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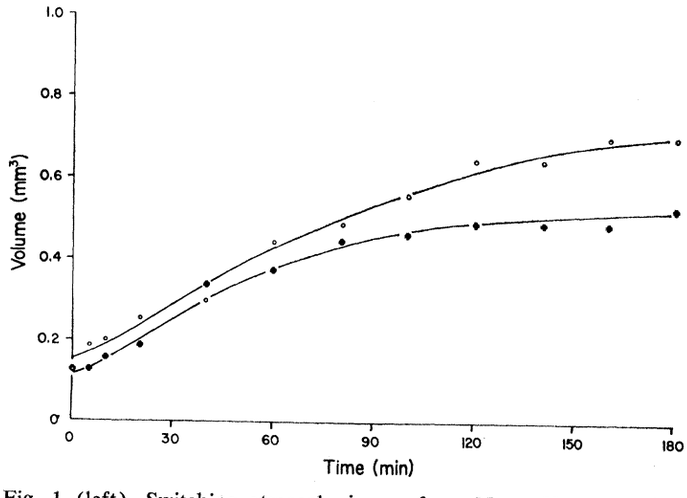
Isobaric Bubble Growth: A Consequence of Altering Atmospheric Gas

Abstract. *During certain treatments of decompression sickness following dives made with compressed air, the U.S. Navy advocates breathing helium-oxygen mixtures. However, stable nitrogen bubbles created within gelatin by decompression have been found to enlarge when the atmosphere was switched from nitrogen to helium without changing ambient pressure. This suggests that decompression sickness would be worsened by switching from nitrogen to helium in the breathing gas mixture.*

Decompression sickness affects divers whose tissues have become supersaturated with gas. The primary cause is generally thought to be the formation and growth of bubbles within tissues and blood (*1*). The treatment of decompression sickness involves (i) increasing the ambient pressure in order to reduce bubble size and (ii) breathing oxygen in order to increase the gradient for inert gas loss from the body and to increase tissue oxygenation. Under certain conditions, such as very high ambient pressures, inert gas as well as oxygen must be breathed in order to avoid oxygen toxicity. The *U.S. Navy Diving Manual* (*2*) advocates that the inert gas breathed be helium, even when decompression sickness has occurred after diving with air or other nitrogen-oxygen mixtures. The rationale appears to be to increase the net rate of loss of nitrogen from the body (*3*). However, switching to helium may be the wrong thing to do. There is considerable evidence that the body is saturated more rapidly by helium than it is desaturated by nitrogen (*3, 4*). During this transition period, bubbles within the body are expected to grow, since helium would diffuse into them faster than nitrogen would diffuse out. We have demonstrated this in a model in which stable bubbles were created in gelatin by decompression. The model simulates the body since diffusion calculations show that the gelatin is saturated by helium more rapidly than it is desaturated by nitrogen.

Experiments were performed in a small pressure chamber having a window in one end through which gelatin and bubbles could be seen and photographed by microscope at any pressure. Gelatin was prepared in a single batch by dissolving 127 g of Knox gelatin crystals in 5 liters of water. Portions were frozen and subsequently thawed at 40°C for each experiment. Thawed gelatin was pipetted into three rectangular glass chambers of horizontal cross section 6 mm by 27 mm, the gelatin depth at the meniscus being 4 mm. Before compression, the gelatin was converted from sol to gel by partly immersing the glass chambers in ice water for 10 minutes. The glass chambers were then placed in a water bath at 21°C inside the pressure chamber, which was pressurized at 13.6 atm/min by adding N_2. The chamber pressure was held at 21.4 atm absolute for 5.25 hours for the gelatin to become saturated with N_2. Pressure was then decreased to 11.2 atm absolute in 10 seconds, following which bubbles ap-



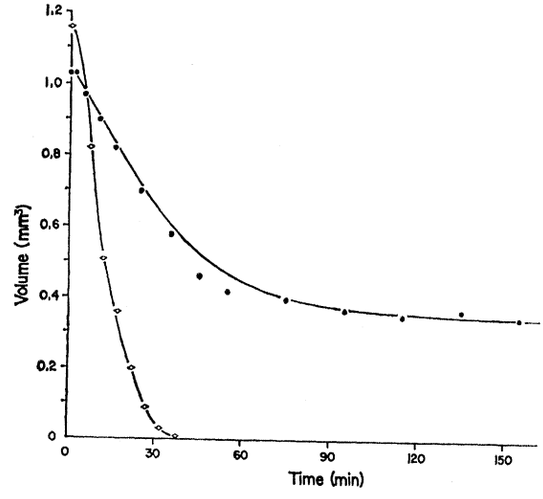


Fig. 1 (left). Switching atmospheric gas from N_2 to He causes bubbles in gelatin to grow. Ambient pressure is unchanged. Fig. 2 (right). Switching atmospheric gas from He to N_2 causes bubbles to shrink or disappear.

peared within seconds and grew for a number of minutes. There were 17.3 ± 1.2 bubbles in the lower 3 mm of gel in each glass chamber. Bubbles within 1 mm of the surface were not counted because they were smaller, more numerous, and difficult to see through the meniscus. The system was allowed to stabilize overnight. The next morning the chamber was flushed with helium for several minutes without altering ambient pressure. Photographs of bubbles were taken serially before and after flushing.

