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Gas bubbles in rats after heliox saturation and different decompression steps and rates

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Skogland, Steffen, Kåre Segadal, Harald Sundland, and Arvid Hope. Gas bubbles in rats after heliox saturation and different decompression steps and rates. *J Appl Physiol* 92: 2633–2639, 2002. First published March 1, 2002; 10.1152/japplphysiol.00795.2001.—Effects of pressure reduction, decompression rate, and repeated exposure on venous gas bubble formation were determined in five groups (GI, GII, GIII, GIV, and GV) of conscious and freely moving rats in a heliox atmosphere. Bubbles were recorded with a Doppler ultrasound probe implanted around the inferior caval vein. Rats were held for 16 h at 0.4 MPa (GI), 0.5 MPa (GII and GIII), 1.7 MPa (GIVa), or 1.9 MPa (GIV and GV), followed by decompression to 0.1 MPa in GI to GIII and to 1.1 MPa in GIV and GV. A greater decompression step, but at the same rate (GII vs. GI and GIVb vs. GIVa), resulted in significantly more bubbles ($P < 0.01$). A twofold decompression step resulted in equal amount of bubbles when decompressing to 1.1 MPa compared with 0.1 MPa. The faster decompression in GII and GVa (10.0 kPa/s) resulted in significantly more bubbles ($P < 0.01$) compared with GIII and GVb (2.2 kPa/s). No significant difference was observed in cumulative bubble score when comparing first and second exposure. With the present animal model, different decompression regimes may be evaluated.

ultrasound; Doppler ultrasound; hyperbaric exposure; helium

DECOMPRESSION MAY INDUCE gas bubble formation in tissue and blood (10, 28) and may lead to decompression illness (DCI). A relationship between DCI and long-term health effects of diving was discussed during a consensus conference in 1993 (13). "Silent bubbles," an expression first defined and used by Behnke (1), do not result in DCI symptoms but have been found to contribute to lung function decrements (9, 25, 27).

In the present rat study, we utilized a method for determining venous gas bubbles produced by decompression by using a Doppler technique described by Watt and Lin (30). Compared with many previous studies in rats where DCI symptoms (or death) were the only measure of decompression outcome in a large number of animals (4, 16, 17), the present method makes it possible to do repeated dives and decompressions in the same freely moving animal. In addition, data on silent bubbles are more interesting from our point of view because the main objective of our animal and human studies is to improve operational dive profiles and procedures to avoid long-term health injuries.

In saturation diving, the breathing gas contains helium instead of nitrogen to reduce the breathing resistance (14) and to avoid the narcotic effect of high nitrogen partial pressures (2). A faster tissue elimination of helium than nitrogen during decompression is also an important factor (28). Divers stay in this hyperbaric heliox atmosphere for periods up to 3–4 wk. A diving bell is connected to the living chamber onboard the diving vessel and brings divers to the work site. The bell cannot always be lowered to the exact depth at which the work in water has to be done. Thus divers have to move usually down, or occasionally up, in the water to get to their work site. Sometimes the pressure change is done by adjusting the gas pressure in the bell. These pressure changes have been named "excursions," and saturation-excursion tables have been developed (22, 23). However, it has been shown that excursions from heliox saturation may result in bubble formation (6).

Norwegian diving procedures permit only minor downward and upward excursions. Operationally, there is a need for wider limits. In this study, we used the Doppler ultrasound method in decompression experiments related to excursions during heliox saturation diving. We compared different decompression steps and rates to and from different saturation pressures, with respect to bubble production. Because it has been claimed that adaptation caused by repeated heliox diving and caisson work may decrease bubble formation (28, 29) and thereby reduce the risk for DCI, we also wanted to determine whether a second exposure would result in fewer bubbles than the first dive.

