SOLUBILITY OF FREON-22 IN BLOOD AND LUNG TISSUE

P. J. FRANKS, R. H. HOOPER AND P. R. M. JONES

Over the past 20 years freon-22 has been used for the inert gas method of measurement of cardiac output. A major drawback to its use is the confusion between Bunsen and Ostwald solubility coefficients; the exact coefficient is critical for an accurate determination of cardiac output.

Several studies report values for solubility coefficients, although none indicates how the measurements of solubility were performed, or their accuracy. Bayley, Clements and Osbahr [1] used a coefficient of $0.71 \text{ ml gas. (ml fluid. atmosphere)}^{-1}$ at 37 °C (which will be represented as $\text{ml ml}^{-1} \text{atmos}^{-1}$) determined in rabbit blood. A value of $0.74 \text{ ml ml}^{-1} \text{atmos}^{-1}$ at 37 °C in neonatal blood was given in another paper [2]. These values are confusing, as it is unclear whether the gas volumes were corrected to STPD (Bunsen coefficients) or to BTPS (Ostwald coefficients). Both these studies failed to provide experimental details of the solubility measurement.

The solubility coefficient for freon 22 (given in [2]) was attributed to Eger and Shargel, but no information was provided. It was stated that they used an adaptation of the Scholander apparatus [3], and exposed blood to pure freon (J. A. Clements, personal communication). Kruhoffer [4] reported, in an abstract, a Bunsen coefficient of $0.71 \text{ ml ml}^{-1} \text{atmos}^{-1}$, although the method of determination was prone to inaccuracy (P. W. Kruhoffer, personal communication). Since then, other determinations by that author have fallen within the range $0.62-0.74 \text{ ml ml}^{-1} \text{atmos}^{-1}$, on exposing blood to pure freon-22. In two later studies the solubility coefficient was $0.73 \text{ ml ml}^{-1} \text{atmos}^{-1}$ [5,6]; both appear to be Bunsen coefficients, despite the latter paper using the symbol $\lambda$, which normally denotes Ostwald solubility coefficients.

The purpose of this study was to examine the solubility of freon-22 in water, blood and lung tissue in order to clear the confusion which has arisen from the application of different solubility coefficients.

METHODS

We used the Scholander apparatus as described elsewhere [3]. This is a relatively simple procedure which requires the calculation of volume uptake of pure freon-22 by a blood sample of a known volume at a known pressure at 37 °C. The Ostwald solubility coefficient ($\lambda$) can be determined:

$$\lambda = \frac{V_g}{V_B \cdot P_g}$$

where $V_g$ is volume uptake of gas (ml), $V_B$ is the volume of blood (ml) into which the gas dissolves.
and $P_*$ is the pressure of the gas (standard atmosphere).

The equations also correct for water vapour pressure [3]. The Bunsen solubility coefficient can be calculated by correcting the difference in temperature using the multiplication factor 0.88 (273 divided by 310).

**Purity of freon-22**

For this method it is important that the soluble gas sample is either pure, or its proportion in a mixture with an insoluble gas is known precisely. To test the purity of the gas used, a sample was taken from a cylinder of 100% freon-22 (BOC Ltd). The gas was passed through a Pye 104 chromatograph with a molecular sieve 5A and a column of Chromasorb 102 which is suitable for the detection of nitrogen and oxygen. The same volume of air was used as a calibration gas. The output was amplified $\times 20$ for the freon sample.

To calculate the percentage of these gases in the sample, the peaks were carefully cut from the chart recorder output and weighed (table I).

As the nitrogen:oxygen ratio demonstrates, there was a small amount of air contaminating the freon-22. The overall purity of the freon-22 was 99.31% after allowing for the small amount of air in the sample (including a calculated value for argon). We assumed that the air caused no interference with the uptake of freon-22 by blood, other than by the reduction of freon partial pressure caused by nitrogen (the minute volume of oxygen would be absorbed by the haemoglobin). Hence, freon partial pressure ($P_*$) was taken to be 0.995.

**Preparation of materials**

Twenty millilitre of venous blood was taken from seven human subjects and six dogs and transferred to heparinized tubes, which were rocked gently before storage overnight at 4°C.

