Gas Nuclei, Their Origin, and Their Role in Bubble Formation

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Gas bubbles are the primary agent in producing the pathogenic effects of decompression sickness. Bubble formation during decompression is not simply the consequence of inert gas supersaturation. Numerous experiments indicate that bubbles originate as pre-existing gas nuclei. Radii are on the order of 1 μm or less. Heterogeneous nucleation processes are involved in generating these gas entities. Musculoskeletal activity could be the main promoter of gas nuclei from stress-assisted nucleation. The half-life and faculty for nuclei to initiate bubble formation during decompression depend on many factors. Oxygen window and surface tension are involved in resolving bubbles. Two factors have been proposed to stabilize gas nuclei against dissolution: gas nuclei trapped in hydrophobic crevices and gas nuclei coated with surface-active molecules such as surfactants. Diffusion and surface tension could play an important role in the formation of gas nuclei crevices. However, while the concept of in vivo hydrophobic crevices remains a theoretical possibility, none have yet been identified in tissues and/or in microcapillaries. Moreover, while surfactants seem present in numerous tissues and could play a role in gas nuclei stabilization, they could be also involved in bubble elimination. The understanding of such mechanisms is of primary importance to neutralize nuclei and for modeling bubble growth. Here we present in a single document a summary of the original findings and views from authors in this field.

Keywords: bubble, gas nuclei, nucleation, diving, decompression sickness.

DURING DIVING WITH compressed gas, inert gas is loaded into tissues at depth and eliminated during decompression. Supersaturation is usually regarded as the driving force leading to the gas leaving solution and forming bubbles. From animal studies, Bert (7) highlighted the relationship between bubble formation and decompression sickness (DCS). Boycott et al. (11) developed the first efficient decompression tables in order to protect the diver from a critical level of supersaturation, preventing bubble formation and DCS. Behnke (6) suggested that these bubbles could occur without DCS. The development of ultrasonic techniques confirmed this notion of "silent" and circulating bubbles in the venous stream, without any clinical sign of DCS. Since Harvey (33), the origin and site of bubble formation have been discussed, since bubbles may not directly arise de novo from supersaturation; they might grow from pre-existing gas cavities called gas nuclei.

The purpose of this paper is to review and critique the evidence and theories concerning the role of gas nuclei in bubble formation in biological systems. We describe: 1) how gas nuclei are formed; 2) how they are stabilized; 3) how they grow; and 4) where they are located. The understanding of these mechanisms is of primary importance to neutralize gas nuclei and prevent decompression sickness.

REVIEW

Bubble Formation from Supersaturation

Pressure difference may be regarded as the driving force for bubble growth. The rate of bubble growth is largely determined by gas diffusion, which depends not only on pressure difference, but also on surface area, diffusion constants, and gas solubility. However, the supersaturation required for bubble formation is unattainable in human hyperbaric exposure. Experimental evidence has confirmed theoretical predictions that de novo bubble nucleation in pure water requires vapor supersaturation (tension or superheat) of about 140 MPa (86). Under most in vivo or in vitro conditions, bubbles grow at low supersaturation. For example, decompression from only 0.135 MPa pressure following a 48-h exposure in an underwater habitat generates bubble formation in 50% of humans (21). This suggests that bubbles grow from pre-existing and stable microscopic gas cavities called gas micronuclei (34).

Evidence for Gas Nuclei

From his studies of the effects of ultrasound, Harvey was the first to suggest that there were gas nuclei present in biological systems (33). Harvey et al. (34) demonstrated that when applying hydrostatic pressure to water, the number of bubbles that appear during subsequent decompression could be reduced. As such, hydrostatic pressure was used as a specific test to indicate the presence of gas nuclei.

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Evans and Walder (22) provided the first evidence for gas nuclei in living organisms using pressure pre-treatment. They submitted 2 groups of 50 transparent shrimp to decompression from normal atmospheric pressure to 60,000 ft of altitude. One group previously had been treated hydrostatically at a pressure of 38.9 MPa. Another group was not pressure-treated. On decompression of the pressure-treated group, bubbles were observed in four shrimp. In the non-pressure-treated group, bubbles were observed in 48 out of 50 shrimp. The marine animal experiments (16,22) provided evidence for gas nuclei but did not demonstrate their role in decompression sickness.

Another link was demonstrated by Vann et al. (70). Their experiment consisted of pre-treating rats at pressure before decompression from a 2-h exposure at 0.9 MPa in air. Without pressure treatment, the incidence of decompression sickness was 83%. With a brief pressure treatment at 3.1 MPa, the decompression sickness incidence was 64%. Thus, pressure treatment seems to reduce decompression sickness in rats similarly to the way pressure eliminated gas nuclei in marine animals.

