believe that these decreases may be due to the acceleration in the extrusion of Ca^{2+} through the sarcolemmal membrane.

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Seal Lungs Collapse During Free Diving: Evidence from Arterial Nitrogen Tensions

Abstract. *Arterial blood nitrogen tensions of free-diving Weddell seals (*Leptonychotes weddelli*) were measured by attaching a microprocessor-controlled blood pump and drawing samples at depth to determine how these marine mammals dive to great depths and ascend rapidly without developing decompression sickness. Forty-seven samples of arterial blood were obtained from four Weddell seals during free dives lasting up to 23 minutes to depths of 230 meters beneath the sea ice of McMurdo Sound, Antarctica. Peak arterial blood nitrogen tensions of between 2000 and 2500 millimeters of mercury were recorded at depths of 40 to 80 meters during descent, indicating that the seal's lung collapses by 25 to 50 meters. Then arterial blood nitrogen tensions slowly decreased to about 1500 millimeters of mercury at the surface. In a single dive, alveolar collapse and redistribution of blood nitrogen allow the seal to avoid nitrogen narcosis and decompression sickness.*

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To learn how marine mammals tolerate deep diving and rapid decompression, we studied the Weddell seal, which can dive to great depths (500 m) and ascend rapidly without encountering either nitrogen narcosis or decompression sickness (the bends) (*1*). Others have described common morphological adaptations of deep diving mammals (*2*) and obtained radiographic studies of restrained Weddell seals in hyperbaric chambers (*3*) and photographs of dolphins taken at depth with analysis of exhaled air after dives (*4*). These studies provided indirect evidence to suggest a mechanism of alveolar collapse at depth to protect these animals from the high arterial blood nitrogen tensions (P_aN_2) which would have otherwise occurred with compression. Measurements of muscle PN_2 in dolphins at the surface after repeated dives (*5*) and of P_aN_2 during forced chamber diving of seals (*6*), suggested that the rise in P_aN_2 is limited to 2000 to 3000 mmHg. However, no direct measurements of P_aN_2 have been made on freely diving animals to verify these estimates. Since both circulatory reflexes and respiratory responses are markedly different between seals that are free swimming and those in forced diving (*7*), we measured blood P_aN_2 during voluntary diving to learn how Weddell seals safely cope with rapid compression to and decompression from high pressures.

Four male Weddell seals (350 to 420 kg) were studied near McMurdo Station, Antarctica (77°S, 166°E). Seals were captured at nearby colonies and sledged to a field research site at which two holes, 1 m in diameter, had been drilled through the 3-m thick ice of McMurdo Sound. The field site was chosen to be at least 6 km from the nearest natural crack in the ice sheet so that when a seal was released into the water, it was obliged to return to the field site to breathe. An observation hut with a hole in its floor was placed over one of these holes; the other allowed the seal to enter and exit from the water. Instruments were placed on anesthetized seals at the field site, a catheter was introduced into a foreflipper artery and advanced to the aorta for blood sampling, and electrocardiogram (ECG) leads were placed for heart-rate monitoring. The ECG leads were connected to an 8-bit microcomputer backpack system (*8*) which also controlled a pressure-resistant submersible peristaltic pump that was used to withdraw blood from the arterial catheter. The microcomputer and blood sampling equipment were glued to the seal's dorsal fur. After 3 to 5 days of monitoring, the arterial catheter and ECG were removed, and the animals were released and were returned to their native colonies.

During the experiment, the instrumented seal was released and surfaced to breathe at the hole in the floor of the observation hut. This hut contained a Zenith Z-90 computer to which data on heart rate, diving depth, swimming velocity, and aortic blood temperature were transmitted while the seal rested at the surface (*8*). The backpack computer was programmed to pump blood into vinyl bags for 90 seconds at indicated depths during descent or ascent, or after a specified diving time. An alternative technique consisted of sequentially filling a sampling device containing nine plastic syringes. Each syringe was filled for 30 seconds; 5 ml of each blood sample was transferred to a glass syringe and, within 2 hours of sampling, injected into a Van Slyke apparatus for the determination of inert gas content (*9*). The few blood samples that could not be analyzed immediately were stored at 0°C. Serial determinations of inert gases from single samples revealed a maximum loss of inert gas content of 8 to 10 percent after 2 hours, presumably due to diffusion of nitrogen through the 1.0-mm thick sampling bag wall.

