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# Effect of air, heliox, and oxygen breathing on air bubbles in aqueous tissues in the rat

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Hyldegaard O, Madsen J. Effect of air, heliox, and oxygen breathing on air bubbles in aqueous tissues in the rat. Undersea Hyperbaric Med 1994; 21(4):413–424.—Our purpose was to examine the behavior of air bubbles in three non-lipid tissues (skeletal muscle, tendon, and the anterior chamber of the eye) during breathing of air, helium–oxygen (heliox, 80:20), or oxygen. Air bubbles were injected into skeletal muscle or tendon in rats after decompression from a 1-h air exposure at 3.5 atm abs (355 kPa) or into the anterior chamber of the rat eye without any previous pressure exposure. The bubbles were studied by photomicroscopy at 1 atm abs (101 kPa) during either air breathing or during air breathing followed by heliox or $O_2$ breathing. Muscle: during air breathing, all bubbles initially increased in size for a period of 55–100 min after decompression and then started to shrink. Both heliox and $O_2$ breathing increased the shrinking rate as compared to air. Bubble size decreased more rapidly during $O_2$ than heliox breathing. Tendon: during air breathing, bubble size decreased at a constant rate; in one bubble the decrease was preceded by a small increase. During heliox breathing most bubbles decreased faster than during breathing of air. $O_2$ breathing caused a short-term increase in bubble size in 4 out of 10 bubbles. Otherwise, the shrinkage rate was increased in six bubbles and uninfluenced in four bubbles during breathing of $O_2$. Rat eye: during air breathing all bubbles shrank in the observation period. When heliox breathing was started, all bubbles transiently grew for 10–35 min, after which they began shrinking faster than during air breathing. When $O_2$ breathing was started, five out of seven bubbles initially grew or stopped shrinking for 5–15 min, after which they decreased in size faster than during both air and heliox breathing. We conclude that breathing of either heliox or $O_2$ will cause air bubbles in aqueous tissues to disappear faster than during breathing of air. Since heliox breathing promoted bubble shrinking in both muscle and tendon, gas exchange was probably not primarily limited by extravascular diffusion in these aqueous tissues. The present experiments suggest that heliox breathing at 1 atm abs may not exacerbate limb bends.

*muscle, tendon, diffusion limitation, eye, perfusion limitation, decompression sickness, limb bends*

In previous communications we have reported that decompression-induced bubbles in adipose tissue (1) and air bubbles injected into spinal white matter (2) of rats decompressed

from air exposures will grow for hours during air breathing at 1 atm abs, but shrink and disappear during helium–oxygen (heliox, 80:20) breathing. During oxygen breathing they will grow initially, then shrink and disappear in about the same time as during heliox breathing (1, 2). After decompression from a 1-h air exposure at 3.8 atm abs, progressive deterioration of spinal conductive function can be demonstrated. This deterioration can be opposed by heliox as well as $O_2$ breathing at 1 atm abs. The effect of heliox seems to be superior to that of $O_2$ (3).

James (4) and Hills (5) explained the effect of heliox in lipid tissues by an outward flux of nitrogen from the bubbles exceeding the inward flux of helium, whether the exchange of gases in the tissue is limited by perfusion or diffusion. In the case of perfusion limitation, because the solubility of He in blood is less than that of nitrogen; in the case of diffusion limitation, because the product of diffusion coefficient and solubility (He diffusibility) in lipid is greater for $N_2$ than for He. According to this reasoning, $N_2$ bubbles in aqueous surroundings would be expected to grow during heliox breathing if the gas exchange between blood and bubbles is limited by extravascular diffusion, since the diffusibility of He in water is 1.4 times that of $N_2$ (1, 6). In the case of perfusion limitation they should shrink.

The purpose of the present experiments was to examine the behavior of air bubbles in three non-lipid tissues (skeletal muscle, tendon, and the anterior chamber of the eye) during breathing of air, heliox, or $O_2$. Muscle was chosen for this study as an easily accessible, low-lipid tissue in which bubbles can be easily injected and observed, and tendon because the bubbles in limb bends probably are located in tendineous or ligamentous tissue (7–9). The experiments in the eye were done to describe the effects of different breathing gases on air bubbles in a situation where gas exchange is known to be dominated by extravascular diffusion in the aqueous humor (10).

