increases in mass-specific O_2 storage in diving birds. Scholander emphasized this in his 1940 monograph. At least two additional factors contribute to that breath-hold capacity. The first is the advantage of increased body size (Noren and Williams, 2000, Noren *et al.*, 2012b). The second is the regulation of the depletion rate of those O_2 stores through changes in heart rate and organ perfusion. The latter is the subject of the next chapter. Increased body size, as noted by Krogh in 1934, confers an advantage in divers through the well-known relationship of metabolic rate to body mass (Kleiber, 1975, Krogh, 1934). Furthermore, not only is the resting or basal mass-specific metabolic rate lower in larger animals, but the metabolic cost of locomotion is also less in larger animals (Heglund *et al.*, 1982a, 1982b , Taylor *et al.*, 1982). While the former is at least partially a function of surface area–volume relationships, the latter is considered dependent on the velocity of shortening of active muscle units and the rate at which those units are activated (both higher in smaller animals). Thus, a given-sized O_2 store will last longer in the larger animal both at rest and during locomotion. Conversely, high metabolic rates secondary to small body size may also account for the high mass specific O_2 stores of the smallest marine mammal, the sea otter.

5 Cardiovascular dive response

The physiological hallmark of diving in marine mammals and birds is a decrease in heart rate relative to the pre-dive or surface heart rate (Irving *et al.*, 1941b, Scholander, 1963). Regulation of heart rate, cardiac output, and the degree of peripheral vasoconstriction during dives is essential to the management and utilization of body O_2 stores because (a) the magnitude and distribution of cardiac output to peripheral tissues contributes to rates of tissue O_2 delivery and tissue O_2 consumption; and (b) cardiac output contributes directly to the rate of blood O_2 uptake from the lungs (Hogan *et al.*, 1993, Kvietys and Granger, 1982, Lutz *et al.*, 1975, Taylor *et al.*, 1987, Valtin, 1973). Although these responses were investigated as early as 1870 by Bert (see Irving *et al.*, 1941b for review) and also examined under conditions of asphyxia (Irving, 1934, 1938, 1939, Irving *et al.*, 1935a), the slowing of heart rate to below resting levels (bradycardia) and the constriction of peripheral blood vessels (vasoconstriction) of seals and penguins during forced submersions were first thoroughly documented in Scholander's 1940 monograph.

During Scholander's forced submersion experiments, heart rates of seals were as low as 10 beats min^{-1} (bpm). Vasoconstriction and the circulatory isolation of muscle were convincingly demonstrated by (a) the depletion of muscle O_2 with a concomitant increase in muscle lactate concentration during the submersion, and (b) the subsequent wash-out of lactate into blood during the post-submersion period (Fig. 5.1). This dive reflex (severe bradycardia in combination with peripheral vasoconstriction) isolated peripheral organs and tissues from the circulation, decreased the rate of blood O_2 depletion (Fig. 5.2), and conserved that blood O_2 for the heart and brain, thus prolonging the duration of the breath hold (Irving *et al.*, 1941b, Scholander, 1940). It was not until the 1960s that Elsner found that the reductions in heart rates of seals and sea lions during trained submersions were not as severe as during forced submersions (Elsner, 1965, Elsner *et al.*, 1964a). Since that time, diving physiologists have continued to investigate the nature, plasticity, and consequences of dive responses in multiple species under different conditions.

Chapter 5 is the longest and probably most detailed section of this book because the dive response represents the core of diving physiology. As such, it is essential that students and researchers understand the physiological mechanisms and implications of the dive response. The chapter will lay the ground work for understanding the role of heart rate and vasoconstriction in (a) the conservation and preservation of O_2 for the heart and brain; (b) the regulation of metabolic rate and the depletion of O_2 stores,

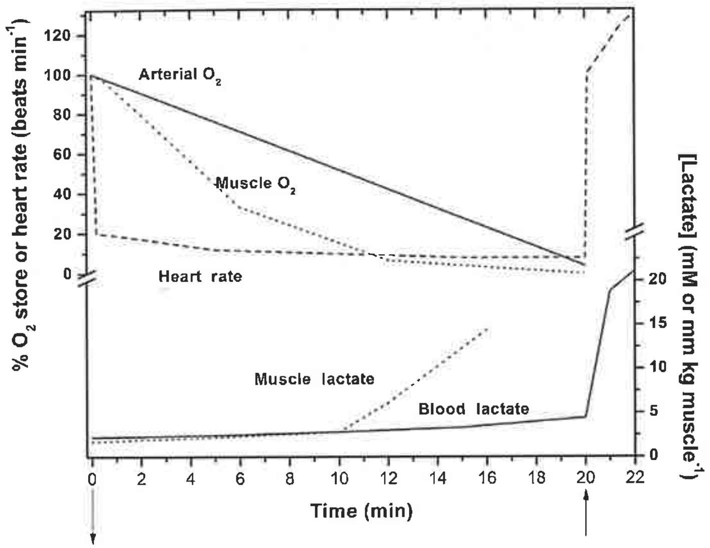

Figure 5.1 Heart rate and blood/muscle oxygen and lactate profiles during forced submersions of seals. Note the depletion of muscle oxygen and isolated intramuscular accumulation of lactate during the severe bradycardia of the submersion with subsequent wash-out of lactate into the blood during the increase in heart rate after the submersion. These profiles provided the evidence of widespread vasoconstriction during the bradycardia of forced submersion. Arrows indicate start and end of submersion. Adapted from Irving et al., (1941b), Scholander (1940, 1963), Scholander et al. (1942a).

especially the muscle O_2 reservoir (Chapters 9 and 10); (c) determination of the duration of aerobic metabolism and aerobic dive limits (Chapter 10); (d) thermoregulation (Chapter 8); and (e) nitrogen uptake/distribution and the risk of decompression sickness (Chapter 12).

In order to review the extensive literature on this subject, mammalian and avian diving cardiovascular physiology are each considered separately in this chapter. The last section of the chapter will outline the neuroregulatory control mechanisms for the dive response in both mammals and birds. Other topics in the cardiovascular physiology of these animals, including unique anatomical adaptations and the optimization of myocardial oxygen supply and demand, will be reviewed in Chapter 6.

