EXPERIMENTAL ATTEMPTS TO INFLUENCE THE BUBBLE THRESHOLD FROM SATURATION DIVES IN ANIMALS


Decompression sickness follows excessive pressure reduction (ΔP) from a prolonged stay at depth and is a result of bubble formation in vivo. Though venous bubbles often can be detected without any indication of decompression sickness during so-called “safe” decompression procedures, the detection of subsymptomatic bubbles remains the meaningful and only objective criterion indicating an in vivo condition of supersaturation. The threshold for bubble detection, the largest ΔP from a given saturation pressure that just produces detectable venous bubbles, has been shown to be free of symptoms indicating decompression sickness (1). More importantly, the threshold for bubble detection has been shown to be the same in rat, cat, and dog (2). This species-independent phenomenon is useful for obtaining the threshold data from an animal of experimental convenience and is applicable to another species, an obvious economical advantage. Whether this threshold can be experimentally altered remains to be tested and is the objective of this study.

In this study, we examined the effect of over-pressure spike prior to decompression and the effect of ambient temperatures on the bubble threshold. The rationales for these studies are based on a) current belief that pre-existing gas nuclei grow, upon inappropriate decompression, to form bubbles in the blood and tissues, and that crushing of these nuclei with brief over-pressure spike prior to decompression reduces the number of bubbles formed during the subsequent decompression (3); b) the process of inert gas elimination can be altered by changes in cardiopulmonary functions and blood flow distribution (4,5). These physiological alterations can be produced appropriately by changes in ambient temperatures, as well as by application of drugs, in the rat
Effect of changes in ambient temperatures on bubble threshold is examined in this study.

METHOD

Male Wistar rats were anesthetized with sodium pentobarbital (40 mg/kg) and surgically prepared by implanting a 3-mm diameter perivascular Doppler flow probe (Parks Electronics, Beaverton, OR) on the posterior vena cava caudad to the renal veins (1). This chronic preparation was chosen over an external probe to eliminate the necessity of anesthetizing the animal during compression and subsequent decompression. The wire leads from the probe were run subcutaneously to the top of the head between the two ears (Fig. 1). The flowmeter probe with frequencies of 8.0–10.0 MHz was tested in vitro prior to implantation for its bubble-detecting ability by introducing bubbles into water flowing through a polyethylene tubing. The ability to detect bubbles in vivo was again confirmed following surgical preparation by injecting small

![Fig. 1. Surgical preparation of a chronic preparation for detecting venous bubbles during decompression. The Doppler flow probe was implanted on the posterior vena cava caudad to the renal veins. The wire leads from the probe were run subcutaneously to the top of the head between the two ears.](image-url)
volumes of air through a 25-G needle into the femoral vein. Rats were allowed to recover for at least 24 h before experimentation.

Intravascular bubbles were detected by a Doppler flowmeter model 803 (Parks Electronics, Beaverton, OR) with the output signals fed to an audio amplifier, a cassette tape recorder, and a pen-writing oscillographic recorder. The bubbles were detected by the distinct Doppler-shifted chirping sounds, or from recorded traces (1,2).

**Determination of Decompression Threshold**

A step increase in ambient pressure was maintained for 2 h with compressed air at a pressure between 3 and 10 ATA ($P_1$). Chamber pressure was then reduced rapidly (8 ATA/min) to a predetermined lower pressure ($P_2$). If there was no indication of venous bubbles within 1 h, the decompression was considered bubble free. For each saturation pressure ($P_1$), an increasing pressure drop ($\Delta P$) to a lower pressure ($P_2$) was tried on different rats until the greatest $\Delta P$ that produced no bubbles was found. The greatest $\Delta P$ that produces no bubble at a given saturation pressure is called bubble threshold.

**Effect of Over-Pressure Spike on Bubble Threshold**

Awake, instrumented rats were exposed to compressed air at 5 ATA for 2 h, followed by rapid pressure reduction ($\Delta P$) of 3.0, 3.5, or 4.0 ATA (Group I). In two other groups, an additional over-pressure spike of 5 ATA with a duration of 5 min was superimposed either at the onset of saturation period (Group II) or at the end of the saturation period (Group III). Decompression was carried out exactly as that in Group I. The procedure along with experimental results are illustrated in Fig. 2. Each group consisted of 12 rats.

**Effect of Ambient Temperatures on Bubble Threshold**

Experiments were carried out with saturation pressure ranging from 3 to 10 ATA compressed air while rats were exposed to either 15, 24, or 35°C environments. Bubble threshold determination was made as described previously at each temperature with first pressure exposure at 24 h after surgery and the repeat exposure within 48 h after surgery.

**Effect of Ambient Temperature on Nitrogen Elimination and Oxygen Consumption**

We determined oxygen consumption and rate of nitrogen elimination to examine a possible relationship between these alterations and temperature-induced threshold changes. Oxygen consumption was determined with a servo-controlled oxygen volume meter (Med-Science Electronics, St. Louis, MO). Carbon dioxide was absorbed by soda lime lining the interior of the animal
chamber. Nitrogen elimination was measured by a whole-body washout method using pure oxygen (3).