## METHODS

### General

Female rats (250–350 g) were anesthetized with sodium thiomebumal (0.1 g/kg) intraperitoneally. A cannula was inserted in the trachea and a catheter was placed in a carotid artery for blood pressure recording. To keep the catheter open, saline was continuously infused at a rate of 1 ml/h. A thermocouple was placed in the vagina for continuous temperature recording. Constant body temperature at about 37°C was maintained as the vaginal thermometer controlled the chamber heating or a heating lamp. After pressure exposure, decompression, and preparation (*see* below), the observed bubble field was illuminated by two flexible fiberoptics and studied though a Wild M-8 stereomicroscope fitted with photographic equipment. The bubbles were photographed at time intervals of 10–15 min, always at ×40. All observations were made at atmospheric pressure. All animals initially breathed air. Some continued air breathing for the entire observation period; others were switched to either heliox or $O_2$ breathing 35–130 min after decompression. Through a T-connection on the tracheal tube the breathing gas was flowing at a constant rate of 1.5–2.0 liters/min. At the end of the observation period the abdomen and thorax were opened and the animals were examined under the microscope for intra- or extravascular gas formation and then killed by exsanguination.

### Muscle and tendon

The musculus obliquus externus or the adductors of the thigh were exposed through skin incisions. The tendons of the tail were exposed through a 3-cm longitudinal and two 1-cm transverse skin incisions. The exposed tissue areas were then covered with gas-impermeable mylar and a 12.5-$\mu$m-thick polyethylene membrane to prevent evaporation during exposure in the pressure chamber.

The animal was exposed to 3.5 atm abs (354.64 kPa) for 1 h, and then decompressed in 7.5 min with short stops at 3.0, 2.0, and 1.5 atm abs. The mylar and plastic covers were removed and one or two bubbles of about 1–1.5 $\mu$l of air were injected superficially into the muscle with a micropipette. In the rat tail tendon, 0.5–1.0 $\mu$l of air was injected (2). The bubbles were covered by a mylar and a polyethylene membrane. In some experiments a thermoprobe was placed under the plastic film to check the temperature of the exposed tissue.

When bubbles are injected into the rat tail tendons the gas distributes itself along the streaks of the tendinous tissue where bubbles are also seen to form in the kangaroo rat tail during decompression sickness (DCS) (7); in muscle the gas will distribute itself along the fibers. Most bubbles injected into muscle and tendon are ellipsoid or cylindrical. Bubbles in tendon may be irregular in shape. Therefore we assessed their size by the bubble area visible on the microphotographs, using an Ott planimeter.

### Eye

The rats were placed on their right sides with the heads elevated and turned so that the circumference of the left cornea was in a horizontal plane. The upper and lower eyelids were fixed by skin sutures on each side of the eye. Atropine (1.0 mg/ml) was then dripped (2–3 drops) onto the surface of the eye to dilate the iris during the experiment. With a micropipette, 10 $\mu$m o.d., the cornea was perforated and an air bubble of 0.1–0.5 $\mu$l was injected into the anterior chamber. Here the bubble would position itself just below the cornea at the highest point of the eye chamber, approximately 2,000–2,500 $\mu$m from the iris. A 1.0-mm-thick glass contact lens was placed on the eye, covering the cornea and its surroundings to prevent gas exchange with the surrounding room air. The rats were then placed under the microscope. From the microphotographs the diameter of the bubbles could be measured and their volume calculated. No animal in the eye experiments was exposed to increased ambient pressures.

### Data analysis and statistics

Average shrinkage rate for a bubble in muscle or tendon was expressed in square millimeters per minute (slope of a line from first observation after gas shift to last observation or disappearance of bubbles). In the air-breathing experiments the bubble shrinkage rate was calculated from the time at which a gas shift from air to heliox or $O_2$ breathing would have been performed to the final observation point or to the time of bubble resolution. In the heliox and $O_2$ experiments the rate was calculated from the time of the gas shift to the time of bubble resolution. The same procedure was applied when calculating the decrease in bubble volume (microliters per minute) in the eye chamber. Average values of bubble shrinkage rates are given $\pm$ SEM.

To examine whether the difference between two mean values of calculated bubble

shrinkage rates was different from zero, analysis of variance (ANOVA) followed by the Newmann-Keuls procedure for multiple comparison of several groups was performed on the difference between mean values in the different treatment groups (11–14). The effect of breathing gases on bubble resolution was also analyzed with 4-fold $\chi^2$ tests dividing the experiments into "bubbles disappeared" or "bubbles not disappeared;" $P < 0.05$ was regarded as the limit for significance.