In this chapter, there are 25 subsections for diving mammals and 12 subsections for diving birds. Each subsection covers a distinct topic and is meant to provide the reader with the research findings in that specific area. Topics include heart rate, blood flow, and organ perfusion patterns during (a) forced submersions, (b) simulated dives, (c) trained submersions, (d) spontaneous breath holds and sleep apnea (breath holding during sleep), (e) surface swimming, and (f) free dives. The variability and intensity of heart rate and peripheral vasoconstriction will be examined in different species and under different circumstances. The physiological and anatomical mechanisms underlying the peripheral vascular response (vasoconstriction) in these animals will also be reviewed. Particular attention will be focused on how heart rate and peripheral blood flow are

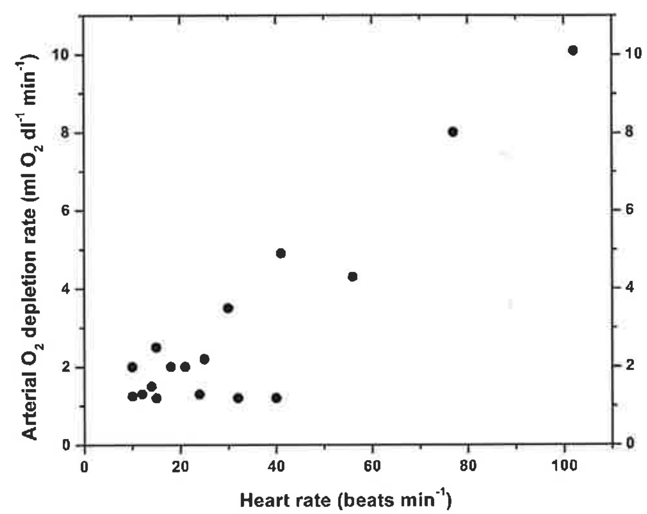

Figure 5.2 Heart rate and the rate of blood oxygen depletion during forced submersion. This graph demonstrates the dependence of the blood oxygen depletion rate on heart rate and represents the end result of the "dive response." Adapted from Irving et al. (1941b).

regulated during different underwater activities of these animals and how the "dive response" might vary as a function of exercise and other parameters during the dive.

5.1 Cardiovascular physiology in marine mammals

5.1.1 Blood flow distribution in terrestrial mammals at rest and during exercise

As a reference for comparison to blood flow distribution during diving, the distribution of cardiac output to peripheral tissues for humans at rest (Williams and Leggett, 1989) can be summarized as follows: brain 12%, heart 4%, kidneys 19%, liver 7% hepatic artery and 15% portal vein (via gastrointestinal tract), gastrointestinal tract 15%, muscle 17%, skin/skeleton 10%, fat 5%, and other 11%. Values vary in different studies as well as different species (Adachi *et al.*, 1976, Behrman and Lees, 1971, Hohimer *et al.*, 1983, Musch *et al.*, 1987a, 1987b, Upton, 2008), but this summary provides a convenient starting point in consideration of the redistribution of blood flow during both diving and exercise.

As will be seen in this chapter, a key question raised by more recent cardiovascular studies of diving animals is the degree of peripheral blood flow reduction and tissue ischemia (low blood flow) during moderate as opposed to severe reductions in heart rate. In particular, regulation of muscle blood flow in an actively swimming, diving animal is especially important in regard to the management of blood and muscle O_2 stores. Do heart rate and muscle blood flow correlate with the stroke rate of propulsive muscle so as to supply blood O_2 to muscle during diving? Is muscle blood flow tailored to muscle O_2 consumption to maximize the duration of aerobic metabolism and allow simultaneous depletion of the blood and muscle O_2 stores (Davis and Kanatous, 1999)? Could there be low-grade or intermittent muscle perfusion independent of muscle workload? Or, is there no muscle blood flow during active diving, and is muscle solely dependent on its myoglobin-bound O_2 store for aerobic metabolism?

An important point to note at this time is that the classic dive response during forced submersion differs radically from the classic exercise response. In contrast to the description at the start of this chapter for the dive response, elevated heart rates during exercise of birds and terrestrial mammals increase cardiac output, and deliver the major portion of that blood flow to exercising muscle (Taylor *et al.*, 1987). The magnitudes of blood flow to non-exercising muscle, splanchnic organs (gastrointestinal tract, liver), and the kidneys are either unchanged or decreased dependent on the species and severity of exercise (Armstrong and Laughlin, 1984, Armstrong *et al.*, 1987, 1992, Butler, 1991, Butler *et al.*, 1988, Hohimer *et al.*, 1983, Laughlin *et al.*, 2010, Musch *et al.*, 1987a, Perko *et al.*, 1998, Sanders *et al.*, 1976). Significant species differences do exist. For example, in contrast to most mammals, splanchnic and renal blood flow during even the heaviest workloads are maintained in Alaska sled dogs, animals which have been selected and trained for long-duration, continuous aerobic exercise (Van Citters and Franklin, 1969). Similarly, there may well be differences in diving heart rate and blood flow distribution patterns in diving animals with different activity patterns (i.e., "divers" versus "surfacers" – see Chapter 1 for this classification).

The physiological processes that underlie the exercise response are also relevant to the mechanisms controlling the dive response. During exercise, activation of the sympathetic nervous system with subsequent vasoconstriction of arterial blood vessels is thought to limit and/or decrease flows to visceral organs and non-exercising muscle, despite the increase in heart rate. In contrast, the increase in blood flow to working muscle occurs despite an increased peripheral sympathetic response. The increased blood flow to working muscle is considered to be mediated by several possible factors, including (a) myogenic relaxation of smooth muscle in blood vessels compressed by contracting muscles, (b) release of endothelial vasodilators in the vasculature of active muscles (nitric oxide), (c) release of "metabolic vasodilators" (adenosine and adenine nucleotides, prostaglandins, kinins, potassium), and (d) a muscular pump mechanism due to contraction of active muscle (expulsion of venous blood and decreased venous pressures, and increased kinetic energy in blood in the vascular bed imparted from contracting muscle) (Laughlin *et al.*, 2010, Saltin, 2007). In addition, hypoxia augments vasodilation even further (Casey *et al.*, 2010, Casey and Joyner, 2011, Park *et al.*, 1992, Skinner and Marshall, 1996). Whether and why blood flow to exercising muscle may or may not occur during dives of diving mammals (and birds) will be the subject of review later in this chapter.