Johnson and Cooke (48) studied seawater and found that the gas nuclei they identified were destroyed by an over-pressure of only several feet of seawater. Yount (81,85) studied water and gelatin. But beware of equating the mechanisms of in vivo and in vitro bubble formation. Radii are on the order of 1 μm or less and can be three orders of magnitude smaller (48,81,85). These results are consistent with theoretical studies that predicted 0.1 μm or less for an initial bubble size (15,74).

The Physics of Gas Nuclei Formation

Homogeneous nucleation: Homogeneous nucleation arises in the liquid phase without the prior presence of additional phases. It is a natural consequence of the distribution of thermal energy among the molecules comprising a volume of material. Because some molecules will be more energetic than others, random processes will occasionally produce groupings of higher energy molecules. If the average energy is high enough, such a grouping of molecules represents an inclusion of the material in a new phase. When the material is a fluid, the inclusion contains vapor. Because statistical fluctuations in the distribution of thermal energy occur continuously, tiny vapor inclusions are constantly forming and disappearing. These inclusions of vapor can, under certain circumstances, become cavitation nuclei in vivo (5,15,25,29,41,71).

Vaporous cavitation concerns the cases where the nucleus grows explosively and contains predominantly vapor. The best controlled conditions for inducing vaporous cavitation are achieved by ultrasonic techniques with high intensity ultrasound. It was through the study of acoustic cavitation that Harvey developed the idea of micronuclei in living systems (33). The theoretical approaches regard the process as essentially a mechanical failure of the internal cohesive forces of the liquid (28). For this reason the negative pressures needed to induce vaporous cavitation are often termed "tensile strengths." However, the various theoretical approaches (described as homogeneous nucleation) have produced estimates of tensile strengths for degassed water ranging from vapor pressure to 35 MPa negative pressure. It is unlikely that bubbles form in the body homogenously, as the magnitude of decompression required for this is far in excess of those experienced under any normal decompression activity (66).

It might be thought that surface tensions in physiologic fluids may be sufficiently low due to the action of surfactants, which may account for homogeneous nucleation at the low gas supersaturations known to elicit DCS occurrence in man (74). However, on the basis of studies of the effects of surfactants on bubble nucleation in water supersaturated with argon, Hemmingsen (37) concluded that the adsorption of surfactants of molecular weight greater than about 330 Dalton fails to occur at the gas-liquid interfaces of nascent bubbles fast enough to affect the spontaneous nucleation event.

Heterogeneous nucleation: In contrast, the generation of bubble nucleation at low supersaturation may be a result of various processes known as heterogeneous nucleation:

1) The consequence of a change in temperature, pressure, or tension can be involved. Sette and Wanderlingh (63), and Walder and Evans (72) postulated that cosmic radiation or the fission products of radioactive isotopes could be a factor contributing to the "normal" level of gas nuclei present in tissues. High-energy ionizing particles, such as neutrons in cosmic radiation or alpha particles from radioactive decay of U238 incorporated in the matrix of bone, deposit energy as they travel through tissue, producing a train of thermal spikes that can rupture with the formation of vapor-filled microcavities.
2) Domains of the new gas phase may also form around ions, impure molecules, or on dust particles. Such centers for growth are named heterogeneous nuclei since they differ in composition from both the new and the parent phases (20,73).

The heterogeneity of tissue compels consideration of this type of nucleation in vivo (66). The heterogeneous bubble nucleation should require lower gas supersaturations than homogeneous nucleation (2). Empirical studies of heterogeneous nucleation at solid surfaces are difficult because solid surfaces at which nucleation should theoretically occur most favorably are also those most able to trap pre-existing nuclei (66).

Stabilization of Gas Nuclei

Hypothesis of hydrophobic crevices: Harvey (34) first showed that when soda water flows over a dry glass surface, bubbles immediately appear. These small gas masses stick to any dirty, especially greasy, and hydrophobic surface, but not to clean wet glass. The sticking of gas bubbles is a matter of contact angles. Seen from the form of bubbles in capillary tubes or on surfaces, contact angles measure the degree of hydrophobicity. De novo formation of gas nuclei may theoretically occur on a surface even if the pressure difference is zero. This necessitates a surface crack or an acute angled cavity,

like that of an inverted cone. Statistical fluctuations in molecular behavior similar to those associated with Brownian movement might produce a very small nucleus at the bottom of the cavity. Once formed, the cavity will become spontaneously filled with gas if the receding contact angle measured between water and solid surface is equal to or greater than $90° + 0.5\ \phi$, where ϕ is the angle of the cone-shaped cavity (34). The sides of a hydrophobic crevice repel water and are not wetted.