METHODS

Intravenous gas bubbles were detected in five groups of conscious, freely moving rats by a Doppler ultrasound method. The first application of this method for detecting circulating gas bubbles generated by decompression appears to have been by Spencer and Campbell (24) and Gillis et al. (11). The technique has been used for bubble detection in animals (7, 11, 24, 30) and humans (6, 10, 26).

The Norwegian Experimental Animal Board approved the experimental procedures described in the present study.

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Animal preparations and ultrasound equipment. Male Wistar rats, weighing 250–350 g, were kept in single cages under 12:12-h light-dark cycles. The 35 rats were anesthetized with Hypnorm (Janssen Pharmaceutica, Beerse, Belgia) and Dormicum (F. Hoffmann-La Roche, Basel, Sveits) in a 1:1:2 solution with sterile water. Anesthesia was given subcutaneously in a dose of 0.25 ml/100 g. Rats were surgically prepared by implanting a 20-MHz perivascular ultrasound, C-formed, cuff probe (model DBF120A-CP-4.0, Crystal Biotech, Hopkinton, MA) made of soft Silastic. Implantation was made through a flank incision, and the probe was placed around the inferior caval vein, below the renal veins. The probe was prepared before the operation by connecting the probe wires to a Lemo chassis contact (ERA 00250 CTL, Lemo, Ecublens, Switzerland), which was mounted in a silicone plate. The silicone plate (~1 cm^2) was implanted subcutaneously in the neck region.

Rats were given 0.2 ml Temgesic (buprenorphinum, 0.3 mg/ml) postoperation for analgesia. During the experimental period, rats had free access to water and standard rat food.

A Doppler flowmeter (EME Pioneer TC 2020, Eden Medizinische Elektronik, Überlingen, Germany), designed for extravascular Doppler registration of blood flow velocity, was used in the 20-MHz pulse-wave mode. The distance between the probe and the ultrasound unit had to be as short as possible, and therefore the preamplifier was placed inside the chamber with a 40-cm-long cable to the probe. Rats were placed in a 40-cm-high Plexiglas cylinder [diameter (D) = 25 cm] 24 h after surgery and instrumentation. A flexible cable, with a quick release coupling to the implanted Lemo contact, connected rats to a swivel (Lehigh Valley Electronics) placed at the top of the cage. The Doppler cable going to the preamplifier was connected to the outlet end of the swivel. The other end of this cable was connected to the equipment on the outside via a penetrator through the chamber wall. Doppler signals were recorded on a digital audio tape recorder (Sony DTC-55 ES) for data saving and postexperimental bubble scoring. A fast Fourier transform analysis of the Doppler signal (Fig. 1) and event detection are both optional with the EME Pioneer system. Event detection is triggered by a sudden elevation of spectrum intensity. The triggering threshold is optional from 1 to 64 dB, and the system also allows automatic saving of both spectra and sound track.

Pressure chamber. Internal volume of the pressure chamber is 130 liters (D = 50 cm). Maximum working pressure is 15 MPa. For inspection and video supervision, there are two windows in the chamber wall, one at the top (D = 50 mm) and one at the end (D = 120 mm). Penetrators for gas inlet/outlet and electrical signals/power supply are run through the chamber's end wall.

A pressure transducer (range: 0–1 MPa; resolution: 5 kPa; Tronic line: 891.13.500; WIKA Alexander Wiegand, Klingenberg, Germany) was connected to the chamber. Internal temperatures were continuously recorded (33AR LH35 CZ, National Semiconductor, Santa Clara, CA). Heating was provided by hot water from a thermostatically controlled reservoir that was pumped (Hetofrig CB7 and 03PF623 Heto Laboratory Equipment) through a copper tube curled around the chamber. An insulation layer around the chamber prevented excessive loss of heat. In this way the temperature inside the chamber was kept constant at a predetermined level. P_{O_2} was measured by an electrode (C3, Middlesbrough, Cleveland, UK) placed inside the chamber. Relative humidity in the chamber atmosphere was recorded with a humidity sensor (Vaisala HM20, Helsinki, Finland).

