than the depth of the cup on the manometric apparatus is now securely fastened to the tip of the syringe by means of a short length of heavy plastic tubing. The free end of this capillary tube, which is somewhat tapered to resemble the tip of an Ostwald-Van Slyke pipette, is fitted with a rubber tip for sealing against the bottom of the cup. The contents of the syringe are carefully expressed to this tip and a little excess is expelled. The capillary is then held vertically, its rubber tip pressed against the bottom of the cup, and, against a slight positive pressure of mercury in the manometric apparatus, the contents of the syringe, by pressure on the plunger, are carefully forced...
into the chamber, stopping accurately at the 2 cc. mark. The stop-cock at
the top of the chamber is now closed, the cup emptied, and the mercury in
the chamber brought down to the 50 cc. mark. 6 cc. of deaerated oxygen
absorber (KOH-hydrosulfite-anthraquinone reagent employed in blood
oxygen analyses (9)) are now added to the cup and the lower 5 cc. admitted
to the chamber in several quick additions to wash the 2 cc. volume clean.
The upper cock is sealed with mercury and the contents of the chamber are
extracted for about 5 minutes. The liquid is then permitted to rise
smoothly to the 2 cc. mark and pressure and temperature readings are taken.
Extraction at 50 cc. is repeated for 5 minute periods until the pressure read-
ing (corrected for any temperature change) remains constant for three
successive determinations. Nitrous oxide concentration is calculated as
follows:

\[
\text{vol. }\% \text{ N}_2\text{O} = f_{N_2O} r_a - (r_0 + C_w)
\]

where \( r_a \) = the manometric reading for the sample and \( r_0 \) = the manomet-
ric reading for the blank. This is run once for each series of analyses, and
consists in deaerating 3 cc. of distilled water, expelling only 1 cc., and
treating the 2 cc. left in the chamber as if it were a sample to be analyzed.
\( C_w \) = the correction for change in water vapor corresponding to any tem-
perature change between the blank and the sample for analysis. \( C_w = (t_a - t_b) \Delta p_w \) where \( t_a \) = the temperature of the sample analysis and \( t_b \) =
the temperature of the blank analysis. \( \Delta p_w \) = the change in water vapor
tension corresponding to 1° of temperature change in the temperature re-
gion of the analysis. \( f_{N_2O} \) = the manometric factor for N\(_2\)O calculated
from a factor \( i \) of 1.03 (Orcutt and Waters (10)) and values of 0.507 and
0.438 for the \( \alpha' \) of the analysis mixture at 20° and 30° respectively.

The Bunsen solubility coefficient (\( \alpha \)) is calculated from the N\(_2\)O concen-
tration in volume per cent in the blood or homogenate as follows:

\[
\alpha = \frac{N_2\text{O vol. }\%}{100} \times \frac{760}{B - T_w}
\]

where \( B \) = the barometric pressure and \( T_w \) = water vapor tension at 37°.

In the case of the brain homogenate the \( \alpha \) obtained above is for the brain-
water mixture as a whole, but since the \( \alpha \) for water can be determined, and
the relative quantities of brain and water are known, the solubility of ni-
trous oxide per gm. of brain can readily be calculated.

\[
\alpha_b = \frac{\alpha_h \left( \frac{W_b}{1.05} + V_w \right) - V_w \alpha_w}{W_b}
\]

where \( \alpha_b \), \( \alpha_h \), \( \alpha_w \) = the Bunsen coefficients for 1 gm. of brain, 1 cc. of
homogenate, 1 cc. of distilled water respectively, all at 37°. \( W_b \) = the weight of the brain sample, \( V_w \) = the volume of water, and 1.05 = the specific gravity of brain.

Determination of Brain and Cerebral Venous Nitrous Oxide Concentrations in Vivo—These studies were performed on dogs anesthetized with sodium pentobarbital. A T-tube, attached to inspiratory and expiratory flutter valves through which gas mixtures could be administered without rebreathing, was introduced into the trachea. The skull was trephined through the occipital protuberance and a threaded brass cannula was screwed into the skull, tapping the torcular Herophili (confluence of the sinuses). A solution of heparin was used in this cannula to prevent clotting. A preliminary period of inhalation of 100 per cent oxygen for 1 hour insured practically complete denitrogenation of the brain. The animal was then permitted to breathe a mixture of 40 per cent nitrous oxide and 60 per cent oxygen. At a variable time after the beginning of nitrous oxide inhalation a sample of cerebral venous blood was collected anaerobically from the cannula in the torcular and immediately thereafter the animal was sacrificed by passage of an electric current through the thorax, a procedure which produced instantaneous ventricular fibrillation. The scalp and muscles were quickly removed from the cranium and two holes 1 inch in diameter were made by means of a trephine on opposite sides of the skull just above the zygomatic arch, with care not to cut completely through the bone. The head was then removed and immersed in a bath of water through which a fine spray of 40 per cent nitrous oxide-60 per cent oxygen had been passing for about 30 minutes. Under the surface of this bath, the trephine holes were completed and the buttons of bone removed. The dura was then cut and a sample of brain was taken anaerobically by means of the following technique. It was found possible to perform all of these operations from the sacrifice of the animal to removal of the brain sample in about 10 minutes, the brain being exposed to the water bath for only 30 seconds.

