Intravascular bubble composition in guinea pigs: a possible explanation for differences in decompression risk among different gases

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Lillo RS, MacCallum ME, Caldwell JM. Intravascular bubble composition in guinea pigs: a possible explanation for differences in decompression risk among different gases. Undersea Biomed Res 1992; 19(5):375–386.—Differences in risk of decompression sickness (DCS) that have been observed among inert gases may reflect differences in gas solubility or diffusivity or both. A higher risk gas might generate a larger volume of evolved gas during decompression, thereby increasing the probability of DCS. If this hypothesis is correct, the composition of bubbles that develop during decompression should reflect such gas differences. Unanesthetized guinea pigs were compressed to depths ranging from 230 to 350 fsw with air, He-O_2 (21% O_2) or one of a number of N_2-He-O_2 or N_2-Ar-O_2 mixtures (21% O_2). Animals were held at depth from 15 to 60 min, then decompressed slowly (60 fsw/min) or rapidly (<15 s) to 5 fsw. If severe DCS developed, as judged by changes in physiologic variables, death usually occurred quickly. Gas/blood samples were then immediately withdrawn from the right atrium or the inferior vena cava, and the gas phase analyzed for He, N_2, Ar, O_2, and CO_2 via gas chromatography. Bubbles from all dives contained 5–9% CO_2, 1–4% O_2, with the balance inert gas. Bubbles after N_2-He-O_2 dives contained substantially more N_2 than He (up to 1.9 times more) compared to the dive mixture; bubbles after N_2-Ar-O_2 dives contained more Ar than N_2 (up to 1.8 times more). For N_2-He-O_2 dives, the actual inert gas makeup of bubbles was dependent on the time-at-depth and the decompression profile. Results may reflect differences among He, N_2, and Ar in tissue solubility/diffusivity and gas exchange rates, and support the rank order of increasing DCS risk (He < N_2 < Ar) and rate of gas exchange (N_2 < He) observed previously during rat dives.

diving 
hyperbaric

decompression sickness
inert gases
gas chromatography

Extensive studies with humans and animals suggest that there are significant differences in risk among gases for causing decompression sickness (DCS) (1–5). Because gas bubbles in tissues are believed to be the major causative factor in DCS, decompression risk may be reflecting, at least partially, differences among gases in tissue solubility or diffusivity or both. Unfortunately, our limited knowledge of how gases interact with tissues at pressure (6, 7) and how bubbles develop and grow (8–11)
prevents easy explanation of why different gases often produce different decompression outcomes. For example, a large diffusion coefficient would not only favor accumulation of gas into a bubble, but also a more rapid loss.

Work in our laboratory with small animals has demonstrated that prediction of risk of DCS can be improved by accounting for two differences among gases: exchange rates and relative potency for causing DCS (12, 13). The exchange rate (i.e., tissue equilibration rate) affects the change in the partial pressures of gases in tissues with time; the potency defines the level of risk based on the estimated partial pressures of the gases in the tissues. One hypothesis to explain the differences in potency is that “riskier” gases generate larger volumes of evolved gas during decompression. Previous workers have suggested that the volume of evolved gas was the important factor affecting the development of DCS (14) and might explain the decompression differences among gases (15). If this hypothesis is correct, the composition of bubbles that develop during decompression should reflect these volume differences. Although no previous study has examined this hypothesis, the composition of intravascular bubbles in animals with DCS has been reported to be quite different from the dive mixture (16). In the present study, we examine the composition of intravascular bubbles in guinea pigs after dives with various inert gas/O2 mixtures and interpret results in terms of decompression differences among gases.

METHODS

Unanesthetized guinea pigs were decompressed to the surface after hyperbaric exposure; death generally resulted after severe DCS. Immediately upon death, blood/gas was collected from a venous catheter using a glass syringe. The gas phase was then analyzed using gas chromatography. Details are presented below.