<table>
<thead>
<tr>
<th>Gas</th>
<th>Air (%)</th>
<th>Freon-22 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_2$</td>
<td>78.04</td>
<td>0.540</td>
</tr>
<tr>
<td>$O_2$</td>
<td>20.93</td>
<td>0.145</td>
</tr>
<tr>
<td>Total ($N_2 + O_2$)</td>
<td>98.97</td>
<td>0.685</td>
</tr>
<tr>
<td>$N_2/O_2$ ratio</td>
<td>3.73</td>
<td>3.72</td>
</tr>
</tbody>
</table>

Haematocrit was determined (an average of three measures) in each sample and 10 ml was spun down to obtain plasma.

The dogs were killed and the lungs removed. The animals had received heparin just before death, to enable blood to drain from the major vessels. It was assumed that any blood remaining in the lung tissues would collect in the capillary bed, and would consequently contribute to the overall solubility of freon-22 in the tissue sample. After storage for 12 h at 4°C, approximately 3 g of lung tissue was removed from various sites. Care was taken to exclude any of the visible hilar structures, and the samples were weighed. A mass of water, approximately 10 times the lung tissue mass, was added and the whole re-weighed. This was found to be the minimum dilution possible to produce a liquid homogenate suitable for use with the Scholander apparatus. The sample was homogenized until all traces of solid had disappeared. Solubility of freon-22 was measured in this sample by the same method as that used for blood, described below.

The samples were degassed. Each was transferred to a small conical flask with several glass beads and placed in a water bath at 37°C (solubility in water was also measured at 20°C), the temperature used for determinations. Highly insoluble gases (argon or helium) were humidified at the test temperature in a water trap and blown continuously over the samples. Air contamination was avoided by use of an enclosed unit submerged in the water bath. The insoluble gas was vented through the water bath to air after it had traversed the conical flask. The flask was shaken vigorously during degassing. As this degassing procedure is less efficient than the rotating tonometer, 4 h was required to clear all traces of oxygen and carbon dioxide from the sample. The haematocrit was checked following degassing to confirm that neither haemolysis nor drying had occurred. The same degassing procedure was used for lung tissue and plasma.

The procedure using the Scholander apparatus was identical to that described by Saidman [3]. After each determination the reaction chamber was washed at least three times with distilled water. This was particularly important when analysing blood, as the red cells adhered to the side of the chamber, making further measurements liable to inaccuracy.

The measurements of solubility were repeated six times on each sample.
Calculations

The calculation of the Bunsen solubility coefficient was identical to that described by Saidman [3], with conversion from the Ostwald coefficient using the multiplication factor described above.

Calculation of the Bunsen solubility coefficient in red cells ($\alpha_R$) was:

$$\alpha_R = \alpha_B \left( \frac{100}{Hct} \right) - \alpha_p \left( \frac{100}{Hct} \right)$$

where $\alpha_B$ = solubility in whole blood, $\alpha_p$ = solubility in plasma, Hct = haematocrit.

Calculation of solubility in lung tissue ($\alpha_L$) was:

$$\alpha_L = \frac{\alpha_{tot} \cdot M_{tot} - \alpha_w \cdot M_w}{M_L}$$

where $M_{tot}$ = total mass of diluted homogenate, $\alpha_{tot}$ = mean solubility of diluted homogenate, $M_L$ = mass of lung tissue, $M_w$ = mass of water, $\alpha_w$ = solubility in water.

Results were analysed using a one-way analysis of variance for data with one criterion of classification [7]. The symbol $V_B$ is the variation in sample quality, indicating inter-sample variation. Within group variation is given by $V_s$ (variance of experimental error), calculated from the six repeated measures.

RESULTS

In one of the seven human subjects, no measurement was made in plasma; in another none was made in whole blood. Hence there are only five results for human red cells (table II). In the dog, blood was collected from various sites following death. In three of the six samples there was some cell fragmentation, making it impossible to determine haematocrit or plasma separation, but these results have been included in the results for whole dog blood.

We found that the Bunsen solubility coefficient of freon-22 in blood was approximately 0.67 ml ml$^{-1}$ atmos$^{-1}$ for the human and 0.66 ml ml$^{-1}$ atmos$^{-1}$ for the dog. The variance estimates were similar for both.