All sensors were connected to a portable computer via an interface box developed at Norwegian Underwater Intervention. Ambient pressure, P_{O_2}, temperature, and humidity could be read from a monitor. A low-voltage fan (Sprofona SJ-80Y12A, Seicoelectronics) mixed the chamber atmosphere to maintain an even gas and temperature distribution. CO_2 content was kept low by use of the CO_2 absorbent Sodasorb (Molecular Products United Drug, Essex, UK) placed in two beakers on the chamber floor. Similarly, the odor absorbent Sofnofil (Molecular Products United Drug) removed odor.

Experimental protocol. The Doppler probe was surgically implanted in two rats at a time at *day 1* of the experimental period. Before pressurizing, rats were placed in the Plexiglas cylinder. The chamber was closed, and an 80% helium-20% oxygen mixture was carefully injected (~20 min) via a gas pipe on the top of the chamber interior. Chamber gas was simultaneously drained via an exhaust pipe at the bottom of the chamber. By this procedure, nitrogen was effectively removed to <0.1% without significantly increasing chamber pressure. Rats were thereafter pressurized to the predetermined depth by the 80–20% heliox mixture. P_{O_2} was kept between 56 and 45 kPa during the experimental period. Time at maximal depth was 16 h.

Animals were divided into five experimental groups, as detailed in Table 1. After 16 h at increased pressure, a linear decompression was performed. Different decompression steps and rates were evaluated with respect to intravascular bubble occurrence. Most animals in *groups I* to *III* were exposed and decompressed identically a second time with 48 h between the two exposures. The time period from surgery to decompression after the second exposure did not exceed 5 days.

Doppler recordings started 10 min before decompression and continued for 2 h after decompression. Doppler signals

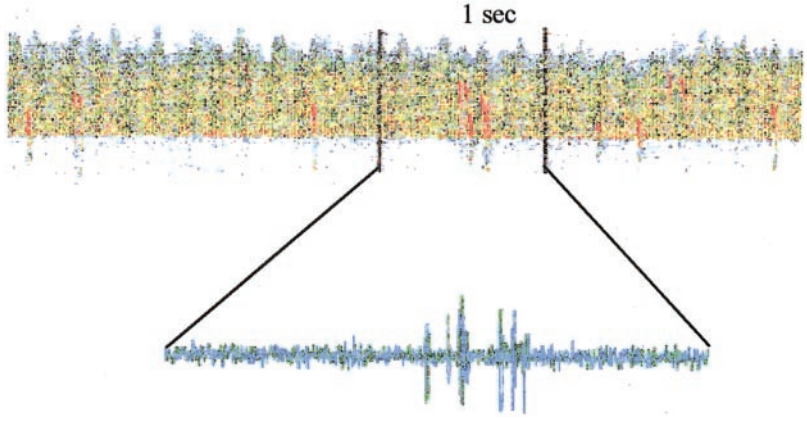

Fig. 1. A fast Fourier transform analysis of a Doppler signal in the caval vein during a typical experiment with gas bubbles appearing as red spots (*top*). A part of the signal (1 s; *top*) is expanded and shown as the analogous signal with 6 auditive and separable bubbles appearing in <250 ms (*bottom*).

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Table 1. *Number of animals and experiments, saturation pressures, decompression procedures, and maximum bubble grades in different groups of rats*

Group No.	No. of Animals	No. of Experiments	Saturation Pressure, MPa	Decompression Time, s	Decompression Step, kPa	Decompression Rate, kPa/s	Bubble Grade
I	5	10	0.4	30	300	10.0	1.4
II	6	10	0.5	40	400	10.0	4.3*
III	7	12	0.5	180	400	2.2	2.2†
IVa		9	1.7	60	600	10.0	0.6
IVb	9	9	1.9	80	800	10.0	2.3‡
Va		8	1.9	80	800	10.0	2.9
Vb	8	8	1.9	360	800	2.2	1.0§

*Significant difference vs. *group I* ($P < 0.01$). †Significant difference vs. *group II* ($P < 0.01$). ‡Significant difference vs. *groups II* and *IVa* ($P < 0.05$). §Significant difference vs. *group Va* ($P < 0.01$).

were visually and audibly observed during the first 30 min after decompression and periodically thereafter.