The solubility of inert gases in seal blood was determined as a function of the hemoglobin concentration by tonometry at 37°C with mixtures of air and CO_2 containing a known inert gas partial pressure (*10*). After appropriate correction for hemoglobin concentration and dilution by flushing fluid (*11*), the inert gas tensions of arterial blood (that is, the nitrogen tension, P_aN_2) were calculated.

Values of P_aN_2 from 29 blood samples obtained during dives by four seals (Fig. 1) show that by the time the seal has descended to 50 to 70 m, the P_aN_2 peaked at approximately 2300 mmHg, then deceased to a range of 1100 to 1800 mmHg as the dive progressed. A comparison of early (<7 minutes) with later (7 to 23 minutes) P_aN_2 values revealed a statistically significant decline (early, 1983 ± 51 mmHg, n = 19; late, 1521 ± 76 mmHg, n = 8).

Sequential 30-second blood samples taken together with heart rate and depth during single dives show that the maximum P_aN_2 of 2079 mmHg at approximately 30 m (Fig. 2A) was followed by a sharp decline of P_aN_2 to 1200 mmHg during further descent to a maximum depth of 89 m. Figure 2B confirms that the P_aN_2 continues to fall during descent, and a greater diving depth was not associated with a higher P_aN_2 value. Two other sets of four sequential samples taken during late phases of dives showed declining P_aN_2 values between 1700 and 1200 mmHg.

Our results provide direct evidence that the free-swimming Weddell seal protects itself from nitrogen narcosis and decompression sickness by both limiting N_2 uptake from the lungs and redistributing N_2 during the dive. Nitrogen uptake is effectively stopped by the collapse of gas-exchanging alveoli, and the maximum P_aN_2 is then reduced by redistribution of N_2 to organs and tissues. Shunting of venous blood through collapsed alveoli will also reduce the peak P_aN_2. The peak P_aN_2 of 2079 mmHg (Fig. 2A) indicates alveolar collapse occurs at approximately 28 m (3.7 atmospheres absolute by 550 mmHg). However, because of the 30-second sampling period, the P_aN_2 values represent averages of blood sampled at depths ranging up to 35 m taking into account the Weddell seals' maximum observed rate of descent or ascent of 70 m/min. Our observation that the P_aN_2 in samples drawn between 200 and 230 m (21 to 24 atmospheres absolute) never rose above 2329 mmHg, explains how the seal escapes nitrogen narcosis during deep diving. The P_aN_2 does not rise to levels that produce narcosis.

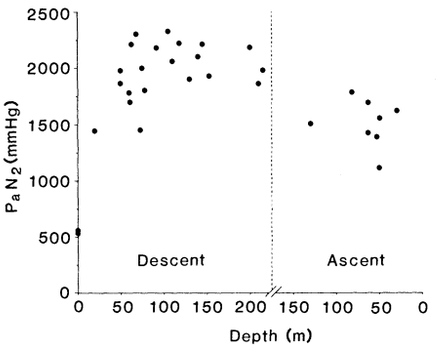

Fig. 1. Arterial blood nitrogen tensions (P_aN_2) in free-diving Weddell seals. Each 90-second sample was obtained during a single dive. P_aN_2 is plotted at the mean depth of the sampling period over various depth ranges, depending upon the seal's rate of descent or ascent.

Seals usually exhale before diving. Kooyman *et al.* measured the average lung volume during diving of adult Weddell seals to be 11.6 liters (n = 4), of which 9.2 liters was N_2 (*12*). Assuming that perfusion during free diving is similar to that in forced diving and is restricted to vital organs, such as the brain, heart, and lungs, only approximately 7.5 kg of tissues and up to 56 liters of blood are available for N_2 distribution in the 350-kg diving Weddell seal (*13*). Thus, the peak P_aN_2 of 2400 mmHg corresponds to absorption of only 2.5 liters of N_2 from the lungs. This provides further evidence that during diving the major fraction of the Weddell seal's lung gas is compressed into nongas exchanging segments of the respiratory tract. At the end of the dive this residual gas can expand to open collapsed alveoli.