The brain sampler (Fig. 1) is simply a 10 cc. all-glass syringe in which the end of the barrel has been cut off and the rim beveled to a fair cutting edge. This end may be closed by means of a snugly fitting rubber stopper through the center of which passes a stout capillary tube of 2 mm. bore, extending 8 cm. beyond the rubber tube and ending in a beveled and tapered tip. This tube must fit the stopper tightly enough to resist being forced up or down in it. The entire unit fits into a metal holder which secures the stopper against the open end and permits the plunger to be forced down the length of the barrel by means of a screw-thread, forcing a fine cord of brain tissue from the capillary tip. To obtain a sample of brain, the syringe is immersed in the water bath and the plunger worked back and forth until it moves very
freely. It is then pushed down just beyond the cutting edge of the barrel. This is now pressed against the brain and, with a slightly twisting motion, forced through the tissue, leaving the plunger free to move out as it is displaced by brain. When the cutting edge reaches the other side of the brain, it is closed by means of the rubber stopper and capillary tube. At this point pressure is applied against the plunger and some of the brain forced down the capillary to the tip. In this manner it is possible anaerobically

![Fig. 1. The modified glass syringe used in obtaining samples of brain anaerobically.](image)

![Fig. 2. The ratio of the nitrous oxide contents of brain and cerebral venous blood plotted semilogarithmically against the time of exposure of the brain before sampling.](image)

...to obtain a satisfactory 6 to 8 cc. sample of brain, representing both gray and white matter, sealed within a syringe.

The brain sampler was then removed from the bath, fastened into its holder, and an analysis for nitrous oxide carried out on 2 cc. samples by the analytical technique described above for blood and brain homogenate. Samples of torcular blood obtained at the time the animal was sacrificed were also analyzed for nitrous oxide by the same technique. The purpose of the water bath equilibrated with a tension of nitrous oxide equivalent to that inhaled by the animal is to minimize the loss of this gas from the brain in the brief time of exposure as the sample is being taken. This pre-
caution was found to be necessary by preliminary experiments in which simply denitrogenated water was used as the bath (Fig. 2). It was found that there was a significant loss of nitrous oxide from the brain with time, if successive samples were taken after the dura was cut. When water equilibrated with 40 per cent nitrous oxide was used, however, it may be seen that this loss of nitrous oxide from the brain was prevented. It is worthy of note that in the earlier trials, in which loss occurred, if an exponential extrapolation is made to time of exposure = 0 the values obtained are comparable to those in which loss is actually prevented.

### Table I

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Dog $\alpha_{37^\circ} \text{N}_2\text{O}$ (per cc. blood)</th>
<th>Man $\alpha_{37^\circ} \text{N}_2\text{O}$ (per cc. blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Red blood cell hematocrit</strong></td>
</tr>
<tr>
<td>9</td>
<td>0.419</td>
<td>28.8</td>
</tr>
<tr>
<td>11</td>
<td>0.419</td>
<td>34.2</td>
</tr>
<tr>
<td>12</td>
<td>0.433</td>
<td>41.0</td>
</tr>
<tr>
<td>13</td>
<td>0.421</td>
<td>44.0</td>
</tr>
<tr>
<td>14</td>
<td>0.435</td>
<td>51.5</td>
</tr>
<tr>
<td>15</td>
<td>0.421</td>
<td><strong>0.412</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>0.425</td>
<td></td>
</tr>
<tr>
<td><strong>s.e.</strong></td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

* $\alpha_{37^\circ} \text{N}_2\text{O}$ (per cc. of blood) = the number of cc. of N$_2$O (reduced to S.T.P.) dissolved by 1 cc. of blood when equilibrated at a nitrous oxide tension of 760 mm.