Bubble scoring. Doppler signals were played back from the digital audio tape after the experiment, and the event detection option was used for detection of bubble signals. The triggering threshold had to be adjusted individually in each experiment depending on the flow signal intensity. Each sudden increase in signal intensity was automatically saved and numbered as an event. Event detection was also visually monitored to minimize artifact detections. Counted events were also audible to the observer, and the number of detections per 30 s was determined.

In addition to bubble number and because most human studies use a bubble-grading system for classifying bubble amount (19), a bubble grade scale (Table 2) was constructed to evaluate differences in decompression outcome between groups.

When bubbling reached a level at which blood flow in the vena caval vein was negatively affected (determined as *grade 5*), animals were recompressed by 180 kPa. In these cases, bubbles disappeared almost immediately. The number of *grade 5* animals was also used as a measure for evaluating differences between groups.

Statistical analysis. Statistical evaluations between groups were based on Wilcoxon's rank-sum test and Mann-Whitney test. Wilcoxon's rank-sum test was performed in analyses where the result from two different procedures in one individual was statistically evaluated. Mann-Whitney test was performed when different procedures in different individuals were evaluated. For all statistical analysis, $P < 0.05$ was considered significant.

RESULTS

The Doppler signals in Fig. 1 are from a typical experiment, with gas bubbles appearing as red spots in the spectrum. A section shows the intensity of the reflected signal with high intensity and easily audible bubble signals. In this example, six separable bubbles were detected in 250 ms (Fig. 1, *bottom*).

Table 2. *Grading of bubbles as a measure of decompression outcome*

Grade No.	Bubble Amount; Decompression Stress
0	No bubbles
1	<1 Bubble/s; sporadic occurrence
2	<1 Bubble/s; periodically continuous occurrence
3	1–5 Bubbles/s; continuous occurrence
4	>5 Bubbles/s; continuous occurrence
5	Recompression necessary; blood flow in the caval vein was markedly decreased due to excessive amounts of bubbles

No animals showed manifest signs of DCI at any time. Bubble amount, expressed as bubble grade, at different decompression steps and rates are presented in Table 1. After saturation at 0.4 and 0.5 MPa (*group I* and *group II*), decompression at 10 kPa/s to 0.1 MPa resulted in significantly more bubbles in *group II* ($P < 0.01$, Table 1). A similar difference in response was obtained after decompression at the same rate from 1.7 and 1.9 MPa to 1.1 MPa (*group IVa* vs. *group IVb*; $P < 0.05$). Significant differences in bubble grade were also observed when comparing decompression rates of 10.0 and 2.2 kPa/s (*group II* vs. *group III*, and *group Va* vs. *group Vb*; $P < 0.01$). This rate dependency was not influenced by decompression step and saturation pressure (*groups II* and *III* vs. *groups Va* and *Vb*; Table 1). Because of deterioration of blood flow in the vena caval vein, 8 of 10 animals had to be recompressed (*grade 5*) in *group II* (10 kPa/s) compared with only 2 of 12 in *group III* (2.2 kPa/s). This difference in number of *grade 5* cases was also highly significant ($P < 0.01$), as determined by analysis of variance and χ^2 approximation (Mann-Whitney). *Grade 5* bubbles were only observed in *groups II* and *III*.

Bubble amount, expressed as event detections in each 30-s period, is shown in Figs. 2 and 3. Differences in decompression steps (*group IVa* vs. *group IVb*; Fig. 2,) and rates (*group Va* vs. *group Vb*; Fig. 3) were both significant ($P < 0.01$).