5.1.2 Forced submersions of seals: bradycardia and vasoconstriction

The initial investigations into diving physiology utilized forced submersion, a technique in which the animal is restrained and submerged involuntarily for a period of time that is unknown to the subject. This approach in Scholander's experiments yielded a maximum dive response as described previously in this chapter. The extreme slowing of heart rate to far below resting levels (bradycardia) and the intense constriction of peripheral arteries (vasoconstriction) slowed the rate of blood O_2 depletion and redistributed blood flow to essentially the heart and brain, two of the most O_2-dependent organs in the body. The intensity of vasoconstriction during the submersion was demonstrated by a lack of bleeding in muscle biopsies, by the inability to draw blood samples through peripheral arteries, and, most significantly, by the concentration profiles of muscle O_2 content/lactate concentration during and after the submersion. As demonstrated in Fig. 5.1, during the submersion muscle O_2 declined more rapidly than blood O_2, resulting in eventual muscle lactate accumulation while at the same time blood O_2 was still preserved and there was only minor elevation in blood lactate concentration (Scholander, 1940, Scholander *et al.*, 1942a). After the submersion, when heart rate increased and blood flow returned to muscle, the wash-out of lactate into the blood confirmed that muscle had been isolated from the circulation during the submersion. Use of a hot wire anemometer demonstrated that muscle blood flow paralleled changes in heart rate during submersions and even during respiratory sinus arrhythmias (spontaneous oscillations in heart rate during the breathing cycle) (Grinnell *et al.*, 1942). Similar muscle blood flow patterns were also later confirmed with tissue laser Doppler flowmetry in submerged harbor seals (*Phoca vitulina*) (Jobsis *et al.*, 2001).

In addition to muscle ischemia during forced submersions, widespread vasoconstriction to other organs was consistent with (a) the maintenance of blood pressure in central arteries, (b) observations of decreased mesenteric blood flow, (c) decreased urine production, and (d) decreased renal clearances of urea, creatine, and p-aminohippurate (Irving *et al.*, 1935a, 1942b, Murdaugh *et al.*, 1961, Schmidt-Nielsen *et al.*, 1959, Scholander, 1940). Because blood pressure is the product of cardiac output (flow) and peripheral vascular resistance (arterial constriction), a constant blood pressure during forced submersions, when heart rate was reduced at least six-fold below resting levels, would suggest that peripheral vasoconstriction had increased resistance by a similar factor.

5.1.3 Forced submersions: angiography and Doppler flow probe measurements

In the 1960s, both angiography and the use of perivascular flow probes demonstrated the dramatic reductions in blood flow in the aorta and peripheral arteries during forced submersions of seals (Bron *et al.*, 1966, Elsner, 1969, Elsner and Gooden, 1983, Elsner *et al.*, 1966a, 1970a, 1985, Van Citters et al., 1965). Decreases in both heart rate and peripheral blood flow were usually nearly instantaneous with the start of submersions in smaller seals such as harbor seals (*Phoca vitulina*). In elephant seals (*Mirounga angustirostris*), now known to be the premier pinniped divers, the decrease in heart rate could be more variable (Elsner *et al.*, 1966a, Van Citters *et al.*, 1965). Perhaps, this may be related to their greater dive capacity or to their dive behavior (i.e., a surfacer versus a diver, see Chapter 1). For example, in one elephant seal heart rate eventually reached 4 bpm late in a 40-min submersion, but in the first minute it only slowly declined about 50% from eupneic levels to 37 bpm (Van Citters *et al.*, 1965). By 5 min, heart rate was still about 22 bpm, and it only reached 10 bpm at 20–25 minutes.

5.1.4 Forced submersions: microsphere studies

The actual decreases in tissue perfusion during the bradycardia and vasoconstriction of forced submersions were documented with radio-labeled microsphere studies in the late 1970s (Blix *et al.*, 1983, Zapol *et al.*, 1979). During these severe bradycardias, 80–100% reductions in tissue blood flow were verified in all tissues except the brain, lung, and adrenal glands. Overall, brain blood flow was preserved during these submersions. Sequential studies revealed that transient reductions in cerebral cortical and cerebellar blood flow occurred early in the submersion, but that, later in the submersion period, flow in these regions and the hypothalamus were increased above pre-submersion levels. Adrenal blood flow was preserved during forced submersions at 40–60% of pre-submersion levels. Maintenance of adrenal perfusion was consistent with the observed elevations in plasma cortisol and catecholamines during the submersion period (Cherepanova *et al.*, 1993, Hance *et al.*, 1982, Liggins *et al.*, 1979, 1993).

5.1.5 Forced submersions: arterio-venous shunts

Lastly, increased microsphere deposition in the lungs, especially early during the submersion, suggested to both teams of investigators that blood circulated through arterio-venous (A-V) shunts early during submersion (Blix *et al.*, 1983, Zapol *et al.*, 1979). The spleen might act as such a conduit, but A-V anastomoses also exist in the skin of seals (Bryden and Molyneux, 1978, Molyneux and Bryden, 1975, 1978). Notably, maintenance of blood flow in the peripheral toe arteries of the hind flipper had been occasionally observed in forcibly submerged seals (Irving *et al.*, 1942b). However, A-V anastomoses have extensive sympathetic nerve innervation and, at least in the tail fluke of the bowhead whale (*Balaena mysticetus*), these anastomoses are especially sensitive to norepinephrine levels (Elsner *et al.*, 2004a). So, it would be expected that these potential shunts would usually constrict during a forced submersion.

On the other hand, flow may continue through A-V shunts in the flippers of seals even during forced submersions (Blix *et al.*, 2010). The foreflipper, for example, has large superficial veins not associated with the deeper countercurrent heat exchanger (Blix *et al.*, 2010). Flipper temperatures were also maintained during forced submersions despite declines in skin temperatures of the back (Hammel *et al.*, 1977). On this basis, Blix and co-workers have hypothesized that such shunting through A-V anastomoses in the flippers could contribute to heat loss and body temperature declines observed during forced submersions (Blix *et al.*, 2010, Hammel *et al.*, 1977, Hol *et al.*, 1975, Odden *et al.*, 1999, Scholander *et al.*, 1942b).