The concave gas-water interface extends into the crevice that cannot be filled because water is repelled. As defined by Laplace's law, the surface tension at a convex gas-liquid interface causes a spherical bubble to dissolve by the outward diffusion of gas: $P_b = P_a + 2\ \gamma/R$, where P_b is gas pressure in the bubble; P_a, absolute pressure; γ, surface tension; π, surface pressure; and R, radius. Gas pockets trapped in the crevices of hydrophobic surfaces are stabilized against dissolution. As liquid enters a crevice, the radius of the concave gas-liquid interface decreases, which reduces the crevice pressure and decreases the outward diffusion of gas.

Surface tension at the concave gas-liquid interface of a gas nucleus in a solid hydrophobic crevice reduces the bubble pressure, stabilizes the gas, and increases its lifetime: $P_b = P_a - 2\ \gamma/R$. The sign of the surface tension is negative due to concave curvature rather than positive as with the convex curvature of a bubble, and this prevents the nucleus from dissolving (unless hydrostatic pressure is applied) (68).

Three phases of bubble growth have been identified with this crevice model (14): 1) increase in the convex curvature of the bubble interface until receding contact angle is reached; 2) rapid growth of the bubble up the crevice until the crevice mouth is reached; and 3) growth of a bubble outside the crevice, whereupon the bubble will be swept into the blood due to the forces applied by the blood flow.

Since hydrophobic surfaces are not identified in vivo, Hills (43) studied hydrophobicity of various endothelial surfaces from sheep and humans. Most surfaces were relatively hydrophilic, but some were distinctly hydrophobic, for instance a sheep's pulmonary vein, left ventricle, and aorta, and the human umbilical vein. Transmission electron microscopy of cerebral vessels demonstrated the evidence of an oligolamellar lining of surfactant on many endothelial surfaces, bridging the "tight" junctions between endothelial cells in many cases. These results are compatible with the theory that hydrophobic surface-active phospholipids (surfactants) migrate from lung tissue into the pulmonary circulation or reach intravascular sites from other sources.

The effectiveness of hydrophobic crevices in trapping and preserving a gas phase has been evaluated theoretically by several investigators. Lago et al. (50) presented a thermodynamic model from the heterogeneous nucleation theory in order to study a single-component system where droplets or bubbles are inside smooth crevices. For Tikuisis (65), the conical crevice model can be used to explain the sharp increase in the number of bubbles observed in shrimp for decompression ratios greater than 4:1. In accordance with the observed attenuating effects of pressure pretreatment on bubble formation in shrimp, the model can also be used to explain: 1) the evolution of the gas nuclei to smaller stable sizes during compression; 2) the return of the nuclei to their original stable configurations when the overpressure is removed; and 3) the necessity of having greater decompressions to generate bubbles from the nuclei as the magnitude and period of pressure pretreatment are increased. Tikuisis also introduced a new geometry of crevices with elliptically shaped walls, thus reducing the height of the crevice needed for bubble emergence and diminishing the constraints for the stability of gas nuclei. This new geometry also significantly reduces the height of crevices required for the prediction of bubble emergence by an order of magnitude when compared with the conical crevice. It at least satisfies the hydrophobic crevice condition as long as the crevice surface has a contact angle greater than 90°. AQ: 2

Chappell and Payne (14) presented a dynamic mathematical model of gas pockets in crevices and their behavior under compression. Although the metabolic gases may only represent a small amount of the gas in the bubble, their presence has a significant effect on the behavior of the bubble under decompression due to the high diffusivity of these gases. They found that the contribution from gas transfer through the bubble interface will be small, primarily because the interface surface area is at least an order of magnitude smaller than the crevice surface area. However, these descriptions have been limited so far to idealized hydrophobic, conical crevices. Clearly, such geometrical configurations would rarely occur in practical settings, while irregular pore geometries are more common.

Ryan and Hemmingsen (62) investigated the bubble formation properties of various porous surfaces at low gas supersaturations. In addition, both hydrophobic and hydrophilic porous surfaces were examined for their ability to initiate the formation of gas bubbles in view of the unique and extensive geometry of the crevices, and the fact that some models do not depend exclusively on hydrophobic crevices. The results suggest that gas not only may be trapped in the main pores of these particles, but also may be dispersed in undefined micropores, which may resist collapse by hydrostatic pre-treatments of the magnitudes and/or durations used in the present study.

It was shown that conical crevice geometry is not required for the effective formation of bubbles at low gas supersaturation since these surfaces actually contained a network of irregular channels. This complex geometry might allow even hydrophilic surfaces to effectively maintain a formed gas phase. However, these hydrophilic surfaces are poor sites for bubble formation, even at moderate gas supersaturation.