## RESULTS

### General condition of animals

*Muscle and tendon*: The animals were observed until the bubble disappeared or for up to 320 min after decompression. Respiration remained stable during the observation period. Four animals died of DCS during the observation period, all with many bubbles present in their veins; three died during air breathing and one during $O_2$ breathing. An occasional extravascular bubble could be observed in the adipose tissue in some animals. Animals that died spontaneously were not included in the data collection.

During 1 h breathing air under pressure, mean arterial pressure (MAP) was between 130 and 180 mmHg. During decompression it fell to 80-140 mmHg. During subsequent air breathing, MAP remained at this level and tended to decrease during the observation period. During heliox breathing the MAP increased gradually, with 120–140 mmHg as the most frequent interval. During $O_2$ breathing, blood pressure was between 120 and 140 mmHg. When $O_2$ breathing was started, an immediate increase of about 20 mmHg in MAP was observed.

*Eye*: The animals were observed until disappearance of the injected bubble. Their respiration remained unaffected. During both air and heliox breathing the MAP was between 120 and 140 mmHg. During $O_2$ breathing, blood pressure increased about 10–20 mmHg and remained at this new level, with the MAP between 130 and 150 mmHg.

### State of tissues

*Muscle*: During the observation period, perfusion in the smaller vessels with a diameter of approximately 10–15 $\mu$m was clearly visible and seemed unaffected throughout the experiment. In all of the experimental groups the tissue temperature varied between 36.7° and 39.5°C, depending on switching on and off of the heating lamp. Occasionally, small muscle fiber twitches could be observed.

*Tendon*: Perfusion in the smaller vessels (10–15 $\mu$m diameter) was clearly visible at all times. Some bleeding from the skin could be seen when it was split from the tendons during the preparation phase. In all groups the temperature of the tissue varied between 35.0° and 36.7°C depending on switching on and off of the heating lamp.

*Eye*: During the entire observation period the iris remained dilated; blood flow in the vessels of the iris was clearly visible at all times.

### Comparability of air, heliox, and oxygen experiments

The three experimental groups (muscle, tendon, and eye chamber) did not differ significantly from each other with respect to the size of injected bubbles, nor did they differ sig-

nificantly with respect to time interval from decompression to bubble injection, first observation and gas shift (muscle and tendon), or from bubble injection to first observation and gas shift (eye).

### Effect of breathing gases on bubbles in muscle

*Air breathing*: During air breathing all bubbles initially grew at a modest rate. Some 55–100 min after decompression the growth changed to an equally slow shrinking in six of seven bubbles (Fig.1 *top*). The mean shrinkage rate of the bubbles during air breathing was $25.83 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$ (SEM: $\pm 5.43 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$).

*Heliox breathing*: Heliox breathing caused all bubbles to decrease in size and disappear at a rate faster than seen during air breathing (Fig.1 *center*). All bubbles had disappeared between 135 and 255 min after decompression. During heliox breathing the mean shrinkage rate was $108.73 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$ (SEM: $\pm 17.75 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$).

*Oxygen breathing*: During $O_2$ breathing bubbles decreased in size at a rate faster than during either air or heliox breathing (Fig.1 *bottom*). All bubbles had disappeared between 90 and 210 min after decompression. During $O_2$ breathing the mean shrinkage rate was $140.45 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$ (SEM: $\pm 27.71 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$).

*Comparison of breathing gas effect on bubbles in muscle*: ANOVA followed by multiple comparisons between the groups showed that heliox breathing was not significantly better than air breathing ($0.10 > P > 0.05$). Oxygen breathing resulted in a significantly faster bubble shrinkage rate as compared to air breathing ($P < 0.01$). There was no significant difference between heliox and $O_2$ breathing ($P > 0.10$). Two hundred and eleven minutes after decompression, no bubbles had disappeared during air breathing ($n = 7$). By this time heliox breathing had caused 10 bubbles to disappear ($n = 11$) and $O_2$ breathing had caused all bubbles to disappear ($n = 11$). Chi$^2$ tests showed that both heliox and $O_2$ breathing were significantly better than air breathing ($P < 0.001$ and $P < 0.001$, respectively) whereas there was no difference between heliox and $O_2$ breathing ($P > 0.1$).

