

The redistribution of vascular bubbles in multiple dives

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Gait, D., K. W. Miller, W. D. M. Paton, E. B. Smith, and B. Welch. 1975. The redistribution of vascular bubbles in multiple dives. *Undersea Biomed. Res.* 2(1):42-50.—The behavior of vascular bubbles in anesthetized, dissected guinea pigs has been studied. The dissection allowed bubbles in the aorta and inferior vena cava to be observed during hyperbaric exposures. The object of the experiments was to seek evidence on the hypothesis that recompression after a prior dive can displace asymptomatic peripheral bubbles through the venous circulation to the lung; bubbles only pass through the lung to the arterial circulation if a critical volume of gas accumulates. This condition may be fulfilled after a severe single dive or after a less severe dive followed by a short dive which redistributes separated gas to the more vulnerable site (i.e. lung). The results showed that after a severe single dive there was a characteristic series of events—bubbles moving centrally in the inferior vena cava, respiratory distress, bubbles in the aorta and subsequently death. After symptomless first dives, however, subsequent dives elicited showers of bubbles in the vena cava which continued to appear for some time at pressure, and which led to the appearance of arterial bubbles on subsequent decompression. Arterial bubbles were followed by death.

bubbles	repetitive diving
animals	decompression sickness

The role of a separated gas phase in the aetiology of decompression sickness is not well understood. Although the immediate efficacy of recompression therapy implicates a compressible gas phase in the symptomatology of decompression sickness, the presence of asymptomatic or *silent* gas bubbles is more difficult to establish and is not acknowledged in the theories upon which decompression tables are based. Hempleman deduced the presence of silent bubbles as early as 1960, and Hills (1970) has challenged the assumption of Haldane that supersaturation of dissolved gas occurs in the tissues without phase separation unless the Haldane ratio is exceeded. The fact that Haldane's supersaturation ratio shows a species variation of an order of magnitude (e.g. 2 for man and 12 for mice) is a further argument against it having physical, as opposed to physiological, significance.

In a previous paper (Griffiths, Miller, Paton, and Smith 1971) we demonstrated that asymptomatic bubbles produced by decompression from an initial symptomless conditioning dive resulted in a heightened susceptibility to decompression sickness as revealed in subsequent test dives. In general as the time spent between dives increased the latent susceptibility first increased rapidly and then declined slowly—qualitatively, but not quantitatively—as would be expected from the predicted extravascular bubble growth and decay pattern. In addition, an extremely brief second dive gave rise to immediate symptoms which could only be explained by the redistribution of asymptomatic bubbles to a new, physiologically sensitive site. The present paper reports experiments in which this

redistribution of bubbles was confirmed both indirectly and by direct observation.

In the previous study mice were used as the experimental animal. For practical reasons guinea pigs have been used in this study and some initial experiments were carried out to establish the time course and degree of severity of the response to experimental dives in this animal. A second series of experiments reports on the direct observations of bubbles in anesthetized, dissected animals. Comparison of the dependence of susceptibility on the time spent at surface between two dives with previously reported data for mice and the direct observations both confirm our original hypothesis.

METHODS

Guinea pigs were exposed in a cylindrical pressure chamber of internal dimensions 60 cm (length) by 28 cm. A door at one end was sealed by a Greylork® seal. One window was located axially at the other end of the chamber and a second was located on the upper part of the cylindrical wall. The experimental apparatus was assembled on a tray bolted to the door before insertion in the chamber. The thermally insulated chamber had a bimetal device which controlled two 150-W heaters to maintain a temperature of 29-31°C. (The fire hazard inherent in the bimetal device does not recommend it for general use.) A powerful fan stirred the chamber gases and CO₂ was removed by soda lime. Experiments were carried out after flushing the chamber with pure oxygen. Pure nitrogen was used for compression, which was accomplished in 30 sec, and the gauge pressure thus registered the partial pressure of nitrogen. Decompression took about 1½ min, oxygen being added as necessary to maintain the PO₂ above 0.2 atm. After decompression animals were maintained on pure oxygen.

Guinea pigs (350-500 g) were used. Symptoms of decompression sickness included gradual loss of muscle tone, paralysis of hind legs, slowing and deepening of breathing with occasional tachypnea, and poorly characterized convulsions generally followed by death. In the guinea pig, paralysis of the rear legs and death provide clear objective criteria of decompression sickness. In quantitative work asymptomatic animals were scored zero, paralyzed animals ½, and dead animals 1. The results for a group of animals were expressed as a percentage of the maximum possible score. Trials showed that animals invariably died from decompression sickness within 20 min after decompression. Consequently in all single-dive routines animals were sacrificed at this point.