Animal preparation

Male albino guinea pigs (Cavia porcellus, Hartley strain), weighing approximately 550–650 g, were obtained from a local supplier and housed locally for at least 10 days before use. Rompun (xylazine, 5 mg/kg) i.m. followed by ketamine HCl (30 mg/kg) i.m. were given to fasted animals to anesthetize them. Surgery was then begun using sterile instruments, surgical gloves, and aseptic techniques to prevent clinical infection. A ventral incision approximately 2.5 cm in length was made in the neck, and a polyethylene catheter inserted into the left carotid artery, advanced 1–2 cm, and sutured in place to allow monitoring of blood pressure. A second catheter was inserted into the external right jugular vein and advanced at least 4 cm but no more than 6 cm to place it in the right atrium or inferior vena cava, and sutured in place; exact location was confirmed postmortem after experiments. This catheter allowed blood/gas samples to be obtained. A 26-gauge needle was then used to puncture a small hole in the ventral side of the trachea approximately 4 cm distal from the larynx. A tiny thermistor (Thermometrics, Edison, NJ, model AB6B8BR14KA132J37C) was then inserted into the hole and advanced several millimeters toward the lung so that the thermistor and its lead rested close to the inside ventral surface of the trachea. Tissue cement was used to fix the thermistor to the trachea and seal the small hole in the airway. The lead was sutured to the fascia and muscle at several spots before
it was threaded under the skin, along with both catheters, and out via a small incision on the dorsal side of the animal, just behind the neck. Three wire leads were then inserted s.c. for ECG monitoring, one on the back of the animal and one on each side of the thorax. Both incisions were sutured closed and all leads braided together and sutured to the skin at point of exit. Animals were allowed to recover overnight. Immediately before experiments, a thermistor probe was inserted into the rectum, advanced approximately 5 cm, and sutured in place using suture ties that had been sewn to the skin close to the anus during the previous surgery. This probe would allow monitoring of body temperature.

Dive procedures

Animals were individually placed into a small wire cage located in a hyperbaric chamber with an inner volume of approximately 7.5 liters (Bethlehem Corp., Bethlehem, PA, model 615-HP); a piece of wire mesh was adjusted inside the cage to gently restrain the animal. Actual gas volume during experiments (i.e., inner chamber volume after subtracting the volume of the animal, heating/cooling shield, cage, and cage tray) is estimated to be approximately 6 liters. All electrical leads from the animal (i.e., ECG and thermistors) were attached inside the chamber to connectors that penetrated the chamber wall to allow outside recording. Arterial and venous catheters were connected to small diameter Teflon tubing that also penetrated the wall. This permitted sampling from the venous line from outside the chamber and periodic flushing of the arterial line with small amounts of heparinized saline (20 IU heparin/ml) to maintain patency.

During experiments, arterial blood pressure was measured via the arterial cannula using a pressure transducer (Gould Inc., Cleveland, OH, model P50) located inside the chamber and vented to chamber pressure by an incision in its electrical lead. The transducer lead was routed to the outside of the chamber again via penetrators in the hull. This transducer was calibrated manometrically using known heights of saline; mean blood pressure was obtained by processing the blood pressure signal using a resistance-capacitance network with a long time constant. Heart rate, rectal temperature, and trachea temperature (used to determine ventilation rate) were monitored using appropriate preamplifiers on a Gould 8-channel recorder (model 2800S).

After hook-up of all leads and catheters, 15–20 min was allowed for animal stabilization. Predive recording of blood pressure, heart rate, ventilation rate, and rectal temperature was then performed with the chamber door open, under quiet conditions. The door was then closed and, before all dives except those in air, the chamber was vented at the surface for 2 min with approximately 25 liters of the gas mixture that would be used to compress the chamber. This procedure switched the chamber atmosphere from air to the dive mixture; the chamber was then compressed at 60 fsw/min to a depth ranging from 250 to 350 fsw with air, He-O₂ (21% O₂), or one of a number of N₂-He-O₂ or N₂-Ar-O₂ mixtures (21% O₂). These mixtures were delivered to the chamber from steel cylinders outside the chamber via high pressure whips. The accuracy of all mixtures was confirmed before use by gas chromatography (GC; details of GC used here are described below).

