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Effect of He-O_2, O_2, and N_2O-O_2 breathing on injected bubbles in spinal white matter

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Hyldegaard O, Møller M, Madsen J. Effect of He-O_2, O_2, and N_2O-O_2 breathing on injected bubbles in spinal white matter. Undersea Biomed Res 1991; 18(5–6):361–371.—Injected air bubbles in spinal white matter in the rat were studied at 1 bar after decompression from an exposure to air at 3.1 bar (absolute) for 4 h. During air breathing all injected bubbles grew for the first 2 h of the observation period. Thereafter three of nine bubbles began to shrink and one of them disappeared. During breathing of heliox (80:20) bubbles consistently shrank and disappeared from view. If the breathing gas was changed from heliox to N_2O-O_2 (80:20), while bubbles still had an appreciable size, they started growing again. If the change to N_2O-O_2 was done after a bubble disappeared from view, it did not reappear. During breathing of 100% oxygen, all bubbles initially grew. Subsequently they all shrank and disappeared at about the same time after gas shift, as during heliox breathing. The effect of heliox treatment on CNS decompression sickness after air dives is discussed.

bubbles
decompression sickness
heliox
myelin
nitrous oxide
oxygen
rat
spinal white matter

The use of heliox breathing during recompression in serious cases of decompression sickness (DCS) after air dives has been recommended by James (1) and successful cases of such treatment have been reported (2). N_2 bubbles in adipose tissue appearing after decompression from hyperbaric air exposures have been observed to shrink rapidly during breathing of heliox at 1 bar (3).

The standard treatment of DCS is recompression combined with oxygen breathing. During transportation to a recompression facility, oxygen breathing is recommended (4). However, in animal experiments we have observed an initial increase in the volume of N_2 bubbles in adipose tissue when oxygen breathing at 1 bar was started. This increase in volume was usually followed by shrinking and disappearance of the bubbles.

The purpose of the present experiments was to examine by direct observation the effect of air, heliox, and oxygen breathing at 1 bar on air bubbles in spinal white matter in rats after decompression from a near-saturation air exposure. To reveal

possible submicroscopic bubbles remaining after heliox treatment, the breathing medium of some animals was shifted to N_2O-O_2, because such bubbles would be expected to grow and become visible under this regimen (5).

METHODS

Female rats weighing 250–350 g were anesthetized with sodium thiomebumal intraperitoneally. A cannula was inserted in the trachea and a catheter was placed in a carotid artery for blood-pressure registration. To keep the catheter open, saline was continuously infused at a rate of 1 ml · h^{-1}. A thermocouple was placed in the vagina for continuous temperature registration. The rat was fixed in a stereotaxic device in the ventral position with the head semiflexed. Through a midline incision the cervical vertebral column was exposed. The ligamentum flavum between first and second vertebrae was divided and adjacent parts of the vertebral arches removed with a dental fissure burr, leaving the dura intact. In this way an opening into the vertebral canal of about 4 × 5 mm was obtained. The incision was closed and the rat exposed to air at 3.1 bar absolute for 4 h. The chamber temperature was set to 25°–28°C, maintaining the vaginal temperature of the animal about 37°C. After 4 h the animal was decompressed in three stages over 20 min. The animal was removed from the chamber and placed under a heating lamp controlled by the vaginal thermometer, to maintain constant body temperature. The incision was reopened and the dura removed exposing the cervical medulla. With a micropipette (10 μm o.d.) mounted in a Leitz micromanipulator and connected to a Gilmont ultraprecision microsyringe filled with silicone oil up to a few microliter from the tip, the pia was perforated and between 1 and 1.5 μl of air injected superficially into the posterior funiculus. The field was illuminated with two flexible fiberoptics and studied through a Wild M-8 stereomicroscope (maximum magnification ×80) fitted with photographic equipment. Figure 1 shows a histologic preparation of a bubble in the posterior funiculus in a rat. The rat was perfusion fixed shortly after the injection. In some experiments a thermoprobe was placed on the surface of the exposed medulla to check the temperature at the surface of the exposed tissue.