### Effect of breathing gases on bubbles in tendon

*Air breathing*: During air breathing, bubbles slowly diminished, one after a small increase (Fig. 2 *top*); the mean shrinkage rate of the bubbles was $20.00 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$ (SEM: $\pm 3.13 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$).

*Heliox breathing*: During heliox breathing 8 out of 11 bubbles shrank at a rate faster than during air breathing (Fig.2 *center*); in three bubbles the shrinkage rate was unchanged or slightly reduced as compared to air breathing. None of the bubbles grew or increased in size after the shift. Heliox breathing caused the bubbles to shrink at a mean rate of $66.91 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$ (SEM: $\pm 14.55 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$).

*Oxygen breathing:* Oxygen breathing caused a 5–15 min increase in bubble size or interruption of shrinkage in 4 of 10 bubbles. Otherwise the rate of disappearance was unchanged in 4 out of 10 bubbles as compared to air breathing, and the other 6 bubbles all shrank faster than during air and some faster than during heliox breathing (Fig. 2 *bottom*). During oxygen breathing the mean shrinkage rate was $54.60 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$ (SEM: $\pm 7.71 \cdot 10^{-4}$ mm$^{-2}$ · min$^{-1}$).

*Comparison of breathing gas effect on bubbles in tendon*: ANOVA followed by multiple comparison between the groups showed that breathing heliox caused a significantly faster