While at depth, the chamber was vented with approximately 10 liters of the dive mixture for 1 min every 10 min to maintain O₂ and reduce CO₂ buildup. Levels of these two gases were monitored every 15 min with an electrolytic O₂ analyzer and
infrared CO₂ analyzer (Beckman Instruments, Fullerton, CA; model 865 infrared analyzer and model OM-11 O₂ analyzer). GC analysis of the chamber atmosphere was performed 5 min after reaching bottom for multiple inert gas dives; in all cases, this confirmed that the chamber inert gas composition at the start of the dive was acceptably close (within 1% relative) to the gas used for compression. Soda lime was placed on a tray below the cage to absorb CO₂. With only rare exceptions, O₂ did not go below 20.4% and CO₂ not above 0.15%. Chamber temperature was kept at 32.0°C ± 0.5°C by a temperature-controlling unit (Yellow Springs Instruments, Yellow Springs, OH); this temperature was adequate to maintain normal rectal temperatures (38°-40°C) during experiments.

Animals were held at depth for 15, 30, or 60 min and then decompressed slowly (1 fsw/s) or rapidly at the maximum rate possible (<15 s) to 5 fsw. Time-at-depth was varied to alter time allowed for gas uptake; decompression rate was varied to alter time allowed for gas elimination. In these ways, the dive profile was manipulated to help resolve differences among the inert gases.

At the surface (i.e., 5 fsw) physiologic variables were monitored for signs of DCS. Usually, severe DCS developed as indicated by a drop of at least 50% in arterial blood pressure, a doubling of ventilatory rate, and severe cardiac arrhythmia. In nearly all cases, these changes were followed within several minutes by death. Immediately upon death, a valve was opened that allowed positive chamber pressure to slowly drive venous blood through the Teflon tubing to the outside of the chamber where it was collected in an upright, 10-ml heparinized glass syringe after purging to remove all air. In many cases, the chamber was recompressed back to 8-15 fsw to facilitate blood collection. The experimental design is detailed in Tables 1 and 2.

Gas analysis

Two 25-μl heparinized gas-tight syringes were immediately filled from the gas phase above the blood after careful needle insertion into the 10-ml syringe. Each 25-μl syringe was filled slowly, removed from the large syringe, and gas expelled to purge the syringe. Each syringe was then filled again and samples analyzed using both isothermal and temperature-programmable gas chromatographs (Shimadzu model GC-9A, Shimadzu Corp., Columbia, MD; Perkin-Elmer model Sigma 4, Perkin-Elmer Corp., Norwalk, CT) with thermal conductivity detection (for O₂ and inert gases) and with methanization/flame ionization detection (for CO₂). Preliminary testing demonstrated that such purging/sampling procedures removed >99% of the air initially present in the syringe; additional purging was not performed due to the very limited amount of intravascular gas that was often collected after a dive. After the first inert gas analysis, a third 25-μl gas sample was drawn and analyzed for O₂ and inert gases to provide replicate measurements; only one CO₂ measurement was performed.

All gas sampling and GC injections were usually completed within 20 min of blood collection from the animal. During this time, any changes in the bubble composition due to gas exchange between blood and gas and to metabolism of the blood are believed to be minimal for several reasons: a) the syringe was maintained in a vertical position at all times to prevent remixing between gas at the top of the syringe and the blood, b) gas transfer between a gas phase and unstirred liquid is extremely slow.
TABLE 1
COMPOSITION OF INTRAVASCULAR GAS FROM GUINEA PIGS WITH DCS AFTER DIVES WITH N₂ OR HE OR BOTH (20.9% O₂ IN ALL CASES)∗