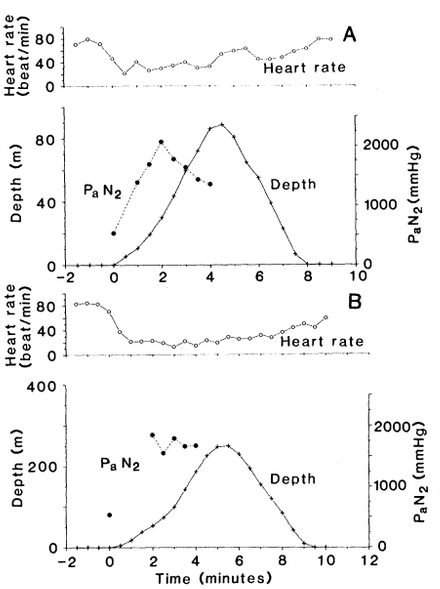

Fig. 2. Heart rate and depth combined with serial determinations of P_aN_2 during two dives. The sampling time was 30 seconds for each sample. (A) P_aN_2 values early during a dive when pulmonary gas exchange spaces are collapsing. (B) Declining P_aN_2 during a later descent phase of a deep dive.

The decline in P_aN_2 from early peak values during the dive is due to mixing within the blood and delivery of N_2 to perfused tissues. During our studies we measured a consistent increase of arterial hemoglobin content from 15 to 25 g/dl during the first 17 minutes of diving. The higher hemoglobin concentration increases N_2 solubility in blood (*10*) by 8 to 10 percent and thus contributes to the decrease of P_aN_2 during the dive. If other major tissues, such as muscle and blubber, the latter with an approximately 4.5 times higher capacity than blood to dissolve inert gases (*14*), are perfused during the dive (*13*), they too will be available for N_2 redistribution and can lower the P_aN_2 to safer levels for surfacing.

The P_aN_2 of 1200 to 1800 mmHg reached near the end of ascent but before breathing allows the seal to return to the surface without risking the bends despite rapid decompression. Although arterial blood samples obtained at depth and left for several hours at ambient pressure often released bubbles, in the swimming seal this gas probably remains in solution long enough to be transported to the lungs and exhaled upon surfacing. The P_aN_2 values measured 4 and 7 minutes after surfacing from a dive to 105 m were 616 and 628 mmHg, respectively.

We do not know all the mechanisms that allow the Weddell seal to dive safely to 500 m and rapidly return to the surface. However, microcomputer-controlled blood sampling has provided direct evidence that, by limiting blood N_2 uptake through alveolar collapse and by redistributing blood N_2 to blood and tissues during the dive the Weddell seal reduces the risk of nitrogen narcosis and decompression sickness.

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11. The arterial catheter, flush lines, and sampling bag were primed with heparinized 0.6*M* saline to

prevent coagulation and freezing of blood samples at the −1.9°C seawater temperature. To calculate P_aN_2 we corrected the inert gas content for dilution. The latter was determined by marking the flush solution with tritiated water (seals 1 and 2) or calculating the hemoglobin ratio of the diluted sample to undiluted blood obtained simultaneously from the sampling line (seals 3 and 4).

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Effects of Genomic Position on the Expression of Transduced Copies of the *white* Gene of *Drosophila*

Abstract. *The* white *gene of* Drosophila *is expressed normally when introduced at many different sites in the genome by P-element–mediated DNA transformation, but is expressed abnormally when inserted at two particular genomic positions. It is now demonstrated that the mutant expression in these two cases is caused by the surrounding chromosomal region into which the* white *gene has been inserted. The* white *gene could be moved from these two positions, where it confers a mutant phenotype, to other positions in the genome where it confers a wild-type phenotype. However, flies in which* white *has been moved to one new location have an unusual mosaic phenotype.*

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The relation between the chromosomal location of a gene and its expression has been studied in *Drosophila* by means of chromosomal rearrangements such as inversions and translocations. Rearrangements can result in the inactivation of genes located at some distance from the rearrangement breakpoints. This inactivation commonly occurs in only a portion of the cells in which the gene is normally expressed, causing the fly to have a mosaic phenotype. Such variegating position effects are usually associated with rearrangements that bring euchromatic genes into the proximity of centromeric heterochromatin (*1*).

