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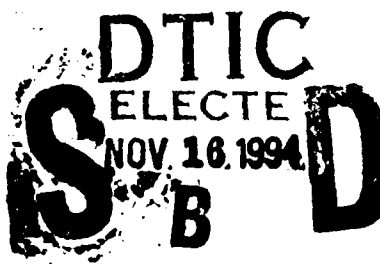
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**SOLUBILITIES OF NITROGEN AND HELIUM IN WATER
AND BLOOD AT 37°C AND PRESSURES OF UP TO 11 ATA**

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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13. ABSTRACT (Maximum 200 words) The solubilities of N₂ and He in water and whole blood were measured at 37 °C using a barometric method described in an earlier report (NMRI Technical Report 90-138). Solubilities have been measured successfully in H₂O at ~2 ATA and ~11 ATA. In addition, protocols for measuring solubilities in whole blood have been explored. Measurements in blood have been unsuccessful thus far (i.e., have disagreed with published results), apparently because efforts have failed to eliminate interphase transport of the metabolic gases O₂ and CO₂ during the experiment. Blood experiments can resume after the experimental assembly has been modified to allow sampling of the gas phase so that its composition can be determined. This information, in conjunction with the pressure measurements that already are made during each experiment, will enable one to eliminate the confounding effect of O₂ and CO₂ transport.				
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INTRODUCTION

The formation of bubbles in tissue during decompression from dives is suspected to be a primary cause of decompression sickness (DCS). Accordingly, the solubilities of diving gases in tissues are of interest to the diving medicine community. This report details the beginning of the compilation of a comprehensive table of solubility coefficients for diving gases.

The technique we have employed is described in detail (1). This barometric technique measures gas/tissue solubility without sampling or analyzing the composition of either phase, thereby eliminating a possible error source. Briefly, the condensed phase is allowed to equilibrate with a gas phase of known composition at or near ambient pressure. The pressure in the vessel (i.e., the barometric pressure or something close to it) is measured. The vessel containing the condensed phase is then compressed by allowing the diving gas of interest to flow into the vessel from a high-pressure supply. After re-equilibration between phases, the pressure in the vessel is measured again. The final pressure is a function of how much of the diving gas dissolved into the condensed phase during the experiment. (Other gases present, such as O_2 , CO_2 , and H_2O vapor, are assumed to undergo no net interphase transport because their partial pressures in the gas phase are not altered when the system is compressed by adding the diving gas, and therefore are assumed not to affect the final pressure.) The solubility of the diving gas can be computed by mass balance (1). The temperature of the system is controlled at 37 °C by immersion in a water bath.

Data are reported as Ostwald coefficients $\Gamma_{2,1}$, defined as follows:

$\Gamma_{2,1}$ = (volume of dissolved gas "2", evaluated at 1 ATA and the experimental temperature) per (volume of condensed phase "1").

Note that $\Gamma_{2,1}$ is never pressure-independent. At pressures low enough for both phases to exhibit ideal solution behavior (that is, low enough for Henry's Law to apply), Γ is proportional to the partial pressure of the solute in the gas phase. In this case, it is convenient to report the value of Γ for a solute partial pressure of 1 ATA, designated $\Gamma_{1 \text{ ATA}}$, and this is the practice followed in this report.

So far we have successfully measured the solubilities of nitrogen and helium in deionized water at solute partial pressures of almost 2 ATA and almost 11 ATA. We have been unable to obtain results with whole blood that are reproducible or that agree with published results. The anomalous results for blood almost certainly stem from an inability to prevent interphase transport of O_2 and/or CO_2 during the experiments, probably because of ongoing metabolism or shifts in the O_2 /hemoglobin and CO_2 /hemoglobin adsorption isotherms (i.e., their dissociation curves). A modified protocol will be described that eliminates the confounding effect of O_2 and CO_2 transport.

METHODS

The protocol for measuring gas solubilities in a condensed phase is covered in some detail (1). Here, we discuss the preparation and analysis of blood.

The experiments were done using whole human blood that had been collected from donors for clinical use, but had exceeded its nominal shelf-life of 28 days. It was collected

in Citrate Phosphate Dextrose Adenine (CPDA) bags that contained sodium citrate (anti-coagulant), dextrose, sodium phosphate, adenine, and heparin. The hematocrit of blood was adjusted to 45% by adding Ringer's Lactate. All anaerobic and aerobic metabolic pathways must be shut-down prior to an experiment so that no gas can be consumed or produced by metabolism during the experiment. Therefore, the following inhibitors were added*: 4 mg/l Amphotericin B anti-fungal agent; 20 mg/l Chloramphenicol and 16 mg/l Vancomycin antibiotics; 84 mg/l (2 mM) sodium fluoride, to inhibit anaerobic glycolysis; and 5 g/l Rotenone, to inhibit aerobic metabolism by blocking electron transfer in the cytochrome. Rotenone was chosen among other aerobic metabolic inhibitors for its low volatility (molecular weight = 394). Before performing any solubility measurements on blood, it was confirmed that there was no measurable pressure change over 7 h in a closed container of treated blood stored at 37 °C, nor any growth in a cultured sample of the same after the 7-hour storage.