### Results

**Solubility of Nitrous Oxide in Blood and Brain in Vitro**—The Bunsen solubility coefficient (Table I) for nitrous oxide in dog blood at $37^\circ$ as determined by this technique was 0.425 (standard error = 0.003); this coefficient for human blood was 0.412 (standard error = 0.004). In the latter case a definite correlation may be noted between the proportion of red cells in the blood and the solubility of nitrous oxide in it. This was found to be the case for nitrogen by Van Slyke, Dillon, and Margaria (11). We found that the blood of a very anemic and a polycythemic individual varied from the mean by only 3 per cent. The values for $\alpha$ obtained by us in blood agree well with the value of 0.416 found by Orcutt and Seevers (12). The solubility of nitrous oxide in brain, however, has not hitherto been investigated. We found identical values for the whole brain of dog (0.437 ± 0.008) and of man (0.437 ± 0.005), the former studied immediately after sacrifice, the
latter obtained in autopsy specimens 4 to 24 hours after death from a variety of diseases (Table II). From these values for blood and brain in both species a brain-blood partition coefficient of 1.03 and 1.06 may be calcu-

**Table II**

**Solubility of Nitrous Oxide in Dog and Human Whole Brain**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Dog $\alpha_{\text{N}_2\text{O}}$ (per gm. brain)</th>
<th>Patient</th>
<th>Condition</th>
<th>Man $\alpha_{\text{N}_2\text{O}}$ (per gm. brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.434</td>
<td>A. K.</td>
<td>Depressive psychosis</td>
<td>0.428</td>
</tr>
<tr>
<td>9</td>
<td>0.406</td>
<td>B. B.</td>
<td>Nephritis</td>
<td>0.434</td>
</tr>
<tr>
<td>11</td>
<td>0.430</td>
<td>S. F.</td>
<td>Apoplexy</td>
<td>0.437</td>
</tr>
<tr>
<td>12</td>
<td>0.420</td>
<td>M. Mc.</td>
<td>Cardiac failure</td>
<td>0.425</td>
</tr>
<tr>
<td>13</td>
<td>0.458</td>
<td>R. C.</td>
<td>Paresis</td>
<td>0.438</td>
</tr>
<tr>
<td>14</td>
<td>0.455</td>
<td>C. H.</td>
<td>Arteriosclerosis</td>
<td>0.432</td>
</tr>
<tr>
<td>15</td>
<td>0.454</td>
<td>J. J.</td>
<td>Hypertension</td>
<td>0.464</td>
</tr>
<tr>
<td>Mean.......</td>
<td>0.437</td>
<td></td>
<td></td>
<td>0.437</td>
</tr>
<tr>
<td>s.e. ........</td>
<td>0.008</td>
<td></td>
<td></td>
<td>0.005</td>
</tr>
</tbody>
</table>

* $\alpha_{\text{N}_2\text{O}}$ (per gm. brain) = the number of cc. of $\text{N}_2\text{O}$ (reduced to s.t.p.) dissolved by 1 gm. of brain when equilibrated at 37° at a nitrous oxide tension of 760 mm.

![Fig. 3. The rate of equilibration of nitrous oxide between brain and cerebral venous blood in vivo.](http://www.jbc.org/)

lated for dog and man respectively, representing the ratio of the quantity of nitrous oxide dissolved in 1 gm. of brain to that in 1 cc. of blood when both are at the same tension.
Partition Coefficient in Vivo for Dog and Rate of Equilibration between Brain and Cerebral Venous Blood—In Fig. 3 are presented the data on the nitrous oxide content of brain and cerebral venous blood obtained simultaneously at times varying from 2 minutes to 2 hours after the onset of inhalation of the 40 per cent nitrous oxide mixture. It is seen that in the early period the brain has not come to equilibrium but that after about 10 minutes equilibrium is apparently established between brain and the blood draining the brain and that the ratio of the nitrous oxide content in the two phases remains unaltered up to 2 hours. The value for this brain-blood ratio in the five experiments with equilibration times from 8.6 to 13.3 minutes (0.970 ± 0.008) is not significantly different from that in the six experiments in which equilibration time was 20 minutes to 2 hours (0.975 ± 0.011). It is therefore possible to conclude that equilibration between brain and its venous drainage with respect to nitrous oxide tension is complete within 10 minutes, which is therefore the value of $u$ in the equation for cerebral blood flow. The value for the brain-blood ratio of nitrous oxide concentrations at equilibrium (0.975) is the in vivo partition coefficient and agrees closely with the value for this constant obtained from in vitro equilibration (1.03 for the dog) (Table III).