The effect of repeated exposure on bubble formation was tested in five and four animals in *groups I* and *III*, respectively. No difference in cumulative bubble detection between *exposures 1* and *2* was observed.

Initial bubble appearance in all bubble-positive experiments is presented in Fig. 4. During decompression to 0.1 MPa (*groups I* to *III*), bubble onset occurred within 1 min postdecompression in 50% of the animals, and in all experiments bubbles were detected within 6 min. Comparing decompression to 1.1 and 0.1 MPa, bubble onset was significantly delayed at the higher pressure (Fig. 4).

DISCUSSION

As mentioned in the introduction, working depth is usually different from saturation depth. A diver's am-

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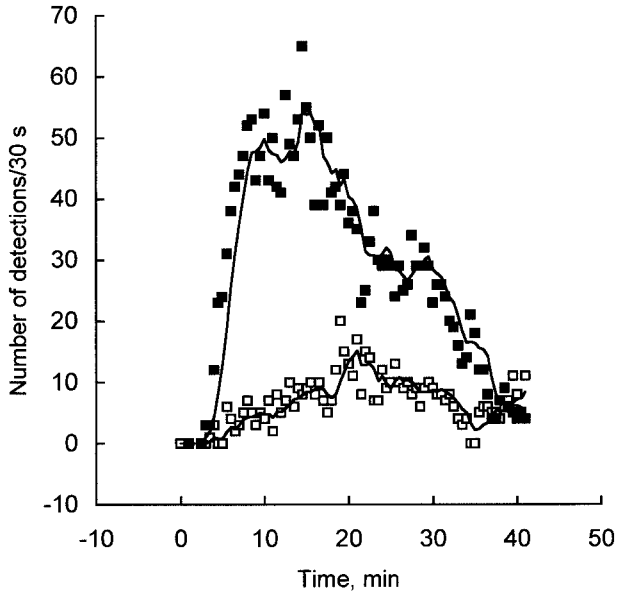

Fig. 2. Average bubble counts, determined as detections per 30 s, after decompression steps of 600 kPa (*group IVa*; □) and 800 kPa (*group IVb*; ■) at 10 kPa/s.

bient pressure must therefore be changed by performing an excursion either by moving vertically in the water or by changing the pressure in the bell. Whereas the US Navy tables allow an upward excursion of ~250 kPa (25 m of seawater) from a saturation depth of 1.2 MPa (28), the equivalent limit in the Norwegian Petroleum Directorate (NPD) tables is only 110 kPa (20). From saturation at 0.4 MPa, respective excursion limits are 180 and 60 kPa.

As reviewed by Berghage et al. (3), different mammalian species have been used as models in decompression studies. Because gas bubbles produced by decompression have been reported in rats (8, 12, 18, 30) and

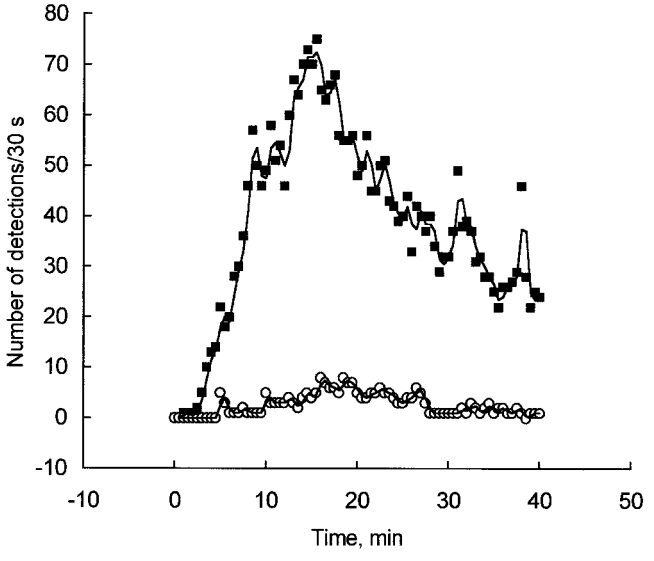

Fig. 3. Average bubble counts, determined as detections per 30 s, after decompression steps of 800 kPa at 10 kPa/s (*group Va*; ■) and 2.2 kPa/s (*group Vb*; ○).