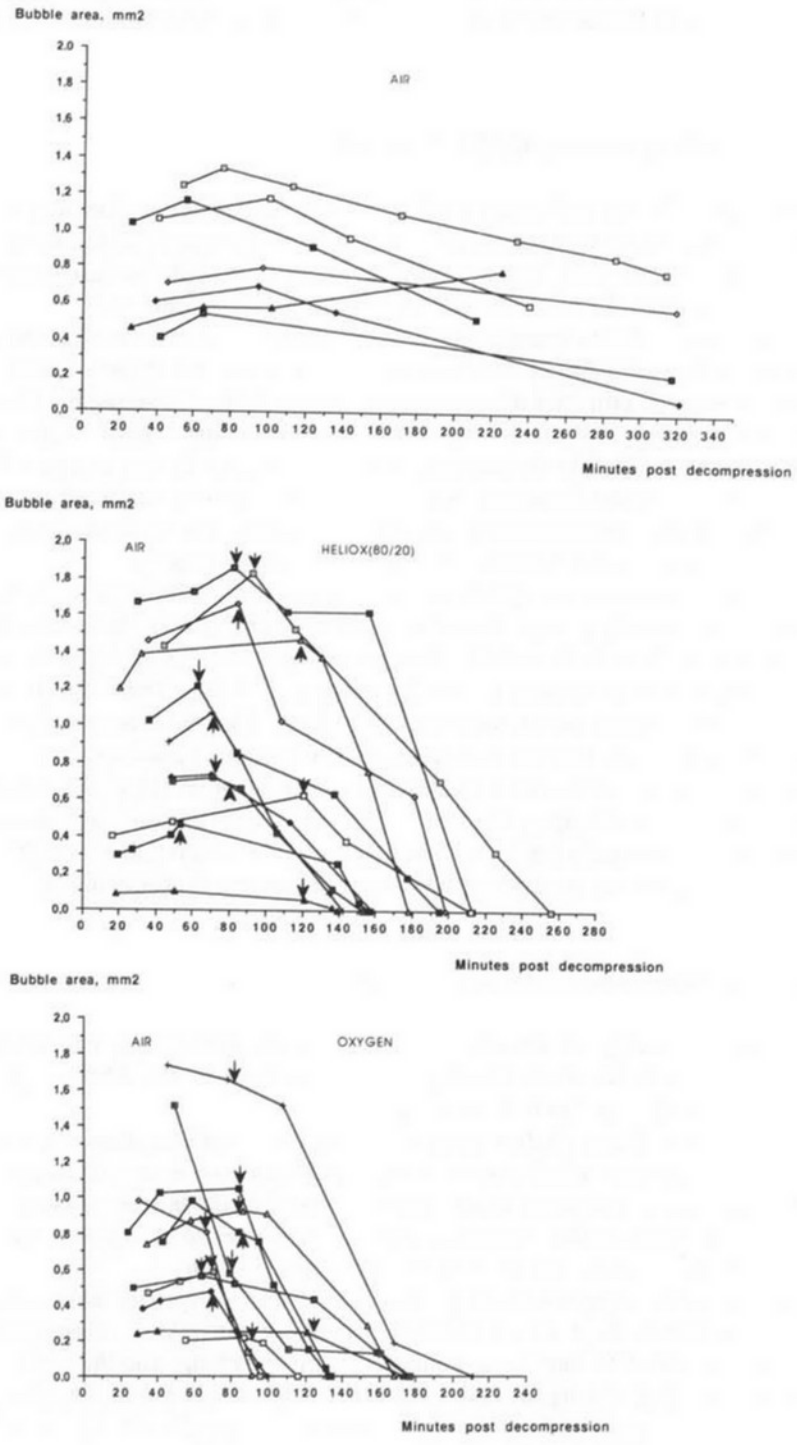



FIG. 1—Skeletal muscle: bubble area vs. time. *Top*, air breathing which began at the first point on curves; *center*, heliox; *bottom*, $O_2$. *Arrows* mark the time of gas shift from air to either heliox or $O_2$ breathing.