Surface-active coatings: The occurrence of stable bubble nuclei is surprising considering that a gas phase larger than 1 μm should rise to the surface of a standing liquid, whereas smaller ones should dissolve rapidly due to surface tension. Several stabilizing mechanisms have been suggested. Fox and Herzfeld (27) and Yount et al. (79,83,84) reviewed these theories and concluded

that gas nuclei could be held intact by surface-active skins that are initially permeable.

Experiments showed that the stabilization of gas phases in water is often attributable to the presence of surface-active substances. Surface-active molecules are amphiphilic, i.e., they contain both hydrophobic and polar constituents. This can lead to the formation of micelles. Surface-active molecules also tend to accumulate at the level of the liquid-gas interfaces, aligning themselves and thus creating monomolecular films. Evidence that gas nuclei are surrounded by films or skins of varying permeability was obtained from a detailed analysis of bubble counts in supersaturate gelatin. For Yount and Hoffman (82), these skins are normally permeable, but they become impermeable if the ambient pressure is increased rapidly to a sufficient extent (varying permeability model, VPM). The skins are permeable during decompression. Nuclear radii range up to about 0.6 μm prior to compression, but they are reduced to significantly smaller sizes, and not eliminated altogether, by rapid increases in ambient pressure. The ability of adsorbent molecules to be "squeezed out" of the layer with sufficient compression as the nucleus diminishes in size is a central feature of the VPM. This behavior is in accord with that observed in other physiologic liquid-gas systems (59).

The life span of a spherical bubble is increased by a shell of surface-active material whose surface pressure (π) reduces surface tension, decreases the outward diffusion of gas, or provides mechanical stability. Surface tension and diffusion are reduced by surface-active molecules at the gas-liquid interface. This stabilizes the bubble (gas nucleus) and increases its lifetime: $P_b = P_a + 2(\gamma - \pi)/R$; with P_b being gas pressure in the bubble; P_a, absolute pressure; γ, surface tension; and π, surface pressure (68).

Biochemical mediators—NO, HSP: Wisloff et al. (77,78) have shown that a single bout of high-intensity aerobic exercise 20 h before the dive suppressed bubble formation and prevented death in rats. This beneficial effect seems essentially related to an increase in vascular endothelial nitric oxide (NO) bioavailability (increase in NO production and/or decrease in NO inactivation). Although the observed effects of NO on vascular bubbles might be explained by the increase in perfusion (and gas nuclei elimination), it has been speculated that NO could reduce hydrophobicity of the endothelial wall, reducing the number of nuclei adhering to the surface. However, it has been shown that bubble production is increased by NO blockade in sedentary but not in exercised rats. This indicates that the exercise effect may be mediated by others factors than NO.

Heat shock proteins (HSP) present in most cells, including endothelial cells, play a key role in normal cellular homeostasis and cell protection from damage in response to stress stimuli. It is well documented that endurance exercise is a stressor that increases the HSP70 expression (49). It has been also demonstrated that heat shock pre-treatment before diving enhanced the expression of HSP70 and protected rats from air embolism-induced lung injury (45). Thus, it is conceivable that exercise-induced HSP70 production affects bubble formation after diving with a different mechanism than the NO pathway (8).

Gas—O_2, CO_2: Once a bubble has nucleated, its content and size will change according to the prevailing difference between the dissolved gas tension and the bubble gas pressure at the gas-liquid interface. A highly soluble gas like CO_2, even at low tension, may play an important role in the early growth of a bubble by diffusion (31,32). For example, a rapid passage of CO_2 out of bubbles as well as entrance into bubbles can be observed 38 times more rapidly than with N_2 in the same conditions (47).

When living animals are in steady state, the sum of the partial pressures of dissolved gas in the tissues is usually less than atmospheric pressure, a phenomenon known as "the oxygen window" or "inherent unsaturation" (6,42). This is because metabolism lowers the partial pressure of O_2 in tissue below the value in arterial blood and the binding of O_2 by hemoglobin causes a relatively large P_{O_2} difference between tissues and arterial blood. At sea level, the nitrogen partial pressure in the bubble is higher than the tissue nitrogen tension, and nitrogen diffuses from the bubble into the tissue, from where circulation carries it to the lungs. At depth, there is a large nitrogen diffusion gradient from the bubble to the tissue, and the bubble begins to dissolve rapidly. The high nitrogen level in the lungs, however, causes tissue to absorb nitrogen, which reduces the bubble resolution rate (68). With DCS bubbles, the window is a major factor in the rate of bubble shrinkage when the subject is in a steady state, modifies bubble dynamics when inert gas is being taken up or given off by the tissues, and may sometimes prevent the transformation of bubble nuclei into stable bubbles (67).