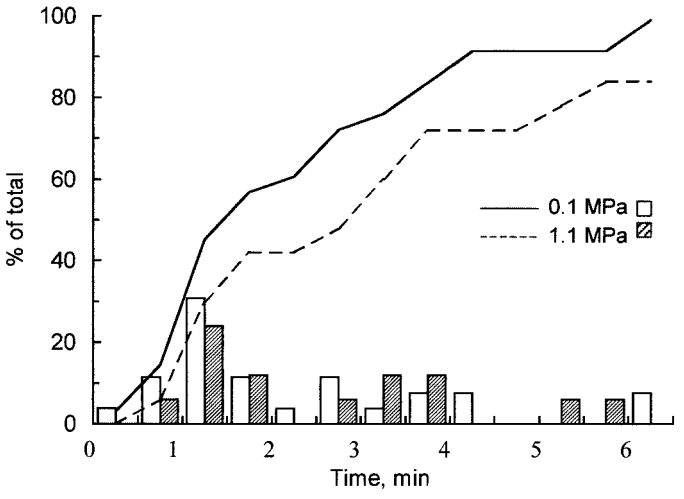

Fig. 4. Time for onset of bubbles after decompression to 0.1 MPa (*groups I* to *III*) and to 1.1 MPa (*groups IV* and *V*). Lines show cumulative values in percent of total.

our laboratory had relevant experimental experience with this animal (5, 12, 21), we wanted to use rats as a model in our decompression studies. However, smaller animals seem to tolerate greater pressure reductions (3, 10, 30), and the reductions applied and evaluated in our rat study were considerably greater than those relevant for humans.

In the present study, we decided to compare excursions, or decompression steps, of 300 and 400 kPa to 0.1 MPa, and 600 and 800 kPa to 1.1 MPa, at two different rates. This is somewhat higher than the limit observed by Watt and Lin (30) during air experiments in rats. They used a method analogous to ours but determined the threshold of decompression-induced intravascular bubbles as "bubbles" or "no bubbles." They concluded that, after 1 h at increased pressure followed by a "rapid" decompression and a 1-h monitoring period, the smallest decompression step necessary to produce bubbles in the rat was ~200 kPa (30). However, because of the faster tissue washout rate and less solubility in lipid tissue of helium compared with nitrogen (28), a greater decompression step from saturation in heliox to produce the same amount of bubbles as in air may be expected.

We observed a significantly higher bubble grade in the 300-kPa excursion to surface (*group I*) compared with the deep 600-kPa excursion to 1.1 MPa (*group IVa*) during the first 15 min after the excursion. Thereafter, no difference was found. Bubble incidence was 80 and 33% in the 300- and 600-kPa excursions, respectively. In the deep (800 kPa) and shallow (400 kPa) excursion, bubble incidence was not statistically different (94 and 100%, respectively), but bubble grade was significantly higher during the 400-kPa excursion (Table 1). Thus, when comparing decompression outcome and bubble production during excursion decompression to 0.1 and 1.1 MPa, it looks as if the pressure drop has to be more than doubled at the higher saturation pressure to produce equal amounts of detectable bubbles as during decompression to normal atmospheric pressure.

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Theoretically, supersaturation after the 800 kPa excursion from 1.9 to 1.1 MPa represents twice the number of gas molecules compared with the 400-kPa excursion from 0.5 to 0.1 MPa (surface pressure), but the volume of a gas bubble will be only one-fifth.

