UNDERWATER PHYSIOLOGY VII

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Construction of a decompression table is based on the fact that animal tissue can tolerate a certain degree of supersaturation without forming bubbles. Formulation of an efficient decompression table depends on determination of pressure reduction ($\Delta P$) from a saturation pressure ($P_1$) to a lower pressure ($P_2$) and depends on determination of the duration required at $P_2$ before subsequent pressure reduction can be made. The duration required at each stage of decompression is a function of the rate of inert gas elimination and thus is species-dependent (1), and metabolic state-dependent (1,2). On the other hand, the maximum $\Delta P$ without forming bubbles is determined by the physics of bubble formation in a given liquid and thus may not be species- and metabolic state-dependent. The objective of this study was to determine and to compare the maximum $\Delta P$ allowable from a saturation dive without forming intravascular bubbles in rats, cats, and dogs.

The decompression schedules currently in use are mainly a result of empirical trials, and to a lesser extent rely on extrapolation from animal models. Animal models should be useful for testing new decompression tables derived theoretically, and for improving existing tables, if interspecies correlation can be made. The convenience of using laboratory animals is often offset by the difficulty in assessing decompression stress, which is less than debilitating or fatal. Evaluation of decompression stress by symptomatic or behavioral observation, or both, in animals is relatively imprecise. Because observed symptoms are results of a major decompression stress and are evaluated subjectively in most cases, it is therefore desirable to determine some presymptomatic parameters by means of an objective criterion. This paper is such an attempt, by monitoring the threshold of intravascular bubble formation. The threshold of
decompression-induced intravascular bubbles was detected by an ultrasonic Doppler flowmeter.

**MATERIALS AND METHODS**

*Rats* (Male Wistar) weighing 475 ± 25 g were anesthetized with pentobarbital sodium (40 mg/kg) and surgically prepared by implanting a 2- to 3-mm diameter perivascular Doppler probe (Parks Electronics, Beaverton, OR) on the posterior vena cava caudad to the renal veins (3). 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. The flowmeter probes with frequencies of 8.0–10.0 MHz were tested for bubble-detecting ability in vitro before implantation 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 volumes of air through a 25-G needle into the femoral vein. Rats were allowed to recover for at least 24 h before experimentation.

*Dogs* weighing between 18.0 and 22.0 kg were also surgically prepared in a way similar to the rat by use of 16- to 20-mm diameter flowmeter probes; however, the probe was located on the posterior vena cava between the heart and the diaphragm. Two weeks were allowed for recovery following surgery. The bubble-detecting ability of the implanted probes was also tested by injecting small amounts of air through a superficial vein in the hind leg.

*Cats* of either sex weighing between 2.3 and 3.2 kg were anesthetized with 50–60 mg/kg sodium pentobarbital. A 3-mm Doppler perivascular flow probe was implanted on the vena cava caudad to the renal veins and the connecting wires exteriorized. The cat was placed in the chamber on its side under anesthesia. The bubble-detecting ability of the probe was tested in a way similar to that of the dog.

Detection of intravascular bubbles was made by a Parks Electronics Doppler Flowmeter Model 803 with the output signal fed to an audio amplifier, a cassette tape recorder, and a pen-writing oscillographic recorder. The bubbles can be detected by the distinct Doppler-shifted chirping sounds, or from recorded traces.

Animals were subjected to a step increase in ambient pressure ($P_1$) under these conditions: unanesthetized rats in a 1-L chamber for 1 h, anesthetized cats in a 50-L chamber for 4 h, and dogs in a human hyperbaric chamber (Hahn and Clay, Houston, TX) for 6 h. Following these exposures, pressure reduction was carried out as rapidly as the system permitted to a predetermined lower pressure ($P_2$). If there was no indication of bubbles within 1 h, the decompression was considered bubble-free. For each saturation pressure ($P_1$), an increasing pressure difference ($\Delta P$) to a lower pressure ($P_2$) was tried on each exposure until the threshold for bubble detection was found. In the rat, repeat exposures were at least 24 h but no longer than 4 days after first
exposures. For the dog, the repeat compression-decompression exposures were made weekly. No repeat exposure was made on the cat. Compressed air was used in all experiments.