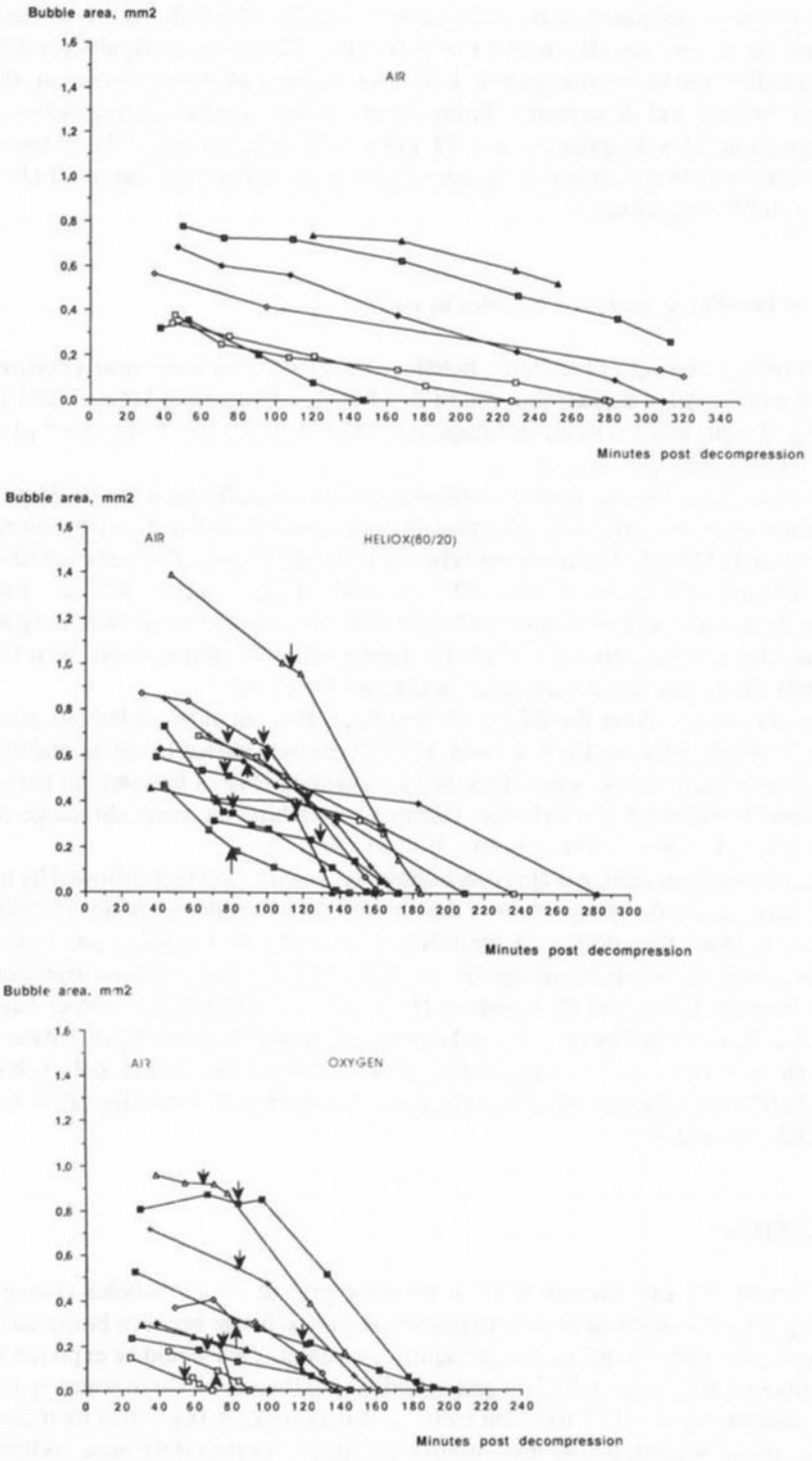



FIG. 2—Rat tail tendon: bubble area vs. time. *Top*, air breathing which began at the first point on curves; *center*, heliox; *bottom*, $O_2$. *Arrows* mark the time of gas shift from air to either heliox or $O_2$ breathing.

shrinkage rate as compared to air breathing ($P < 0.05$). The difference between $O_2$ and air breathing was not significant ($0.10 > P > 0.05$). There was no significant difference between heliox and $O_2$ breathing ($P > 0.10$). At 300 min after decompression, three out of seven bubbles had disappeared during air breathing, whereas during heliox and $O_2$ breathing all bubbles disappeared ($n = 11$ and $n = 10$, respectively). The $\chi^2$ test showed that the effects of both heliox and $O_2$ were significantly better than that of air ($P < 0.05$ and $P < 0.05$, respectively).

### Effect of breathing gases on bubbles in eye

*Air breathing*: During air breathing, bubbles consistently shrank at a nearly constant slow rate in the observation period; four out of five bubbles disappeared between 250 and 325 min (Fig. 3 *top*), and the mean shrinkage rate of the bubbles was $8.20 \cdot 10^{-4}$ $\mu l \cdot min^{-1}$ (SEM: $\pm 1.07 \cdot 10^{-4}$ $\mu l \cdot min^{-1}$).

*Heliox breathing*: During heliox breathing all bubbles initially grew for 10–35 min, after which they began to shrink and disappear at a rate faster than during air breathing (Fig. 3 *center*); all bubbles had disappeared between 130 and 186 min. The mean shrinkage rate during heliox breathing was $19.20 \cdot 10^{-4}$ $\mu l \cdot min^{-1}$ (SEM: $\pm 2.20 \cdot 10^{-4}$ $\mu l \cdot min^{-1}$).

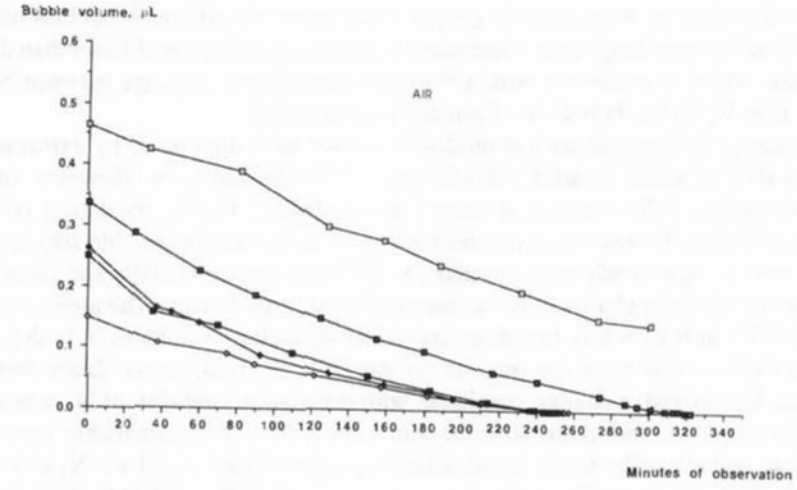
If exactly the same experiment is conducted without covering the eye with the glass contact lens, this transient growth of bubbles during heliox breathing is not seen ($n = 5$); apparently He is lost to the surroundings through the cornea.