Bubble onset was significantly delayed at the higher pressure (Fig. 4). If a bubble has to grow to a certain size before it unfastens and becomes detectable in the caval vein, this volume difference could explain the observed delay in bubble occurrence. Furthermore, Berghage et al. (3) concluded that the time to onset of DCI symptoms in 50% of the rats was 6 min postdecompression. In our shallow experiments with decompression to 0.1 MPa, initial bubble appearance in 50% of the animals occurred within 1 min postdecompression. Within 6 min, all experiments had resulted in bubbles (Fig. 4). Thus, from our data on silent bubbles and the experiments resulting in DCI (3), it seems to be a delay of ~5 min from bubble onset until DCI symptoms appear in rats.

Rate of decompression is another relevant factor with respect to bubble production. In the 10 kPa/s experiments in *group II* (400-kPa excursion), recompression was necessary in 8 of 10 exposures. All animals in this group were recompressed in at least one of the two exposures. When the rate was reduced to 2.2 kPa/s (*group III*), recompression was necessary in only 2 of 12 experiments. This difference in recompression frequency (equal to bubble *grade 5*) was highly significant ($P < 0.01$). At higher saturation pressures, a similar and statistically different ($P < 0.01$) rate dependency was observed (*group Va* vs. *group Vb*; Table 1 and Fig. 3).

As reviewed by Vann and Thalmann (28), it has been claimed that acclimatization (or adaptation) to decompression outcome may occur in caisson work and heliox diving. Walder (29) observed that the incidence of DCI fell from 12 to 3% during the first 10 days of caisson work during construction of the Tyne tunnel. As observed by Watt and Lin (30), no significant difference in bubble quantities was found when comparing *exposures 1* and *2* in our *groups I* and *III*. The discrepancy between Walder's data (29) on DCI and the data on silent bubbles could be explained by a reduced response to bubbles with time in the caisson workers. Alternatively, less bubbles are produced at the end of a 10-day period with daily diving, and only one repeated exposure is not enough to detect such a decrease in bubble production. Species differences may also be involved.

In earlier decompression studies in the rat, classification of decompression outcome has mainly been qualitative, i.e., symptoms or no symptoms (4, 15–17), whereas in this study a method for quantitative analysis (bubbles/min) was implemented. Furthermore, by doing repeated exposures in the same animal, the total number of animals can be markedly reduced. The Doppler method makes it possible to detect differences in decompression outcome and bubble load without causing paralysis and death of the animals. Although this method does not determine all bubbles produced in the body, detection by the caval vein probe measures bubbles from the hind part of the body, representing a mixture of tissues like skin, subcutaneous fat, muscle, bones, and joints.

The time period from surgery to the last exposure did not exceed 5 days in the present experiments. By exceeding this time period, we have sometimes experienced that the Doppler signal is lost because of accumulation of adipose and connective tissue in the space between the crystal and vascular wall. Partial degeneration of the vena cava and formation of collateral venous vessels were also observed. Moreover, there was an increasing tendency of local inflammation in the silicone implant area as a function of time after surgery.

The high-frequency (20-MHz) pulse-wave Doppler used in the present study has the advantage of higher signal resolution than lower frequencies. On the other hand, high frequencies also have the disadvantage of a lower penetration. However, this factor is of minor importance in the present model because a proper flow signal and spectrum quality is a prerequisite for carrying out the experiment and, therefore, ascertains acceptable conditions. In experiments with high bubble intensity, each single-bubble detection could cover more than one bubble, and the scores should be considered as minimum estimates of the real bubble number. For the other experiments, the detected numbers are considered to be correct. The recordings in Fig. 1 illustrate the unmistakable bubble signals, both in the

