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Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals

Jessica U. Meir,¹ Cory D. Champagne,² Daniel P. Costa,² Cassondra L. Williams,¹ and Paul J. Ponganis¹

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Meir JU, Champagne CD, Costa DP, Williams CL, Ponganis PJ. Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. Am J Physiol Regul Integr Comp Physiol 297: R927–R939, 2009. First published July 29, 2009; doi:10.1152/ajpregu.00247.2009.—Species that maintain aerobic metabolism when oxygen (O₂) supply is limited represent ideal models to examine the mechanisms underlying tolerance to hypoxia. The repetitive, long dives of northern elephant seals (Mirounga angustirostris) have remained a physiological enigma as O₂ stores appear inadequate to maintain aerobic metabolism. We evaluated hypoxemic tolerance and blood O₂ depletion by 1) measuring arterial and venous O₂ partial pressure (PO₂) during dives with a PO₂/temperature recorder on elephant seals, 2) characterizing the O₂-hemoglobin (O₂-Hb) dissociation curve of this species, 3) applying the dissociation curve to PO₂ profiles to obtain %Hb saturation (SO₂), and 4) calculating blood O₂ store depletion during diving. Optimization of O₂ stores was achieved by high venous O₂ loading and almost complete depletion of blood O₂ stores during dives, with net O₂ content depletion values up to 91% (arterial) and 100% (venous). In routine dives (>10 min) PO₂, and PaO₂ values reached 2–10 and 12–23 mmHg, respectively. This corresponds to SO₂ of 1–26% and O₂ contents of 0.3 (venous) and 2.7 ml O₂/dl blood (arterial), demonstrating remarkable hypoxemic tolerance as PaO₂ is nearly equal to the arterial hypoxemic threshold of seals. The contribution of the blood O₂ store alone to metabolic rate was nearly equivalent to resting metabolic rate, and mean temperature remained near 37°C. These data suggest that elephant seals routinely tolerate extreme hypoxemia during dives to completely utilize the blood O₂ store and maximize aerobic dive duration.

PO₂: aerobic metabolism; %Hb saturation; O₂-Hb dissociation curve; hypoxia

ANIMALS OPERATING IN EXTREME environments often push the limits of their physiological capacity and thus represent unique examples of physiological adaptation. Species that must maintain aerobic metabolism when oxygen (O₂) supply is limited represent ideal models in which to examine the physiological, cellular, and biochemical mechanisms underlying tolerance to hypoxia. In addition, investigation of adaptations to hypoxia in animals at high altitude, during hibernation, or in diving environments may provide insights to the understanding and treatment of clinical conditions, such as circulatory collapse, ischemia, and hypoxemia.

The northern elephant seal (Mirounga angustirostris) is a consummate pinniped diver, with routine 10- to 30-min dives to depths of 400 to 800 meters and mean surface intervals of only 2 min (51, 52). The repetitive, long duration dives of this pelagic predator remain a physiological enigma in that O₂ stores appear insufficient to account for these frequent, long dives (34). This diving behavior makes the elephant seal an excellent model of hypoxemic tolerance and regulation of tissue O₂ delivery and consumption.

The aerobic dive limit [ADL; duration beyond which blood lactate concentration increases above resting levels (43)] and the concept that most dives in the wild are aerobic has dominated the interpretation of diving physiology and diving behavior for the past 25 years. Although an ADL has not been measured in elephant seals, their diving metabolism has been considered predominantly aerobic in nature because surface intervals are only 2 min in duration (51, 52). Furthermore, >90% of their time at sea is spent submerged during 2-mo foraging trips throughout which elephant seals gain an average of 1 kg/day body mass (51, 52). With the exception of post molt females, most dives of elephant seals are within their theoretical ADL (34, 51). The physiological mechanisms that account for the sustained, continuous diving of this species, however, are unresolved.

Hypothermia has been suggested as a mechanism of metabolic rate reduction (10, 14). In addition, moderate bradycardias of 30–40 beats/min, regulated organ perfusion during dives (5, 33), a hydrodynamic body shape (26), and prolonged gliding (92) undoubtedly contribute to the conservation of blood and muscle O₂ stores during dives. Another strategy that has been proposed to conserve O₂ during dives is partitioning of the metabolic demands of travel, foraging, and digestion into different dive types and extended surface intervals (34, 53, 80). We postulate in the present study that extreme hypoxemic tolerance in the elephant seal allows for nearly complete blood O₂ depletion with every dive and that it is this tolerance that especially allows the seal to make such frequent, repetitive, long dives.

The breath-hold capacity of air-breathing animals is dependent on increased O₂ stores, the management and depletion of those stores, and hypoxemic tolerance. In phocid seals, such as the elephant seal, approximately two-thirds of the total body O₂ store is in the blood (65). The contribution of the lung to total O₂ stores is small (<5% of total) due to a small diving lung volume following exhalation prior to submersion and also due to lung collapse at depth (25, 46). High myoglobin concentrations, especially in the primary locomotory muscles [>15× the human value (86)], account for ~25% of the total store. The blood O₂ store constitutes 65–71% of the total and is based upon a large blood volume (~3 times the human mass-specific volume) and a high Hb concentration (almost twice the human value) (65). Although the management of the blood O₂ store is central to the understanding of diving physiology, there have been few studies that quantify the rate and...
mammals; 20.6 (56, 73, 79, 91); /H11002

elephant seals are an excellent model for investigation of blood O2 contents are diminished in a breath-hold diver with collo-

sopheric rate during dives of translocated juvenile northern elephant seals. We hypothesized that maintenance of metabolism during routine dives of these seals is due to extreme hypoxemic tolerance and almost complete depletion of the large blood O2 store. The objectives of this project were to 1) measure arterial and venous O2 (PaO2 and PVO2) continuously during dives, 2) characterize the O2-hemoglobin (O2-Hb) dissociation curve, 3) apply the O2-Hb dissociation curve to the PO2 profiles to obtain %Hb saturation (SO2) during dives, and 4) calculate the magnitude of blood O2 store depletion during diving and estimate its contribution to the meta-

bolic rate during the actual dive.

Marine mammals typically have P50 (PaO2 at which Hb is 50% saturated) values similar to those of humans and other terrestrial mammals (25–31 mmHg) (54, 56, 73, 79, 91). The complete O2-Hb dissociation curves of whole blood of ele-

phant seals at a temperature of 37°C and at various pHs have not been published previously. Documentation of the entire dissociation curve in whole blood is essential to estimate in vivo SO2 changes during the dive on the basis of the PO2 profiles collected in this study.

We hypothesized that: 1) end-of-dive PaO2 and PVO2 values would be ≤ 10 mmHg, far below hypoxemic limits of other mammals; 2) the elephant seal O2-Hb dissociation curve would be similar to those of other marine mammals with a P50 near 30 mmHg and a Bohr effect (Alog P50/APH) between −0.5 to −0.6 (56, 73, 79, 91); 3) application of the O2-Hb dissociation curve to PO2 profiles would demonstrate that end-of-dive SO2 is routinely < 10% in all dives and that the rate of desaturation will vary according to dive duration (i.e., the rate of desaturation will be slower during longer dives); and 4) the contribution of the blood O2 store alone to diving metabolic rate during routine (10–30 min) dives of juvenile elephant seals would be equivalent to or greater than the resting metabolic rate for this species.

