Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

Classics

AFTER 73 YEARS, STILL THE FOUNDATION OF DIVING PHYSIOLOGY RESEARCH

Paul Ponganis discusses Per Scholander's classic monograph, 'Experimental investigations on the respiratory function in diving mammals and birds', published in *Hvalrådets Skrifter* in 1940.

Per Scholander's 1940 monograph (Scholander, 1940) has been the foundation of diving physiology research for the past 73 years. Scholander addressed two basic questions: (1) how can a diving animal hold its breath for so long, and (2) how can a diving animal withstand exposure to pressure at great depths? Earlier studies by Laurence Irving in the 1930s had emphasized the importance of a decreased heart rate (bradycardia), constriction of peripheral blood vessels, and a slow depletion of oxygen stores in determining the breath-hold capacity of diving animals (Castellini, 2012; Irving, 1939). But Scholander put all these factors together by simultaneously investigating cardiovascular responses, oxygen store depletion patterns and blood/tissue lactate formation during the breath hold. Although best demonstrated in seals, he also extended these studies to porpoises, ducks, penguins and a beaver. Documentation of the magnitude and depletion of oxygen stores also allowed comparison with pre-submersion oxygen consumption measurements, and the conclusions that (1) the seal incurred an oxygen debt during the breath hold, (2) the debt was not fully repaid by glycolysis/lactate formation during the breath hold or by excess oxygen uptake during recovery, and (3) oxygen consumption (metabolic rate) was therefore reduced from pre-submersion levels during the breath hold. All these findings supported Irving's earlier postulates. As if this work were not enough for one monograph, Scholander set out to investigate pressure tolerance in diving animals by studying the anatomy and diving behavior of whales. This work formed the basis of his hypothesis of lung collapse at depth for the prevention of decompression sickness (the 'bends').

This paper led to the collaborative, ground-breaking investigations of diving physiology by Irving and Scholander in the 1940s, and, it could be argued, to the eventual construction of Scholander's Physiological Research Laboratory at Scripps Institution of Oceanography in the 1960s and the establishment of the two foremost diving physiologists of more recent times, Robert Elsner and Gerald Kooyman. And since that time, diving physiologists have attempted to examine the nature of these physiological/metabolic/biochemical responses in free-diving animals in order to better understand the mechanisms of hypoxemic (low oxygen) tolerance, ischemic (low blood flow) tolerance and pressure tolerance, as well as explore the physiological limits of the foraging behavior and ecology of these animals. The findings and hypotheses of this monograph have remained the core of diving physiology research, as evidenced in major reviews of diving physiology, both past and recent (Blix and Folkow, 1983; Butler and Jones, 1982; Butler and Jones, 1997; Elsner and Gooden, 1983; Kooyman, 1989; Kooyman and Ponganis, 1998; Ponganis, 2011; Ponganis et al., 2011; Ramirez et al., 2007; Zapol, 1996).

In order to address the physiological responses underlying the diver's breath-hold capacity, Scholander utilized a forced submersion protocol, in which the restrained animal was submerged underwater for an unknown (to the animal) duration. In addition, he was an innovative researcher, who developed new techniques and instruments to monitor physiological and biochemical changes before, during and after the submersion period. The techniques and equipment used in these studies included: (1) a newly designed respirometer that allowed for measurement of respiratory volumes, ventilation, and changes in oxygen and carbon dioxide content; (2) electrocardiogram and temperature recording; (3) blood volume measurement; (4) arterial blood sampling for oxygen, carbon dioxide and lactate measurements; (5) muscle biopsies; and (6) analysis of muscle for oxygen content and lactate concentration.

Applying the forced submersion protocol to seals, porpoises, ducks and penguins, Scholander documented heart rate, temperature, the size of respiratory, blood and muscle oxygen stores, the rate and magnitude of oxygen store depletion, and the contribution of oxygen stores to metabolic rate during the submersion. With results from these studies, he concluded that oxygen consumption during the submergence was depressed below the pre-submersion rate. Underlying these findings were the