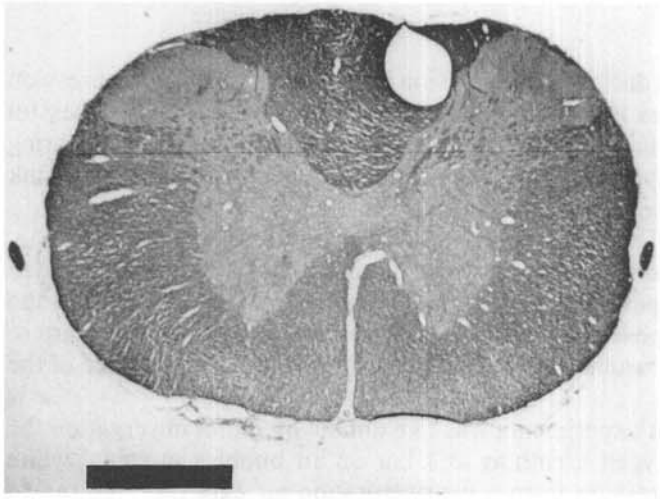

Fig. 1. Cervical section of rat spinal cord showing bubble injected into the posterior funiculus. Bar = 1 mm.

After injecting the bubble, one of the following types of experiments was performed, all at 1 bar: a) air breathing; b) breathing of heliox (80:20%), c) heliox (80:20%) breathing followed at various time intervals by breathing of N_2O-O_2 (80:20%), or d) breathing of O_2. The gas shift was done 30–80 min after decompression.

At intervals the field was photographed, always at ×40. Since the bubbles were usually of irregular shape (Fig. 2) their volumes could not be calculated and the size was evaluated by the bubble areas on the photographs. Measurements were performed with an Ott planimeter giving bubble areas in square millimeters. At the end of the observation period the animals were killed by exsanguination.

Statistics

Average values are given ± SEM. Comparison of means is done by simple *t* test (6).

RESULTS

General condition

The animals were observed for up to 4 h after decompression and remained apparently unaffected as evaluated by arterial mean blood pressure and respiration. Occasional bubbles might be seen in the abdominal adipose tissue at the termination of the experiment. No bubble formation was observed in blood vessels or in the medulla. Seven out of 38 experiments had to be discarded for technical reasons.

Mean arterial pressure (MAP)

During the 4 h air breathing under pressure, MAP was in the range of 140–180 mmHg. During decompression it fell to a level between 80–150 mmHg, the most frequent interval being 120–140 mmHg. In the air and heliox breathing experiments

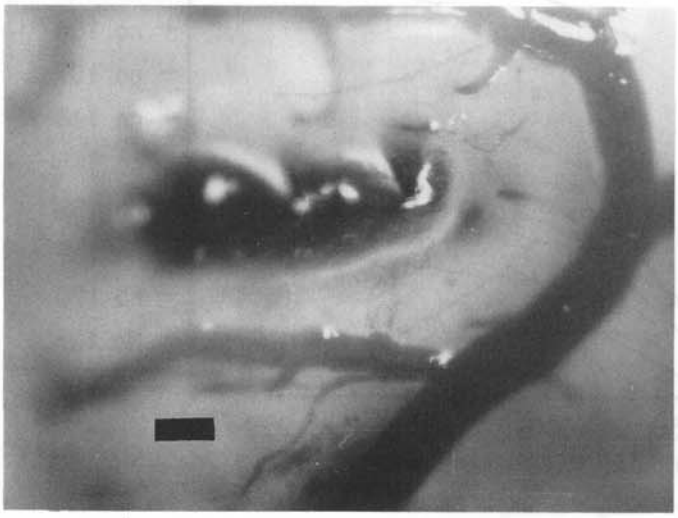

Fig. 2. Photomicrograph of bubble as seen at the surface of the medulla. Bar = 200 μm.

blood pressures remained at this new postdecompression level. During O_2 breathing the blood pressure increased about 20 mmHg and remained at this new level. If the breathing medium was changed from heliox to N_2O-O_2, a transient increase of about 10–15 mmHg was seen.