<table>
<thead>
<tr>
<th>Dive profile</th>
<th>Dive gas/diver depth</th>
<th>n (no. animals)</th>
<th>%CO₂</th>
<th>%O₂</th>
<th>%N₂</th>
<th>%He</th>
<th>%N₂:He Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 fsw/min decompression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air/250 fsw</td>
<td>60 min</td>
<td>10</td>
<td>5.0</td>
<td>5.6</td>
<td>89.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>He-O₂/300 fsw</td>
<td>60 min</td>
<td>8</td>
<td>5.5</td>
<td>2.2</td>
<td>2.1</td>
<td>90.4</td>
<td>—</td>
</tr>
<tr>
<td>50% N₂-50% He/275 fsw</td>
<td>60 min</td>
<td>10</td>
<td>6.2</td>
<td>1.7</td>
<td>61.8</td>
<td>30.1</td>
<td>1.9</td>
</tr>
<tr>
<td>75% N₂-25% He/275 fsw</td>
<td>60 min</td>
<td>6</td>
<td>6.3</td>
<td>1.6</td>
<td>76.8</td>
<td>15.0</td>
<td>1.9</td>
</tr>
<tr>
<td>50% N₂-50% He/325 fsw</td>
<td>30 min</td>
<td>5</td>
<td>8.8</td>
<td>1.7</td>
<td>56.9</td>
<td>32.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Rapid decompression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% N₂-50% He/275 fsw</td>
<td>60 min</td>
<td>7</td>
<td>7.3</td>
<td>1.9</td>
<td>57.8</td>
<td>33.7</td>
<td>1.6</td>
</tr>
<tr>
<td>50% N₂-50% He/325 fsw</td>
<td>30 min</td>
<td>5</td>
<td>8.2</td>
<td>2.4</td>
<td>52.6</td>
<td>36.2</td>
<td>1.5</td>
</tr>
<tr>
<td>50% N₂-50% He/350 fsw</td>
<td>15 min</td>
<td>7</td>
<td>6.2</td>
<td>4.0</td>
<td>51.8</td>
<td>37.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Values are means (SD).

particularly for inert gases that have relatively low solubility in blood (7), and c) the 10-ml syringe containing blood/gas was stored in ice between gas samplings.

Helium was used as the GC carrier gas except in cases where the dive mixture contained He; in those instances, inert gas analysis was performed using Ar as the carrier gas that allowed detection of He. Molecular sieve 5A packed columns were used for separation of inert gases and O₂. A carbosieve-packed column was used for analysis of CO₂; generally, a small amount of methane was also detected using this column. For dives using mixtures containing Ar, a molecular sieve 5A PLOT (porous, lined, open, tubular, 25-m long, 0.53 mm i.d.) capillary column was used which enabled the very difficult GC separation of Ar and O₂ under cryogenic conditions (i.e., −50°C).
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TABLE 2
COMPOSITION OF INTRAVASCULAR GAS FROM GUINEA PIGS WITH DCS AFTER DIVES WITH 50% N₂:50% AR (20.9% O₂ IN ALL CASES)*

<table>
<thead>
<tr>
<th>Time-at-depth</th>
<th>%CO₂</th>
<th>%O₂</th>
<th>%N₂</th>
<th>%Ar</th>
<th>%Ar:%N₂ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 fsw/min decompression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>275 fsw</td>
<td>7.0</td>
<td>1.9</td>
<td>33.0</td>
<td>57.8</td>
<td>1.7</td>
</tr>
<tr>
<td>60 min</td>
<td>(1.8)</td>
<td>(0.6)</td>
<td>(1.3)</td>
<td>(1.6)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>n = 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rapid decompression

<table>
<thead>
<tr>
<th>Time-at-depth</th>
<th>%CO₂</th>
<th>%O₂</th>
<th>%N₂</th>
<th>%Ar</th>
<th>%Ar:%N₂ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>275 fsw</td>
<td>6.3</td>
<td>2.1</td>
<td>33.2</td>
<td>58.1</td>
<td>1.8</td>
</tr>
<tr>
<td>60 min</td>
<td>(0.4)</td>
<td>(0.9)</td>
<td>(1.1)</td>
<td>(0.7)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>n = 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>275 fsw</td>
<td>8.6</td>
<td>2.1</td>
<td>32.2</td>
<td>56.7</td>
<td>1.7</td>
</tr>
<tr>
<td>30 min</td>
<td>(4.7)</td>
<td>(0.9)</td>
<td>(0.5)</td>
<td>(3.8)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>275 fsw</td>
<td>8.0</td>
<td>0.8</td>
<td>33.9</td>
<td>56.9</td>
<td>1.7</td>
</tr>
<tr>
<td>15 min</td>
<td>(2.1)</td>
<td>(0.8)</td>
<td>(0.9)</td>
<td>(1.5)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are means (SD).