Because the conventional differences in arterial and venous O2 contents are diminished in a breath-hold diver with collapsed lungs (due to the lack of addition of O2 from the respiratory system and continual decline in blood O2 during a dive), we also hypothesized that PaO2 and PVO2 values would become indistinguishable, particularly toward the end of longer dives. Accordingly, hepatic sinus PO2 values should approxi-

mate those in the artery. Indeed, Elsner’s early work (23) with elephant seals reported higher O2 contents in the posterior vena cava than the aorta at the end of forced submersions. This resulted from the slow metering of blood from the hepatic sinus and posterior vena cava back into the general circulation via the caval sphincter, the lack of O2 uptake from the lung, and the return of desaturated blood via the anterior vena cava (23). Equalization of end-of-apnea PaO2 and PVO2 values has also been observed in sleep apnea studies (82).

MATERIALS AND METHODS

This research was conducted on juvenile (1- to 3-yr-old) elephant seals [body mass = 195 kg (SD 46), n = 13] from the Año Nuevo colony (San Mateo County, CA) during the April molt season using the translocation method (5, 60) over three field seasons (2006, 2007, 2008). Seals were captured from their haul-out site on the Año Nuevo beach, transported by truck to the Long Marine Laboratory (University of California, Santa Cruz, CA) for instrumentation, allowed to recover overnight, and then transported by truck to the other side of Monterey Bay (Hopkins Marine Station) or by boat for offshore release. Thirteen seals were instrumented with a PO2 electrode and thermistor (3 seals with electrodes in the extradural vein, 4 in the hepatic sinus, and 6 in the artery (5 brachial artery, 1 femoral artery)), and blood was drawn from 15 seals for O2-Hb dissociation curve analysis. All procedures were approved by a University of California Santa Cruz Chancellors’ Animal Research Committee and a National Marine Fisheries Service marine mammal permit (no. 87-1743-02).

Po2 Electrode Deployments

Seals at the Año Nuevo colony were immobilized with an intra-

muscular injection of a mixture of tiletamine HCl and zolazepam HCl (Telazol) at an approximate dose of 1 mg/kg. At the laboratory, immobilization techniques included either ketamine (3 mg/kg im or 0.5–1 mg/kg iv) or telazol (0.4–0.8 mg/kg im) followed by the administration of isoflurane and 100% O2 (61, 66, 67, 69, 82) for all hepatic sinus and arterial deployments. An extradural vein catheter (14 g) was inserted postinduction for blood sample collection and was removed prior to transport.

A PO2 electrode (model Licox C1.1 Revoxode; Integra Life Sciences, Plainsboro, NJ) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH) were inserted percutaneously through a peel-away catheter (16–20 g) into one of the following sites (1 site per animal): 1) extradural vein, 2) hepatic sinus-inferior vena cava, or 3) aorta (via the brachial or femoral artery). Catherization techniques, the PO2 electrode, and thermistor have been described previ-

ously (61–63, 69, 71, 81). The electrode and thermistor were connected to a custom-built microprocessor (UFI, Morro Bay, CA) in a backpack unit (5 × 16 cm, 570 g), and mounted on the middorsal region of the seal using 5-min epoxy (Loctite). PO2 and temperature were sampled every once or 15 s, depending on the specific recorder used for that deployment. A Mark 9 time-depth recorder (TDR; Wildlife Computers, Redmond, WA; sensitive to 0.5 m, 30 g, 6.7 × 1.7 × 1.7 cm) was attached to document the dive profile, with a 1-Hz sampling rate. The PO2/temperature recorder and TDR were synchronized to the same PC clock, which was automatically synchronized to an Internet time server. A SPOT 4 satellite transmitter (4 × 3.5 cm footprint, 160 g, monitored via ARGOS; Wildlife Computers) was secured to the animal’s head to track the seal during the transit back to the colony and a VHF radio transmitter (148–150 MHz, 5 × 2 cm, 34 g; Wildlife Computers) was attached to the animal’s back to relocate it for recorder recovery (5, 16). Returning seals were immobilized as during capture, and all instruments removed at the beach haul-out location.

Po2 Electrode Calibration and Verification

As stated above, the PO2 electrode and thermistor, the associated calibration procedures, and verification testing have been described previously (70, 71, 81). In addition to these protocols, additional testing was conducted to verify maintenance of electrode function (response time and potential drift) over the range of data collection in the deployments of this study. PO2 electrodes were placed in saline-filled test tubes with various concentrations of bubbling N2 and O2 (maintained at 37°C in a water bath) and deployed for a 9-day period (equivalent to the longest recorded data in this study. Note, this is not equivalent to the number of days the animal spent at sea due to battery failure/electrode breakage.) Recalibration postdeployment was also conducted in some cases.

O2-Hb Dissociation Curve Characterization

O2-Hb dissociation curves on fresh whole blood were determined with the mixing technique of tonometered blood (77). Blood samples

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were obtained from the seals immediately after immobilization, placed on ice, and processed immediately upon return to the laboratory. All dissociation curve analyses were completed within 6 h after blood collection to prevent depletion of labile organic phosphates, such as 2,3-DPG, which would influence the dissociation curve. The mixing technique consists of the volumetric mixing of 0% O2-saturated blood and 100% O2-saturated blood to achieve desired SO2 at various points (i.e., 90, 70, 50, 40, 20, 10, 5% SO2) along the curve with subsequent measurement of the PO2 of the resulting mixture using an i-STAT blood gas analyzer (Abbott Point of Care, Princeton, NJ) (7, 38, 59, 73). Use of an i-STAT and Tucker chamber (87) also allowed verification of pH, partial pressure of carbon dioxide (PCO2), and blood O2 content. The CO2 Bohr effect was determined by changing the CO2 concentration of the gas in the tonometer to adjust pH. Dissociation curves were determined at pH values of 7.4, 7.3, and 7.2. The log[SO2/(100 − SO2)] vs. log(PO2) was plotted, and linear regression analysis performed to generate the equation for the O2-Hb dissociation curve at each pH (all saturation points, all seals combined) (58). The Bohr coefficient was derived from linear regression of the log PO2 on pH (each point averaged from all data of all seals for pH = 7.4, 7.3, and 7.2) (58, 91). In addition, the fixed acid Bohr effect was determined by titrating with HCl to pH 7.3 and 7.2, while PCO2 was maintained at the level required for the standard pH = 7.4 curve in that seal (91). The effect of lactic acid on the dissociation curve was also evaluated in whole blood by determining additional dissociation curve points after adding 5 and 10 mM concentrations of lactic acid to the blood (58).

To validate the specific equipment and methods used in this study, dissociation curves were also determined with blood from pinnipeds with previously published O2-Hb binding data [Leptonychotes weddellii, Phoca vitulina, Zalophus californianus (54, 55)]. Mixing technique tests were also conducted to verify that no hemolysis occurred during mixing.

**SO2 Calculations**

Percent Hb saturation (SO2) values were obtained by applying PO2 data to the linear regression equation generated by the dissociation curve, log[SO2/(100 − SO2)] vs. log(PO2) plot, and solving for SO2 at the appropriate pH, see below.