Size of bubbles and state of spinal cord tissue

Although the injected volume of air was 1–1.5 μl in all experiments, the visible area of the injected bubble varied from about 0.02 to 0.6 mm^2 at the beginning of the observation period. This suggests that a variable fraction of the injected volume escaped from the bubble, most likely during the injection or when the microneedle was withdrawn. An alternative explanation could be that air might distribute more or less deeply into the tissue, although the near circular shape of bubbles cut transversely (Fig. 1) is evidence against this possibility. During the whole observation period, circulation in the bigger pial vessels was lively whereas the blood stream in the smallest vessels close to the bubble was impeded. No bubble formation was observed in blood vessels or in the medulla. The temperature at the surface of the tissue varied between 36° and 39.5°C, depending on the switching on and off of the heating lamp.

Effect of air breathing on injected bubbles

The average bubble area at the first observation was 0.171 ± 0.052 mm^2. All injected air bubbles increased in size for the first 2 h after decompression (Fig. 3). Thereafter three of nine bubbles began to shrink, and one of them disappeared.

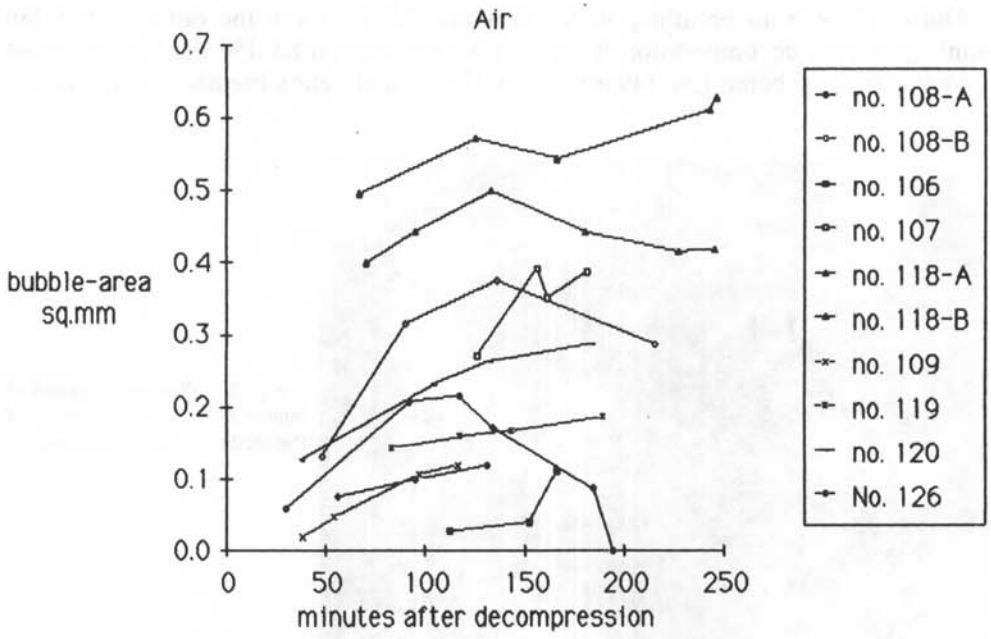

Fig. 3. Changes in bubble area during breathing of air.

Effect of heliox breathing on injected bubbles

These bubbles measured at an average 0.209 ± 0.029 mm^2 at the beginning of the observation period. During heliox breathing all bubbles consistently shrank and disappeared (Figs. 4 and 5 *right*). The time from the beginning of heliox breathing to disappearance of the bubbles averaged 104 ± 11 min.

Effect of N_2O-O_2 after heliox breathing

Bubbles that were shrinking during heliox breathing grew rapidly if the breathing medium was switched to N_2O-O_2 (Fig. 5 *left*). If the shift was done within 1 min after the disappearance of the bubble, it did not reappear (Fig. 5, *right*).