Animals were anesthetized with ethyl carbamate (1.6 mg/g injected intraperitoneally) for all operative procedures.

To enable vascular bubbles to be observed in the inferior vena cava and descending aorta the following procedure was carried out (Fig. 1). The anterior abdominal wall was incised; the rectum and the mesenteric artery were ligatured and cut close to the pelvis; the coeliac and anterior mesenteric arteries and the hepatic portal vein were ligatured and divided; the esophagus was ligatured below the diaphragm and cut; and the alimentary canal was then removed. The ureters were also ligatured and cut. The aorta and inferior vena cava were gently separated from the vertebral muscles and the ilio-lumbar artery and vein were ligatured and cut to enable a specially designed wedge-tipped *light pipe* to be inserted without occluding the blood vessels. Light was provided by a quartz iodide projector lamp located in the chamber. The light was filtered through a water tank before passing down the light tube to back-illuminate some 2 cm of the aorta and inferior vena cava, thus enabling small bubbles to be visualized clearly with a 3-D microscope outside the chamber.

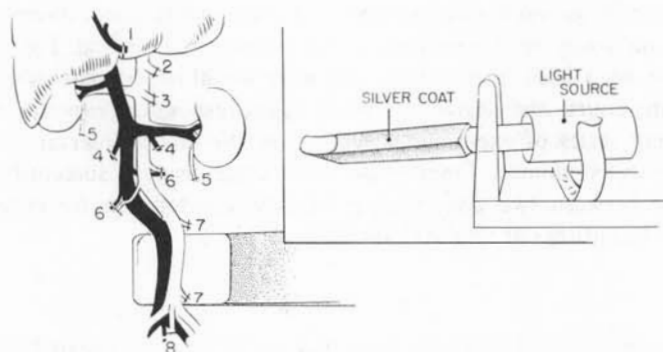


Fig. 1. Sketch showing the position of the tip of the light pipe used to back-illuminate the aorta and inferior vena cava (see text under *Methods*). The complete light pipe is shown inset. Key: 1, portal vein; 2, coeliac artery; 3, anterior mesenteric artery; 4, spermatic vein; 5, ureters; 6 and 7, ilio-lumbar arteries and vein; 8, posterior mesenteric artery.

RESULTS

Dose-response curves obtained with unanesthetized (A) and anesthetized (B) guinea pigs are presented in Fig. 2. The ordinate represents the percentage of animals that died following

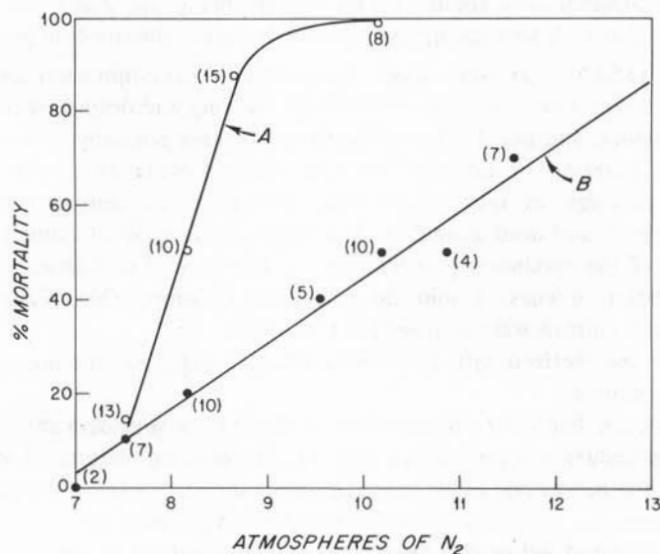


Fig. 2. The relation between exposure pressure (in atm of N₂; 1 atm O₂ was also present at all times) and percentage mortality of unanesthetized, ○, and anesthetized guinea pigs, ●, subject to a 15-min dive. Numbers against each point are sample size.

a 15-min exposure to hyperbaric nitrogen. The anesthetic confers a slight protection ($LD_{50} 10.25 \pm 0.55$ [S.E.] atm of N₂ compared to 8.1 ± 0.1 atm analyzed by the method of Waud [1972]), and reduced the slope of the dose-response curve.