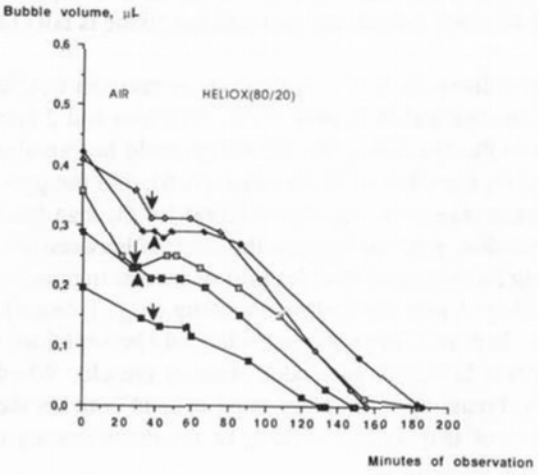
*Oxygen breathing*: After the shift to $O_2$ breathing, five out of seven bubbles transiently grew or stopped shrinking for 5–15 min, after which they started to shrink and disappear at a rate faster than during either air or heliox breathing (Fig. 3 *bottom*); all bubbles had disappeared between 95 and 161 min. During $O_2$ breathing the mean shrinkage rate was $27.00 \cdot 10^{-4}$ $\mu l \cdot min^{-1}$ (SEM: $\pm 3.46 \cdot 10^{-4}$ $\mu l \cdot min^{-1}$).

*Comparison of breathing gas effect on bubbles in the eye*: ANOVA followed by multiple comparisons among the groups showed that the shrinkage rate during heliox breathing was significantly faster than during air breathing ($P < 0.05$). $O_2$ breathing was significantly faster as compared to air breathing ($P < 0.01$), whereas there was no significant difference between heliox and $O_2$ breathing ($P > 0.1$). At 200 min no bubbles had disappeared during air breathing ($n = 5$), and during heliox and $O_2$ breathing all bubbles disappeared ($n = 5$ and $n = 7$, respectively). $\chi^2$ tests showed that heliox and $O_2$ breathing caused bubbles to disappear significantly faster than during air breathing ($P < 0.02$ and $P < 0.01$, respectively).

## DISCUSSION

The present eye experiments show a transient growth of air bubbles during heliox breathing (Fig. 3 *center*) in circumstances where gas exchange between blood and bubble is determined mainly by diffusion in an aqueous medium. This would be expected because the product of diffusion coefficient and solubility coefficient of He in water is 1.4 times greater than for $N_2$ (1, 6). A transient inert gas supersaturation (i.e., total inert gas partial pressure) of the aqueous humor may contribute to bubble growth if the aqueous humor saturates faster with He than it desaturates with $N_2$ because of the difference in diffusibilities of the two gases (15, 16). The flow of aqueous humor in the anterior chamber of the rat eye may contribute to gas exchange (10) but would further reduce shrinkage of the bubble



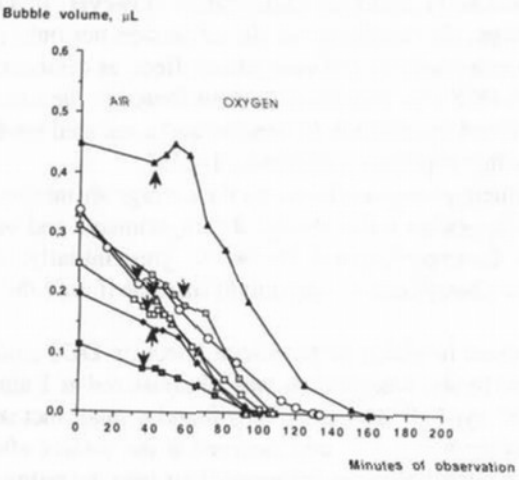




FIG. 3—Rat eye: bubble volume vs. time. *Top*, air breathing which began at the first point in curves; *center*, heliox; *bottom*, $O_2$. *Arrows* mark the gas shift from air to either heliox or $O_2$ breathing.

because the solubility of $N_2$ in water is greater than that of He. Although bubbles initially grew during heliox breathing, they subsequently shrank and disappeared faster than during air breathing, which is consistent with a diffusion-limited gas exchange between bubble and blood after $N_2$ in the bubble has been replaced with He.