The development of the technique of P-element–mediated DNA transformation in *Drosophila* has expanded our ability to study genomic position effects. A gene, carried within a P transposable element, can be introduced at many genomic positions, and its expression at these different positions can be compared (*2*). Several genes that have been transformed into the germline by this method show proper developmentally regulated expression at a variety of euchromatic positions (*2, 3*).

The *white* gene confers an essentially wild-type (red-eyed) phenotype (*4*) when transduced to any of at least 20 different chromosomal locations by P-element vectors (*5, 6*). In contrast, three cases have been reported in which a transduced *white* gene confers a mutant mosaic eye color (*5, 6*). Flies carrying one copy of the transduced *white* gene A^R4-3 are more darkly pigmented in the anterior portion of their eyes than in the posterior portion. This transduced gene is located near the centromeric heterochromatin of chromosome arm 2L. Another transformant, A4-4, has a transduced *white* gene at the end of the right arm of chromosome 3 and has a yellow eye color with scattered flecks of red (*5*). Gehring *et al.* (*6*) have reported a third mosaic transformant, $P(w^{var})$, that has red-orange variegated eyes, and a transduced *white* gene at the left end of chromosome 2.

We have suggested that the mutant phenotypes of A^R4-3 and A4-4 are the result of genomic position effects because we could not detect alterations of their transduced *white* DNA that might account for the mutant phenotypes (*7*). Furthermore, the *white* gene in the A^R4-3 transformant is located adjacent to centromeric heterochromatin, which is capable of eliciting variegating position effects (*1*). However, their mutant phenotypes might be due to mutations within the transduced genes that were not detectable by the DNA blotting methods used. To test this possibility we have moved the transduced *white* gene in A^R4-3 and A4-4 to new positions. Relocating the gene should result in a wild-type eye color at most new positions if its mutant phenotype is due to a position effect, but not if it is due to a mutation intrinsic to the gene.

To relocate the transduced *white* gene in A^R4-3 and A4-4, we mobilized the P-*white* transposon in these two strains (Fig. 1). The P-*white* transposon cannot catalyze its own transposition, but can be induced to transpose by injecting a nondefective P element. Embryos of each strain were injected with plasmid DNA containing the "wings-clipped" P element, which is a P element with a deletion in a portion of one of the terminal inverted repeats (*8*). The "wings-clipped" P element can induce the transposition of P-element transposons, but does not itself integrate into the chromosomes (*8*). Therefore, the P-*white* transposon should transpose in the germ cells of some of the injected embryos but should be stable in subsequent generations.

Progeny of the injected embryos having eye colors different from A^R4-3 and A4-4 were chosen for further analysis and are referred to as revertants. Seventeen independent revertants were recovered from the progeny of injected A^R4-3 embryos and five independent revertants were recovered from the progeny of the injected A4-4 embryos (*9*). Hybridization of *white* gene DNA to polytene chromosomes (*10*) resulted in the identification of 13 new chromosomal locations of the P-*white* transposon among the revertants of A^R4-3 and two new locations among the revertants of A4-4 (*11*). The sites to which the P-*white* transposon moved were distributed over the euchromatic portions of all of the major chromosome arms (Fig. 2). Restriction digest analyses (*12*) of the DNA of these revertants are consistent with there being a single intact *white* gene at each of these new sites. An example of a DNA blot hybridization which was part of this analysis is shown in Fig. 3. Flies in which the *white* gene from A^R4-3 has been inserted at the 24CD site (*13*) have a new mosaic pattern of pigmentation (described below). Flies having a mobilized *white* gene at any one of the other new sites had a wild-type eye color, as judged by visual inspection (*14*). We conclude that the mutant phenotypes of the parental transformants A^R4-3 and A4-4 are the result of genomic position effects, as the same copy of the *white* gene that confers a mutant phenotype in each of these parental transformants could confer a wild-type phenotype at other sites.

In addition to the revertants described above, the transduced *white* gene remained within the same chromosomal region as in the original mutant