Bubble reduction after decompression in the prawn *Palaemon elegans* by pretreatment with hyperbaric oxygen.

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Arieli Y, Katsenelson K, Arieli R. Bubble reduction after decompression in the prawn Palaemon elegans by pretreatment with hyperbaric oxygen. Undersea Hyperb Med; 34(5):369-378. On the theory that bubbles originate from preexisting micronuclei, we previously demonstrated that pretreatment with hyperbaric O_2 (HBO_2) reduced the number of bubbles in the prawn decompressed from 203 kPa. In the present study, we examined the effect of two HBO_2 pretreatment pressures (405 and 709 kPa) on prawns decompressed from a range of pressures between 203-810 kPa. Prawns from the experimental groups were pretreated with O_2 at 405 or 709 kPa for 5 min (*series A* and *series B*, respectively). Prawns from the control groups were exposed only to air. Following pretreatment, prawns were exposed to air at the desired pressure until saturated with nitrogen, then subjected to rapid decompression and examined under a light microscope. *Series A*: HBO_2 pretreatment at 405 kPa for 5 min significantly reduced the number of bubbles after decompression from 203, 304 and 405 kPa ($p<0.05$). The total volume of accumulated gas was not affected by HBO_2. *Series B*: Pretreatment with HBO_2 at 709 kPa significantly reduced the number of bubbles after decompression from 203, 304, 507 and 608 kPa ($p<0.05$). Total gas volume after decompression from 507 and 608 kPa was reduced as a result of pretreatment with O_2. This study demonstrates that HBO_2 pretreatment at 405 kPa is sufficient to reduce the number of bubbles that will emerge on decompression from several levels of compression.

INTRODUCTION

Decompression sickness is a concern in underwater activity such as recreational, professional and military diving, in aviation when flying non-pressurized aircraft, and in hyperbaric medicine (1). During sudden or too rapid decompression, supersaturated fluids release gas in the tissues in the form of bubbles, which might cause decompression sickness (DCS). Extensive efforts have therefore been made towards understanding the mechanisms by which bubbles emerge and grow in decompressed tissues, in the hope that uncovering the mechanisms may lead to an intervention that will reduce bubble production.

It is widely accepted that bubbles which grow during decompression originate in tissue either at a point of friction between solid structures immersed in a liquid solution, i.e., tribonucleation (2, 3), or from preexisting gas micronuclei (4-10). On the basis of the latter assumption, we hypothesized that pretreatment with hyperbaric oxygen (HBO_2) will shrink some of the gas micronuclei, thus reducing the number of emerging bubbles. This is due to a process that comprises two steps. During HBO_2 pretreatment, oxygen replaces the inert gas in the micronuclei. When HBO_2 is replaced by hyperbaric air, the oxygen tension in the tissue is reduced. This leads to diffusion of oxygen (O_2) from the micronuclei to the tissue, where it will be consumed to supply the tissue's

metabolic requirements. Due to the proximity of the mitochondria and the micronuclei, the oxygen should be consumed at a rate faster than that at which nitrogen will be loaded into the micronuclei. Thus some micronuclei will subsequently disappear, or be shrunk to a volume ineffective for bubble generation. In the hemolymph of crustaceans, the affinity of hemocyanin for O_2 is only three times greater than that of the plasma, compared with the much higher affinity of hemoglobin (11). Thus, most of the O_2 in the whole prawn will be in dissolved form. We therefore assumed that consumption of the O_2 would reduce the oxygen partial pressure (PO_2) linearly with time. Assuming a metabolic rate of 0.176 ($ml \times gr^{-1} \times hr^{-1}$) for a small crustacean (12), and tissue solubility of 2.4×10^{-4} ($ml \times gr^{-1} \times kPa^{-1}$) at 25°C (13), the total dissolved O_2 in a prawn on air at 203 kPa will be consumed in 3 min. Consequently, after inert gas loading, there will be less micronuclei on decompression with the potential to produce bubbles. We had previously defined optimal HBO_2 pretreatment as the minimum time and PO_2 required for a maximum reduction in the number of bubbles. This pretreatment with HBO_2 at 405 kPa for 5 min led to a significant reduction in bubble density and total gas volume in transparent prawns decompressed from 203 kPa (14, 15). Whether this optimal pretreatment holds for other decompression ratios bearing a closer resemblance to those employed in human diving is under investigation at the present time. Decompression from higher pressures may result in the formation of bubbles by micronuclei that did not emerge as bubbles on decompression from 203 kPa.