RESULTS

Effect of Over-Pressure Spike Bubble Threshold

The number of rats that showed bubbles following decompression is shown in Fig. 3 and summarized in Table I. As the ΔP increased, the number of rats showing intravascular bubbles increased similarly in all three groups. For a given magnitude of pressure reduction, rats in the over-pressure groups not only showed no reduction in frequency of bubbles, but there tended to be a higher number of rats indicating bubbles. For example, at the threshold (ΔP = 3.0 ATA, in this instance), that is, the greatest ΔP that produces no bubbles, there was no indication of bubbles existing intravascularly in Group I, but there was one out of four rats in Groups II and III indicating bubbles (Table I).

Effect of Ambient Temperature on Bubble Threshold

First exposure. At the higher end of saturation pressures (P₁), the decompression threshold was reduced at both 15 and 35°C environments as compared to the 24°C condition (Fig. 3). No significant differences were found at lower saturation pressures. These data are summarized in Table II.
First exposure. Regression lines are the great pressure reduction that produces no detectable intravascular bubbles at 15, 24, and 35°C. The maximum possible pressure reduction is shown by $\Delta P = P_1 - 1$, where $P_1$ is the saturation pressure. See Table II for numerical data.

Second exposure. With repeat exposure, decompression threshold was lowered in the cold environment. However, the decompression threshold was no different between 24 and 35°C conditions (Fig. 4). These data are summarized in Table II.

First vs. second exposure. Comparison of the bubble threshold of the first and the repeat exposures at three ambient temperatures is shown in Fig. 5. Increased decompression thresholds were observed in repeat exposures at 24 and 35°C environments. No difference was found between the first and the repeat exposure at 15°C environments (Fig. 5).

Oxygen Consumption and Nitrogen Elimination

Oxygen consumption increased from 25.5 mL/min/kg at 24°C ambient temperature to 43.3 mL/min/kg at 15°C. Oxygen consumption decreased to
TABLE I

The Number of Rats in Which Venous Gas Bubbles Were Detected following Decompression

<table>
<thead>
<tr>
<th>P, ATA</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>0/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>3.5</td>
<td>2/4</td>
<td>3/4</td>
<td>3/4</td>
</tr>
<tr>
<td>4.0</td>
<td>3/4</td>
<td>4/4</td>
<td></td>
</tr>
</tbody>
</table>

Rats were exposed to compressed air at 5 ATA for 2 h, followed by rapid pressure reduction (ΔP) of 3, 3.5, or 4 ATA (Group I). In two other groups, an over-pressure of 5 ATA with a duration of 5 min was superimposed at the onset of the saturation period in one group (Group II), and in the other at the end of the saturation period (Group III).

20.6 at 35°C (Table III). Lowering ambient temperature from 24 to 15°C reduced nitrogen elimination by 37% (Table III). At the elevated temperature where oxygen consumption was reduced, no change in rate of nitrogen elimination was recorded (Table III).

DISCUSSION

Intravascular gas emboli are expected to occur during excessive rate of reduction in ambient pressure either from normobaric (aviation, aerospace) or from hyperbaric conditions (diving). Decompression-induced intravascular gas bubbles have indeed been demonstrated in man (8–10), swine (11,12), sheep (13,14), and in the rat (1,2). However, at the threshold of bubble detection the rat shows no symptom indicating decompression sickness. With pressure reduction exceeding the threshold, the animal exhibits behavioral indications of decompression sickness such as respiratory distress, irritability, limping and

TABLE II

Effect of Ambient Temperature on Decompression Threshold

<table>
<thead>
<tr>
<th>Exposures</th>
<th>Temp,°C</th>
<th>n</th>
<th>Regression equation</th>
<th>r</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>15</td>
<td>15</td>
<td>( P = 1.0596 + 0.4564 P_t )</td>
<td>0.9709</td>
<td>0.2691</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>19</td>
<td>( P = -0.4147 + 0.6930 P_t )</td>
<td>0.9704</td>
<td>0.4474</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>17</td>
<td>( P = 1.0983 + 0.4281 P_t )</td>
<td>0.8996</td>
<td>0.5216</td>
</tr>
<tr>
<td>Repeat</td>
<td>15</td>
<td>13</td>
<td>( P = -0.1729 + 0.6504 P_t )</td>
<td>0.9937</td>
<td>0.1573</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>18</td>
<td>( P = -0.1870 + 0.7266 P_t )</td>
<td>0.9735</td>
<td>0.3263</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>10</td>
<td>( P = 0.6216 + 0.5946 P_t )</td>
<td>0.9936</td>
<td>0.1501</td>
</tr>
</tbody>
</table>

\( n \): number of rats in each threshold determination. \( \text{SEE} \) is the standard error of estimate.
Fig. 4. Effect of ambient temperatures on bubble threshold for rats exposed to pressure for the second time within 24 h following the first exposure. The maximum possible pressure reduction is shown by $\Delta P = P_1 - 1$; $P_1$ is saturation pressure. See Table II for details.

inability to maintain posture, and the like. It is not unusual that death of the animal follows shortly after appearance of these symptoms. Thus, detection of low-grade bubbles is an important indicator which precedes symptoms of decompression sickness.