An oxygen window in a crevice model will mean that if the bubble is in equilibrium with the tissue, it will be undersaturated as compared with the blood. Hence, gas transfer would be expected across the bubble interface. However, this component is likely to be small since the interface surface area is smaller than the crevice surface area (14).

Gas Nuclei Growth by Hydrodynamic Cavitation

Nucleation can be facilitated at relatively low apparent gas supersaturation by hydrodynamic or mechanical effects that decrease the hydrostatic pressure and increase the prevailing gas supersaturation in small, localized regions of tissues (66). Thus, musculoskeletal activity has long been recognized as facilitating DCS (75). Normal day-to-day ambulation and exercise is thought to produce populations of nuclei that persist as the product of a dynamic equilibrium between nuclei production and dissolution.

The most telling evidence of this concept comes from the work of Powell et al. (61) at NASA. This research involved depressurizations from sea-level pressure (saturation), thus eliminating gas uptake as a variable. The work was started following the observation that during extravehicular activity astronauts appeared to be experiencing DCS at a much lower incidence than predicted from the studies employed to develop the decompression protocols on Earth. Powell put forth the

hypothesis of stress-assisted nucleation from hydrodynamic cavitation being much less in null gravity than in the 1-G environment on the ground. Powell et al. (61) decompressed subjects from 0.1 MPa to 0.043 MPa for 3 h after either being fully ambulatory at unit gravity or being hypokinetic and adynamic (simulated microgravity of 3-d bed rest). The results indicated a reduction in whole-body gas phase formation in individuals after bed rest when compared with being fully ambulatory. These results are compatible with a hypothesis relating stress-assisted nucleation to the continual formation of tissue gas nuclei and their gradual depletion with hypokinesia. Additional experiments lead to the development of NASA decompression schedules for astronauts during extravehicular activity. The main forms of stress-assisted nucleation are described below.

Reynolds' cavitation: Osborn Reynolds (17,34) described the water flow through a tube which has a constriction. The velocity is increased at the constriction point and, by Bernouilli's Law, the pressure must decrease. Under the proper conditions of fluid velocity, low pressures will be produced and the water will suddenly break into cavities while producing a sound. Reynolds called the phenomenon "the boiling of water in an open tube at ordinary temperature." Reynold's cavitation might play a role in the streaming of extracellular fluid during musculoskeletal activity.

Viscous adhesion: Viscous adhesion (3,13,35,46) is a mechanism for the temporary production of low pressures with motion. This is sometimes referred to as tribonucleation. It causes bubble formation as a result of large negative pressures generated by viscous adhesion between surfaces separating in liquid. It occurs when two closely opposed surfaces separated by a thin film of viscous liquid are pulled rapidly apart. Viscosity prevents the liquid from filling the widening gap, resulting in negative pressure. The bubble formation is found to be in proportion to the product of the viscosity of the fluid and to the velocity of the separation from the solid surfaces (46). Viscous adhesion could be involved in different situations.

1.) Bubble formation from movements: McDonough and Hemmingsen (55,56) studied young specimens of trout, catfish, sculpin, and salamanders. They were equilibrated with elevated gas pressures, and then rapidly decompressed to ambient pressure. The newly hatched forms tolerated extremely high gas supersaturations; equilibration pressures of 8–12 MPa argon or 15–25 MPa helium were required for in vivo bubble formation. During subsequent larval development, the equilibration pressures required decreased to just 0.5–1 MPa and bubbles formed in the fins. By anesthetizing older fish before decompression, the bubble formation was prevented in the fins. This suggests that swimming movements mechanically initiate bubbles, possibly by a hydrodynamic cavitation mechanism.

   McDonough and Hemmingsen (53,54) also studied bubble formation in various crustaceans equilibrated with high gas pressures and rapidly decompressed to atmospheric pressure. The species varied widely in tendency to bubble formation, and adults were generally more sensitive than animals at larval stage. Bubbles formed in the leg joints of megalopa and adult crabs following decompression from only 0.3–1 MPa argon; the stimulation of limb movements tended to increase this bubble formation, whereas the inhibition of movements decreased it. High hydrostatic compressions applied before gas equilibration or slow compressions did not affect bubble formation. The authors concluded that stress-assisted nucleation appeared to be the primary cause of the bubbles in crabs.

2.) Bubble formation from joints: Fick (24) ascribed the vacuum phenomenon to reduced pressure in the joints as a result of movement, a mechanism currently recognized as viscous adhesion. The collapse of a bubble from vaporous cavitation is associated with the sound of a "cracking joint." As the dissolved gas content increases or the hydrostatic pressure decreases, a transition occurs from vaporous to gaseous cavitation, which is soundless and leaves a stable bubble. The vacuum phenomenon is associated with aging joints, injury, or structural pathology and is frequently seen in intervertebral disks, but also has been observed in the epidural space surrounding the spinal cord. Ford et al. (26) found the gas in a lumbar disk to be 90% nitrogen. Generally, these large gas volumes are not associated with any DCS, however.