Before the pressure chamber was flushed with helium, there was no change in bubble size. Soon after it was flushed all the bubbles grew, and subsequently stopped growing, as indicated for two bubbles in Fig. 1. The change in bubble volume appeared to depend on the initial bubble volume and the bubble's distance from the gel surface and from other bubbles. The growth of five bubbles in three separate experiments was measured with similar results. These data suggest that switching from breathing nitrogen-oxygen to breathing helium-oxygen during the treatment of decompression sickness would make the disease worse rather than better. This conclusion is also supported by studies of the change in size of subcutaneous pockets containing various gases in rats (*5*). Other related experiments in animals have involved venous air embolism (*6*), pneumothorax (*7*), and bowel obstruction (*7*).

The converse experiment was performed, in which the initial saturation was by helium and the chamber atmosphere was subsequently changed to nitrogen. Bubbles near the surface became smaller and those deep in the gel disappeared. The histories of two bubbles are shown in Fig. 2. A total of four bubbles were measured in three experiments with similar results. These data support work by Keller and Buhlmann (*8*), in which the decompression time in humans was shortened by switching from helium-oxygen to nitrogen-oxygen breathing during decompression.

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Pancreatic Ribonuclease: Enzymic and Physiological Properties of a Cross-Linked Dimer

Abstract. *Monomeric ribonuclease A has very low activity toward typically double-stranded RNA's; the dimeric form of ribonuclease A obtained by cross linking the enzyme by dimethyl suberimidate has more than 78 times the activity of the monomer toward polyadenylate · polyuridylate and 440 times the activity of the monomer toward the double-stranded RNA of a virus from* Penicillium chrysogenum. *The half-life of the dimer in the bloodstream of the rat is 12 times that of the monomer.*

Interest in the properties of dimeric ribonucleases has been stimulated by the finding of D'Alessio and Leone and their colleagues (*1*) that the ribonuclease of bovine seminal plasma is a dimer. The seminal enzyme is similar in some of its properties to the more readily dissociable dimer that is formed (*2*) when solutions of pancreatic ribonuclease A in 50 percent acetic acid are lyophilized. A special property (*3*) of such dimeric structures, with two active sites, is the ability to hydrolyze double-stranded RNA's at an appreciable rate under conditions of salt concentration where the action of the pancreatic monomer is minimal. Since there is increased interest in the roles of double-stranded RNA's in animal cells (*4*, *5*) and in bacteria (*6*), we have studied the enzymic properties of a cross-linked dimeric enzyme that is readily prepared from bovine pancreatic ribonuclease A.

The cross linking, through specific reaction with NH_2 groups of ribonuclease A, has been accomplished with the bifunctional reagent dimethyl suberimidate $[CH_3OC(=NH)(CH_2)_6C(=NH)OCH_3]$ under conditions similar to those described by Hartman and Wold (*7*) for cross linking of ribonuclease A by adipimidate. To a stirred solution of 100 mg of ribonuclease A (Worthington, RAF grade) in 10 ml of 0.1*M* sodium phosphate, *p*H 10 at 25°C, 10 mg of dimethyl suberimidate dihydrochloride (Pierce Chemical) was added in 2-mg portions at 5-minute intervals; the *p*H was maintained at 10 by additions of 0.1*N* NaOH. After 1 hour, the reaction was stopped by the addition of 0.5 ml of 0.2*M* ammonium acetate. The dimeric fraction was isolated by gel filtration on a column (1.5 by 80 cm) of Sephadex G-100 with 0.9 percent NaCl as eluent [figure 3 in (*7*)]. The yield of dimer (eluted between 75 and 100 ml) was about 50 mg. The solution was frozen for storage.

A molecular weight of about 27,000 for the dimeric fraction was obtained by sodium dodecyl sulfate gel electrophoresis (*8*). The number of free NH_2 groups remaining in the dimer was measured photometrically by the picryl sulfonic acid method of Satake (*9*). Ribonuclease A (*10*) has one alpha NH_2 group and ten epsilon NH_2 groups of lysine residues; the photometric method gave 11 groups for the monomer; the cross-linked dimer contained 19 free NH_2 groups, which indicated that the suberimidate had imidylated three NH_2 groups in the dimeric structure. This result is comparable to that obtained by Hartman and Wold (*7*) with dimethyl adipimidate; they found that two NH_2 groups reacted with the bifunctional reagent to form a bridge between the two monomers and that two additional NH_2 groups reacted monofunctionally with the reagent. Their dimer had 135 percent of the activity of the monomer toward 2′,3′-cyclic cytidylate.

The enzymic activities of the present dimer toward a variety of polynucleotides have been measured by the procedure of Anfinsen *et al.* (*11*). The *p*H-activity curve was first established for the dimer against yeast RNA and found to be the same as that of the