In the present study, we investigated the effect of two levels of HBO_2 pretreatment (405 and 709 kPa) on prawns decompressed from a range of pressures between 203-810 kPa.

METHODS

Animals

The prawn *Palaemon elegans* (35 ± 4 mm long and weighing 1.54 ± 0.52 g, calculated as in ref. 15) is very common in the water along the rocky shores of the Mediterranean Sea. Due to its transparent shell, it is possible to observe its tissues using a microscope without any invasive manipulation. Prawns were kept at a temperature of 20-25°C in a 100-liter aquarium of aerated sea water.

Determination of nitrogen loading

We have previously determined the time for inert gas saturation (nitrogen) by exposure of the prawns to hyperbaric conditions (16). The calculated rate constant *(k)* was 0.32 min^{-1}, and the following equation was derived:

(Eqn 1)

$$PtN_2 = PamN_2 - (PamN_2 - PtN_2(0))e^{-0.32t}$$

where PtN_2 is the partial pressure of inert gas in the tissue (essentially both nitrogen and argon) at exposure time *t*, and $PtN_2(0)$ and $PamN_2$ are the tissue N_2 tension at the start of the exposure and the ambient N_2 tension, respectively. We used this equation to calculate N_2 loading and unloading in the prawn's tissues and the percent saturation when the ambient pressure of N_2 was constant. Pressure changes were carried out on air and oxygen, linearly with time. When air was used, the nitrogen pressure could be described by the equation:

(Eqn 2)

$$PamN_2t = PamN_2(0) + a \times t$$

where *a* is the compression or decompression rate in kPa nitrogen/min. We calculated N_2 loading (or unloading) during the linear change

of pressure on air by the following equation (15):

(Eqn 3)

$$PtN_2 = PamN_2(0) + a \times t - a/0.32 + (PtN_2(0) - PamN_2(0) + a/0.32) \times e^{-0.32t}$$

In all the exposures before the final explosive decompression, N_2 loading or unloading reached 98-102% of complete saturation in air-aerated water at pressures in the range 203-810 kPa ($PtN_2/PamN_2(0)$ ranging from 0.98-1.02, PtN_2 from *Eqn 1*).

The total gas volume for each prawn was calculated by summation of all the bubble volumes in the prawn. Bubble volume was calculated on the assumption that the bubbles were ellipsoids with a circular cross section.

EXPERIMENTAL PROCEDURES

The prawn was placed in a small jar (9.5 × 5.5 × 5.5 cm) filled with continuously aerated sea water, inside a temperature-controlled (25ºC) 150-liter experimental hyperbaric chamber (102 × 52 cm in diameter; T.C.A.H.O., La Spezia, Italy).

The experimental procedure consisted of hyperbaric exposure, decompression, and the subsequent assessment of bubbles by video-microscope at atmospheric pressure. *1)* Pressure was increased linearly (at 101 kPa/min) to 405 or 709 kPa in separate runs (*series A* or *series B*, respectively), with oxygen (experimental group) or air (control group) bubbling into the prawn's jar; *2)* The experimental prawn was pretreated with HBO_2 at the desired pressure for 5 min for the assumed diminution of gas micronuclei, whereas air was used in the control prawn; *3)* The O_2 was switched to air for the experimental prawn, at which point the pressure was changed at a rate of 101 kPa/min to the desired bottom pressure (203-810 kPa) for both experimental and control prawns; *4)* The prawn remained at this pressure with bubbling air until the calculated nitrogen saturation reached 98-102%; *5)* The prawn was subjected to explosive decompression at 304 kPa/min.

Following decompression, the prawn was immediately transferred into a small transparent cuvette supplied with a flow of aerated sea water, and was examined for bubble density and size under a light microscope (model CH 40, Olympus, Tokyo, Japan) 15 min after the decompression, as described earlier (14). Fig. 1 shows a picture of bubbles in the decompressed prawn.