3.) Bubble formation from heart valve: Clinical studies using transcranial Doppler ultrasonography have shown the presence of emboli in the cranial circulation of some mechanical heart valve patients (30). Meanwhile, transesophageal echocardiography of mechanical heart valve patients has shown images of bright, mobile signals (also considered to be gas bubbles) near the valve. However, implanted heart valves have been exposed to air, thereby potentially causing the formation of gas-containing crevice nuclei that could result in bubble formation unrelated to bubbles following physiological decompression. In vitro studies performed to investigate the relationship between dissolved gas concentration and the incidence of bubble formation after valve closure (10) indicated that stable gas bubbles can form during mechanical heart valve operation. The bubbles likely form from the combined effects of gaseous nuclei formed by cavitation, low-pressure regions associated with regurgitant flow, and the presence of CO_2, a highly soluble gas. Hennessy (40) has proposed that arterial microbubbles, nucleated primarily in circulatory turbulence at the tips of the cusps of the pulmonary valve, are the primary cause of the common forms of DCS, although other mechanical effects must also be considered. However, if Hennessy's hypothesis of arterial microbubbles were correct, one would expect that arterial bubbles and arterial gas embo-

lism would be a major factor in DCS. This does not seem to be so (66).

Reservoir of Gas Nuclei in vivo

Bubbles are not formed in single living cells, such as Euglena, Paramecium, Amoeba, Arbacia and Asterias eggs, and the alga, Nitella, when submitted either to high vacuum or on decompression after previous exposure to high gas pressures (34). Hemmingsen et al. (39) observed the lack of bubble formation in different hypobarically decompressed cells. Suspensions of human erythrocytes or of unicellular microorganisms (*Tetrahymena pyriformis*, *Euglena gracilis*, *Escherichia coli*, and *Microcyclus aquaticus*) were equilibrated with nitrogen gas pressures up to 20 MPa and rapidly decompressed to hypobaric pressures below the vapor pressure of water. None, or only a few cells, were damaged in each case, and bubbles were never observed intracellularly after decompression. Hemmingsen (38) studied the bubble formation of hydrophobic particles in water and cells of *Tetrahymena*. Particles that were particularly effective as generating bubbles were added to suspensions of ciliates. On their ingestion, all of the particles lost their ability to induce bubble formation in the supersaturated cells. Apparently, surface nuclei were lost. In view of such extreme tolerances, it is doubtful that bubbles originate intracellularly during decompression of multicellular organisms, in which bubbles occur with far lower gas supersaturations.

The venous blood is the easiest place to detect bubbles, but experiments suggested that they do not form there. Lee et al. (52) investigated bubble formation in the inferior vena cavae of dead rats after 6–15-h exposures to air at 12.3 MPa and decompression to 0.1 MPa at 1.36 MPa $\cdot$ min^{-1}. Bubbles were detected by light microscopy, buoyancy, and underwater dissection. No bubbles were formed in 42 blood-filled vena cavae that were isolated from the minor circulation (capillaries) by ligatures, but bubbles were always observed in unisolated vena cavae. These results strongly indicate that nuclei are not present in blood, even at supersaturations that are significantly higher than those experienced in vivo. This clearly demonstrates that bubbles observed in blood using ultrasonic techniques or the nuclei from which they may be formed, could originate from tissues and/or microcapillaries and migrate into blood circulation. The contact between adjoining endothelial cells on the capillary walls could be a site for crevice nuclei. The extravascular space could be an alternative location: as extravascular nuclei (small bubbles) expanded, they might rupture capillaries, thereby seeding the blood with gas (68).

Blood may be resistant to bubble formation, but skin is not, and there are gas nuclei in the outermost layer of the skin that cavitate when they are irradiated by ultrasound (58). The only other tissues in which bubbles (vacuum phenomena) are routinely observed are the joints, including the spine. These are the structures most frequently affected by DCS and, except for the spinal cord itself, are likely sites for viscous adhesion. Although vacuum phenomena are common and would be expected to expand in response to decompression, no direct evidence links them to DCS (68). Tissues in which bubbles do not form at physiologic supersaturations would be expected to be affected only by vascular bubbles that originate at other sites, i.e., lungs, brain.

Obstruction of the venous drainage of the spinal cord by bubbles has been observed in animals and has been proposed as a mechanism for spinal injury. This mechanism depends on the sluggish nature of the venous circulation of the cord, which is predisposed to stagnation when bubbles are present. However, spinal cord lesions in human and animal decompression injury occur most often in the extravascular white matter (68). In fact, experiments suggested that extravascular or "autochthonous" bubbles probably arise as an artifact and that intravascular bubbles can occur in spinal vessels after decompression (60).