Effect of O_2 breathing on injected bubbles

In these experiments the bubble areas averaged 0.150 ± 0.031 mm^2 at the first observation. During breathing of 100% O_2 all bubbles initially grew (Fig. 6); 51 ± 8.5 min after the start of oxygen breathing they began to shrink and disappeared 104 ± 8 min after oxygen breathing was begun. In the initial phase the bubble areas increased by an average factor 1.9 ± 0.25, which must imply a relatively greater increase in volume.

Comparison of air, heliox, and O_2 experiments

The 3 groups did not differ significantly ($P > 0.1$) from each other with respect to the time from decompression to bubble injection nor with respect to first observed

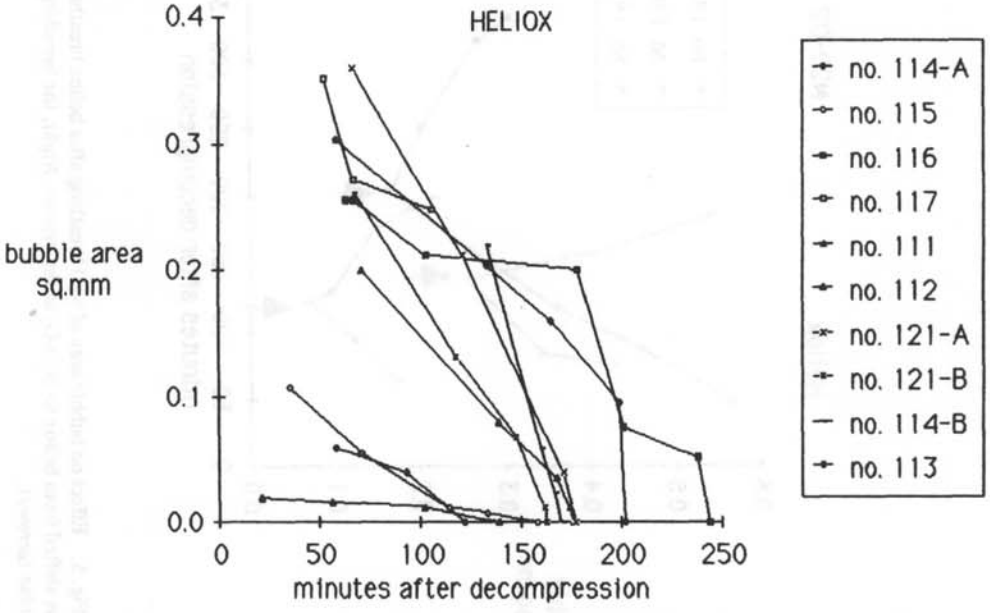

Fig. 4. Effect of heliox breathing on bubble area. Heliox breathing started at the first point of each curve. Experiment 117 was discontinued for technical reasons before disappearance of the bubble.