**Blood O2 Store Depletion Calculations**

Blood samples taken for the O2-Hb dissociation curve were also used for Hb analyses [cyanomethemoglobin technique (63)] to obtain Hb concentration. Use of such blood samples for this purpose is appropriate because Hb concentration is high in blood samples obtained from seals immediately after sedation (13) (P. J. Ponganis and D. P. Costa, unpublished observations); the large decrease in Hb content observed during inhalational anesthesia (63) does not occur.

Oxygen content for initial (based on the maximum PO2 value during initial descent of dive) and end-of-dive time points (minimum PO2 during the dive) for each dive were calculated from the corresponding SO2 and PO2 values, using a Hb concentration of 25 g/dl [this study, agrees well with previous findings (45, 78)] with the formula: O2 content (ml O2/dl blood) = (1.34 ml O2/g Hb) × [Hb](g/dl) × SO2 + (0.003 × PO2). Maximum SO2 was estimated with the pH 7.4 dissociation curve, and the minimum SO2 value was estimated with the pH dissociation curve at 7.4 or 7.3, depending on the duration of the dive (pH = 7.4 for dives < 15 min, pH = 7.3 for dives > 15 min). The effect of pH is critical in estimation of end-of-dive SO2. For example, from data in Weddell seals (73), a PO2 of 10 mmHg corresponds to 8% SO2 at pH 7.4, but only 2% at pH 7.0. However, since pH values were not < 7.3, PO2 values not > 55 mmHg, and lactate levels not elevated during even long dives of Weddell seals (29, 72), it is expected that this pH effect will be small and that the SO2 profile obtained with the pH 7.4 dissociation curve will reflect the general pattern of change in SO2. This minimum change in pH is also supported by data from forced submersions of Weddell seals in which pH decreased to only 7.28 after 30-min submersions (22). Nonetheless, for greater accuracy, the pH 7.3 dissociation curve was used to estimate the end-of-dive SO2 value for blood O2 store depletion calculations in dives > 15 min. This duration was selected based on pH data from diving Weddell seals and sleep apnea of elephant seals, demonstrating little to no change in pH for the first 15 min of diving in both short and long dives or apneas (72, 82).

The %O2 depletion in either the arterial or venous system (dependent on the site of insertion) for each dive of each seal was calculated as: %O2 content depletion = [(maximum O2 content − minimum O2 content)/maximum O2 content] × 100. The rate of O2 content depletion, (maximum O2 content − minimum O2 content)/dive duration, was also calculated for the arterial system, hepatic sinus, and extrapulmonary vein. This provides an overall depletion rate, not an instantaneous measure of O2 consumption. Data from seals with PaO2 electrodes provided an estimate of the net depletion of O2 in the arterial system; those with venous probes provided estimates of net venous O2 depletion.

**Data Analysis and Statistics**

The time required for PO2 to return to a venous SO2 (SvO2) of 75% (PO2 = 46 mmHg) or arterial SO2 (SaO2) of 90% (PO2 = 70 mmHg) upon completion of the dive was determined. These values were chosen as representative of SvO2 and SaO2, using a conservative 90% SO2 as typical for that of the arterial system and employing an arterial-venous O2 content difference of 5 ml/dl to determine the venous value. This is because there is no typical “resting” value for these animals since PO2 is constantly oscillating during continuous diving and during sleep apnea, while on land, and because surface intervals are not extended rest intervals in this species. As outlined above, the dissociation curve, calculated using linear regression, was applied to PO2 values to yield SO2 values, and calculations of O2 depletion rate and percentage of O2 depletion were made. Differences between results at the three electrode insertion sites were determined with ANOVA. Correlation between dive duration and the variables of minimum PO2, minimum SO2, maximum SO2, %O2 content depleted, and depletion rate was addressed with Spearman rank order correlation tests. Statistical significance was assumed at P < 0.05, and the significance level is quoted in the text. Values are expressed as means (SD). PO2 values are expressed in mmHg (as measured). Figures include the corresponding values in kPa, assuming 1 mmHg = 0.133 kPa.

**RESULTS**

**Behavior**

Seals in this study often dived with transit patterns (travel dives) consistent with the translocation method (5, 60); however, some seals did exhibit prolonged bouts of diving consistent with foraging and drift dives of free-ranging juveniles (53). Most seals returned to Año Nuevo within 1–3.5 days (Table 1). Mean dive duration and depth data are given in Table 1.

**Temperature**

Continuous temperature measurements from the thermistor were obtained from 13 seals (3 extradural vein, 4 hepatic sinus, 6 arterial) and provided correction of PO2 electrode output. Average temperature during dives remained near 37°C (mean temperature of all dives of all seals pooled = 37.4°C (SD 1.3); n = 3,213 dives from 13 seals). This justifies the use of O2-Hb dissociation curves obtained at 37°C for the SO2 calculations.

**Table 1.** Mean dive duration and depth data are given in minutes and meters, respectively. Standard deviation (SD) is shown for each value. The %O2 depletion was calculated from the change in PO2 electrode output, and the %O2 content depletion was calculated from the change in SO2 from the pH 7.4 curve. Minimum PO2 and minimum SO2 refer to the lowest values achieved during dives. Maximum PO2 and maximum SO2 refer to the highest values achieved during dives. Temperature was measured using a thermistor placed on the pinnipeds for the duration of their submersion.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Temperature</th>
<th>Data Analysis and Statistics</th>
<th>Results</th>
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</table>
R930 BLOOD O2 DEPLETION IN DIVING ELEPHANT SEALS

Individual dive, PO2, SO2, and depletion rate data for all seals

<table>
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<tr>
<th>Seal</th>
<th>Length of Deployment, days at sea</th>
<th>PO2, mmHg</th>
<th>SO2, %</th>
<th>Depletion Rate, ml O2/dl/breath</th>
<th>% O2 Content</th>
<th>Min PO2, mmHg</th>
<th>Max PO2, mmHg</th>
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<tr>
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<td>267</td>
<td>13 (SD 7)</td>
<td>13 (SD 6)</td>
<td>1.246</td>
<td>13.1 (SD 14.3)</td>
<td>65.3 (SD 5.9)</td>
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<td>81</td>
<td>11 (SD 5)</td>
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<td>0.74</td>
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<td>Roberta</td>
<td>480</td>
<td>15 (SD 6)</td>
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<td>0.876</td>
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<td>Larry</td>
<td>160</td>
<td>1.2</td>
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**O2-Hb Dissociation Curve**

Complete O2-Hb dissociation curves were determined with blood from 10 seals, with P50 values obtained in an additional five seals. The P50 was 30.5 mmHg at a pH of 7.4 (SD 1.2; n = 15 seals) (Fig. 1). The CO2 Bohr effect (slope of log P50 vs. pH) was $-0.56$ (y = $-0.56 x + 5.63$, $r^2 = 0.99$, $P < 0.0001$). The fixed acid Bohr effect (addition of HCl or lactic acid) was not significantly different from the CO2 Bohr effect.