Interestingly, the early opening of an A-V shunt during a submersion could also account for the observations by Van Citters *et al.* (1965) of a large reduction in iliac artery blood flow in the presence of a constant heart rate and blood pressure at the start of forced submersions of elephant seals. For example, iliac artery blood flow could

decrease by 50% at the onset of a submersion even before significant changes occurred in heart rate (Van Citters *et al.*, 1965). In one instance, iliac artery flow decreased by 1200 ml min^{-1}, a 75% reduction from eupneic levels, despite no changes in heart rate or blood pressure. Such changes were consistent with an intense vasoconstriction response in the iliac artery. However, remember that blood pressure is the product of cardiac output and peripheral vascular resistance, and that cardiac output is the product of heart rate and stroke volume. Arterial vasoconstriction and a reduction in iliac blood flow of 1200 ml min^{-1} in the presence of a constant blood pressure and heart rate would argue that either stroke volume decreased at the start of the submersion and/or that vasoconstriction to other tissues decreased. Otherwise, blood pressure should increase. In this regard, it is notable that during breath holds of human divers, skin sympathetic nerve activity decreased while that in muscle increased, consistent with vasodilation to skin and vasoconstriction to muscle (Fagius and Sundolf, 1986). Thus, those reductions in iliac artery blood flow in the presence of a constant heart rate and blood pressure are consistent with the use of A-V shunts during forced submersion. These findings also suggest that the magnitude of vasoconstriction to a particular tissue and the subsequent reduction in that tissue's blood flow may not always be quantitatively proportional to the change in the heart rate profile. Such a lack of correlation between heart rate and a given tissue's blood flow during a dive has potential limitations on assumptions used in the numerical modeling of tissue blood flow distribution and oxygen consumption during diving.

5.1.6 Forced submersion: the physiology and anatomy of peripheral vasoconstriction

In regard to the widespread vasoconstriction that occurred during the forced submersion of seals, it is notable that local vasodilation and muscle blood flow did not occur in ischemic, hypoxic muscle that had elevated tissue lactate concentrations during the latter segments of forced submersions. The release of local vasodilators in hypoxic or exercising muscle in most mammals usually represents a mechanism to overcome increased sympathetic vasoconstriction at the arteriolar level during exercise and to increase blood flow selectively to working muscle (Casey and Joyner, 2011, Joyner and Wilkins, 2007, Laughlin and Armstrong, 1987, Laughlin *et al.*, 2010, Saltin, 2007).

The maintenance of intense vasoconstriction in the presence of oxygen depletion and lactate accumulation in muscle during forced submersions is considered secondary to the dense distribution of sympathetic nerve fibers penetrating into the walls of major supply arteries that are far from the capillary beds of skeletal muscle and other major organs in seals (White *et al.*, 1973). It is the differential distribution of arterial sympathetic nerve innervation that most probably contributes to the pattern of redistribution of blood flow during the submersion. Dense sympathetic innervation occurs in the arteries supplying spinal/hindlimb muscles, splanchnic organs, and the kidneys of seals. Activation of sympathetic nerve fibers in the proximal segments of these arteries would constrict these vessels independent of the accumulation of local vasodilators in distal tissue, i.e., the extramuscular throttle of Gooden and Elsner (Cherepanova *et al.*, 1993, Gooden and Elsner, 1985). In addition, such constriction in the proximal arteries may also be enhanced because of the elevated circulating catecholamine levels during the submersion and the presence of adrenergic receptors in those arterial segments. In contrast to the arteries supplying the kidneys, gastrointestinal tract, and hind flipper muscles, such innervation patterns of sympathetic fibers were not observed in the aorta, carotid arteries, coronary arteries, or pulmonary arteries. The lack of such fibers would allow for the maintenance of blood flow to the brain and heart despite intense activation of the sympathetic nervous system.

Differences in sympathetic innervation of major supply arteries to various muscle groups may also account for the differential muscle blood flow patterns observed in Baikal seals (*Phoca sibirica*) during forced submersions (Cherepanova *et al.*, 1993, Matyukhin *et al.*, 1988, Neshumova and Cherepanova, 1984). During these submersions, the decline in muscle blood flow was most intense to muscles with the highest myoglobin concentrations, namely the muscles of the back and hind limb. The decline in blood flow to muscles of the pelvis and forelimb was intermediate, while there was no change in blood flow to muscles of the neck. Further investigation of the physiological basis of this differential muscle blood flow pattern during forced submersion has not been conducted. Perhaps there are differences in the densities of sympathetic nerve fibers to arteries supplying different muscle groups. It is also noteworthy that muscle "electrical" activity during swimming was greatest in the muscles with the highest myoglobin concentrations (Neshumova *et al.*, 1986). Thus, the most active swimming muscles, those with the highest myoglobin concentrations, have the most intense arterial vasoconstrictor responses during forced submersions. Although hidden away in the Russian literature, these observations have important implications not only for understanding regulatory mechanisms, but also for the better understanding and modeling of oxygen store utilization during diving (Chapters 9 and 10).

During dives or forced submersions, sympathetic vasoconstriction presumably overrides effects of any potential vasodilators, such as nitric oxide or carbon monoxide, that may be circulating in the blood. However, as discussed in Chapters 11 and 13, such compounds may contribute to maintenance of blood flow in non-constricted or partially constricted blood vessels, such as those to the brain, lung, heart, and, in pregnant animals, the placenta.

5.1.7 Forced submersion: the physiology and anatomy of pulsatile myocardial blood flow

In contrast to the complete ischemia of skeletal muscle, it is notable that pulsatile flow patterns have been observed in seal myocardial blood flow during forced submersions (Elsner *et al.*, 1985). Such pulsations in coronary flow may be due to the lack of sympathetic innervation in the proximal coronary artery and the competing effects of distal coronary arteriolar constriction and transient, localized, hypoxia-linked release of vasodilators at the arteriolar level. In addition, distal coronary arteriolar constriction in the seal appears to be mediated by cholinergic fibers and acetylcholine release, and not by the sympathetic nervous system (Elsner and de la Lande, 1998). Such cholinergic activity mediated by the parasympathetic system via the vagus nerve provides a mechanism to match myocardial oxygen supply (blood flow) and demand (cardiac work) during the vagally induced bradycardia of diving (Elsner and de la Lande, 1998).