Before the solubility measurement, it is necessary to (1) equilibrate the blood with a gas of known composition at ambient pressure and 37 °C and (2) titrate the blood to pH 7.4. These operations involved alternately contacting the blood with the desired gas mixture and titrating it with 0.1 M NaOH and 0.1 M HCl, in iterative cycles. It is necessary to repeat the iterations several times because the dissolved CO₂ tension and pH are mutually dependent.

In some experiments, the dissolved gas tensions and pH were measured during the above pre-equilibrations, and immediately before and after the experiments, using a Ciba-Corning model 170 Blood Gas Analyzer (this analysis was not available for all of the

* The concentrations of these additives were lower in our initial experiments, but were increased because of experimental results suggesting that metabolism might not be completely eliminated at the original dosages.

experiments). The total concentration of Hb and the percent oxygenated Hb (%O₂-Hb) was measured spectrophotometrically immediately before and after the experiments using an Instrument Laboratory model 282 co-oximeter.

Initially, "the gas of known composition" with which we attempted to equilibrate blood prior to experiments was 5% CO₂ in air, or roughly the composition of gas with which blood equilibrates *in vivo* in a person breathing air at 1 ATA. Because N₂ is adsorbed onto hemoglobin (Hb) in amounts that are significant compared with the amount of N₂ in free solution (2), it is desirable to maintain at physiologic values any parameters such as the concentration of dissolved CO₂ and O₂ that might affect the spatial conformation of Hb and thereby shift the adsorption isotherms. When anomalous results were obtained in these experiments, a new series of experiments performed with the pure diving gas was substituted for the 5% CO₂/air mixture.

There has been one other addition to the methodology since the first technical report was written (1). Appendix B of that report describes a barometric method for measuring the volume of the test vessel. That method was subsequently discarded in favor of a method believed to be more accurate, which consists of filling the vessel with water from a graduated cylinder. This technique is described in the Appendix of this report.

RESULTS AND DISCUSSION

The means and standard deviations of our measured solubilities for N₂ and He are listed in Table 1. Also shown are corresponding average published values from references 3-12. Our average experimental values for both He and N₂ in water, measured at ~11 ATA,

are within 1% of the average published values. On the other hand, our average experimental values for He in H₂O, measured at ~2 ATA, are about 9% higher than published values, which is statistically significant.

One error analysis suggests that leakage to the atmosphere during the experiment is potentially the most troublesome source of error (1). The leak rate therefore was measured at 2 ATA and 11 ATA, in the absence of a condensed phase. The predicted *total* uncertainty for solubility measurements, based on the average measured leak rate and on manufacturers' claimed precision of the equipment we used, is less than 5%. The standard deviations on the H₂O data are indeed less than 5%. However, as noted already there seems to have been a significant *biased* error present in the He/H₂O experiments at 2 ATA -- perhaps, in this case, the average leak rate during solubility measurements differed from the average rate during leakage measurements.

The error analysis predicts that the total measurement precision improves with increasing solubility and increasing pressure.* Therefore, one might expect somewhat better agreement between the mean measured solubilities and published results for N₂ than for He, and at 11 ATA than at 2 ATA. These expectations are borne out by the data, albeit usually not with statistical significance.

The measured solubility of gas in blood depended strongly on the gas mixture with which the blood was equilibrated before the experiment. When blood was equilibrated with 5% CO₂ in air (the "A" group of blood experiments), the measured solubilities for He and N₂ were consistently about two times higher than previously reported. The likely explanation is

* This is true assuming that the fraction of gas lost to leakage is the same at both pressures, as has proven true within experimental uncertainty in direct measurements of leakage.

that the tensions of O₂ and/or CO₂ in free solution in the blood declined, causing a net transport of one or both gases from the gas phase into the blood. For blood with a 45% hematocrit that is equilibrated with 5% CO₂ in air at 1 ATA and pH 7.4, the amount of CO₂ and O₂ in blood, including the portion that is bound to Hb, is one order of magnitude greater than the amount of dissolved N₂ or He present in our experiments. Therefore, it is obvious why interphase transport of O₂ and CO₂ can easily confound the experimental results.

Table 1: Measured solubilities of diving gases in H₂O and blood.

$\Gamma_{1 \text{ ATA}}$ (cm ³ gas)/(condensed phase @37 °C & 1 ATA)				
Condensed Phase	Gas	Average Published Value ³⁻¹²	2 ATA	11 ATA
water	He	0.0098	0.0107 ± 0.0004	0.0099 ± 0.0002
water	N ₂	0.0143	0.0145 ± 0.0003	0.0143 ± 0.0006
whole blood	He	0.0094	Inconsistent with published values (see text).	
whole blood	N ₂	0.0148	Irreproducible and inconsistent with published values (see text).	