The resulting regression equations from the plots of log[SO2/(100 – SO2)] vs. log(P50), all saturation points, for all seals combined, were: pH (7.4): log[SO2/(100 – SO2)] = 2.60211 × log(P50) – 3.84507 (n = 63, $r^2 = 0.99$, $P < 0.0001$); pH (7.3): log[SO2/(100 – SO2)] = 2.59256 × log(P50) – 3.97523 (n = 33, $r^2 = 0.98$, $P < 0.0001$); and pH (7.2): log[SO2/(100 – SO2)] = 2.622 × log(P50) – 4.11264 (n = 24, $r^2 = 0.98$, $P < 0.0001$).

Samples from other species (L. weddelli, P. vitulina, Z. californianus) run for dissociation curve method validation agreed with previously published values (54, 55). Mixing technique verification tests confirmed that no hemolysis occurred while mixing blood samples in the syringes, based on clear plasma color in hematocrit tubes after centrifugation. The mean Hb concentration in this study [24.9 g/dl (SD 3.4), $n = 454$] agreed with previously published values (54, 55). Mixing electrodes or battery failure partway through the deployment.

**PO2 Profiles, SO2, and O2 Depletion**

PO2 profiles were obtained for 2,165 dives from 11 seals (extradural vein = 3 seals, 454 dives; hepatic sinus = 4 seals, 1,050 dives; arterial = 4 seals, 661 dives). The variability in the number of dives between seals is explained by the differences in their time spent at sea and, in some cases, breakage of electrodes or battery failure partway through the deployment.

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**Table 1. Individual dive, PO2, SO2, and depletion rate data for all seals**

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<td>106.3 (SD 30.0)</td>
<td>0.1 (SD 0.4)</td>
<td>0.1 (SD 0.4)</td>
<td>2.5 (SD 1.1)</td>
</tr>
<tr>
<td>Jonesie</td>
<td>70</td>
<td>3.5</td>
<td>3.5</td>
<td>0.415</td>
<td>10.5 (SD 2.9)</td>
<td>10.5 (SD 2.9)</td>
<td>106.3 (SD 30.0)</td>
<td>0.1 (SD 0.4)</td>
<td>0.1 (SD 0.4)</td>
<td>2.5 (SD 1.1)</td>
</tr>
</tbody>
</table>

**Fig. 1. Oxygen-hemoglobin (O2-Hb) dissociation curves from one seal at pH 7.4, 7.3, and 7.2. PO2, partial pressure of oxygen.**
The shape of the PO2 profile was generally consistent among all seals in all three blood compartments. PO2 continued to increase (from the predive surface interval) at the start of the dive and then exhibited a progressive decline throughout the remainder of the dive (Figs. 2 and 3). The decrease of PO2 generally became more rapid coincident with the start of ascent, often most pronounced in the final 15–45 s of the dive. The PO2 continued to decrease during the dive, often most pronounced in the final 15–45 s of the dive (Figs. 2 and 3). The decline in PO2 also continued briefly for ~5–25 s after the dive. After this brief postdive decline, PO2 increased quickly during the surface interval.

Minimum PO2, maximum PO2, and blood O2 depletion rates during diving were significantly different between the three PO2 electrode sites (extradural vein, hepatic sinus, and arterial) (one-way ANOVA of seals grouped according to electrode site: P < 0.001 for all; F values = 932.38, 1,298.76, 308.81, respectively) (Table 2). Minimum PO2 and minimum SO2 had a significant negative correlation with dive duration for all seals, while maximum SO2 had a significant positive correlation with dive duration in all but one seal (Spearman rank order correlation, Table 3). The %O2 content depleted (%total venous or arterial O2 content depleted during the dive) had a significant positive correlation with dive duration in all but one seal (Spearman rank order correlation, Table 3).

The minimum pV02, in the extradural vein was ≤ 10 mmHg in 51% of dives (reaching as low as 2 mmHg), and ≤ 5 mmHg in 2% of dives (Table 2). In the hepatic sinus, minimum pV02 was ≤ 10 mmHg in 21% of dives and ≤ 5 mmHg in 9% of dives. Minimum PaO2 was ≤ 30 mmHg in 46% of dives and ≤ 20 mmHg in 10% of dives. When only considering dives ≥ 10 min (routine dives for this species), minimum PaO2 was ≤ 20 mmHg in 29% of dives.

Maximum PO2 (the peak value obtained during the dive), minimum SO2, maximum SO2, %O2 content depleted, and PO2 depletion rates are displayed in Tables 1 and 2. The depth and elapsed time (from dive start) at which maximum PO2 occurred was variable [extradural vein: mean = 35.8 m (SD 24.6) and 68 s (SD 31), range = 0.5–113.5 m and 1–326 s; hepatic sinus: mean = 68.5 m (SD 51.3) and 108 s (SD 66), range = 1–336.5 m and 3–757 s; arterial: mean = 20.0 m (SD 15.4), and 31 s (SD 13), range = 1–82.5 m and 1–96 s].

The average surface interval was 91 s (SD 48; n = 2,093). Although the PO2 increased rapidly during the surface interval (Figs. 2 and 3), the time required for PO2 to return to an SvO2 of 75% (PvO2 = 46 mmHg) or 90% SaO2 (PaO2 = 70 mmHg) was variable. Furthermore, PO2 did not always reach this level during the surface interval or even during the next dive. For dives that did reach these SaO2 and SvO2 criteria, either during the surface interval or within the following dive (46% of dives for the extradural vein, 59% of dives for the hepatic sinus, and 86% of dives for the arterial side), the mean time to return to these SvO2 and SaO2 levels was 123 s (SD 23; range = 8–289 s) in the extradural vein, 149 s (SD 72; range = 1–695 s) in the hepatic sinus, and 82 s (SD 39; range = 1–356 s) for the artery.

**PO2 Electrode Calibration and Verification**

Response of the PO2 electrode to a change in O2 concentration is immediate. The near complete response time, as specified by the manufacturer and verified in this study and in Stockard et al. (81), is 60 s. Postdeployment verification tests of the probes revealed no significant changes in response time or between the original and postdeployment calibrations and no baseline drift.

**DISCUSSION**

**PO2 and SO2 extremes**. The minimum PaO2 values (12–23 mmHg) documented for elephant seals in this study correspond to routine SaO2 of 8–26%, the lowest values ever measured in a freely diving seal (Table 1, Figs. 4 and 5). The extreme PO2 values in this study are less than that previously measured in free-diving Weddell seals (L. weddellii) (lowest PaO2 = 18.2 mmHg, corresponding to 28% SaO2) (72) and during sleep apnea of elephant seals (82). These exceptionally low values demonstrate remarkable hypoxic tolerance for the elephant seal compared with most mammals. For example, shallow

**Table 2. Dive, PO2, SO2, and depletion rate data for seals grouped by PO2 electrode location (extradural vein, hepatic sinus, arterial)**