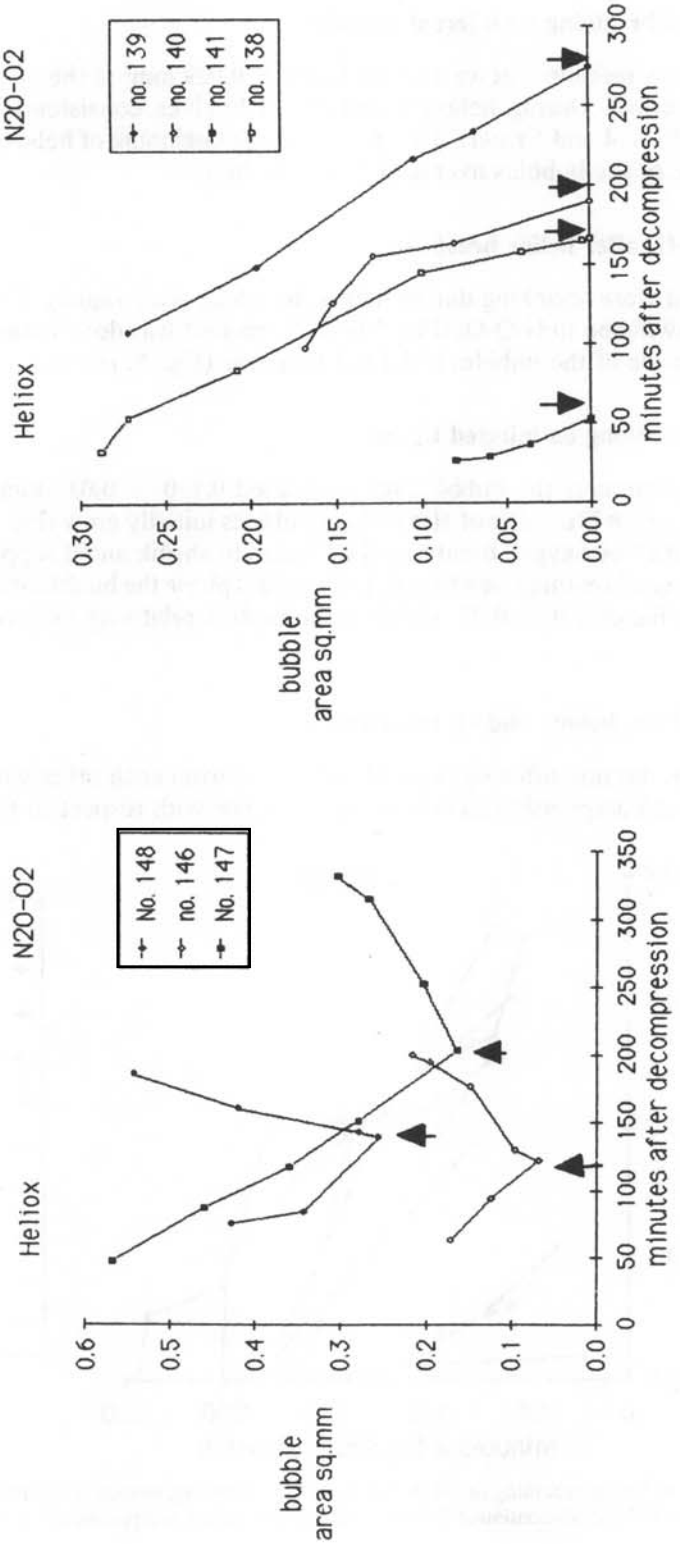

Fig. 5. Effect on bubble area of N_2O breathing after heliox breathing. Heliox breathing started at the first point of each curve. *Left*, the breathing medium was shifted from heliox to N_2O-O_2 at the *arrows*. *Right*, the breathing medium was shifted from heliox to N_2O-O_2 a few minutes after disappearance of the bubble (*arrows*).