RESULTS

Rat A total of 54 decompression trials were made on 39 rats. The data shown on Fig. 1 are the greatest $\Delta P$ values not producing bubbles and the smallest $\Delta P$ values where bubbles were detected during the first pressure exposures. Each point represents one rat for one pressure exposure. Trial points with values greater than the lowest $\Delta P$ with bubbles (circles) and less than the highest $\Delta P$ without bubble (squares) have been omitted. The true threshold for Doppler-detected intravascular emboli can be considered to lie between the paired bubble and no-bubble lines. Upper dashed lines indicate incidence levels for decompression sickness based on behavioral observations of Berghage et al. (4). Data on the repeat pressure exposures for the rat are similarly summarized in Fig. 2. The paired lines without data points indicate the regression lines of the first exposure results as seen in Fig. 1. It is important to note that

![Graph](image-url)

Fig. 1. Doppler-determined decompression sickness thresholds based on the detection of venous gas emboli in the rat during the first pressure exposure. Circles represent the minimum pressure reduction from saturation that produces intravascular bubbles. Squares represent the maximum pressure reduction from saturation that produces no intravascular bubbles. The true decompression-induced bubble threshold can be considered to lie between the bubble and no-bubble regression lines. $P_i$ is the saturation pressure, $\Delta P$ the pressure reduction from $P_i$ to a predetermined lower pressure $P_f$. Upper dashed lines indicate incidence levels for decompression sickness based on behavioral observation by Berghage et al. (4).
no animals represented by the threshold boundary lines exhibited any overt
signs or behavioral changes indicating decompression sickness, even if intra-
vascular bubbles were detected.

The thresholds of intravascular bubble detection by the present method
can be represented by a linear equation of the form $P_1 = aP_2 + b$ where $P_1$
is saturation pressure and $P_2$ the reduced pressure. Regression equations de-
scribing the threshold lines (Table I) show a greater slope value for repeat ex-
posures, indicating that an increased pressure differential is tolerable without
forming bubbles upon repeat exposures.

Fig. 2. Doppler-determined decompression sickness thresholds based on the detection of venous gas em-
bolli in the rat during repeat exposures. The blank rectangular area represents the bubble threshold for
the first exposure experiments as shown in Fig. 1. For further explanation, see legend for Fig. 1.
Maximum No-Bubble Decompression

TABLE I
Regression Equations Describing Bubble Detection Thresholds

<table>
<thead>
<tr>
<th>Exposure</th>
<th>No Bubble</th>
<th>Bubbles</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>$P_1 = 1.71 P_2 + 1.25$</td>
<td>$P_1 = 1.57 P_2 + 2.21$</td>
</tr>
<tr>
<td></td>
<td>$r = 0.99$, $P &lt; 0.001$, $n = 8$</td>
<td>$r = 0.99$, $P &lt; 0.001$, $n = 8$</td>
</tr>
<tr>
<td>Repeat</td>
<td>$P_1 = 2.34 P_2 + 0.77$</td>
<td>$P_1 = 2.51 P_2 + 0.61$</td>
</tr>
<tr>
<td></td>
<td>$r = 0.97$, $P &lt; 0.001$, $n = 8$</td>
<td>$r = 0.99$, $P &lt; 0.001$, $n = 7$</td>
</tr>
</tbody>
</table>

The experimental points included in the calculation of the regression lines are the least pressure reduction that produced bubbles and the largest pressure reduction that produced no bubbles. See text for further explanation.

Dog. Sixteen compression-decompression experiments were performed in three surgically prepared dogs. Experimental protocols were similar to those for the rat. The first trial began with the average critical pressure for humans (5), in which the $\Delta P$ was much smaller than for the rat as presented above (Fig. 3, line $C$). It became apparent that intravascular bubbles do not form for this small $\Delta P$. We made subsequent trials using the first exposure bubble threshold line of the rat and detected no bubbles in all trials in dogs (Fig. 3, line $B$). However, use of the repeat rat pressure exposure line in two out of four exposures in the dogs resulted in intravascular bubbles, although no observable symptoms appeared (Fig. 3, line $A$).