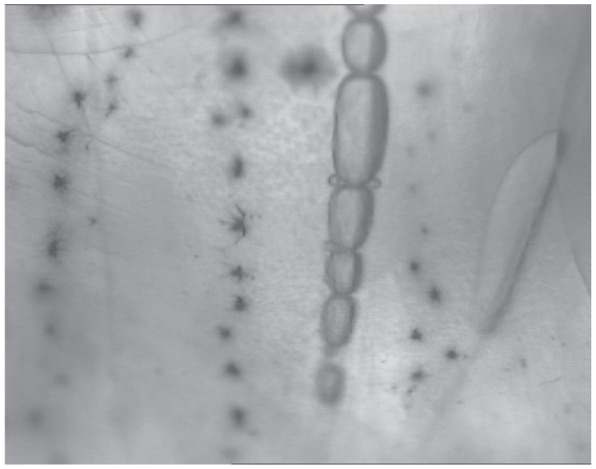

Fig. 1. Bubbles in the decompressed prawn Palaemon elegans.

Experimental protocol

Series A: We conducted *series A* to examine the effect of the 405 kPa HBO_2 pretreatment from our previous study (15) on prawns exposed to decompression from a range of bottom pressures. Each of the prawns was assigned to 14 experimental exposures consisting of two components: HBO_2 pretreatment at 405 kPa for 5 min and air control, and decompression from each of seven bottom air pressures (203, 304, 405, 507, 608, 709, and 810 kPa).

Series B: Pretreatment with HBO_2 at

405 kPa, which prevented micronuclei from growing to form bubbles on decompression from 203 kPa (15), may not be sufficient to do so on decompression from higher pressures. We therefore conducted *series B*, in which pretreatment with HBO_2 was given at 709 kPa. Each of the prawns was assigned to 12 experimental exposures consisting of two components: HBO_2 pretreatment at 709 kPa for 5 min and air control, and decompression from each of six bottom pressures (203, 304, 405, 507, 608, and 709 kPa).

To eliminate the influence of one exposure on the outcome of the next, the time interval between any two exposures of the same prawn was at least 48 h. This has previously been shown to be a sufficient length of time for a subsequent compression not to be affected by a previous exposure (14, 17). Fig. 2 shows a scheme of the experimental procedure.

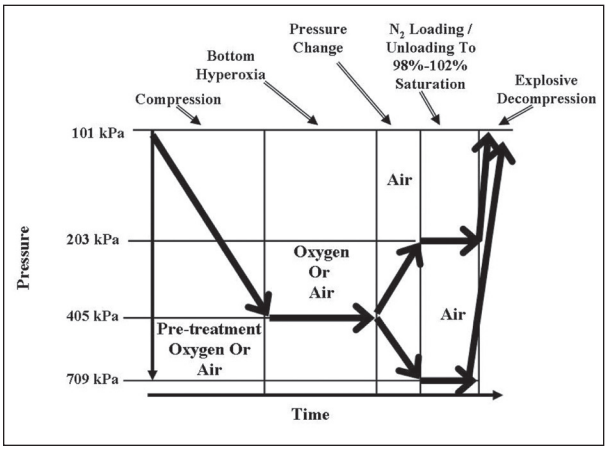

Fig. 2. General scheme of the experiment.

Prawns were checked before each high pressure exposure for their viability. Under even the slightest stress of any kind, the prawns lose their transparency and become opaque, so that any damage to their tissues would have been seen immediately. For the experiments we took only those prawns that looked totally transparent, not under stress and with no evidence of tissue damage. Before each set of exposures, prawns were examined under the microscope to confirm that there were no gas bubbles in their tissues.

Data analysis and statistics

Our previous attempts to mark prawns individually were unsuccessful. Although the experimental and control procedures were conducted on the same group of prawns, we could not use a paired test for statistical analysis. No correlation was found between body size of the prawn and the number of bubbles for any of the exposures (Spearman correlation test). Therefore, although prawns were of variable size, bubble density is expressed per prawn without volume correction.

Two parameters were compared in the 26 exposures for 11 prawns. Total gas volume per prawn was compared by one-way, two-way, and three-way ANOVA using SAS 6.03 (SAS Institute, Cary, NC), and the number of bubbles by a linear Poisson model. $p<0.05$ was considered significant.

RESULTS

In most cases, bubbles were seen proximal to the border between the segments, from the head to the abdomen. No bubbles were seen inside the gut or in the leg joints. The highest bottom pressure was 810 kPa. After decompression from this pressure, large numbers of cracks were observed in the shells of all the prawns from both the experimental and control groups, and most of them died. Some of the prawns exposed to 709 kPa also died a couple of hours after decompression. This phenomenon may be attributed to the evolution of high gas volumes on decompression from the higher pressures. The results of decompression from 810 kPa are therefore not included in our analysis.