Gas chromatographs were calibrated with gravimetric standards similar in composition to the gas samples being analyzed and certified to ±1% relative or better. Precision for 25-μl syringe injections was <5% (for repeat injections, 1 SD was less than 5% of the mean) although generally much better for most analysis; linearity of GC response with changing gas concentrations was excellent over the ranges of calibration and sample gases involved. Blank GC injections (100% He) made during each experiment determined the degree of air leakage during GC injections; in cases of excessive leakage, the problem was corrected usually by septum replacement. Combined error in reported measurements due to imprecision, nonlinearity, and residual gas left in the syringe after purging is estimated <10% relative. However, in most cases, combined error is believed to be substantially lower than this.

After determination of absolute percentages of gases in the bubble, the values were normalized so the total sum of inert gases, O₂, CO₂, and methane equaled 100%. This corrected for discrepancies in injection volumes due to a) blood in the injected sample resulting in an injection volume <25 μl and b) blood in the needle, which interfered with pressure equilibration between the atmosphere and syringe interior before injection and resulted in an injection volume >25 μl. The normalization generally changed absolute values up to ±15% relative.

Data analysis

For dives using multiple inert gas mixtures (i.e., N₂-He-O₂ and N₂-Ar-O₂), inert gas composition of bubbles was compared using ratios corrected for the composition of the dive mixture:
corrected bubble $\frac{N_2}{He} = \frac{\text{measured bubble } \%N_2/\%He}{\text{dive mixture } \%N_2/\%He}$

and

corrected bubble $\frac{Ar/N_2}{= \frac{\text{measured bubble } \%Ar/\%N_2}{\text{dive mixture } \%Ar/\%N_2}}$

The rationale was that a corrected ratio other than 1.0 would indicate that one of the inert gases contributed disproportionately to the bubbles relative to the composition of the dive mixture. Because all but one group of multiple inert gas dives used mixtures that contained approximately equal amounts of two inert gases, the correction in the ratio generally adjusted for only slight deviations from 50:50% in the dive mixture. The one exception, 60-min dives on 75% $N_2$:25% He, allowed testing the effect of a change in the dive mixture on the bubble ratio. The effect of dive time, decompression rate (i.e., rapid or slow), and the dive time-decompression rate interaction on the bubble ratio was examined by analysis of variance (ANOVA) using a least-squares fitting program with the following model:

$$\text{Ratio} = B_0 + B_1 \cdot (\text{dive time}) + B_2 \cdot (\text{decompression rate}) + B_3 \cdot (\text{dive time}) \cdot (\text{decompression rate})$$

where decompression rate was coded as 1 = slow and 2 = rapid and dive time was coded in minutes. Significance of parameters was tested by calculation of $F$ values based on sum of squares.

Cardiovascular/respiratory variables (i.e., heart rate, blood pressure, ventilatory rate) will not be reported here.

**RESULTS**

**Data selection**

Sampling and analysis protocol (detailed in Methods) produced two measurements of $O_2$ and inert gases and one measurement of CO$_2$. In most cases, the two $O_2$/inert gas measurements agreed within 5% relative, and the first set of values are reported. When first and second measurements disagreed by more than this, the reason(s) was usually obvious (e.g., blood clot, sample contaminated by air as indicated by high $O_2$, blood in the sample injected as indicated by GC plot). In these cases, the problem set of values was thrown out and the other set reported. In a few cases, disagreement between values could not be explained; here, the set of values with the lowest $O_2$ was reported, assuming the discrepancy was due to minor air contamination.

**Composition of bubbles**

Tables 1 and 2 indicate that the composition of intravascular bubbles was very different from the dive mixture. Bubble CO$_2$ ranged from 5 to 9%, and $O_2$ ranged from 1 to 4%. Less than 1% methane was usually detected (not given in tables), and the balance was inert gas. The relatively higher percent $O_2$ in bubbles after air dives is not believed to be real but thought to be due to co-elution of Ar (taken up by the
guinea pig from the dive air, which contained 0.9% Ar) with O₂ during GC analysis, thus erroneously elevating the O₂ measurement.

Animals dived on He-O₂ for 60 min showed only trace amounts (on average 2%) of N₂ in bubbles, of which probably half can be attributed to residual air left in the syringe after sampling (see Methods). This suggests that 60 min was sufficient time for nearly all N₂ initially in the animal at the surface to wash out and not influence bubble composition.