It is assumed that a population of nuclei already exists in the tissues, but the distribution of these nuclei could be modified in specific sites. As we described above, surface-active phospholipids like surfactants could play a role in determining the gas nuclei coating and its stabilization. Hills studied spinal cord DCS, which is more frequent than cerebral DCS, with a ratio often quoted as 3:1 (44). Hydrophobic protein (HP) was discovered in sheep spinal tissue at roughly three times (3.3:1) the level in the brain, by several times higher than in skeletal muscle or plasma. Extravascular lamellar bodies of largely phospholipid (PL) were also found in spinal tissue by electron microscopy using a special fixative. In this case, the population was 4.1 times higher than in brain tissue, where some lamellar bodies were found adjacent to vascular endothelium. Extracts of spinal surfactant (HP+PL) were found to be particularly surface-active. The PL/HP surfactant complex was found to render surfaces hydrophobic when they were able to initiate "strings" of bubbles in supersaturated gases solutions. These results could highlight the link between gas nuclei from which bubbles are formed and spinal DCS exacerbated by the large quantities of spinal surfactant present.

DISCUSSION

While the concept of in vivo hydrophobic crevices remains a theoretical possibility, none have yet been identified. Hills (43) studied contact angles (not bubble formation) on endothelial surfaces and reported contact angles consistent with hydrophobicity. These studies were done with specimens that had been removed from their natural environment (blood) and exposed to air. No bubble formation was observed, however, when isolated endothelium in continuous contact with blood was decompressed (52). Perhaps contact with air (which denatures proteins) rendered the endothelium hydrophobic in Hills' studies. Hydrophobic crevices, on the other hand, are clearly identified and described in the case of in vitro bubble formation. Brubakk (12) has postulated that hydrophobic sites could exist on the surface of the endothelium in the form of caveolae. Caveolae are little invaginations made of lipids in the cell membrane connected to the plasmalemma by a neck-like structure. Molecules from the medium enter the indentation, which then closes itself off into a bub-

ble that migrates into the cell interior. Caveolae are present in continuous capillary endothelia, including central nervous system microvasculature. The geometry of these sites is likely to be more akin to cylinders than the conic crevices. The effects on the behavior of the bubble for more complex geometries than the conical crevice must be considered in future work (14).

Surfactants preferentially populate gas-liquid interfaces. They stabilize the microbubble and increase its lifetime. It has been suggested that musculoskeletal activity could generate surface-active molecules with a shift of surfactant species on the gas nuclei interface (43). On the other hand, there have been studies that demonstrate the beneficial effect of surfactants on bubble elimination. It has been suggested that the addition of surfactants to blood makes it feasible to manipulate interfacial stresses and prevent or reduce formation of the adhesion responsible for trapping intravascular gas bubbles. In vivo studies have shown that the addition of surfactants favorably alters the patterns of deposition and accelerate the rates of clearance of embolic bubbles. Experiments are consistent with the concept that bubble surface coverage with the surfactant out-competes protein adsorption and reduces formation of adhesion interactions with the vessel wall. Surfactants seem to preserve basic endothelial structure and vasodilator function despite attempts to damage the endothelium (64). Finally, while surfactants could play a role first in microbubble stabilization, they could also be involved at last in vascular bubble elimination.

In summary, we believe that many gas nuclei have a limited lifetime in the blood stream because they are dissolved by oxygen window and surface tension; however, viscous adhesion resulting from physical activity continuously generates new gas nuclei. Observations in crabs and shrimps suggested that gas nuclei are mainly generated during motion. Thus, there would be a dynamic equilibrium between the creation and resolution of gas nuclei (61,68). On the other hand, we believe that crevice nuclei are probably present in the microvasculature wall and are able to resist collapse under both normal and raised atmospheric pressure. Diffusion in crevice surface area and/or viscous adhesion could be involved in crevice nuclei growth. The effect of muscular contraction on crevices might be expected to squeeze the gas pocket and potentially cause the release of free bubbles (14).

Control of Gas Nuclei and Practical Applications in DCS Prevention

Pressure before exposure: Short high pressure application before exposure can reduce bubble formation and decompression sickness in animals. In man high pressure pre-treatment has not been evaluated in DCS prevention.