Series A

Pretreatment with HBO_2 at 405 kPa for 5 min significantly reduced the number of bubbles after decompression from 203, 304 and 405 kPa ($p<0.05$, Fig. 3). Please see pages 374-375 for Figures 3-6. No difference was found between the control and experimental groups after decompression from 507, 608 and 709 kPa. The total amount of accumulated gas increased after decompression from pressures greater than 405 kPa. The volume of gas released was not affected by HBO_2 pretreatment (Fig. 4).

Series B

In both the control and the experimental groups, there was an increase in the number of bubbles per prawn and total accumulated gas volume after decompression from pressures greater than 405 kPa, with a reduction in both parameters after decompression from 709 kPa. Pretreatment with HBO_2 at 709 kPa for 5 min significantly reduced the number of bubbles after decompression from 203, 304, 507 and 608 kPa ($p<0.05$, Fig. 5). No difference was found between the control and experimental groups for the other decompression ratios. Pretreatment with HBO_2 at 709 kPa significantly reduced the total volume of accumulated gas after decompression from 507 and 608 kPa ($p<0.05$, Fig. 6).

Comparison between series A and B

The number of bubbles and total gas volume after decompression from the different pressures were greater following pretreatment at 709 kPa compared with 405 kPa ($p<0.05$, treatment and control). For example, the average bubble count per prawn in the experimental groups after decompression from 304 kPa was 3.9 in *series A* compared with 9.1 in *series B*. After decompression from 507 kPa, the total gas volume per prawn in the experimental groups was 0.6 µl in *series B* and only 0.3µl in *series A*.

DISCUSSION

In our previous reports, the effect of HBO_2 was investigated on the shrinking of gas micronuclei for a decompression ratio of 2, i.e., decompression from 203 to 101 kPa (15). The purpose of this study was to investigate the effect of HBO_2 pretreatment on other decompression ratios. Two HBO_2 pretreatments were selected, 5 min at 405 or 709 kPa, followed by exposure to a range of bottom pressures between 203-810 kPa. We found that HBO_2 pretreatment at 405 kPa significantly reduced the number of bubbles in the prawn after decompression from 203, 304 and 405 kPa, whereas pretreatment at 709 kPa produced a significant reduction in the number of bubbles after decompression from 203, 304, 507 and 608 kPa.

A puzzling finding is that for some bottom pressures the number of bubbles was not affected by HBO_2 pretreatment. Statistical evaluation indicated that it would make no difference if we increased the sample size for those decompression ratios. It is possible that shrinking of gas micronuclei by HBO_2 is limited to well-defined ranges of decompression ratios, most probably due to the existence of micronuclei having different thresholds for bubble generation. There are a number of possible mechanisms by which bubbles may emerge and grow at various sites: crevices in hydrophobic surfaces (7), surface-active molecules surrounding micronuclei (18), tribonucleation (2, 3), and contact zones between hydrophobic surfaces and aqueous solutions (19). HBO_2 pretreatment at 405 kPa and at 709 kPa may not have the same effect on the different mechanisms for bubble growth or