Figure 3 illustrates the incidence of decompression sickness following a second dive as a function of the time spent between dives. As observed with mice, the incidence first

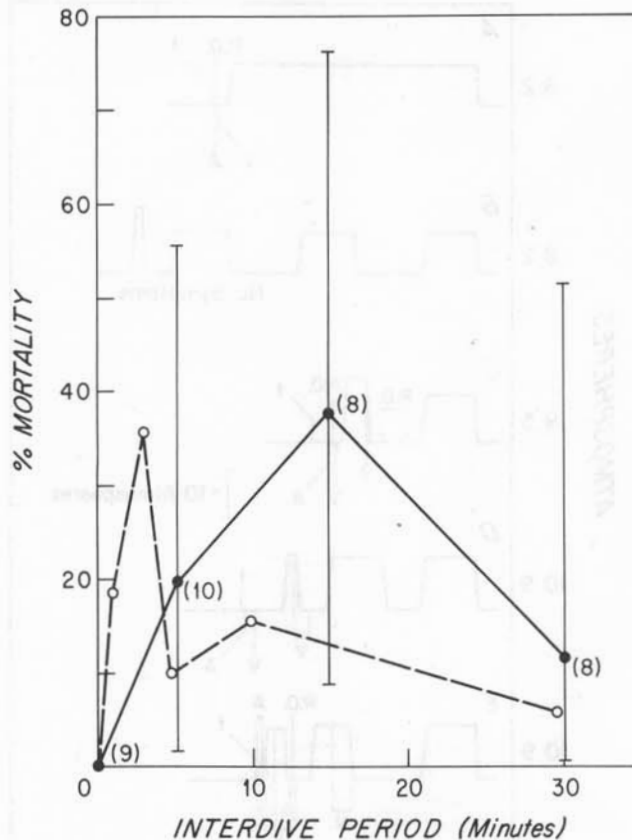


Fig. 3. Solid line: Mortality in guinea pigs after exposure to 6.9 atm N_2 for 15 min, a variable interdiving period, and a second exposure to 5.5 atm N_2 for 15 min. Animals breathed 1 atm O_2 throughout. Numbers against each point are sample size. Bars indicate 95% confidence limits.

Dashed line: Data from Griffiths et al. (1971) for mice exposed to two 5-min dives to 10.2 atm N_2 with a variable interdiving period as before.

increases and then decreases. Controls showed that the depth of the second dive had little influence on incidence and this data may therefore be compared to the dashed curve representing similar data for mice exposed to double dives of equal depth (reported in a previous publication, Griffiths et al. [1971]). Note that in both cases the incidence after the second decompression remains above that for a single dive of twice the duration (i.e. two single dives with zero interval between them) for an extended period of time, a period that apparently increases with the size of the animal studied.

The results of experiments on five anesthetized, dissected guinea pigs subjected to a variety of exposures are summarized schematically in Fig. 4. As noted below there is no evidence from this small group of animals to suggest that our dissection radically altered susceptibility.

The first of these experiments (Fig. 4A) shows the results of a 75-min exposure to 8.2 atm of nitrogen. Control experiments showed that this is a saturation dive with an expected mortality of over 80%. Venous bubbles were observed moving centrally, 2½ min after