5.1.8 Forced submersion of other mammals

In addition to the studies above on seals, this "dive reflex" has also been confirmed in forced submersion studies of other diving mammals, including manatees, fur seals, muskrats, beavers, mink, nutria, and even neonatal and fetal seals (Elsner *et al.*, 1970a, Ferrante, 1970, Ferrante and Opdyke, 1969, Folkow *et al.*, 1971, Hammond *et al.*, 1969, Irving *et al.*, 1963, Liggins *et al.*, 1980, McKean, 1982, Neshumova and Cherepanova, 1984, Scholander, 1940, Scholander and Irving, 1941, West and Van Vliet, 1986). As already reviewed in Chapter 2, this pattern of bradycardia and vasoconstriction is a general response to asphyxia; it occurs to some degree not only in humans and non-diving mammals such as armadillos and sloths, but also in a wide range of other vertebrates, including reptiles, amphibians, and even fish out of water (Garey, 1962, Irving *et al.*, 1942a, Johansen, 1959, Murdaugh and Jackson, 1962, Scholander, 1963, Scholander and Irving, 1941, Shelton and Jones, 1965, White and Ross, 1966, Wilber, 1960).

5.1.9 Simulated dives of pinnipeds

Simulated dives differ from forced submersions in that the animal is also exposed to increased ambient pressure. Such studies in pinnipeds have been conducted in pressure chambers designed by G. L. Kooyman in the 1960s (Kooyman and Sinnett, 1982, Kooyman *et al.*, 1976a, 1970, 1973b, Sinnett *et al.*, 1978). The goals of these experiments have primarily focused on the respiratory system and blood gas analyses. Some heart rate profiles were available, however, and the data demonstrated that the heart rate response during simulated dives was similar to that during forced submersions. Heart rates during simulated dives of Weddell seals, northern elephant seals, harbor seals, and sea lions were reported in the range of 5–20 bpm, similar to those in forced submersions of pinnipeds (Kooyman and Sinnett, 1982, Sinnett *et al.*, 1978).

5.1.10 Trained submersions of pinnipeds: moderate bradycardia

Trained submersions differ from forced submersions in that the animal is trained or conditioned to hold its breath for fixed durations. Under such conditions, the intensity of the bradycardia is usually much less, i.e., the decline in heart rate is not as great. This was demonstrated in sea lions and seals trained to immerse their heads in water (Elsner, 1965, Elsner *et al.*, 1964a), and also in sea lions trained to hold their breath in air (Ridgway *et al.*, 1975a). As will be reviewed below and later in the chapter, trained submersions as well as spontaneous breath holds are invaluable in that they provide the opportunity to examine these physiological parameters with sophisticated techniques in a non-moving and often dry animal. Elsner and co-workers, for example, were able to demonstrate that stroke volume, the volume of blood ejected from the heart per heartbeat, was constant between apnea (breath-hold period) and eupnea (breathing period) in the sea lion (Elsner *et al.*, 1964a). In 1977, Dormer and colleagues confirmed that cerebral blood flow actually increased during the bradycardia of trained head immersions of sea lions (Dormer *et al.*, 1977). As will be reviewed in Chapter 6, trained submersions of young elephant seals in a magnetic resonance imaging (MRI) scanner allowed assessments of cardiac output, stroke volume, aortic flow, and contraction/relaxation of the spleen (Thornton *et al.*, 1997, 2001, 2005).

5.1.11 Trained submersion: muscle blood flow during moderate bradycardias

The observation of higher heart rates during a trained breath hold raised the question as to the degree of vasoconstriction under such conditions, and, importantly, the isolation of muscle from the circulation and from the blood O_2 store during the breath hold. This question became even more relevant when heart rates during free dives and spontaneous breath holds of harbor seals were found to be quite variable and again often not as low as during forced submersions (Jones *et al.*, 1973).

In actuality, evidence for less intense vasoconstriction to muscle during higher heart rates was noted as early as the 1940s in a study on muscle blood flow during forced submersions (Grinnell *et al.*, 1942). Notably, the authors reported that the seal exhibited an anticipatory increase in both heart rate and muscle blood flow when the experimenter's hand was raised at the end of the submersion. During the course of the study, the seal appeared to have become conditioned to the raising of the experimenter's hand prior to elevation of its head above water at the end of the submersion. It was demonstrated that this increase in heart rate and muscle blood flow even occurred when the hand was raised in the middle of a submersion; however, both heart rate and muscle blood flow declined quickly when the breath hold was not ended.

Further investigation of the relation of heart rate, muscle blood flow, and muscle oxygenation was conducted in the 1990s during naïve and trained three-minute submersions of harbor seals (Jobsis *et al.*, 2001). In comparison of trained vs. naïve submersions (Fig. 5.3), submersion heart rate almost doubled to 35 bpm, muscle blood flow as measured by laser Doppler flowmetry increased three-fold, the deoxygenation rate of muscle, as determined by near-infrared spectroscopy, slowed by about 25%, and the rate of decline in extradural vein P_{O_2} increased. Although the seals were still bradycardic during the trained submersions, the relative increase in muscle blood flow presumably allowed for blood-to-muscle O_2 transfer, accounting for both the decrease in the rate of muscle deoxygenation and the increase in the rate of venous P_{O_2} decline during the submersion. In contrast to a forced submersion, muscle was not completely isolated from the circulation in the trained submersions of this study. However, despite supplementation from the blood O_2 store, the muscle O_2 store still declined, albeit at a slower rate. The frequency and magnitude of such blood O_2 supplementation of muscle metabolism during free diving remain key questions in understanding the management of O_2 stores during dives.

As in Grinnell's study almost 60 years earlier, the conscious, exquisite control of heart rate and peripheral vasoconstriction was also demonstrated in the Jobsis study when the last trained submersion of the study was not ended at the usual three minutes (Fig. 5.4). Without any cues from the investigators, the seal immediately lowered its heart rate and muscle blood flow to naïve levels at the three-minute mark, and maintained those low levels until the end of the submersion at five minutes.