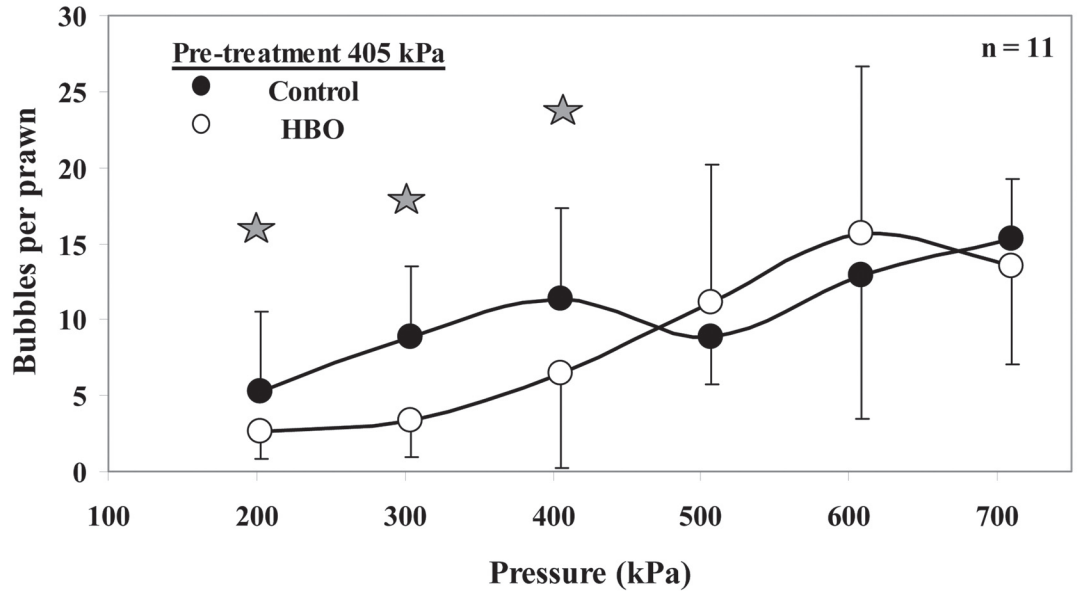

Fig. 3. Effect of HBO_2 pretreatment at 405 kPa for 5 min (series A) on bubble count (mean ± SD) after decompression from various pressures. Asterisks indicate a significant difference between the experimental and control groups only when bubble count in the experimental group was lower than in the control.

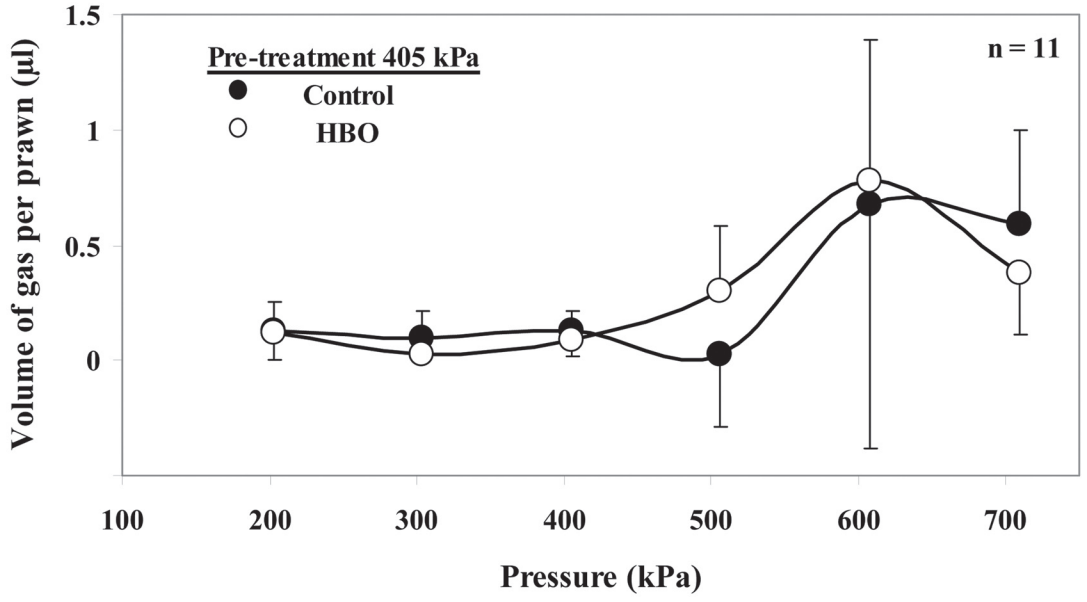

Fig. 4. Effect of HBO_2 pretreatment at 405 kPa for 5 min (series A) on the total gas volume (mean ± SD) after decompression from various pressures. No significant difference between the experimental and control groups.