Cat. The results of six trials on six anesthetized cats are presented in Fig. 3. There were no bubbles detected in all four trials using the first exposure schedule for the rat. In two cats decompressed by the repeat exposure schedule, intravascular bubbles were detected. Both cats died shortly (within 10 min) following detection of bubbles.

DISCUSSION

The results of this investigation demonstrate the feasibility of using an implanted Doppler probe in laboratory animals of varying sizes for detection of decompression-induced intravascular bubbles. The intravascular bubble thresholds indicate a mild, previously undetectable level of decompression sickness in animals. Thus, the present method offered a clear and non-subjective criterion for detection of the onset of decompression sickness which is still sub-symptomatic. At the threshold of intravascular bubble formation induced by decompression, the rat and the dog showed no indication of decompression sickness. This procedure is clearly more sensitive and advantageous than the behavioral determination of decompression sickness.

The ultrasonic Doppler flowmeter has been used to detect intravascular gas emboli induced by decompression in both animals and man. Larger animals, such as swine (6-7), and sheep (8) have had Doppler probes implanted directly on the vessels; it has been necessary to use external probes on small
Fig. 3. Doppler-determined decompression sickness threshold based on the detection of venous gas emboli in the unanesthetized dog at weekly exposures, and in the anesthetized cat. Lines A and B are the bubble threshold regression lines for the rat at the repeat exposure and at the first exposure, respectively (Figs. 1 and 2 and Table I). Line C is the averaged critical reduction pressure for humans according to Yount (5). Solid circles represent no bubbles (●), and open circles indicate detection of intravascular bubbles (○) during decompression in the dog. Solid triangles represent no bubbles (▲), and open triangles indicate detection of intravascular bubbles (△) during decompression in the cat.

animals (10-11), a technique that requires anesthesia or restraint. The present method of Doppler bubble detection in small laboratory animals, as described in this paper, is an improvement over past methods in that it allows a sensitive determination of decompression sickness thresholds based on a well-defined, objective endpoint; it can be used on awake unrestrained animals; and it does not result in debilitation or death, and thereby allows repeat studies on the same animal. Repetitive measures of decompression sickness in the same animal provide direct evidence for many past observations and speculations that greater pressure reductions can be tolerated on repeat dives than on first dives (12-15). The most likely explanation may be that pressurization alters the number, conformation, or distribution of gas micronuclei shown to be necessary for bubble formation upon decompression (16). Two out of four trials on dogs pressurized 1 week apart showed intravascular bubbles by using the repeat schedule for the rat. It may be that the regeneration of micronuclei requires less than 1 week.

The results of the present study indicate that the maximum ΔP without forming intravascular bubbles is the same in the dog, the cat, and the rat, which represents a significant finding. Dogs and rats differ in body weight by
Maximum No-Bubble Decompression

a factor of about 40, yet according to our criterion the maximum $\Delta P$ was the same in these two species. However, according to behavioral criteria, species differences in maximum allowable pressure from saturation exist and, in general, a larger animal tolerates a smaller magnitude of pressure reduction from saturation (1). Methodological differences in determining decompression sickness may be of fundamental importance and should be resolved by further experimentation.

When the pressure reduction from saturation is less than maximum, there will be infinite decompression pressure-time combinations. Consequently, comparisons of decompression tables are extremely difficult. The present study suggests that decompression from saturation be made with a maximally-allowable pressure difference, then it becomes a matter of determining the duration to reach equilibrium at the lowered ambient pressure. This duration is, of course, a function of inert gas elimination, and is species-dependent as well as metabolic state-dependent. Extrapolation of experimental data between species becomes less burdensome in this single-variable approach as compared to the multiple-variables scheme currently in use.

In summary, the results of this investigation demonstrate the feasibility of using an implanted Doppler probe in laboratory animals of varying sizes for detection of decompression-induced intravascular bubbles. These intravascular bubbles indicate a mild, previously undetectable level of decompression sickness in the rat and in the dog. The findings showed that the maximum no-bubble pressure reduction from a saturation dive appears not to be a species-dependent phenomenon. We anticipate that this finding will facilitate the extrapolation of decompression schedules among species, since only the consideration of a species-specific parameter is required, namely, the rate of inert gas elimination.

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References