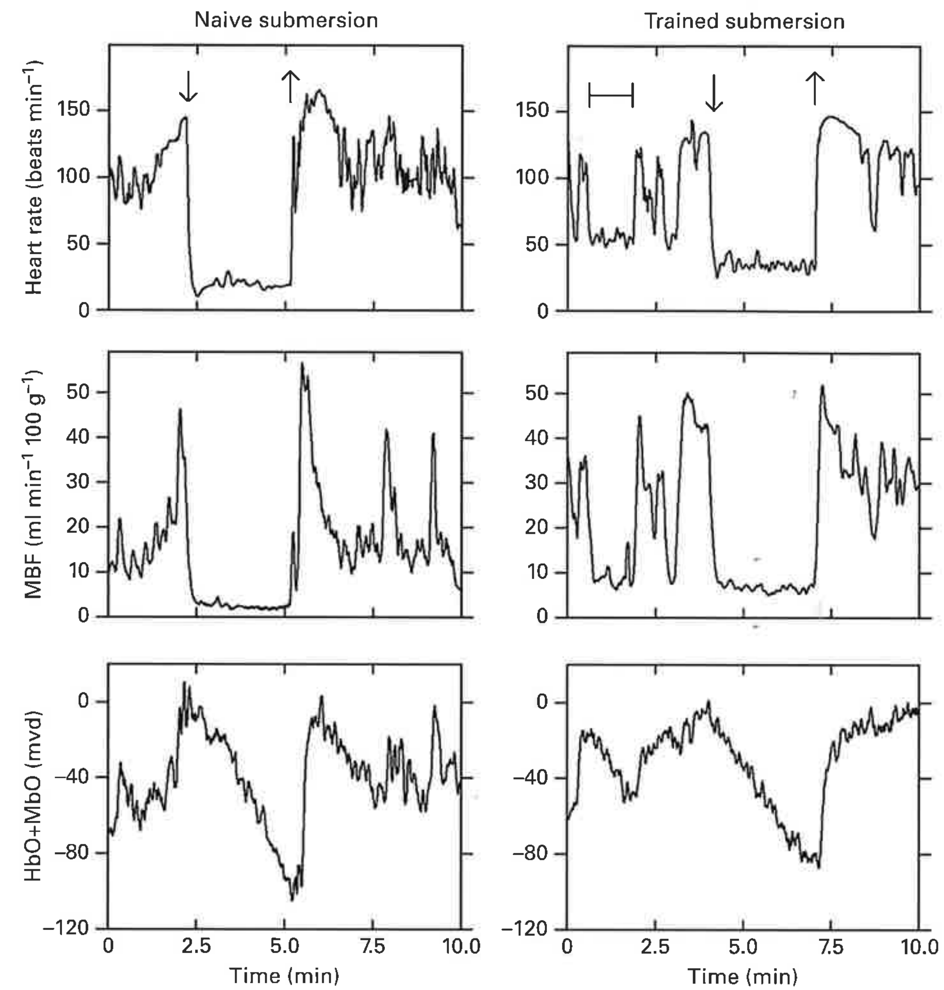

Figure 5.3 Heart rate, muscle blood flow, and muscle oxygenation during naïve and trained three-minute submersions of a harbor seal (*Phoca vitulina*). Heart rate and muscle blood flow are greater and muscle desaturation rate less during the trained submersions. Arrows indicate start and end of submersion. Bracket indicates spontaneous breath hold. Muscle oxygenation (near-infrared spectroscopy) is the saturated myoglobin and hemoglobin (MbO_2 + HbO_2) signal in vander units. Reproduced with permission from the *Journal of Experimental Biology* (Jobsis et al., 2001).

5.1.12 Spontaneous breath holds and sleep apnea

Marine mammals breathe intermittently, and it had long been known that less intense declines in heart rate occurred during short, spontaneous breath holds in seals, dolphins,

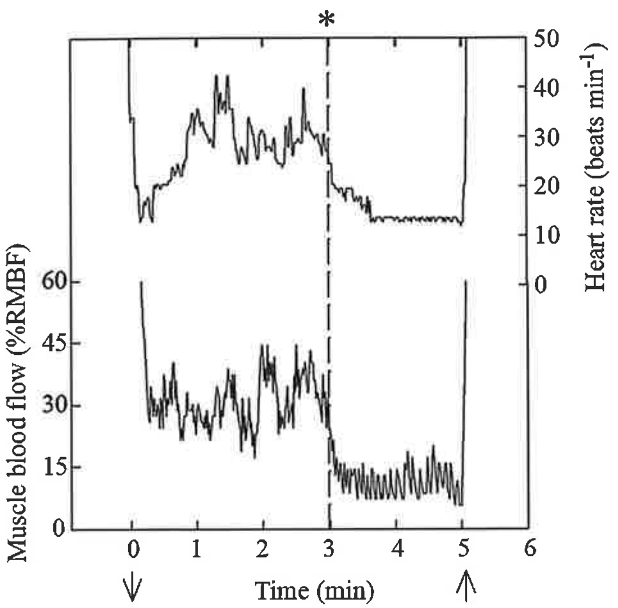

Figure 5.4 Regulation of heart rate and muscle blood flow in a young harbor seal (*Phoca vitulina*) during a trained submersion when submersion was not ended at the usual three minutes (*). Immediate declines in heart rate and muscle blood flow occurred at three minutes, and were maintained at forced submersion levels until the end of the submersion. Reproduced with permission from the *Journal of Experimental Biology* (Jobsis et al., 2001).

and manatees (Grinnell *et al.*, 1942, Irving *et al.*, 1941a, Scholander and Irving, 1941). Muscle blood flow also decreased slightly as heart rate declined during the respiratory arrhythmia of the seal (Grinnell *et al.*, 1942). Such heart rate patterns during the breathing cycle are classified as respiratory sinus arrhythmias, and are associated with higher heart rates during inspiration and slower heart rates during exhalation or during the breath hold. Sinus arrhythmias were reviewed in diving mammals and birds as early as 1939 (Irving, 1939). Similar respiratory variations in heart rate also occurred in cormorants, penguins, porpoises, and whales (Enstipp *et al.*, 1999, Halsey *et al.*, 2008, Meir *et al.*, 2008, Ponganis and Kooyman, 1999, Reed *et al.*, 2000).

The sleep apneas of phocid seals (long, spontaneous breath holds during sleep) are probably the best-known and most-studied spontaneous, non-diving breath holds in marine mammals (Andrews *et al.*, 1997, Bartholomew, 1954, Blackwell and Le Boeuf, 1993, Castellini, 1994, Castellini *et al.*, 1986, 1987, 1994a, 1994b, Falabella *et al.*, 1999, Kenny, 1979, Kooyman and Campbell, 1972, Ponganis *et al.*, 2006a, 2006b, 2008, Ridgway *et al.*, 1975b, Williams and Bryden, 1993). In sleeping sub-adult and adult northern elephant seals, mean apneic durations ranged from 7.4 to 9.7, minutes with an observed maximum duration of 23.1 min (Blackwell and Le Boeuf, 1993). Breathing only occurred during slow-wave sleep (Castellini *et al.*, 1994a).