**DISCUSSION**

An exact value for the solubility of nitrous oxide in the living human brain is of course not directly obtainable, although preliminary experiments in this laboratory have indicated that such values for radioactive gases are capable of direct estimation. Nitrous oxide solubility has, however, been measured in samples of human brain obtained at autopsy. We have demonstrated furthermore that this solubility in human brain tissue is identical with that in dog brain after death which, in turn, is remarkably close to the solubility in the living brain of that animal. It is, therefore, very likely that the same is true for man and that the solubility constant obtained in vitro is applicable to the living state. The experimentally determined values for the brain-blood partition coefficient of nitrous oxide

<table>
<thead>
<tr>
<th>Technique</th>
<th>Species</th>
<th>$S$</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Dog</td>
<td>1.03</td>
<td>0.020</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>Man</td>
<td>1.08</td>
<td>0.016</td>
</tr>
<tr>
<td>&quot; vivo, 8-13 min. equilibration</td>
<td>Dog</td>
<td>0.970</td>
<td>0.008</td>
</tr>
<tr>
<td>&quot; &quot; 20 min. to 2 hrs. equilibration</td>
<td>&quot;</td>
<td>0.975</td>
<td>0.011</td>
</tr>
</tbody>
</table>
lie on either side of and only slightly removed from unity and it seems fair to accept this value as a best approximation. The identity of the values found in dog and man speak for the dependence of this coefficient only on gross physicochemical constitution, which varies within extremely narrow limits despite major pathological changes (13–15). The small deviations found in the studies on brains from patients dying of a variety of diseases are further evidence for the constancy of this partition coefficient in different patients and diverse pathological states. This value of unity for the partition coefficient is less than the value of 1.3 tentatively accepted on the basis of a few preliminary measurements (7). The numerous refinements in technique herein reported have undoubtedly yielded a more accurate evaluation of this constant.

In view of the high lipide content of brain tissue and the more than 3-fold greater solubility of nitrous oxide in common fats and oils over that in water or blood, it is surprising that the solubility of this gas in brain is not significantly greater than its blood solubility. In fact in two determinations on white matter (which has twice the lipide content of cortex) the nitrous oxide solubility was found to be within the range of that in whole brain (0.427 and 0.468). These findings are, however, not without precedent. In fact the majority of investigators who have studied, by somewhat cruder techniques, the contents in brain and blood of various volatile anesthetics have arrived at similar conclusions (2–6). Especially noteworthy are the studies of Campbell and Hill (16) who found the nitrogen content of the whole brain at the usual atmospheric tensions to be 1.08 volumes per cent, not significantly different from the blood nitrogen content; yet these workers found the solubility of nitrogen in adipose tissue to be 5 times its solubility in blood. The conclusion is inescapable that brain lipides do not behave as do the usual fats and oils or even adipose tissue in their capacity for nitrous oxide and probably other gases. This does not necessarily vitiate the lipide theory of anesthesia, whose proponents may still reasonably ascribe a preferential absorption of volatile anesthetics to certain important lipides in the surface layers of nerve cells, even though the bulk of the lipides of central nervous tissue is indifferent to these gases.

The authors wish to acknowledge the cooperation of Dr. Helena E. Riggs, neuropathologist of the Philadelphia General Hospital, through whom the human brain samples were obtained.

**SUMMARY**

1. Techniques are described for the determination of the solubility of gases in brain in vitro and in vivo.
2. The Bunsen coefficient expressed as cc. of nitrous oxide (converted
to standard temperature and pressure) dissolved by 1 gm. of brain when equilibrated at a nitrous oxide tension of 760 mm. and 37° equals 0.437 ± 0.008 and 0.437 ± 0.005 for the mixed brain of dog and man respectively.

3. The brain-blood partition coefficient for nitrous oxide at 37°, expressed as the solubility per gm. of brain divided by the solubility per cc. of blood, was found to be 1.03 and 1.06 for dog and man in vitro, respectively, and 0.98 for the dog in vivo.

4. After approximately 10 minutes of inhalation of a constant tension of nitrous oxide there is complete equilibrium in the dog between brain and cerebral venous blood with respect to nitrous oxide tension.

5. The pertinence of these determinations to a method for measurement of cerebral blood flow in man and to the lipide theory of anesthesia is discussed.

BIBLIOGRAPHY
THE SOLUBILITY OF NITROUS OXIDE
IN BLOOD AND BRAIN
Seymour S. Kety, Merel H. Harmel, Hannah T.
Broomell and Clara Belle Rhode


Access the most updated version of this article at
http://www.jbc.org/content/173/2/487.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/173/2/487.citation.full.html#ref-list-1