**Effect of dive time and decompression rate**

After N₂-He-O₂ dives, the bubble ratio of N₂ to He was up to 1.9 times greater than that of the breathing mixture. After N₂-Ar-O₂ dives, the bubble ratio of Ar to N₂ was up to 1.8 times greater than that of the breathing mixture (Tables 1 and 2). For N₂-He-O₂ dives, bubble composition was significantly (P < 0.01) affected by dive time and decompression rate as indicated by analysis of variance (ANOVA); the dive time-decompression rate interaction was not significant. As dive length increased, the amount of N₂, relative to He, in the bubble rose; this was true for both slow and rapid (Fig. 1) decompression. The amount of gas taken up was directly related to time at depth, suggesting that N₂ has a slower uptake rate than He and may take more than 30 min to equilibrate. Slow decompression resulted in bubbles with more N₂ relative to He compared to rapid decompression for dives of equal length (Fig. 1). Rapid decompression minimizes gas elimination, suggesting that N₂ also has a slower elimination rate than He.

![Graph showing bubble ratio of N₂ to He for slow and rapid decompressions](image)
Results from dives using a 3:1 N$_2$:He mixture demonstrated that the corrected N$_2$:He bubble ratio was independent of the actual dive mixture (i.e., N$_2$:H$_2$ ratio = 1.9 for 60-min dives with either 50% N$_2$:50% He or 75% N$_2$:25% He followed by 1 fsw/s decompression). Bubbles after N$_2$:Ar-O$_2$ dives do not show the effects of dive time and decompression rate as the Ar:N$_2$ ratio seemed constant for the dive profiles performed.

DISCUSSION

Our results support the rank order of a) increasing DCS risk (He < N$_2$ < Ar) via increased gas evolved and b) increasing rate of gas exchange (N$_2$ < He) observed previously during rat dives (12, 13); gas exchange rates of N$_2$ and Ar seem similar for the dive profiles used. However, the risk differences estimated in the rat studies were substantially less than would be expected if risk related directly to bubble composition as found in the present study: Helium was found to be approximately 10% less risky than N$_2$ for rats, whereas the N$_2$:He bubble ratio from 60-min/rapid decompression profiles was 1.6; Ar was found approximately 30% more risky than N$_2$ in rats even though the Ar:N$_2$ ratio was 1.8. Because a 60-min/rapid decompression profile minimizes the loss of gas during decompression from presumably a saturation exposure, these bubble ratios should reflect relative amounts of evolved gas per unit of pressure of each inert gas. However, this reasoning is invalid if saturation has not been achieved. N$_2$ washout seemed to be completed by 60 min, based on results from dives with He-O$_2$. If the rate of N$_2$ uptake is similar to that of washout, N$_2$ should be at equilibrium after a 60-min pressure exposure. Helium, with an apparently faster exchange rate than N$_2$, should also be equilibrated by 60 min. The situation for Ar is unknown. However, if Ar is far from equilibration, tissue may provide a significant sink for this gas after decompression, which would be expected to lower the Ar:N$_2$ bubble ratio.

Disagreement between relative potency and bubble composition may indicate that the occurrence of DCS as we measure it in rats cannot be related to intravascular bubble volume in this simple manner. Unfortunately, the relationship between risk estimates for rats and bubble composition for guinea pigs will be confounded by any significant animal differences relative to DCS (17, 18). Another problem is that we are analyzing only intravascular bubbles from the venous system and do not include the effect of extravascular bubbles. Although correlation between intravascular bubbles and symptoms of DCS, other than death in small animals, is poor (19, 20), we would expect intravascular gas to play an important role in the whole animal response to DCS, as we and others have previously discussed (20, 21). We believe that the gas being collected is generated in the lower part of the animal (i.e., primarily the hind limbs) and gradually moves into the venous system from which we sample. Few or no gas bubbles were seen in the blood during the initial period of sampling (i.e., first 3–5 ml of blood); thus, the bulk of the gas does not seem to come from that which dissolves in the blood at depth. This would be expected given the low solubility of inert gases in blood (7).