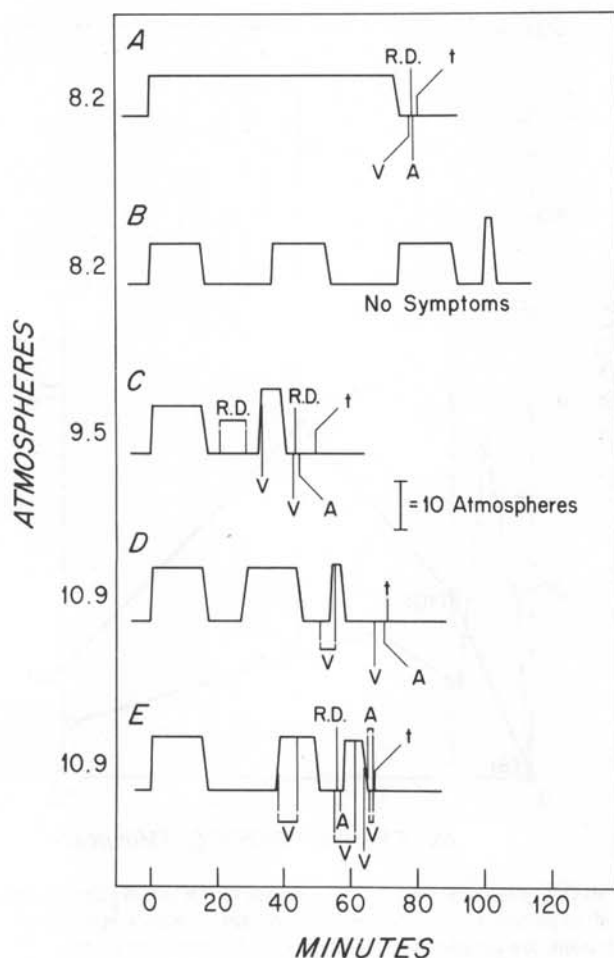


Fig. 4. Pressure time profiles of five experiments on dissected anesthetized guinea pigs. Pressures are in atmospheres gauge and represent the partial pressure of N_2 present. 1 atm O_2 was also present. Symbols: A, arterial bubbles; V, venous bubbles; R.D., respiratory distress; †, death.

decompression, respiratory difficulty (2 min 50 sec), arterial bubbles moving peripherally (3 min), then backing up centrally (4 min); and death (4-5 min) followed the initial observation in a characteristic sequence. The sequence of centrally moving venous bubbles, respiratory distress, arterial bubbles, death, has been observed by previous workers in bubble trap experiments (Gramenitskii and Savish 1965), while Emerson, Hempleman, and Lentle (1967) reported arterial backup of gas in the terminal stages of decompression sickness. Thus our dissection and visualization techniques appear to introduce no unusual artifactual effects.

The succeeding experiments (Fig. 4B-E) explore the effects of increasingly severe multiple-dive procedures.

Figure 4B shows the result of three 15-min exposures to 8.2 atm of nitrogen separated by periods of 20 min in 1 atm of oxygen. No symptoms or bubbles were observed even after a final short exposure to 13.3 atm of nitrogen. The expected incidence for intact animals was

somewhat greater than 40%; so, as judged by the decreased susceptibility of this single animal, it appeared safe to conclude that no gross increase in susceptibility had been incurred by our dissection procedure.

Figure 4C provides the first evidence for compression-induced redistribution of bubbles. The first decompression from 9.5 atm nitrogen for 15 min produced only transient respiratory distress. Normally death would not be observed in guinea pigs later than 20 min postdecompression; nonetheless, during the second recompression a substantial shower of venous bubbles moving centrally was observed, presumably released from some peripheral site by the recompression. After the second decompression the same characteristic set of events leading to death were observed as following the saturation exposure.

The first exposure in Fig. 4D was sufficient to produce death from decompression sickness in 50% of exposures (Fig. 2B). The first two exposures together were virtually certain to produce death. Seven minutes after the second decompression bubbles were observed moving centrally in the inferior vena cava. About 1 min later, when a copious flow of venous bubbles was established, therapeutic recompression was instituted. The flow of bubbles did not cease until a minute later (cf. the previous experiment). After a further 1½ min at pressure decompression was instituted and subsequent venous bubbles were only observed 8 min postdecompression.

In Figure 4E both of the phenomena observed in the previous two cases were observed in a single animal. Venous bubbles first appeared during the second *compression* over 20 min after prior decompression and only ceased after 5 min at pressure. The second decompression produced, after some 4 min at 1 ATA, the characteristic sequence of venous bubbles, respiratory distress, arterial bubbles. On this occasion therapeutic recompression was only instituted after the appearance of arterial bubbles. On recompression, arterial bubble flow ceased immediately, while characteristically venous bubble flow continued for several minutes, after which breathing appeared normal. Two minutes later decompression was initiated. On reaching 2.2 ATA a few small bubbles moved centrally up the vena cava, but no more were seen until after 1.0 ATA was reached. In contrast arterial bubbles appeared at 1.7 ATA in large numbers moving peripherally and continued to do so until death occurred at about 1½ min. postdecompression.