In contrast to humans (Dempsey *et al.*, 2010), this was not a pathological condition. Such long, spontaneous breath holds in a sleeping, non-mobile, and approachable animal provided the opportunity to examine apneic cardiovascular responses in great detail.

In young elephant seals, heart rate during sleep apnea, although variable, was near 40–50 bpm, and was essentially an extension of the baseline (minimum) heart rate of the seal's sinus arrhythmia during respiration (Castellini *et al.*, 1994a; see Fig. 5.5a). Overall, mean heart rate, cardiac output, and muscle blood flow during sleep apnea were about 50% of the mean eupneic values; stroke volume was not significantly different between apnea and eupnea (Ponganis *et al.*, 2006b, 2008,). Notably, apneic cardiac outputs, although less than eupneic values, were typical of similarly sized terrestrial mammals at rest. With maintenance of a "normal" cardiac output and some muscle blood flow during sleep apnea, most organs were probably well-perfused and muscle was not isolated from the blood O_2 store during the breath hold. This maintenance of tissue perfusion is in stark contrast to the situation during forced submersions, and is more similar to that just reviewed for trained submersions. On the basis of heart rate and blood flow patterns, one would predict that blood O_2 depletion should be faster and muscle O_2 depletion slower during sleep apnea than during forced submersion. And, indeed, that is what was found (Ponganis *et al.*, 2008, Stockard *et al.*, 2007) (see Chapter 11).

Captive, wild Baikal seals in an aquarium also exhibited long, spontaneous breath holds, even when they were not asleep (Ponganis *et al.*, 1997b). When these shy animals were aware of human presence, they remained submerged at rest for up to 25 min. Apneic heart rates were 30–50 bpm during short breath holds of less than 10 min, but heart rate progressively decreased during longer breath holds – to 5–10 bpm for the last five minutes of a 25-minute breath hold. A wash-out of lactate into the blood after submersions greater than 15 min was also consistent with vasoconstriction and tissue O_2 store depletion. Although access to Baikal seals is difficult, this range of breath-hold durations and cardiovascular adjustments make this smallest of phocid seals a potentially valuable model for future studies.

5.1.13 Surface swimming: heart rate, cardiac output, and O_2 consumption

Cardiovascular responses and swim patterns during surface swimming in a swim flume have been most studied in pinnipeds. As workload increased in phocid seals, percentage time submerged decreased, but surface heart rate and submerged heart rate remained constant and distinct (Fedak *et al.*, 1988, Williams *et al.*, 1991). For example, in harbor seals, submerged and surface heart rates averaged 50 and 137 bpm, respectively, regardless of workload (Williams *et al.*, 1991). The high surface heart rate and an increased stroke volume at the surface resulted in a four-fold greater cardiac output at the surface (Ponganis *et al.*, 1990). However, similar to sleep apnea in seals, the submerged (apneic) cardiac output and stroke volume remained typical of seals and other mammals at rest.

Maintenance of significant cardiac output in shallow-swimming phocid seals was also supported by specific activity decay curves of bolus injections of labeled substrates (Castellini *et al.*, 1985). The decay curves were similar to those in seals at rest. The similarity of submerged heart rate during surface swimming to that during sleep apnea of phocid seals again would imply that there is some muscle blood flow during surface swimming. However, unlike exercise in terrestrial mammals (Musch *et al.*, 1987a), submerged heart rate, and presumably muscle blood flow, did not increase with workload (Williams *et al.*, 1991). Under such conditions, muscle blood flow during submergence could only increase if there were a selective redistribution of blood flow away from central organs and toward working muscle. On a conceptual basis, enhanced muscle blood flow might occur if there were only partial sympathetic vasoconstriction of proximal artery segments in combination with dilation of distal intramuscular arterial segments secondary to localized vasodilator release in working muscle.

In flume-swimming sea lions (*Zalophus californianus*), the swim pattern and heart rate responses differed from those in harbor seals in that percentage time submerged did not decrease with workload (Williams *et al.*, 1991). In addition, both surface and submerged heart rates increased with workload. There was a constant, but small, difference between surface and submerged heart rates. Another difference was that submerged heart rates in sea lions were near 100 bpm, much higher than in harbor seals. Cardiac output, which at rest was equivalent to that of harbor seals, increased with exercise workload in the sea lion (Ponganis *et al.*, 1991).

The increase in submerged heart rate of the sea lion in relation to workload contrasted with the constant submerged heart rate of the harbor seal. The sea lion's response was more typical of exercise in terrestrial mammals. This raised the question of whether muscle blood flow and oxygen delivery during the submergence differed between the two pinniped species. Theoretically, the higher myoglobin concentrations in the longer-diving harbor seal might make it less reliant than the young sea lion on muscle blood flow for O_2 supply during submergence. The differences in heart rate also emphasized potential differences in the magnitude of pulmonary gas exchange during submergence between the two species. Higher cardiac outputs and more gas exchange during submergence may be necessary in the sea lion due to its short surface periods at high workloads.

Regardless of the differences in cardiovascular responses in flume-swimming harbor seals and sea lions, they both exhibited about an 8–10-fold metabolic scope, which was normal to high for most mammals but less than those of "elite" athletes such as dogs and horses (Ponganis *et al.*, 1990, 1991, Taylor *et al.*, 1987, Williams *et al.*, 1991). Maximum O_2 consumption in the flume was in the range of 35–40 ml O_2 kg^{-1} min^{-1}.

Exercise research on cetaceans has focused on the bottlenose dolphin (*Tursiops truncatus*). Maximum O_2 consumption in exercising dolphins (pushing against a load cell) was about 30 ml O_2 kg^{-1} min^{-1} (Williams *et al.*, 1993). At low workloads and swim speeds, heart rate oscillated between a short post-inspiratory tachycardia and an apneic bradycardia near 50 bpm (Williams *et al.*, 1992c, 1993). This difference disappeared as heart rate increased at higher workloads and swim speeds. This cardiovascular response paralleled that in the sea lion. Notably, both surface and submerged heart rates during wave-riding were less than those during surface swimming at similar speed in the dolphin. This finding provided evidence for the lower energetic costs of wave-riding.