Our results agree with previous reports of a disparity between the composition of the dive mixture and that of intravascular bubbles resulting from DCS (in and cited by refs. 16 and 22). In general, these other studies report that intravascular gas from
both live and dead animals contains lower amounts of \( O_2 \) and higher amounts of \( CO_2 \) compared to the gas mixture breathed at depth. This is as expected because bubble gas presumably is equilibrated with metabolic levels of \( CO_2 \) and \( O_2 \) in blood and tissue (23). In addition, the composition of intravascular bubbles, particularly that of \( O_2 \) and \( CO_2 \), has been reported to change in live rabbits with postdecompression time (22) and would be expected to change with time after death. For these reasons, the importance of the \( O_2 \) and \( CO_2 \) measurements in the present study is uncertain. Our \( O_2 \) measurements (i.e., 1–4% \( O_2 \)) agree with previous analysis of bubbles from the inferior vena cava from dead rats after decompression (16). However, other studies cited by Ishiyama (22) reported \( O_2 \) levels in intravascular bubbles, mainly from dead animals, that ranged from a trace to over 10% of the bubble composition; in general, \( CO_2 \) levels were substantially higher than reported here.

Interestingly, the relative proportion of bubble \( N_2 \) to \( He \) after correction for the dive mixture was 2.3 in the only other trimix (i.e., \( N_2-He-O_2 \)) bubble study known (16). This ratio based on bubbles taken from dead rats within 5 min after decompression was similar to our value of 1.9 for a 1-h/slow decompression profiles. Although those animals were dived much deeper (21 atm abs) for 1 h, the total decompression time was similar to that allowed here. Before the start of our dives, the animal was equilibrated with 1 atm abs air and thus had dissolved \( N_2 \) in its tissues. However, this \( N_2 \) probably had little if any effect on the bubble data due to the combination of high dive pressures (8.5–11.6 atm abs) and dive lengths, which should allow significant amounts of gas to be taken up by the guinea pig even during the shortest dive (e.g., 15 min).

Bubble composition would be expected to be affected by differences among gases in tissue solubility or diffusivity or both. Using published solubility values for gases in some liquids, some very simple comparisons can be made. For example, coefficients for \( He \), \( N_2 \), and \( Ar \) solubility in water at 37°C [0.0099, 0.0145, and 0.0300 ml gas/ml fluid, respectively; (24)] are in relative proportion of 1.5 for \( N_2/He \) and 2.1 for \( Ar/N_2 \). Such coefficients from the same report for \( He \), \( N_2 \), and \( Ar \) solubility in oil at 37°C (0.0168, 0.0760, and 0.1600 ml gas/ml fluid, respectively) are in relative proportion of 4.5 for \( N_2/He \) and 2.1 for \( Ar/N_2 \). Thus, with the exception of the oil ratio for \( N_2/He \), these coefficient ratios agree closely with the bubble ratios observed. However, it is unknown whether these coefficients relate well to the amount of gas that actually dissolves in animal tissues under pressure.

Surprisingly, there was relatively little variability in bubble compositions for any given profile (i.e., note the small standard errors of the mean values in Tables 1 and 2), although the length of time between decompression and death (and thus, sampling) varied from several minutes to approximately 20 min. Additional examination of bubble data within each profile indicated no apparent difference between dives producing death soon after surfacing and those where the animal died considerably later (example, 10–15 min after surfacing). This was contrary to our expectation that a longer postdecompression time would have allowed greater washout time of the inert gases, thus affecting the inert gas makeup of the bubble.

This investigation does not specifically model a type of human DCS. Rather, we take a simplistic approach with these experiments in terms of gas loading and elimination and how these processes relate to DCS. In this way we avoid having to make assumptions regarding the etiology of DCS that is not well understood and is probably very complex and varied, depending on the tissues involved. The only
assumption we make is that an excess of gas in the animal after decompression produces a severe physiologic response. The similarity in the rat and guinea pig models (i.e., both are severe DCS presumably leading to cardio-respiratory collapse) provides rationale for our efforts to relate guinea pig bubble data to previous observations of gas differences in rat DCS. The significance of these findings for human diving is unknown. However, these data certainly suggest that the amount of gas that evolves in specific tissues prone to DCS (e.g., cartilage in joints; spinal cord) may be different for nitrogen compared to helium and that this may affect the risk of DCS in man.

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The experiments reported herein were conducted according to the principles set forth in the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animals Resources, National Research Council, DDH, Publ. No. (NIH) 85-23.

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