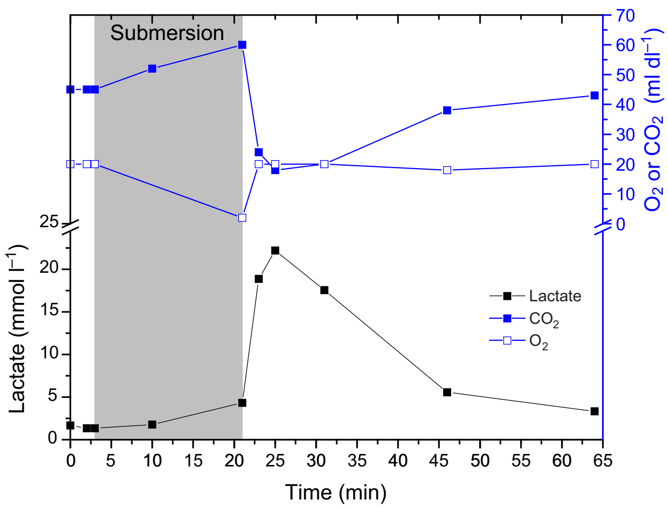

Fig. 1. Arterial oxygen, carbon dioxide and lactate content profiles before, during and after an 18 min forced submersion of a young grey seal (*Halichoerus grypus* Nilsson). Adapted from Scholander's monograph (Scholander, 1940).

bradycardia and peripheral vasoconstriction during the breath-hold period, the so-called 'dive reflex'. These cardiovascular responses were demonstrated by declines in heart rate to less than 10 beats min^{-1} and by the rate and pattern of change in blood oxygen and lactate contents (Fig. 1). In particular, peripheral vasoconstriction was demonstrated by the inability to draw blood from peripheral arteries during submersion and by the isolated accumulation of lactate in muscle during a submersion. Washout of the large concentrations of intramuscular lactate into blood only occurred during the post-submersion period when heart rate increased and muscle blood flow returned. The bradycardia and peripheral vasoconstriction of the submersion were found to be common cardiovascular responses to asphyxia in many vertebrates, even occurring in cases of human fetal distress (Elsner et al., 1966; Scholander, 1963).

Scholander also conducted research on whaling vessels, where he determined the oxygen contents of whale muscle and blood as well as measured whale lung volumes. This work prompted his hypothesis that lung collapse and lack of gas exchange at depth were a mechanism to avoid the bends. He observed that the airways of the whales were reinforced with cartilage, leading him to the concept that pressure at depth would compress the collapsible alveoli (the sites of gas exchange in the lung) and transfer air into the reinforced airways (bronchi and trachea), thus preventing the excess absorption of nitrogen at depth. This hypothesis has since generated landmark studies of lung function and nitrogen absorption in diving animals (Falke et al., 1985; Kooyman et al., 1970; Kooyman et al., 1973a; Kooyman et al., 1973b; Kooyman and Sinnett, 1982; Ponganis et al., 1999; Ridgway and Howard, 1979) and, to this day, remains an active subject of investigation, especially in relation to the potential role of decompression sickness in the stranding of beaked whales after exposure to naval sonar (Hooker et al., 2012; Houser et al., 2010; Jepson et al., 2003; McDonald and Ponganis, 2012).

In addition to addressing these two major topics in diving physiology, there were many other insights and contributions in this monograph that have often been overlooked or forgotten. Scholander logged surface-dive times of captive penguins, seals and dolphins, and even whales at sea (from whalers' reports), to examine dive behavior. He also developed a capillary tube maximum depth gauge to attach to whales, dolphins, seals and penguins to determine dive depths – a technique later used to first document maximum dive depths of birds, including emperor penguins, *Aptenodytes forsteri* Gray (Kooyman et al., 1971). His techniques to extract and analyze oxygen content in blood and muscle resulted in development of the famous Scholander one-half cubic centimeter respiratory gas analyzer commonly used throughout the last half century in physiological and medical research (Scholander, 1947).

In summary, Scholander's 1940 monograph laid the groundwork for modern diving physiology research. Today, laboratory studies of diving adaptations range from the anatomical to molecular level and current bio-logging investigations of divers in the wild extend from small seabirds to the largest whales. Scholander's work has certainly been an inspiration to my research career and has stimulated my interests in (1) end organ adaptations to hypoxemia and ischemia, (2) oxygen store management and cardiovascular responses in free-diving animals, and (3) mechanisms of pressure tolerance in both marine mammals and penguins.

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