<table>
<thead>
<tr>
<th>PO2 Electrode Location</th>
<th>No. Seals/Dives</th>
<th>Duration, min</th>
<th>Dive Depth, m</th>
<th>Max PO2, mmHg</th>
<th>Min PO2, mmHg</th>
<th>Max SO2, %</th>
<th>Min SO2, %</th>
<th>%O2 Content Depleted</th>
<th>PO2 Depletion Rate, ml O2·dl⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extradural vein</td>
<td>3/454</td>
<td>10.2 (SD 6.3)</td>
<td>71.7 (SD 70.9)</td>
<td>44 (SD 12)</td>
<td>12 (SD 7)</td>
<td>69 (SD 15.8)</td>
<td>9.7 (SD 12.6)</td>
<td>83.8 (SD 21.2)</td>
<td>2.4 (SD 0.9)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>1–43.7</td>
<td>5–300</td>
<td>16–75</td>
<td>2–48</td>
<td>16–3–16.6</td>
<td>0.1–77.6</td>
<td>4.3–99.9</td>
<td>0.3–6.8</td>
</tr>
<tr>
<td>Hepatic sinus</td>
<td>4/1,050</td>
<td>12.0 (SD 5.8)</td>
<td>139.0 (SD 135.9)</td>
<td>50 (SD 16)</td>
<td>17 (SD 8)</td>
<td>74.6 (SD 13.3)</td>
<td>19.6 (SD 16.0)</td>
<td>72.2 (SD 23.1)</td>
<td>1.8 (SD 1.3)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>1–29.3</td>
<td>5–699</td>
<td>22–124</td>
<td>0–40</td>
<td>30.8–97.6</td>
<td>0–67.8</td>
<td>4.0–100</td>
<td>0.2–2.19</td>
</tr>
<tr>
<td>Arterial</td>
<td>4/661</td>
<td>8.8 (SD 5.4)</td>
<td>79.5 (SD 97.4)</td>
<td>88 (SD 20)</td>
<td>36 (SD 16)</td>
<td>93.0 (SD 34)</td>
<td>53.9 (SD 24.5)</td>
<td>41.7 (SD 26.8)</td>
<td>1.5 (SD 0.7)</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>1–30.5</td>
<td>5–474</td>
<td>45–171</td>
<td>12–76</td>
<td>74.1–98.9</td>
<td>8.4–91.8</td>
<td>0.7–91.0</td>
<td>0.2–4.4</td>
</tr>
</tbody>
</table>

The mean (SD) and range are given for each location.
BLOOD O₂ DEPLETION IN DIVING ELEPHANT SEALS

Table 3. Spearman rank order correlation test data

<table>
<thead>
<tr>
<th>Seal</th>
<th>No. Dives</th>
<th>Min PO₂/Duration</th>
<th>Min SO₂/Duration</th>
<th>Max SO₂/Duration</th>
<th>%O₂ Depleted/Duration</th>
<th>Depletion Rate/Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick</td>
<td>267</td>
<td>-0.809 &lt;0.001</td>
<td>-0.825 &lt;0.001</td>
<td>0.616 &lt;0.001</td>
<td>0.894 &lt;0.001</td>
<td>-0.679 &lt;0.001</td>
</tr>
<tr>
<td>Starburst</td>
<td>106</td>
<td>-0.298 0.002</td>
<td>-0.490 &lt;0.001</td>
<td>0.674 &lt;0.001</td>
<td>0.602 &lt;0.001</td>
<td>-0.933 &lt;0.001</td>
</tr>
<tr>
<td>Patty</td>
<td>81</td>
<td>-0.720 &lt;0.001</td>
<td>-0.743 &lt;0.001</td>
<td>0.737 &lt;0.001</td>
<td>0.826 &lt;0.001</td>
<td>-0.844 &lt;0.001</td>
</tr>
<tr>
<td>Bodil</td>
<td>192</td>
<td>-0.793 &lt;0.001</td>
<td>-0.868 &lt;0.001</td>
<td>0.584 &lt;0.001</td>
<td>0.881 &lt;0.001</td>
<td>-0.037 0.297</td>
</tr>
<tr>
<td>Larry</td>
<td>218</td>
<td>-0.392 &lt;0.001</td>
<td>-0.456 &lt;0.001</td>
<td>0.464 &lt;0.001</td>
<td>0.537 &lt;0.001</td>
<td>-0.485 &lt;0.001</td>
</tr>
<tr>
<td>Per</td>
<td>160</td>
<td>-0.715 &lt;0.001</td>
<td>-0.716 &lt;0.001</td>
<td>0.120 0.130</td>
<td>0.779 &lt;0.001</td>
<td>-0.719 &lt;0.001</td>
</tr>
<tr>
<td>Roberta</td>
<td>480</td>
<td>-0.869 &lt;0.001</td>
<td>-0.881 &lt;0.001</td>
<td>0.447 &lt;0.001</td>
<td>0.903 &lt;0.001</td>
<td>-0.929 &lt;0.001</td>
</tr>
<tr>
<td>Jerry</td>
<td>132</td>
<td>-0.937 &lt;0.001</td>
<td>-0.938 &lt;0.001</td>
<td>0.826 &lt;0.001</td>
<td>0.951 &lt;0.001</td>
<td>-0.596 &lt;0.001</td>
</tr>
<tr>
<td>Sammy</td>
<td>70</td>
<td>-0.707 &lt;0.001</td>
<td>-0.721 &lt;0.001</td>
<td>0.726 &lt;0.001</td>
<td>0.772 &lt;0.001</td>
<td>-0.375 &lt;0.001</td>
</tr>
<tr>
<td>Knut</td>
<td>242</td>
<td>-0.898 &lt;0.001</td>
<td>-0.912 &lt;0.001</td>
<td>0.825 &lt;0.001</td>
<td>0.922 &lt;0.001</td>
<td>-0.685 &lt;0.001</td>
</tr>
<tr>
<td>Jonesie</td>
<td>217</td>
<td>-0.838 &lt;0.001</td>
<td>-0.838 &lt;0.001</td>
<td>0.576 &lt;0.001</td>
<td>0.883 &lt;0.001</td>
<td>0.255 &lt;0.001</td>
</tr>
</tbody>
</table>

Shading indicates the only 2 instances in which there was no significant correlation between the 2 variables.

The increase in PaO₂ during the initial phase of descent (Fig. 3) is consistent with the maintenance of pulmonary gas exchange, and the transfer of O₂ from the lungs to the blood at depths that are shallower than that at which lung collapse or cessation of gas exchange is predicted to occur (8, 24, 25, 46, 48). Maximum PaO₂ values in this study were slightly lower than that of a previous study on Weddell seals [max PaO₂ = 171 mmHg in this study and 232 mmHg for the Weddell seal (72)]. In part, this difference in absolute values may be due to the response time of the PaO₂ electrode. Although lower PaO₂ values might indicate a shallower depth of “lung collapse” in the present study, the absolute value of PaO₂ will be a function not only of ambient pressure, but also of the O₂ fraction in the lung, ventilation perfusion matching, and the degree of the pulmonary shunt (24, 25, 46, 47). All things considered for these four variables, the ambient pressure (depth) for the peak PaO₂ value is probably the best and most reliable estimate of the depth of lung collapse during dives in the wild.

The depth at which the maximum PaO₂ occurred was highly variable. If the depth at which peak PaO₂ occurs is indicative of the minimum depth at which there is cessation of gas exchange, then gas exchange does not always cease at the 30- to 50-m depth suggested by prior studies (25, 46). These findings are more likely consistent with a wider range of depths at which lung collapse could occur (24, 48). This may be secondary to differences in diving lung volume. Considerable variability in diving lung volume has been demonstrated in simulated dives of harbor seals and sea lions (48). One of the seals in the present study (Jonesie) had generally higher values of minimum SaO₂ and consistently high maximum SaO₂ (and consequently a lower percentage of arterial O₂ content depletion during dives) than other seals, even for dives of similar durations and depths [mean minimum PaO₂ = 54 mmHg (SD 11); mean maximum PaO₂ = 87 mmHg (SD 12), Table 1]. It is possible that this seal dived with a larger lung volume than the other seals. A larger lung volume should lead to increased PaO₂ in the blood, especially during shallow dives in which the lungs have not undergone collapse.