If gas exchange in ligamentous and tendinous tissues were dominated by extravascular diffusion, a shift to heliox breathing should cause air bubbles to grow. However, in both muscle and tendon, heliox breathing caused an accelerated bubble resolution (Figs. 1 *center* and 2 *center*). Heliox breathing did not induce an increase in bubble size in either of the two tissues. Apparently extravascular gas diffusion between bubble and blood does not limit gas exchange under the circumstances of these experiments. The absence of initial growth after shift to heliox breathing and faster shrinking of bubbles in both muscle and tendon indicate that from the time of the gas shift, $N_2$ disappeared faster from the bubbles than He entered, a finding consistent with the higher solubility of $N_2$ than of He in blood. Since gas saturation and desaturation in these tissues is presumably limited by the perfusion, and since the blood/tissue solubility ratios ($\lambda$) are equal for $N_2$ and He in aqueous tissues ($\lambda$ values for He and $N_2$ for aqueous tissue 1.042 and 0.966, respectively), the total inert gas pressure gradient between bubble and surrounding tissue is not changed in these experiments.

When the breathing gas was shifted from air to $O_2$, a transient increase in bubble size or impaired shrinkage was seen in the eye and in tendon (Figs. 3 *bottom* and 2 *bottom*). The initial growth of bubbles seen in the eye during $O_2$ breathing could be explained by the 1.5 times greater diffusibility of $O_2$ than that of $N_2$ in water (1, 6), and the growth in tendon could be ascribed to the greater transport capacity of blood for $O_2$ than for $N_2$. It was not observed in all bubbles in tendon, perhaps because the interval between observations was too long. Oxygen breathing increased the bubble elimination rate in muscle (Fig. 1 *bottom*) as compared to both air (Fig. 1 *top*) and heliox breathing (Fig. 1 *center*). The observed increases in bubble volume in tendon but not in muscle could be explained if the ratio of $O_2$ supply to $O_2$ consumption is higher in tendon than in muscle. Whether a transient volume increase during $O_2$ breathing takes place must depend both on the perfusion rate and the metabolic activity of neighboring tissues, so the inconsistency is not surprising.

A growth of bubbles, although transient, is obviously undesirable. However, in a damaged tissue with edema and hemorrhage, $O_2$ breathing has the advantage not only of $O_2$ delivery at an increased partial pressure but also an anti-edematous effect, as demonstrated in the spinal white matter of rats with DCS (3). This effect was attributed to the vasoconstriction and blood flow reduction elicited by the high $O_2$ tension and a reduced tendency of leukocytes to block microvessels after exposure to bubbles (17–19).

Our statistical comparisons of treatment groups are based on the average shrinkage rate, which does not reflect changes in disappearance rate during the experiments and underestimates the rate in the later part of the experiments if the bubble grew initially. Thus, the significance of differences between disappearance rates might increase if only the later part of bubble life were considered.

Animal experiments have been reported in which no beneficial effect on DCS could be demonstrated with heliox as compared to air, when heliox was administered at 1 atm abs during DCS of the pulmonary "choke" type. Catron et al. (20) found a small increase in pulmonary artery pressure in dogs, when heliox was administered at the surface after an air dive, corresponding to the effect reported after the infusion of air into the pulmonary vasculature (21). Presson et al. (22) found transient growth of intravascular air bubbles injected into the pulmonary vasculature of dogs after shift to heliox or $N_2O$–$O_2$ at normobaric conditions. The initial increase in bubble size seen in their experiments was followed

by a steeper slope in the elimination phase of the bubbles as compared to air breathing. Helium diffuses into bubbles rapidly but also diffuses out rapidly. This may explain why the rate of elimination of bubbles is steeper (i.e., faster) during heliox breathing than during air breathing and is in agreement with our present results of bubbles in the eye disappearing faster on heliox breathing than during air breathing.

Our previous findings in lipid tissues (1–3) suggest that heliox breathing may be beneficial during transportation at sea level of a patient with spinal DCS. However, on theoretical grounds we stated that heliox breathing might cause $N_2$ bubbles in an aqueous medium, such as tendon or muscle, to grow in the case of diffusion-limited gas exchange. Our present results go against reservations regarding heliox treatment of neurologic DCS for fear of exacerbating limb bends, because injected air bubbles in tendon and muscle did not grow during heliox breathing. Bubbles in the anterior chamber of the eye are no problem in DCS. However, the optimal heliox composition remains to be established.

Reservations are appropriate when recommending DCS treatment based on studies of injected bubbles. Consequently, experiments should be done on animals in which limb bends can be produced by decompression. In view of reports of successful recompression treatments of spinal DCS after air dives with heliox breathing (4,5,23–26), experiments on bubble disappearance during recompression are also warranted.

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