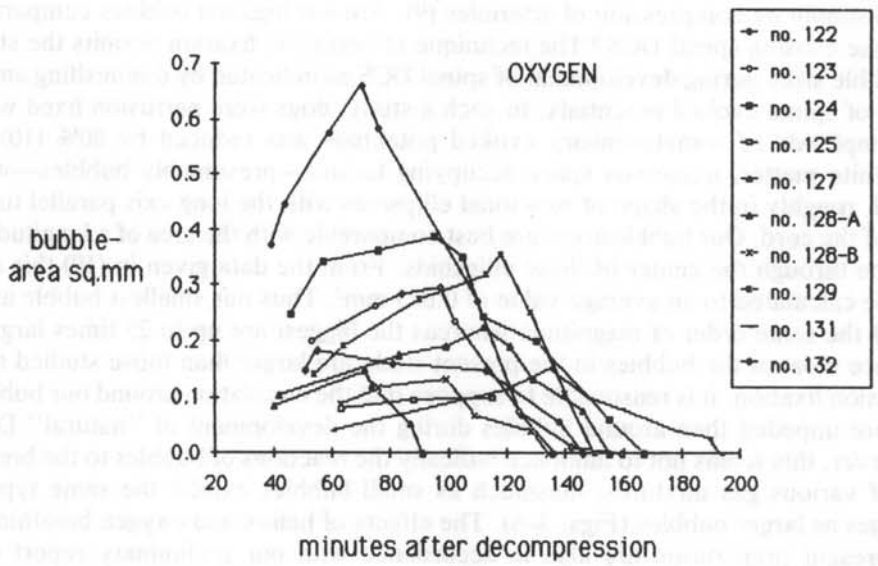

Fig. 6. Effect of oxygen breathing on bubble volume. Oxygen breathing was started at the first point of each curve. Experiment 125 was discontinued when the oxygen supply ran out.

size of the bubbles. Nor did the time from bubble injection to gas shift differ significantly from the heliox (34.6 ± 5.8 min) to the oxygen experiments (24.1 ± 2.4 min). The average time from gas shift to disappearance of bubbles was identical in the heliox and oxygen experiments.

DISCUSSION

Injection technique

Although bubbles in *adipose* tissue are nearly always spherical (3), air injected into the spinal medulla will distribute along the neurons. Consequently, the injected bubble is usually oblong in the cranio-caudal direction, its shape can be irregular, and it is impossible from the surface to measure how deep into the medullary substance air has penetrated (Figs. 1 and 2). Thus it is not possible to calculate the volume of the bubble. Instead we assess bubble size by the area the bubble presents on the surface of the medulla. The fact that the visible surface area may not always have the same relation to the volume adds an uncertainty to our results. However, the fact that each of the 3 groups of experiments presents a rather uniform picture supports the validity of the results.

Injected bubbles and spinal DCS

Boycott et al. (7) reported the presence of gas bubbles mainly in the white substance of the medulla of animals that died with spinal DCS. It has been proposed that such bubbles are the result of arterial gas embolism (7), are secondary to venous obstruction (8), or arise extravascularly from supersaturation and compromise the local

blood supply by compression of arterioles (9). Are our injected bubbles comparable to those causing spinal DCS? The technique of perfusion fixation permits the study of bubble sizes during development of spinal DCS as indicated by diminishing amplitudes of spinal evoked potentials. In such a study, dogs were perfusion fixed when the amplitude of somatosensory evoked potentials was reduced by 80% (10). In the white matter, numerous space-occupying lesions—presumably bubbles—were found, roughly in the shape of rotational ellipsoids with the long axis parallel to the axis of the cord. Our bubble areas are best comparable with the area of a longitudinal section through the center of these ellipsoids. From the data given in (10) this area can be calculated to an average value of 0.025 mm^2. Thus our smallest bubble areas are of the same order of magnitude whereas the biggest are up to 25 times larger.

Since most of the bubbles in the present study are larger than those studied after perfusion fixation, it is reasonable to suppose that the circulation around our bubbles is more impeded than around bubbles during the development of "natural" DCS. However, this seems not to influence radically the reactions of bubbles to the breathing of various gas mixtures, inasmuch as small bubbles exhibit the same type of changes as larger bubbles (Figs. 3–6). The effects of heliox and oxygen breathing in the present preparation are also in accordance with our preliminary report of a highly significant preventive effect of heliox as well as of oxygen breathing on the deterioration of spinal evoked potentials during the development of DCS in rats after an air dive (11). Of the two treatments, heliox was the more effective.

There is considerable variation in the rates with which spinal bubbles grow or shrink, probably corresponding to variation in the perfusion of the surrounding tissues. The tendency for big bubbles to change *volume* at a faster rate than small bubbles observed in adipose tissue (3) is less apparent in the present experiments where *bubble areas* are studied. This is not surprising considering the relationship between bubble volumes and areas as well as the shortcomings of the present method discussed above.

Effect of heliox and O_2 breathing on injected bubbles

The effects of varying breathing gas mixtures on air bubbles injected into spinal white matter were similar to those found on decompression-induced bubbles in adipose tissue (3).