It should be noted that presence of detectable bubbles does not automatically mean that there will be symptomatic expression of decompression sickness. Eatock detected bubbles in 360 out of 585 dives, but only 28 of these resulted in bends (see Ref. 15). We have demonstrated the same phenomenon (earlier) by comparing the bubble threshold of rats with the results of Berghage et al. (16). They determined pressure-reduction limits from saturation at 6 to 60 ATA for rats based on behavioral observation. The bubble-defined threshold was below the 5% incidence level of decompression sickness based on behavioral end points. The pressure-reduction limits allowed by the bubble detection were much less than that defined by behavioral criteria (1). Whether decompression tables should be constructed to eliminate bubble detection or to
reduce symptoms of decompression sickness has not yet been agreed upon generally.

Results obtained in this study indicate that brief over-pressure is ineffective in altering bubble threshold in the protocol we used. It may be that the over-pressure used in this study was insufficient in magnitude. As compared to the study done by Vann et al. (17), their over-pressure ranged from 12 to 26 ATA. They reported that over-pressure spikes were somewhat effective in reducing incidence of decompression sickness. Their criterion for decompression sickness was death of the rat. In 1969, Evans and Walder (19) demonstrated bubble formation during decompression in transparent shrimp. Bubble formation was drastically reduced by treating the shrimp with brief over-pressure of 389 ATA prior to decompression. Their results imply the dissolu-

### TABLE III

Oxygen Consumption and Nitrogen Elimination in Rats

<table>
<thead>
<tr>
<th>Temp, °C</th>
<th>$\text{O}_2$ Consumption (mL/min/kg)</th>
<th>$\text{N}_2$ Elimination (mL/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>$43.3 \pm 1.6^*$</td>
<td>$8.73 \pm 0.67^*$</td>
</tr>
<tr>
<td>24</td>
<td>$25.5 \pm 1.0$</td>
<td>$13.94 \pm 0.86$</td>
</tr>
<tr>
<td>35</td>
<td>$20.6 \pm 0.9^*$</td>
<td>$13.22 \pm 0.68$</td>
</tr>
</tbody>
</table>

$X \pm \text{SE}$, 5 rats in each group weighing 350 g on the average. Nitrogen elimination was summed for the first 30 min following the onset of washout. *Indicates statistical significance at $P < 0.05$ compared to 24°C conditions.
tion of micronuclei in vivo under pressure. Yount and Strauss (18) have demonstrated in an in vitro system that increasing crushing pressures reduce the number of bubbles formed in subsequent decompression. These results showed promise but the magnitude of the over-pressure required becomes impractical for treating bend patients in common recompression facilities.

The effectiveness of altering cardiopulmonary functions in changing bubble threshold is also marginal. In any event, in comparison to the bubble threshold that was obtained in 24°C ambient temperature, the altered temperature reduced rather than increased the magnitude of pressure reduction. This is especially clear at the higher end of the saturation pressures (Figs. 3 and 4). Oxygen consumption was elevated by lowering ambient temperature and was depressed by increasing ambient temperature (Table III). But, the end result is the same, i.e., bubble threshold is reduced. Determination of rate of nitrogen elimination did not help explain the bubble threshold obtained in this study. Lowering of ambient temperature depressed the rate of nitrogen elimination. But, this group exhibited bubble thresholds no worse than those in higher ambient temperatures. The mechanism by which ambient temperature affects bubble threshold requires further study.

Repeat exposure to pressure appears effective in elevating bubble threshold. This means that greater pressure reduction is permitted at a given level of pressure saturation after initial exposure (Fig. 5). This result substantiates previous findings in the rat (1) and in man (20–23) that greater pressure reductions can be tolerated on repeat dives better than on first dives. Because the partial pressures of the ambient gases were the same on first and repeat dives and because the solubilities of gases do not change, gas transport and bubble formation must effect the difference. The mechanism of this effect is unclear at present. Hematological changes initiated by circulating bubbles have apparently no effect in this acclimatization process because the effect of an increased threshold seemed to be consistent regardless of the outcome of the initial decompression. Rats undergoing decompression with pressure reduction below bubble threshold (no bubble formed), as well as those demonstrating bubbles, became acclimatized following the first pressure exposure (1). The most likely explanation is an alteration of the number, conformation, or distribution of gas micronuclei shown to be necessary for bubble formation upon decompression. We have no explanation at the present as to why the brief over-pressure of 5 ATA superimposed on saturation pressure at 5 ATA was ineffective, but prolonged initial 5-ATA exposure was effective in elevating bubble threshold 24 h later.

In summary, brief over-pressure superimposed on saturation pressure of 5 ATA is found to be ineffective in altering the bubble threshold. Exposure of the rats in 15 and 35°C consistently depressed bubble threshold as compared to 24°C ambient temperature, especially at the higher range of saturation pressures. Explanation of these changes is not currently available.

Acknowledgment

This study (HP/R2) was supported in part by University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-00070 from NOAA Office of the Sea Grant, Department of Commerce, and a grant from the Hawaii Heart Association.
References