Implications for Blood Flow

Arteriovenous shunts and the spleen. PaO₂ increased during the early phase of the dive (Figs. 2 and 3). This is similar to previous findings in diving emperor penguins (Aptenodytes forsteri) (70). The magnitude of the increase in PaO₂ was variable, but at times reflected arterial values. This was especially evident in some dives of one seal (Roberta, Table 1), where PaO₂ reached 124 mmHg in the hepatic sinus. Such high PaO₂ values are not consistent with blood extraction by tissues and are, indeed, indicative of arterialized blood and potential use of an arteriovenous shunt. Shunting could potentially occur in the well-described arteriovenous anastomoses in the skin (11) or perhaps through splenic blood vessels, although the spleen itself is considered to be contracted during diving (37, 85). The spleen of the phocid seal has been proposed to act as a “scuba tank” and significantly support the hypothesis that the spleen is important for O₂ storage during both diving and exercise (93, 94).
increase Hb concentration (and O2 content) during dives, but the magnitude of such an effect in elephant seals is probably minimal due to the insufficient amount of time for splenic expansion during the very short surface intervals of these seals (37, 72, 85). In addition, this scuba tank effect would only be seen in the first dive of a bout when the spleen first contracts.

Muscle blood flow. Increases in PvO2 during the initial period of descent are also consistent with a lack of blood flow to muscle. The initial descent consists of a period of continuous stroking for 30–200 s when the elephant seal must overcome its positive buoyancy (92). This range of time overlaps completely with the time in which PO2 continues to increase toward the maximum PO2 during the dive [mean time to maximum PO2 = 31 s (SD 13) in the arterial system, 68 s (SD 31) in the extradural vein, and 108 s (SD 66) in the hepatic sinus]. If active locomotory muscles were being perfused during this period of high activity, PvO2 would be expected to decline due to O2 extraction, not to increase as we observed here (Figs. 2 and 3). One might consider the subsequent decline in PvO2 during the later part of descent as evidence for a resumption of muscle blood flow and muscle O2 extraction, especially in the extradural vein since it drains the paraspinal muscles. During this phase of the dive, however, near-infrared spectroscopy studies in Weddell seals provided no evidence of routine muscle O2 resaturation, which would be expected if muscle blood flow resumed (30). In summary, these PvO2 and SvO2 data do not provide evidence for maintenance of muscle blood flow during the descent phase of the dive, and therefore have major implications for modeling convective O2 transport in seals (18, 19).

Extradural vein flow. Minimum PO2 in the extradural vein reached values between 2 and 10 mmHg routinely in all seals (Table 2). These final PO2 values were generally lower than those in the hepatic sinus, perhaps reflecting flow patterns and return of more desaturated blood within the extradural vein. This could include blood flow from the intracranial drainage and paraspinal muscles (31, 39). If there is an increase in muscle blood flow and delivery of O2 to muscle at the end of the dive coincident with the ascent tachycardia in these animals (5), the PvO2 and SvO2 of the extradural vein blood that has drained locomotory muscle would reflect this increased O2 extraction. Because minimum PvO2, maximum PvO2, and blood O2 depletion rates were significantly different between the extradural vein and hepatic sinus, it does not seem justified to conclude that the extradural vein can be considered representative of either the entire venous system or the hepatic sinus. With the drainage of blood from the brain and other tissues into
this vein, the extradural vein may be indicative of how low venous O2 decreases in the peripheral veins, but not in the central hepatic sinus venous reservoir during diving.

Ascent tachycardia and tissue perfusion. The rate of \( P_{\text{VO}_2} \) (and often \( P_{\text{A}_2} \)) and \( S_{\text{vO}_2} \) decline generally became more rapid coincident with the start of ascent (Figs. 2, 3, and 5), often becoming even steeper in the final 15–45 s of the dive. The declines in \( P_{\text{VO}_2} \) are concurrent with the increase in heart rate which occurs during ascent in this species (5). This ascent or “anticipatory” tachycardia is characterized by a gradual increase in heart rate upon ascent; the rate of increase becomes most pronounced in the last 15 s of the dive. It has been hypothesized to increase blood flow and O2 delivery to depleted tissues, thereby lowering the \( P_{\text{O}_2} \) in the blood and providing a larger gradient to maximize O2 uptake at the surface (84). Such a function for the ascent tachycardia would be consistent with the \( P_{\text{vO}_2} \) profile. This decline in \( P_{\text{VO}_2} \) during the ascent is also congruent with the continuous stroking that occurs during the initial ascent in dives of elephant seals (92). Thus, the steeper decline of \( P_{\text{VO}_2} \) during ascent supports the concept of blood flow to locomotory muscle and muscle O2 uptake during this period of intense stroking. Blood flow to muscle during this period also agrees with previous studies of Weddell seals that demonstrated the temperature of the primary locomotory muscle does not increase during diving (29, 30, 64).

Alternatively, because gas exchange has presumably resumed at shallower depths, the steeper decrease in \( P_{\text{A}_2} \) and subsequently \( P_{\text{VO}_2} \) in the final 15–45 s of the dive might also reflect the decline in alveolar \( P_{\text{O}_2} \) due to the large decreases in ambient pressure during the final phase of ascent. In addition, if alveolar \( P_{\text{O}_2} \) during final ascent were less than \( P_{\text{VO}_2} \), O2 would diffuse from the lung capillary into the alveolus and result in a more rapid decline in \( P_{\text{A}_2} \) (35, 50).

The continued decline in \( P_{\text{O}_2} \) after the end of the dive is likely explained by a combination of: 1) the response time of the \( P_{\text{O}_2} \) electrode (81), 2) the sampling rate of the \( P_{\text{O}_2} \) electrode (once every 5- or 15-s, depending on the recorder), 3) the lag time in circulation from the heart through the arterial system and into the veins, 4) a delay in inhalation due to presumed maximal expiration that immediately follows the dive, and 5) increased blood O2 extraction by previously ischemic tissues. In a study of \( S_{\text{aO}_2} \) during apnea in humans, this continual decline occurred until \( \sim 30 \) s after breathing resumed (1), compared with \( \sim 5–25 \) s after the dives of the elephant seals in this study. A similar decline in \( P_{\text{VO}_2} \) that continued into the postdive period was also observed in diving emperor penguins (70).

**Implications for Blood O2 Store Management**

**Hypoxemic tolerance and the hepatic sinus-blood O2 reservoir.** There was considerable overlap between minimum hepatic sinus \( S_{\text{vO}_2} \) values and minimum \( S_{\text{aO}_2} \) values in this study (Fig. 4), with the exception of one of the seals with a hepatic sinus electrode (Roberta). Arterial and venous blood O2 equilibration was expected due to the diminishing differences in arterial and venous O2 contents in a breath-hold diver with collapsed lungs and is similar to results from previous forced submersion and sleep apnea studies (23, 82).