The finding that N_2 bubbles in spinal white matter shrink during heliox breathing at a time when they would grow during breathing of air is consistent with the amelioration of spinal DCS during treatment with heliox reported by James (1), Douglas and Robinson (2), and others. Catron and coworkers (12) and Lillo and coworkers (13) found an aggravation of DCS during heliox breathing after experimental air dives of dogs and guinea pigs. However, in both these reports DCS seemed to be of the pulmonary "choke" type with no indication of spinal involvement, so an ameliorating effect was not to be expected.

It is remarkable that nearly all air bubbles grew after the switch to oxygen breathing, several growing for more than an hour and some more than doubling the visible area. In clinical practice, heliox treatment is combined with recompression, whereas our observations were carried out at 1 bar. The effect of pressurization on the gas exchange between blood and bubbles will be to increase the pressure gradients for N_2 as well as for He and thus to speed up the changes observed. At the same time,

the bubble volume will be reduced corresponding to the increase in pressure. This may prevent growth of bubbles during oxygen breathing. However, shrinking of bubbles may be delayed.

Our observations can be taken as evidence in favor of treating spinal bends with heliox rather than with oxygen breathing. It may also be advantageous to administer heliox rather than pure oxygen to a patient with spinal DCS during transportation to a recompression facility. Comparing the advantages of heliox and pure oxygen breathing in the treatment of spinal DCS, it can be argued that high oxygen partial pressures in the arterial blood promotes tissue oxygenation. However, as shown by Bergø and Tyssebotn (14), this effect may, at least in some regions of the CNS, be offset by vasoconstriction and blood flow reduction elicited by the high oxygen tension.

Gas exchange in spinal white matter

James (1) and Hills (15) have explained shrinking of N_2 bubbles in lipid tissues during heliox breathing by an outward flux of N_2 from the bubbles exceeding the inward flux of He, 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 N_2; in the case of diffusion limitation because the product of diffusion coefficient and solubility in lipid is greater for N_2 than for He.

James (1) also pointed out that at equal partial pressure differences the flux of O_2 in fat is twice that of N_2 and 4 times that of He, a fact that might cause growth of N_2 bubbles during O_2 breathing and be responsible for the occasional worsening seen when O_2 is used in the treatment of DCS.

Our previous observations on bubbles in adipose tissue (3) are consistent with the hypothesis of James (1) and Hills (15). In rats decompressed from a saturation exposure at 3.27 bar we observed that N_2 bubbles in adipose tissue grow during breathing of air, whereas they shrink and disappear during breathing of heliox. During breathing of O_2 most N_2 bubbles initially grow and then shrink and disappear.

In contrast, a N_2 bubble would be expected to gain He faster than it loses N_2 if it is situated in an *aqueous tissue* under circumstances where the gas exchange is limited by diffusion, because the product of solubility coefficient and diffusion coefficient in water is greater for He than for N_2 [physical constants for gases in (3)]. Since spinal white matter contains only 18% lipid (16, 17), our results suggest that the gas exchange in spinal white matter is predominantly limited by the blood perfusion. However, it can be objected that most of our bubbles are larger than the volume of a bubble that has separated from supersaturation. Consequently it can be expected to create more disturbance in the local circulation, and it cannot be excluded that the injected air creates a condition of perfusion limitation that would not exist around a smaller bubble. If this were the case, minute bubbles might grow until gas exchange became limited by the perfusion of the surrounding tissue, whereas bigger bubbles would shrink until perfusion was no longer limiting. Between these two situations a semistable bubble size might exist in which the opposing effects of diffusion and perfusion limitation would balance each other.