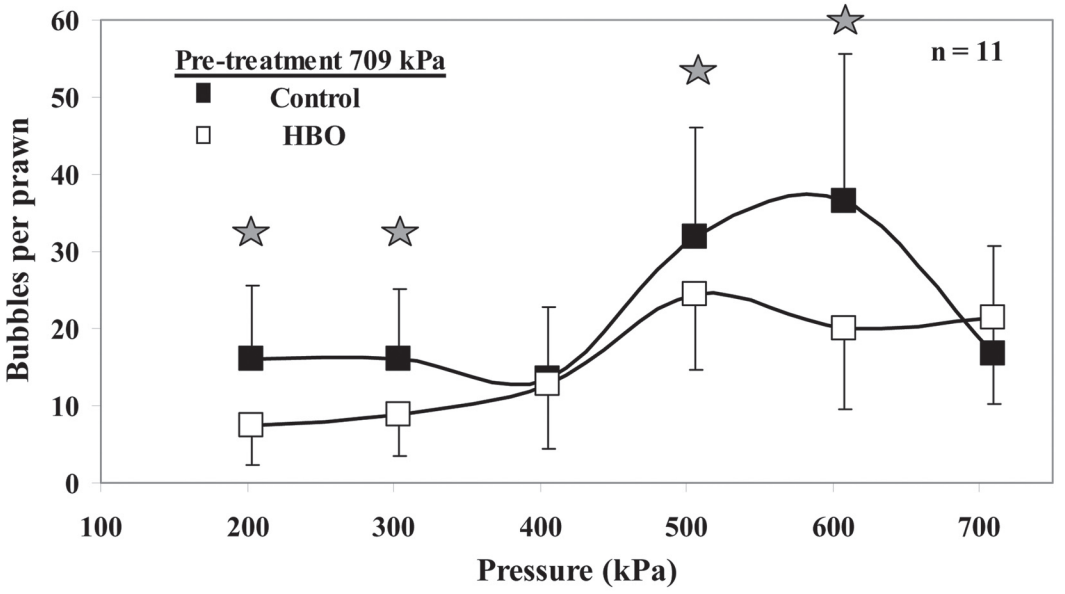

Fig. 5. Effect of HBO_2 pretreatment at 709 kPa for 5 min (series B) on bubble count (mean ± SD) after decompression from various pressures. Symbols as in Fig. 3.

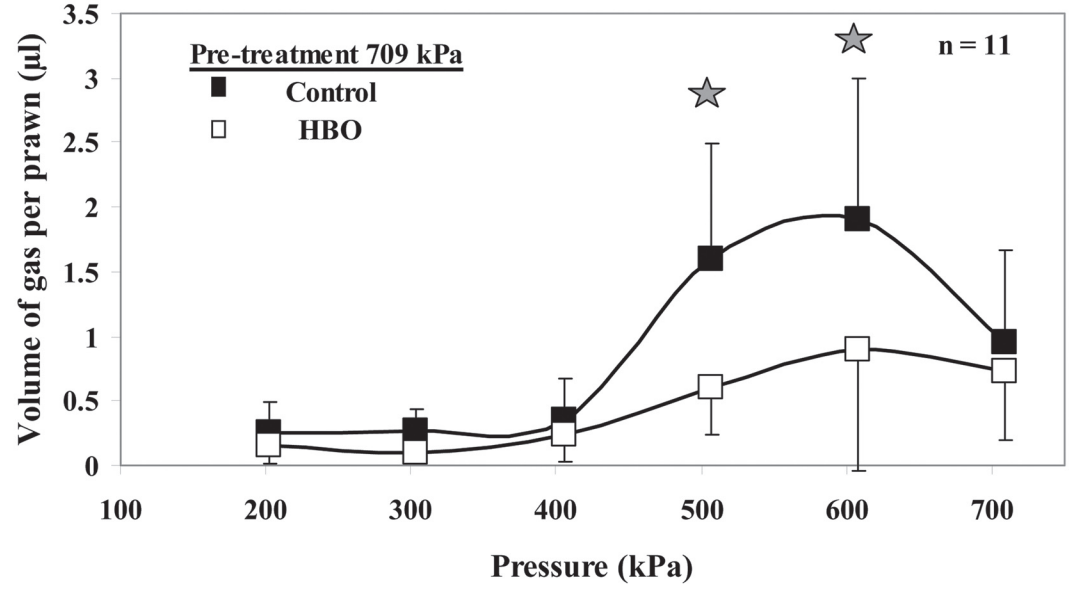

Fig. 6. Effect of HBO_2 pretreatment at 709 kPa for 5 min (series B) on the total gas volume (mean ± SD) after decompression from various pressures. Symbols as in Fig. 3.

on the various gas micronuclei populations.

Higher decompression ratios yielded increased numbers of bubbles. As the bottom pressure and gas saturation increase, more gas micronuclei with smaller radii are activated, as well as the bubbles with bigger radii that emerged at the lower bottom pressures (7, 20). In spite of this, however, with the highest decompression ratios (7 and 8) there was a reduction in bubble count and gas volume. An explanation for this may be gas leakage from unobserved cracks in the prawn shell. Such structural damage probably caused the later death of some of these prawns.