The reduction in the dissolved O₂ or CO₂ tension could have resulted from either aerobic metabolism or a pH decrease caused by lactic acid production during anaerobic metabolism. A pH decrease would reduce the affinity of Hb for O₂ and for CO₂ (13,14), and would accompany a shift to the right of the [bicarbonate ⇌ carbonic acid ⇌ CO₂ gas] equilibrium; all of which imply transport *from* the blood *to* the gas phase, causing an error of sign opposite to that observed. Anaerobic metabolism would have generated gas, rather than consumed it, and therefore does not explain the results, either. Aerobic metabolism may provide at least a partial explanation: although about 20% fewer molecules of CO₂ are

generated than O_2 molecules are consumed, the solubility of CO_2 is high enough to allow a net pressure drop in the gas phase. This, however, does *not* explain why the same phenomenon was not observed during replicated measurements of the pressure in closed containers of treated blood stored at 37 °C. Also, the experimental results were not improved by increasing the concentrations of inhibitors and antibiotics, casting doubt on aerobic metabolism as the cause of experimental error.

We attempted to eliminate CO_2 and O_2 from the blood prior to some experiments (the "B" group) by purging it with pure inert gas. These trials yielded irreproducible data. The measured solubilities varied between zero and 2/3 of those reported elsewhere. An anomaly associated with these experiments is that, even after several hours of contact (with agitation) between the blood and the inert gas, blood/gas analysis showed there still remained substantial amounts of O_2 and CO_2 . It is likely that at least some of the blood gas analyses were spurious, perhaps because of clotting.

It is significant that the blood darkened during *all* experiments from both the A and the B groups. This indicates an ongoing dissociation of O_2 , which might result from a decline in pH or a shift in the O_2/O_2 -Hb equilibrium caused by depletion of dissolved O_2 , as would result from ongoing aerobic metabolism. If some of this newly freed O_2 ended up in the gas phase, it would at least partially explain the low measured solubilities in the group B experiments. In fact, the co-oximeter data from those trials consistently indicated a decrease in the % O_2 -Hb during the experiments (why *any* O_2 -Hb would remain after hours of contact between agitated blood and a stream of pure inert gas is not clear). There also was a

measured pH decrease of 0.2-0.6 units during two of those experiments, but not during the remaining five.

To eliminate a possible cause of changes in the amounts of dissolved O_2 and CO_2 , we have considered using some of the active reagents containing cyanide (CN^-), which are known to bind much more strongly to Hb than do O_2 or CO_2 . These could be used to competitively displace those metabolic gases, and shifts in the O_2/O_2 -Hb and CO_2/CO_2 -Hb isotherms would thus be rendered irrelevant. (This would not solve the problem, if any, of metabolism.) Attempts to treat whole red blood cells with ferricyanide were unsuccessful: spectrophotometry did not reveal the characteristic adsorption peak of methemoglobin (met-Hb) after contact between the cells and ferricyanide. The met-Hb peak was seen after treating lysed red blood cells, indicating that the cell membrane is not permeable to ferricyanide. Treatment of whole red blood cells with potassium cyanide (KCN) was successful, so that this may be a good candidate as an agent for displacing O_2 and CO_2 . However, the adsorption of CN^- to Hb is easily reversed, so that its effective use would entail continuously maintaining a partial pressure of HCN in the gas phase. Besides the obvious safety risks, this would present yet another soluble gas to confound the experimental results.

An alternative solution is simpler, and somewhat safer as well: A port can be installed to make it possible to sample the gas phase at the same time as the hydrostatic pressure is measured (once at the start of the experiment and once at the close). This will enable measurement of the composition of the gas phase at those two time points using gas chromatography. Therefore, the partial pressure of the diving gas will be known at the

relevant times, even if constant partial pressures of O_2 and CO_2 cannot be maintained. It then will be a straightforward matter to compute the solubility via mass balance.

CONCLUSIONS

The solubility measurements for N_2 and He in water and blood were performed at 37 °C and pressures of 2 ATA and 11 ATA. For water, our measured values are in excellent agreement with the average published values. Measurements in blood yielded apparently false results, probably because of the difficulty of eliminating physico-chemical processes in blood that lead to unwanted interphase transport of gases during these experiments.

We have discussed how the difficulty with measurements in blood might be overcome, and have proposed a relatively modest alteration to the apparatus, designed to enable samples to be withdrawn from the test vessel for chromatographic analysis. The in-house capability already exists for this analysis.

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APPENDIX: MEASUREMENT OF THE VOLUME OF THE TEST VESSEL

An obvious method of measuring a volume is by filling it with an incompressible fluid from a graduated container. Whereas the test vessel for our experiments includes some small-diameter tubing that is somewhat tortuous, filling it with liquid at ambient pressure inevitably would result in trapped air pockets and an incorrect measured volume.

Our procedure was to evacuate the vessel to a pressure of < 10 mm Hg and then allow the vessel to fill itself by suction with water from a graduated cylinder kept at ambient pressure. After filling, some time was allowed for visible bubbles to disappear before the volume of water remaining in the cylinder was read (the water was degassed under vacuum before use, to minimize bubble growth in the first place). The tube leading from the graduated cylinder to the test vessel was already filled with water before each measurement, so that the volume of water leaving the cylinder equalled the volume entering the test vessel alone (not the volumes of the vessel + the tube). The above technique yielded replicate measurements that agreed to within $\pm 0.3\%$.