We have never observed shrinking bubbles reach such a stable "equilibrium volume" during heliox breathing. One reason for this might be that such a volume is below the resolving power of our microscope (bubble diameter ~10–20 μm). N_2O can be used as an amplifier of N_2 and He bubbles (5) because it dissolves 30 and 45

times better in blood than N_2 and He, respectively, and diffuses much faster through both lipid and aqueous tissues into a bubble than either N_2 or He can diffuse the opposite way given similar partial pressure differences (3). We have therefore tried to switch the breathing medium from heliox to an 80% N_2O-O_2 mixture before or just after the disappearance of the bubbles. Although visible bubbles grew after such a change, as seen in Fig. 5 *left*, they never reappeared if the change was done after the disappearance of the bubbles, indicating that bubbles did not persist at a submicroscopic volume (Fig. 5 *right*). Apparently they disappear from existence when they disappear from view. We take these observations as evidence against the gas exchange in spinal white matter being limited by diffusion in a predominantly aqueous medium.

CONCLUSION

Nitrogen bubbles in spinal white matter are eliminated in about the same time whether the breathing medium is oxygen or heliox. The finding that bubbles initially grow during oxygen breathing suggests that breathing of heliox may be advantageous in the treatment of such bubbles.

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REFERENCES

1. James PB. Problem areas in the therapy of neurological decompression sickness. In: James PB, McCallum RI, Rawlins JSP, eds. Report of proceedings of symposium on decompression sickness. Cambridge: European Undersea Biomedical Society, 1981:127–142.
2. Douglas JDM, Robinson C. Heliox treatment for spinal decompression sickness following air dives. Undersea Biomed Res 1988; 15:315–319.
3. Hyldegaard O, Madsen J. Influence of heliox, oxygen and N_2O-O_2 breathing on N_2 bubbles in adipose tissue. Undersea Biomed Res 1989; 16:185–193.
4. U.S. Navy Diving Manual, rev. 2, chapter 8. Washington, DC: Navy Department, 1988.
5. Van Liew HD. Dissolved gas washout and bubble absorption in routine decompression. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:145–150.
6. Kemp T. Statistiske metoder i medicin og biologi. Copenhagen: E. Munksgaard, 1941:103–107.
7. Boycott AE, Damant GCC, Haldane JS. The prevention of compressed-air illness. J Hyg 1908; 8:342–443.
8. Hallenbeck JM, Bove AA, Elliott DH. Mechanisms underlying spinal cord damage in decompression sickness. Neurology 1975; 25:308–316.
9. Hills BA, James PB. Spinal decompression sickness: mechanical studies and a model. Undersea Biomed Res 1982; 9:185–201.
10. Francis TJR, Griffin JL, Homer LD, Pezeshkpour GH, Dutka AJ, Flynn ET. Bubble-induced dysfunction in acute spinal cord decompression sickness. J Appl Physiol 1990; 68:1368–1375.
11. Hyldegaard O, Møller M, Madsen J. Protection against spinal decompression sickness by oxygen and heliox breathing. In: Sterk W, Geeraedts L, eds. Proceedings of the XVIth annual meeting of the European Undersea Biomedical Society. The Netherlands: University of Nijmegen, 1990:131–135.
12. Catron PW, Thomas L., Flynn ET, McDermott JJ, Holt MA. Effects of He-O_2 breathing during experimental decompression sickness following air dives. Undersea Biomed Res 1987; 14:101–111.

13. Lillo RS, McCallum ME, Pitkin RB. He-O_2 recompression treatment of decompression sickness in guinea pigs. Undersea Biomed Res 1988; 15:283–300.
14. Bergø GW, Tyssebotn I. Regional cerebral blood flow during exposure to 1, 3 and 5 bar oxygen. Undersea Biomed Res 1989; 16 (suppl):75.
15. Hills, BA. Scientific considerations in recompression therapy. In: James PB, McCallum RI, Rawlins JSP, eds. Report of proceedings of symposium on decompression sickness. Cambridge: European Undersea Biomedical Society, 1981:143–162.
16. McIlwain, H. Biochemistry and the central nervous system, 2nd ed. Boston: Little, Brown & Co. 1959:25.
17. Tower DB. Chemical architecture of the central nervous system. In: Field J, Magoun HV, Hall VE, eds. Neurophysiology. Handbook of physiology, sect. 1, vol. III. Baltimore: Williams & Wilkins, 1960:1795.