DISCUSSION

Our freon-22 solubility value is somewhat lower than that reported previously, but there are uncertainties concerning the source of the earlier data. If the value of 0.74 ml ml$^{-1}$ atmos$^{-1}$ used in a previous study [1] refers to the Ostwald solubility coefficient of this gas, the Bunsen coefficient would have been 0.65 ml ml$^{-1}$ atmos$^{-1}$.

In a comparative study [8] using two gases for rebreathing experiments in man, a Bunsen coefficient of 0.74 ml ml$^{-1}$ atmos$^{-1}$ was used, as suggested by Kruhoffer to these authors in a personal communication. They found that freon-22 significantly underestimated cardiac output compared with an acetylene rebreathing method. They showed that the cardiac output values corresponded very closely to each other if the freon-22 solubility was taken as 0.67 ml ml$^{-1}$ atmos$^{-1}$. It is

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>n</th>
<th>$\alpha$</th>
<th>CV %</th>
<th>$V_B \times 10^{-5}$</th>
<th>$V_s \times 10^{-5}$</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>20</td>
<td>10</td>
<td>0.793 (SD 0.011)</td>
<td>1.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>10</td>
<td>0.476 (SD 0.007)</td>
<td>1.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Whole blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>37</td>
<td>6</td>
<td>0.673</td>
<td>—</td>
<td>8.82</td>
<td>13.8</td>
</tr>
<tr>
<td>Dog</td>
<td>37</td>
<td>6</td>
<td>0.662</td>
<td>—</td>
<td>2.78</td>
<td>16.1</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>37</td>
<td>6</td>
<td>0.479</td>
<td>—</td>
<td>7.23</td>
<td>12.8</td>
</tr>
<tr>
<td>Dog</td>
<td>37</td>
<td>3</td>
<td>0.437</td>
<td>—</td>
<td>2.33</td>
<td>11.0</td>
</tr>
<tr>
<td>Red cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>37</td>
<td>5</td>
<td>0.899</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dog</td>
<td>37</td>
<td>3</td>
<td>0.890</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lung tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>37</td>
<td>6</td>
<td>1.077</td>
<td>—</td>
<td>818</td>
<td>1074</td>
</tr>
</tbody>
</table>
interesting to note that this is the same as the value we found by measurement of the Bunsen solubility coefficient in human blood. It is possible that the value suggested by Kruhoffer was an Ostwald and not a Bunsen coefficient.

Whatever the errors involved in measurements of cardiac output in man involving gas uptake measurements and using the lungs as tonometers, our evidence suggests that the hypothetical value devised by Bonde-Petersen was correct and supports the use of freon-22 in place of acetylene for indirect measurement of cardiac output.

The value obtained in plasma was similar to that found in water at the same temperature, although the solubility in red cells was significantly higher ($t = 13.7, P < 0.001; \text{ratio} 0.54:1$). This may be a result of high lipid solubility, or of binding with haemoglobin. The solubility in red cells is similar in man and the dog, but a species difference in haematocrit leads to different whole blood solubilities.

As we used methods given elsewhere [3] to achieve comparability, the conditions did not mimic the situation in vivo. In particular, the blood was degassed, leading to lack of oxyhaemoglobin and an increased pH. It is possible that both factors could alter freon uptake. Further, we used pure freon, as did the previous workers. It is not known if freon-22 obeys Henry's law and so its use at low concentrations for blood flow measurement may be questionable.

Human cardiac output has been measured by estimating pulmonary blood flow using gases such as acetylene and nitrous oxide. These gases have a whole blood: lung tissue solubility ratio of approximately unity [9]. For freon-22 this is not the case, the ratio being 0.61:1.00 in the dog. The whole blood: red cell solubility ratio is slightly greater (0.74:1.00). It is not clear why lung tissue should have such a high solubility coefficient. Unfortunately, there was considerable variation in the measurement of freon-22 solubility in lung tissue samples (table II). This might have resulted from the degree of dilution of the homogenate necessary for success when using the Schölander apparatus.

The measurement of blood flow through the lungs would not be affected seriously by this inaccuracy. The lung tissue volume is small compared with the alveolar gas volume and the total flow of blood through the lungs over the period of measurement. Consequently, the calculation of pulmonary blood flow should not be compromised seriously by this degree of variation in the tissue solubility measurement.

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REFERENCES