Despite the existing overlap of arterial and hepatic sinus \( P_{\text{O}_2} \) values, minimum \( P_{\text{A}_2} \) and \( S_{\text{aO}_2} \) values at the end of the dive were slightly higher than those in the hepatic sinus (Fig. 4, Table 2). The mean of minimum \( P_{\text{A}_2} \) was also significantly different from the \( P_{\text{VO}_2} \) in the hepatic sinus. These slightly elevated values on the arterial side could reflect resumption of lung gas exchange and O2 uptake from the lungs during the final phase of the seal’s ascent. Increased perfusion of the lung secondary to the ascent tachycardia could enhance the potential effect of the resumption of gas exchange in maintaining higher \( P_{\text{A}_2} \) at the end of the dive. As discussed previously, this hypothesis assumes that some lung O2 was preserved during the dive due to the cessation of gas exchange at depth. This could serve to increase the arterial O2 content at the end of the dive, preventing the \( P_{\text{O}_2} \) from dropping too low and mitigating the risk of shallow water blackout (17).

![Fig. 4](image-url)

**Fig. 4.** Minimum \( P_{\text{O}_2} \) during the dive vs. dive duration in seals with electrodes in the hepatic sinus and in the artery (A) and calculated \( S_{\text{vO}_2} \) (B) for these dives. Note the overlap of hepatic sinus and arterial minimum \( S_{\text{O}_2} \) values with the exception of lower values in one seal with an electrode in the hepatic sinus (Roberta). Minimum \( S_{\text{O}_2} \) was determined at \( \text{pH} = 7.4 \) for dives < 15 min, and \( \text{pH} = 7.3 \) for dives > 15 min. Minimum \( S_{\text{O}_2} \) had a significant negative correlation with dive duration in all seals (Spearman rank order correlation, see Table 3).
The small differences observed between PaO₂ and hepatic sinus PvO₂, however, may simply be secondary to variation among different dives by different seals. If PaO₂ and hepatic sinus PvO₂ do indeed equalize during dives, then the hepatic sinus PvO₂ profiles of Roberta (appropriately named after Robert Elsner) reflect arterial values, and demonstrate extreme in vivo cerebral hypoxemic tolerance in the elephant seal. Many of the hepatic sinus PvO₂ values in this seal often approached 0, and were far less than 10 mmHg, the threshold for electroencephalographic (EEG) changes indicative of hypoxemic brain damage (39).

Surface interval. As expected from hyperventilation and tachycardia during the surface interval (2, 5), the PO₂/SO₂ increased rapidly during this period (Figs. 2, 3, and 5). These surface interval PO₂/SO₂ data are similar to PaO₂ values obtained by blood samples during the recovery period in previous studies of Weddell seals (49, 72). The time required for PO₂ to return to representative arterial and venous values (90% SaO₂ and 75% SvO₂) was variable and did not always reach this level during the surface interval or even during the next dive. The continued increase in PO₂ during the initial descent emphasizes the importance of moderate heart rates and continued gas exchange during this early portion of the dive (5, 69). These resaturation times may reflect the time necessary to recover from the frequently low PO₂/SO₂ values at the end of the dive and, especially in the venous system, may be secondary to the circulatory lag time and the reloading of O₂ in tissues as previously discussed.

The mean time for PaO₂ to reach 70 mmHg (90% saturation) in these elephant seals was 82 s. In Weddell seals, end-tidal PO₂ of the first postdive exhalation, which presumably approximates the end-of-dive PaO₂, declined significantly with dive duration, reaching a PO₂ of 14 mmHg after a 32-min dive (44, 63). Although end-tidal PO₂ increased greatly during the second breath, the time required for end-tidal PO₂ to reach 60 mmHg after long dives (>20 min) of Weddell seals (63) ranged from 0.8 to 2.5 min, similar to the recovery time for PaO₂ in the present study. In comparison, the lowest mean end-tidal PO₂ values in grey seals and harbor porpoises were relatively high, at ~70 mmHg after mean dive durations of < 4 min and 1 min, respectively (9, 74). In these previous studies, Reed et al. (74) found that end-tidal PO₂ returned to baseline within the 0.8-min surface interval, and Boutillier et al. (9) hypothesized that reoxygenation of arterial blood occurs rapidly, within the first three or four breaths postdive. These end-tidal PO₂ findings contrast with the surface interval PaO₂ profiles in the present study and with the surface interval blood sample PO₂ data from Weddell seals in prior studies (49, 72).

There are likely several reasons why end-tidal PO₂ values during the surface interval may not accurately reflect PaO₂. During the surface interval, ventilation, heart rate, and cardiac output are at their peak in elephant seals (2, 5, 44, 69). In addition, mixed PvO₂ is probably extremely low as reflected by our extradural vein and hepatic sinus PO₂ data. The surface interval is a period of maximum oxygen uptake for the seal, analogous in many ways to exercise at maximum O₂ consumption for an athlete (89). Exercise-induced hypoxemia with a widening of the alveolar-arterial PO₂ difference as much as 30 mmHg frequently occurs under such conditions (89). This relative hypoxemia is considered secondary to 1) diffusion limitation due to increased cardiac output and decreased pulmonary transit time, 2) ventilation-perfusion inequality, and 3) intra- and extrapulmonary shunts (36, 75, 89). With low mixed PvO₂, any shunt of venous blood to the arterial side will lower the PaO₂ and contribute further to arterial hypoxemia (89). Although shunting constituted only 0.5% of cardiac output in exercising athletes (88), the shunt in harbor seals prior to submersion was 8% (48). Therefore, we hypothesize that all of these factors (diffusion limitation, ventilation-perfusion inequality, shunting, extremely low mixed PvO₂) contribute to widening of the alveolar-arterial PO₂ difference in seals during the surface interval. This hypothesis would account for the differences between prior end-tidal PO₂ profiles and the PaO₂ profiles during the surface interval in this study. In addition, after repeated deep dives, Weddell seals often cough up copious foamy secretions (presumably surfactant) during surface intervals (P. J. Ponganis, unpublished observation). Some impairment of gas exchange may occur prior to clearance of these secretions from the airway in these deep divers.

O₂-Hb Dissociation Curve

The O₂-Hb dissociation curve for the elephant seal (Fig. 1) is similar to that of other pinnipeds and equivalent to the P₅₀ quoted for this species from previously unpublished observations listed in a book chapter (54–56). These data are also in agreement with the theory that long-duration divers like many pinnipeds may benefit from Hb with relatively lower O₂ affinity (slightly lower than terrestrial animals and short-duration divers) (54, 79). Since these species generally dive upon expiration and the lungs do not comprise a significant portion of total O₂ stores, lower affinity Hb could promote diffusion of O₂ into the tissues to enhance tolerance to apnea. As expected, the Bohr effect was similar to that of other pinnipeds (56, 73, 79, 91).