Oxygen before exposure: Oxygen breathing is a common procedure employed to reduce the risk of DCS before high altitude exposure (4) or before extravehicular activity in space (69). Latson et al. (51) used oxygen to denitrogenize humans after a simulated air saturation dive in a study of safe escape from a disabled submarine. They showed that prolonged decompression with oxygen reduced the incidence of DCS only when the subjects breathed oxygen at the saturation pressure of 250–280 kPa for a period of 2–3 h beforehand. The authors explained this reduction in the incidence of DCS as being due to the elimination of nitrogen from the body tissues and the possible elimination of gas nuclei. Arieli et al. (1) studied the possibility that hyperbaric oxygen could replace the resident gas in the nuclei by oxygen and, because of the metabolic role of the latter, eliminate the nuclei themselves. After pre-treatment with oxygen, prawns were saturated with nitrogen to 203 kPa before explosive decompression at 30 $m \cdot min^{-1}$. The authors found that pre-treatment with hyperbaric oxygen significantly reduced the number as well as the volume of bubbles and suggested that hyperbaric oxygen eliminates bubble nuclei in the prawn.

Surface tension: Hjelde et al. (36) showed that bubble formation after decompression was inversely proportional to pre-dive serum surface tension. A small surface tension difference between individuals may influence vascular bubble production. Fluid balance at the time of decompression significantly influences the incidence and the onset of DCS. Fahlman and Dromsky (23) showed that, after direct ascent from saturation conditions, dehydrated swine manifest severe DCS sooner and more often than their hydrated counterparts. It has been suggested that dehydration may reduce blood flow to poorly perfused tissues and/or that dehydration may decrease surface tension and thereby facilitate bubble formation and DCS risk. Changes in hydration status and surface tension may offer a relatively easy means of reducing DCS risk.

Exercise before exposure: Studies in rats have shown that a single bout of high-intensity aerobic exercise 20 h before the dive suppressed bubble formation and prevented death with no effect at any other time (48, 10, 5, and 0.5 h prior to the dive) (77,78). In man, a single bout of aerobic exercise 24 h and 2 h before a dive significantly reduced venous gas emboli and consequently could have a preventive effect on occurrence of DCS (8,19). The mechanisms underlying the protective effect of exercise on bubble formation remain unclear. They could be related to vasodilator substance production increasing blood flow and gas nuclei elimination.

It was also observed that the incidence of bubbles decreased when the rest interval from an anaerobic exercise (150 deep knee squats over a 10-min period) to altitude depressurization lengthened and was performed 1–2 h before exposure (18). These results are perhaps understandable based on the creation of gas nuclei by deep knee bends and their resolution within several hours as a result of the oxygen window and surface tension.

Chemical treatment before exposure: Wisloff et al. (78) speculated that both exercise and NOS hinder bubble formation via alteration in vascular endothelial properties since pre-existing gas nuclei are probably attached to the endothelium, where they grow into bubbles that are dislodged into the blood stream. An attractive hypothesis is that it may be possible to use either exercise or NO-releasing agents before a dive to inhibit bubble

formation and thus protect against DCS. They studied chronic and acute administration of a NO-releasing agent in rats. NO given for 5 d and then 20 h prior a dive to 700 kPa lasting 45 min breathing air significantly reduced bubble formation and prevented death. The same effect was seen if NO was given only 30 min before the dive.

Decompression models (76,80,82) indicate that decompression safety might be improved by adding "deep stops," timed pauses during ascent at greater depths than those included in conventional tables. Deep stops could be more efficient than shallow stops in reducing micro-bubble growth. Marroni et al. (57) suggested that introduction of a deep stop during human decompression after a repetitive dive (25 msw with a 3.5 h surface interval) reduces bubbles and fast-tissues gas tensions. However, the utility of deep stops in human decompression has yet to be confirmed for air dives between 50–60 msw (9).

Conclusions

Bubble formation during decompression is not simply the consequence of inert gas supersaturation. A large body of evidence indicates that bubbles originate as persistent bodies of undissolved gas called pre-existing gas nuclei. Heterogeneous nucleation processes are involved first for generating these gas entities. Musculoskeletal activity could be the main promoter of gas nuclei from stress-assisted nucleation, such as Reynolds' cavitation and/or viscous adhesion. Oxygen window and surface tension are involved in resolving bubbles. Two major factors have been proposed to stabilize gas nuclei against dissolution, one involving adsorption of surface-active molecules like surfactants, and the other involving geometrical factors such as intercellular crevices. Diffusion and viscous adhesion could play an important role in the formation of crevice nuclei. Surfactants seem present in numerous tissues and on many endothelial surfaces they could play a role in gas nuclei stabilization, but also in bubble elimination. Further experiments are needed to elucidate the role of such "stabilization factors" in vivo. The understanding of nucleation processes is of primary importance for modeling bubble growth. Pressure, metabolic gas, surface tension, exercise, and chemical treatment before exposure are able to play a role in controlling gas nuclei and preventing DCS.

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