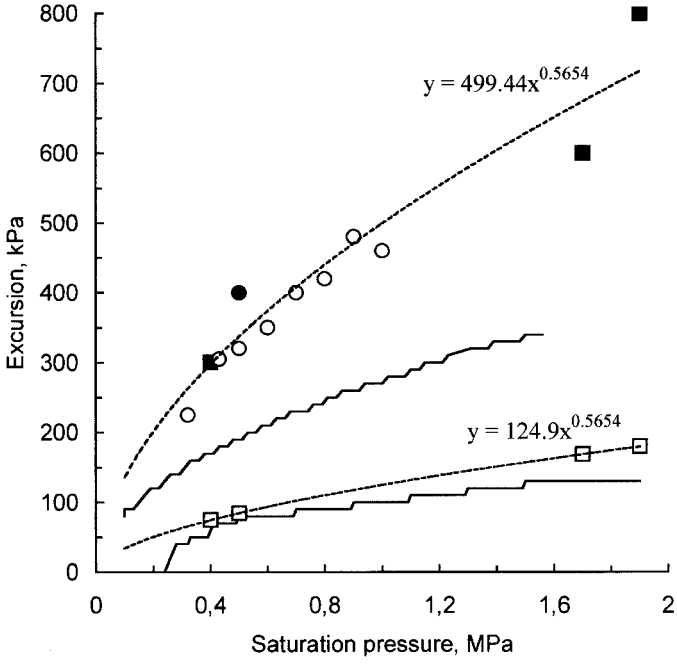

Fig. 5. Calculated trend curve for excursion distances with low (and acceptable) bubble grades in *groups I*, *IVa*, and *IVb* (*top* dashed line, ■). *Bottom* dashed line (□) shows the same line but corrected for the rat-to-human ratio of 4:1 (see text). Excursion distances are compared with human excursion tables from the Norwegian Petroleum Directorate (*bottom* solid line) and US Navy (*top* solid line). Also shown are data from *group II* (●) and rat data from Watt and Lin (○; Ref. 30).

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flow spectrum and as analog audible signals. The latter are quite similar to those observed by Butler et al. (7) after decompression in the anaesthetized dog and clearly demonstrate that small animals can also be used in the detection of circulatory bubbles.

There seems to exist a relationship between decompression step, animal size, and DCI occurrence (3). The smaller the animal, the greater the step needed for producing DCI symptoms. A similar relationship is seen in intravascular gas bubble formation (10, 30). Differences in physiological parameters like peripheral blood flow, heart rate, body weight, and respiratory exchange could explain this (3). Thus literature data indicate that, for bubbles to form during rapid decompression, an ~4:1 ratio in pressure reduction can be found when rats and humans are compared (3, 10, 30). Therefore, both absolute pressure reduction and decompression rate were significantly increased in the present rat study compared with those normally producing bubbles in humans.

A comparison of silent bubbles in groups I and IV, corrected for the 4:1 ratio, with the US Navy and NPD tables is illustrated in Fig. 5. A highly significant correlation is obtained between the corrected rat curve and the NPD table ($r = 0.94$). The corrected rat curve indicates that the NPD excursion limits from a saturation pressure of 1.2 MPa could be increased from today's 110 kPa to ~135 kPa.

The present results on bubble formation in heliox decompression show that small animals like the rat can also give valuable information in regard to decompression outcome and may be useful in the effort to improve decompression procedures in human diving. Our data indicate that the NPD excursion limits could be somewhat extended and that the US Navy limits are too wide. The established animal model and these initial findings in the conscious rat make a basis for future studies aimed at getting data on bubble formation during different decompression regimes.

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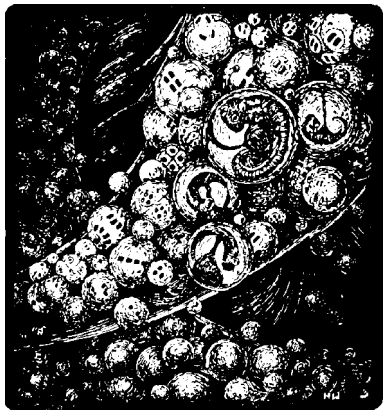

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