The protocols used in this study cover the range of PCO₂ and pH values found in previous studies of diving Weddell seals (72). Since the blood pH changes in free-diving Weddell seals are most often secondary to elevations in PCO₂ (a respiratory acidosis) and because no difference was seen in the Bohr effect between fixed acid and CO₂ for the elephant seals in this study, the protocol used should be sufficient for calculations of SO₂ from PO₂. Most importantly, based on these arguments, the whole blood tonometry protocols for these O₂-Hb dissociation curve studies should mimic the external blood environment (i.e., the range of pH, PCO₂, and PO₂ values) of red blood cells during diving. Since the concentration of 2,3-DPG, a modulator of Hb affinity, is affected by changes in SO₂ and intracellular pH, the tonometry protocol should cause similar intracellular changes in 2,3-DPG concentration.

SO₂ Profiles

Implications to O₂ store utilization. The maximum SVo₂ was highly variable both between different seals and between dives of individual seals, indicating differences in O₂ loading during the surface intervals preceding dives (Tables 1 and 2; Fig. 5). It should also be noted that maximum saturation values in this study were likely slightly underestimated, due to the lack of determination of a true, critical Po₂ value at 100% SO₂. To ensure complete saturation in the tonometry of the 100% O₂-saturated blood for O₂-Hb dissociation curve determination, a high PO₂ (>200 mmHg) was used for that sample. Thus, the actual inflection point in PO₂ at which saturation first
reaches 100% was not determined. The lack of this data point in the regression equations results in a slightly lower calculated SO2 for PO2 values that approach the inflection point of 100% SO2. In turn, this implies that net depletion values and O2 depletion rates are slightly underestimated.

Particularly high maximum SvO2 in the hepatic sinus (almost 100%) was evident in one of the seals (Roberta) (Table 1, Fig. 5). This individual was also the seal with extremely low minimum PO2 and SO2 in the hepatic sinus (Table 1, Fig. 4). Not only was the blood O2 store maximized in this seal by the arterialization of its venous blood, but it made full use of the large O2 store by decreasing its PO2 to near zero. This pattern of maximal O2 loading and depletion may be due to a difference in the diving behavior of this animal. This seal remained at sea for an extended amount of time (15 days), performing dives indicative of foraging as opposed to more typical behavior of a direct route of transit dives across the bay and back to the colony. A complete analysis of dive type and behavior is beyond the scope of this report, but would serve to reveal such differences in O2 store loading and utilization between different dive types.

In general, the elephant seal is almost completely depleting its blood O2 stores during routine dives, with net O2 content depletion values as high as 91 and 100% (arterial and venous, respectively) (Table 2). This highly efficient use of available O2 stores is much greater than that previously predicted by models or measured in studies of sleep apnea. For example, in a computer model of O2 transport in Weddell seals, the end-of-dive limits of arterial and venous blood O2 depletion were 38 and 27%, respectively (18). In a typical 7-min sleep apnea of a young elephant seal, with a final PO2 near 25 mmHg, only 56% of the blood O2 store was utilized (82). Oxygen depletion data from this study have implications to a variety of topics in diving physiology and physiological ecology, particularly those which rely on accurate estimates of the extent of usable O2 stores and will provide valuable input for recent modeling studies focused on diving animals (18, 24). As in the above case of the seal making full use of its venous O2 store, the true maximum value for the blood O2 store may be the entire blood volume with completely oxygenated Hb.

**Blood O2 Store Depletion**

**Blood O2 depletion rates.** It is realized that the lack of simultaneous PO2 data from more than one site in the same seal...
Blood O$_2$ Contribution to Metabolic Rate During the forced submersion (20). Bradycardia (and reduced cardiac output) is associated with equivalent to those at rest (67), and apnea is maintained at resting levels (69), example of Roberta, the seal that demonstrated optimum loading is arterial and two-thirds venous in distribution (42). Using the blood volume, with the assumption that one-third of blood volume [(maximum O$_2$ content - minimum O$_2$ content)/dive duration] × blood volume, with the assumption that one-third of blood volume is arterial and two-thirds venous in distribution (42). Using the example of Roberta, the seal that demonstrated optimum loading and utilization of the blood O$_2$ store during diving, the mean value of the contribution of her venous blood O$_2$ store alone to the O$_2$ needs of her dives was 3.4 ml O$_2$·kg$^{-1}$·min$^{-1}$ (mean of calculated blood O$_2$ store contribution for all dives of this seal. This represents the venous O$_2$ store contribution only as this seal had an electrode in the hepatic sinus). This value is equivalent to: I) 76% of the resting metabolic rate of a juvenile elephant seal, quietly breathing at the water’s surface (also 76% of the diving metabolic rate for an adult Weddell seal) (63, 90); 2) the metabolic rate of a juvenile elephant seal diving in a metabolic chamber (90); and J) 117% of the allometrically predicted basal metabolic rate for an elephant seal of this mass (41).

In examining individual dives of this seal, the contribution of the venous blood O$_2$ store to metabolic rate is J) 3.6 ml O$_2$·kg$^{-1}$·min$^{-1}$ in a 7-min dive, 2) 3.0 ml O$_2$·kg$^{-1}$·min$^{-1}$ in a 12.2-min dive, and J) 1.7 ml O$_2$·kg$^{-1}$·min$^{-1}$ during an extended dive of 27.5 min. Even for dives equivalent to the mean dive duration for this seal (12.2 min), the contribution of the venous blood O$_2$ store toward total metabolic rate is > 100% of the allometrically predicted basal metabolic rate. Since the venous blood O$_2$ store contribution alone to metabolic rate (even without considering the arterial O$_2$ store or the significant muscle O$_2$ store for this animal) represents such a large percentage of resting metabolic rate and because mean temperature during dives remained near 37°C, these data suggest that at the level of the whole animal, juvenile elephant seals are not “hypometabolic” while diving and that they do not require any significant anaerobic metabolism during routine dives. Comparatively, during a typical 7-min breath hold during sleep apnea of an elephant seal, blood O$_2$ depletion (arterial and venous) contributed 4.2 ml O$_2$·kg$^{-1}$·min$^{-1}$ to total body metabolic rate during apnea (82). The range of blood O$_2$ depletion values in this study reinforce the fact that cardiovascular adjustments and, consequently, O$_2$ depletion during routine dives are intermediate between those of sleep apnea and forced submersion.

**Perspectives and Significance**

These blood O$_2$ data are the first ever measured in a truly free-diving seal with unrestrained access to the surface. The results of this study indicate that elephant seals possess exceptional hypoxemic tolerance. In dives > 10 min (routine dives for an elephant seal), P$_{vO_2}$ declined to as low as 2–10 mmHg, and P$_{aO_2}$ decreased to between 12 and 23 mmHg in all seals. These values correspond to So$_2$ values of 1–26% and O$_2$ contents as low as 0.3 ml O$_2$/dl blood (venous) and 2.7 ml O$_2$/dl blood (arterial). The underlying mechanisms of this extreme hypoxic tolerance remain to be revealed, but may include intrinsic neuronal tolerance as demonstrated in the hooded seal (Cystophora cristata) (27), increased brain capillarization (39), increased glycogen content in the brain and/or heart (32, 40), alterations in reactive O$_2$ species production or scavenging systems (21), increased tissue buffering capacity (15), or potential roles of the neurally located neuroglobin or more widespread cytoglobin O$_2$-binding proteins (12). The elucidation of these mechanisms may also provide applications relevant to enhancing the survival of humans experiencing circulatory collapse, ischemic injury, and other trauma.

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