RESULTS OF CARDIAC MONITORING

After decompression the electrocardiogram consistently showed a progressive decrease in heart rate, which was restored on recompression. Thus in the profile represented in Figure 4D, the rate fell from 4.0 to 3.25 beats/sec during the period after the first decompression. The second decompression produced a subsequent decline from 3.8 to 2.7 beats/sec, as did the third decompression. The heart rate and electrocardiogram changed simultaneously with the observation of bubbles moving centrally in the inferior vena cava, while the appearance of bubbles moving peripherally in the descending aorta coincided with an arrhythmic trace. The rise in heart rate previously reported during decompression (Buckles 1968) was confirmed in this work.

DISCUSSION

In a previous paper (Griffiths et al. 1971) we discussed in detail the interpretation of results obtained by the exposure of mice to double- and multiple-dive procedures (see for example, Fig. 3). We concluded that, while the increase and decline of susceptibility as a

function of the time between two dives was qualitatively in accord with a model based on the growth and decay of an extravascular silent or asymptomatic bubble, this model had to be modified for two major reasons. First, calculations showed that the decline in susceptibility took place too rapidly to be accounted for by bubble decay; and second, very short (15-25 sec) second exposures produced an immediate increase in mortality. To explain this we invoked the behavior of vascular bubbles, hypothesizing (in accord with several earlier workers reviewed by Elliott 1969) that bubbles released at a primary site pass down the venous system to the lung. Here they are filtered and gradually eliminated. However, if a critical quantity of separated gas is achieved in the pulmonary arterial tree, bubbles are released and appear in the arterial circulation. Thus the observed rapid decay in susceptibility may be explained because gas may be eliminated from the tissues as bubbles as well as in the form of dissolved gas. The lethality of a rapid second dive then results from the release by compression of trapped bubbles into the vascular system and their subsequent entrapment in the lungs, where, if a rapid decompression is then made, a critical volume of gas is exceeded and separated gas escapes to the arterial circulation.

Our data in Fig. 3 further support the conclusion that the time course of susceptibility cannot be wholly explained by the physics of extravascular bubble formation. The lengthening of the time to maximum susceptibility during the interdive period for the guinea pig (15 min compared to 3 min in mice) probably reflects the slower rate of perfusion-limited gas exchange in the larger animal which permits bubble growth to proceed for a greater time.

Our results for direct observation of vascular bubble behavior are fully consistent with the model proposed here. The observations following a single saturation dive are in accord with earlier experiments (Elliott 1969) and suggest that the dissection technique has introduced no detectable qualitative artifact. Nor is the incidence after the first dive in Figure 4B and C quantitatively different from that expected from the control dose-response curve (Fig. 2, curve B) within the limits imposed by the size of our dissected group.

In all of our experiments with guinea pigs none died later than 20 min postdecompression. In two out of three multiple-dive routines the occurrence of venous bubbles during recompression 15 and 20 min after a prior decompression must therefore be associated with the recompression. Rubissow and MacKay (1974), using noninvasive ultrasonic techniques, have demonstrated that recompression may produce bubble showers by release of bubbles trapped in the capillary beds of leg tissues and lungs. This is in accord with our explanation of double-dive related decompression sickness, which assumed that peripheral bubbles *bled* off into the active venous circulation on recompression. One mechanism for this would be the shrinkage of bubbles in occluded vessels allowing re-establishment of blood flow, but our experiments provide no direct evidence on this point. The venous bubbles usually, but not always (of four occasions in three animals, the incidence was three out of four), continued to appear at pressure for some time after compression. This finding offers an explanation for the fact that in mice the incidence of decompression sickness immediately following a second dive increases as this dive is extended from 16 to 25 sec in duration—the lungs are still assimilating separated gas during this interval at pressure.

The appearance of arterial bubbles was usually preceded by visible respiratory distress but the latter was not always preceded by the observation of bubbles in the inferior vena cava, suggesting that bubbles are also contributed to the lungs from other peripheral sources. This is in accord with the work of Lever (1969) who observed initial bubbles in the veins of the head and neck as well as in the inferior vena cava.

We have assumed that a critical volume of separated gas accumulated in the right side of the heart and pulmonary arterial vessels results in the opening of arterial-venous shunts and in the release of gas into the pulmonary veins and thence into the systemic arterial circulation. The common observation of arterial bubbles together with the electrocardiogram changes provide some support for this assumption. Further, if this is so then recompression during the appearance of arterial bubbles should immediately relieve this situation, as indeed we observed during the third recompression in exposure (E). Also subsequent decompression allowed arterial gas to be released even though only a few small bubbles had appeared moving centrally in the inferior vena cava. Unless other peripheral areas had contributed significant gas during decompression, the appearance of this arterial gas must have been triggered by the expansion of gas already present in the right heart or pulmonary arteries. By way of contrast, the less severely affected animal (D), which had also experienced therapeutic recompression, required additional peripheral gas after the third decompression before this event was triggered.