The bubble count in *series B* (both HBO_2 and control) was higher than in *series A*. Whereas the increase in the number of bubbles in the control group of *series B* is due to the increased air pressure during "pretreatment" at 709 kPa, in the HBO_2 group this may be related to the additional O_2 load that hinders the elimination of gas micronuclei. High PO_2 during pretreatment will result in faster elimination of the resident gas from the micronuclei, but the slower reduction of PO_2 as a consequence of N_2 loading will reduce the rate at which O_2 is eliminated. The role of O_2 as an inert gas has been shown in mammals (21, 22). This effect may have been exacerbated in the present study, where the removal of O_2 due to the metabolic rate of the prawn was slower than its loading rate. After exposure to 709 kPa oxygen, it will take 50 min for the prawn to consume the whole O_2 load; after pretreatment with 405 kPa O_2, the oxygen load will be lower. However, the rate of O_2 washout from the prawn's tissues is much faster than its removal due to the animal's metabolic rate, and this would therefore be the main route. The half-time for removal of O_2 from the prawn is less than 6 min (O_2 diffuses faster than N_2), thus most of the O_2 will be washed out during the N_2 loading. However, after O_2 pretreatment at 709 kPa some of the O_2 might remain in the tissues and serve as an inert gas load, contributing to bubble formation more than it would after pretreatment at 405 kPa. This mechanism counteracts the beneficial effect of O_2 in micronuclei elimination, and may explain the higher number of bubbles at all exposure pressures in *series B*. Extension of the N_2 loading period might prevent O_2 from acting as an inert gas after pretreatment at the higher pressures.

The total gas volume represents that part of the supersaturated gas which changed phase during decompression (from soluble to gaseous). HBO_2 pretreatment at 405 kPa did not have a significant effect on the total gas volume of the bubbles (Fig. 4). In fact, no difference could have been expected in the total gas volume of the bubbles between the control and experimental groups, because the amount of dissolved gas was the same in both. The result was that the same volume of dissolved gas entered a smaller number of micronuclei in the experimental group, producing larger bubbles, as entered a larger number of micronuclei in the control group, producing smaller bubbles. Thus there was great variability in bubble size between the control and pretreatment groups, i.e., although there were fewer bubbles in the experimental group, they were much bigger. In contrast to the 405 kPa pretreatment, HBO_2 at 709 kPa reduced total gas volume for all the decompression ratios, more notably after decompression from 507 and 608 kPa (Fig. 6). For those pressures, HBO_2 pretreatment at 709 kPa had a considerable effect on bubble count that was not observed following pretreatment at 405 kPa. When the number of gas micronuclei is greatly reduced, more of the supersaturated gas will be transferred to the irrigating water than to the growing micronuclei due to diffusion distances.

A comparison of the two HBO_2 pretreatments (*series A* and *series B*) shows that after pretreatment with 405 kPa, there were fewer bubbles and less total bubble

volume compared with *series B*. Thus it may be preferable to administer HBO_2 pretreatment at 405 kPa for 5 min, which can be considered less dangerous and more efficient.

Our results support the hypothesis that at least some gas bubbles are formed from preexisting micronuclei. The present study used HBO_2 pretreatment to reduce the number of bubbles emerging from such micronuclei after decompression from pressures much lower than those in previous studies (3, 9, 17), demonstrating that this pretreatment is effective in the pressure range of recreational and professional diving. Prawns have no vascular system and may therefore be referred to as one-compartment. This is in contrast to mammals, which are multi-compartment. The mechanism of bubble reduction works well for this simple, one-compartment model, but may be more complicated when applied to mammals. In their study on humans, Landolfi et al. (23) used methods similar to our procedures (14) to show that HBO_2 pretreatment before N_2 loading reduces the number of bubbles recorded by Doppler ultrasound following decompression. Based on the present results, we are now examining the efficacy of HBO_2 pretreatment in rats. The possibility of CNS oxygen toxicity must be taken into account when these procedures are being applied to mammals. If HBO_2 pretreatment is as efficient in shrinking gas micronuclei and reducing the risk of DCS in rats, its use may be justifiable in humans.

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