The relation of the behavior of vascular bubbles directly observed in this work to the human situation is difficult to assess with confidence. The situation of most direct relevance is the therapeutic treatment of *chokes*. Our results in this and previous work (Griffiths et al. 1971) indicate the source of danger from terminating therapeutic decompression too soon to be (1) the presence of enough separated gas remaining in the right heart and pulmonary arterial tree to exceed the critical condition upon decompression, or (2) sufficient gas remaining in the periphery to enable this condition to be re-established sometime postdecompression. Possibly the arterio-venous pressure difference would provide a means of monitoring for this situation (Niden and Aviado 1956).

The suggestion in Fig. 3 that the duration of elevated susceptibility increases markedly with animal size could be of significance to human diving safety. Indeed the extra margins empirically allowed in decompression routines for repetitive dives seem to reflect this fact. How long this heightened susceptibility remains after an initial dive by a human diver is difficult to estimate. It is interesting to note that the period of heightened susceptibility roughly parallels the time required for saturation (30-40 min for mice; 60-90 min for guinea pigs) and on this empirical basis one might expect a figure of 12-24 hours for men. No reliable method for such scaling is known, however, and the problem should repay further experimental study. One recently reported study (Spencer, Hong, and Strauss 1974) using a precordial ultrasonic bubble detector on two human subjects performing four 15- to 36-min dives with 35- to 110-min surface intervals showed bubbles after one each of the second, third and fourth dives, but none after either of the first dives.

The occurrence of chokes is related to the balance between the rate of supply of separated venous gas to the lung and its ability to eliminate it. We know little about the latter, but this work shows the influence of compression on the former rate. The earlier work of Nims (1951) indicates the role of exercise, although direct observations on, for example, the effects of vasodilation and constriction have not been reported. This work provides no direct information on the critical processes involved in accumulation and release of gas at the periphery and at the lung. The development of noninvasive ultrasonic techniques for bubble monitoring may eventually answer such questions.

Our exposures are relatively severe ones but, as we have argued before, Hempleman's results (1960) for the much milder *joint* bends in goats are fully consistent with ours. It therefore seems probable that heightened susceptibility in multiple-dive procedures similar to ours would be observed in manned diving if they were sought in a controlled series of

experiments. The current practice of calculating decompression routines for multiple dives by assuming a dive equal in length to the sum of diving and surface time clearly reflects inadequacies in our understanding. Current trials with Doppler-shift decompression monitors may well go some way towards vindicating our point of view.

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Gait, D., K. W. Miller, W. D. M. Paton, E. B. Smith, and B. Welch. 1975. La redistribution de bulles de gaz dans les vaisseaux sanguins au cours de plongées multiples. *Undersea Biomed. Res.* 2(1):42-50.—Le comportement des bulles gazeuses dans les vaisseaux sanguins du cobaye anesthésié et disséqué a été étudié. La dissection a rendu possible l'observation de bulles dans l'aorte et dans la veine cave inférieure au cours de l'exposition à l'hyperbarie. Le but des expériences était de vérifier l'hypothèse que la récompression après une plongée puisse déplacer des bulles asymptomatiques périphériques à travers la circulation veineuse jusqu'au poumon. Les bulles ne traversent le poumon pour entrer dans la circulation artérielle que s'il y a accumulation d'un volume critique de gaz. Cette condition peut être remplie après une seule plongée difficile, ou après une plongée moins difficile suivie d'une plongée courte au cours de laquelle le gaz séparé se redistribue à un lieu plus vulnérable, c'est à dire, le poumon. Après une seule plongée difficile nous avons retrouvé une série typique de faits: des bulles traversant la veine cave inférieure, détresse respiratoire, bulles dans l'aorte, et puis la mort. Au cours de plongées effectuées après de premières plongées asymptomatiques nous avons observé des bulles dans la veine cave qui continuaient à apparaître pendant un certain temps en compression; des bulles de gaz ont été observées dans les artères après la décompression, et la mort est survenue peu après.

bulles de gaz
cobayes

plongées répétées
maladie de décompression

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