5.1.14 Free dives: early studies of marine mammals

Despite the significance of heart rate and cardiovascular responses to the regulation of O_2 consumption and dive duration, investigation of these physiological parameters in free-diving animals has been limited. Measurements of heart rate and other indices of cardiovascular function in free dives of aquatic mammals began with the use of a standard electrocardiogram (ECG) recorder and application of long ECG leads to allow acquisition of the ECG while the animals swam in relatively close confines nearby. Demonstration of a diving bradycardia with heart rates as low as 40 bpm were actually reported from partial ECG records as early as 1941 during shallow dives of dolphins in tanks and at sea (Irving *et al.*, 1941a). Elsner later documented that heart rate decreased from pre-dive rates of 100 bpm to submerged heart rates of 20–40 bpm in a dolphin diving in a tank (Elsner *et al.*, 1966b).

Reports of heart rate in unrestrained, diving seals were based on this same technique and were limited to short-duration, shallow dives of Weddell seals (*Leptonychotes weddellii*) and harbor seals and to the initial descent of long dives in Weddell seals (Jones *et al.*, 1973, Kooyman and Campbell, 1972). Both of these studies found that diving heart rates were variable, often about 50% the eupneic heart rate, and much greater than the 10 bpm rates reached in early forced submersion studies. The higher heart rates in unrestrained shallow dives of seals again raised the question as to the nature of the peripheral vascular response and the degree of restriction of blood flow to tissues under such conditions. In contrast to short dives, Kooyman and Campbell also reported that initial heart rates of longer dives were lower than those during shorter dives, consistent with more intense vasoconstriction and greater conservation of blood O_2 during the longer dives.

5.1.15 Weddell seals: heart rate and splanchnic/renal blood flows

In a later study designed by Kooyman's team to assess the degree of vasoconstriction and magnitude of organ perfusion in free-diving Weddell seals (Davis *et al.*, 1983), the clearances of inulin and indocyanine green (ICG) were determined as indices of renal glomerular filtration rate (GFR) and hepatic blood flow, respectively. Both inulin and ICG clearances were maintained during shorter-duration dives, suggesting that GFR and hepatic perfusion did not decrease during those dives in which Kooyman and Campbell had observed higher initial heart rates. In addition, the plasma became lipemic, indicative of maintenance of gastrointestinal blood flow. In longer dives, inulin clearance (GFR) decreased, again consistent with the observed lower heart rates and presumed greater vasoconstriction during such dives. However, ICG clearance did not decline in long dives, which suggested that hepatic perfusion was maintained even during long dives. Davis and co-workers pointed out that there was one caveat to this conclusion – namely, it was unknown if hepatic extraction of ICG was constant under conditions of decreased liver perfusion. If the extraction efficiency increased during hypoperfusion, then the ICG clearance technique would overestimate hepatic blood flow under such conditions. Thus, the issue of hepatic blood flow during longer dives was unresolved.

These studies of heart rate and perfusion were conducted on the sea ice of McMurdo Sound, Antarctica, with use of an isolated dive hole as pioneered by Kooyman in his behavioral and respiratory physiology investigations of free-diving Weddell seals (Kooyman, 1968, 1981). In this approach, a seal was allowed to dive in a man-made ice hole to which it had to return because there were no other cracks or holes in the ice which it could reach to escape. This technique, which allowed data collection from dives with a wide range of depths and durations, provided the basis to further investigate cardiovascular responses during diving in the 1980s. Such data collection became feasible with the development of microprocessor-based heart rate recorders and blood samplers (Hill, 1986). In a series of studies in the 1980s through 1990s, Zapol and associates assessed heart rate profiles and perfusion patterns of the kidneys, liver and muscle in diving Weddell seals (Zapol, 1996). These findings will be reviewed in detail below as these projects, together with work by the Kooyman teams, provide the most detailed assessment available of cardiovascular responses in any free-diving marine mammal.

Heart rates during dives of 10–15-min duration of free-diving Weddell seals were 35–45 bpm, and those of dives longer than 20 minutes were 29–36 bpm (Hill *et al.*, 1987). Heart rates during post-dive periods were 66–98 bpm, and at rest heart rates ranged from 60 to 78 bpm. Diving heart rates were variable and clearly below resting heart rates (Fig. 5.5b); in addition, heart rate decreased with dive duration. However, even during long dives, heart rates were twice the 15 bpm value of Weddell seals during forced submersions (Zapol *et al.*, 1979). Notably, the decline in fetal heart rate during free dives of pregnant seals was also not as severe and abrupt as during forced submersions (Hill *et al.*, 1987, Liggins *et al.*, 1980).

In order to assess the degree of vasoconstriction and perfusion/function of the liver and kidneys during these moderate bradycardias in diving Weddell seals, renal and hepatic clearance rates were assessed with use of radio-labeled tracers and sequential blood sampling in two studies. In a companion study to Hill *et al.*'s heart rate investigation, serial blood samples were obtained with use of a backpack microprocessor-controlled blood sampler (Guppy *et al.*, 1986, Hill, 1986). In long exploratory dives, prolonged equilibration times and markedly decreased clearance rates of p-amino-hippuric acid and inulin were consistent with decreased renal perfusion and GFR, respectively. Although measurements were only obtained for one dive, the results agreed with the prior findings of Davis *et al.* and with the heart rate observations of Hill *et al.* Clearance of cholate, considered an index of hepatic blood flow (Shrestha *et al.*, 1997), was successfully measured in one short-duration dive. Cholate clearance during the dive was slower than during rest periods at the surface, suggesting decreased hepatic function even during short dives. This contrasted with Davis *et al.*'s work, in which ICG clearance was maintained during short dives. However, Guppy *et al.* also reported that the equilibration time for cholate during the short dive was short in comparison to the longer equilibration times for tracers in longer dives, and that this was consistent with more flow and less vasoconstriction of the liver during short-duration dives. Thus, precise evaluation of liver blood flow and function during dives was again difficult to achieve.

Nonetheless, the combined work of both the Davis and Guppy papers remain remarkable achievements, and even 25–30 years later remain the only studies of renal and hepatic perfusion in free-diving animals. These papers demonstrated that splanchnic (hepatic and gastrointestinal) and renal perfusion were maintained in short-duration, aerobic dives of Weddell seals, and that significantly decreased perfusion was primarily associated with the lower heart rates found in longer dives (Davis, 2014). In addition, maintenance of aerobic metabolism even at lower heart rates may be facilitated by the high O_2 capacity of Weddell seal blood, which potentially allows for adequate O_2 extraction at lower tissue perfusion rates (Davis and Kanatous, 1999).

5.1.16 Weddell seals: heart rate, myoglobin saturation and muscle blood flow

Indirect evaluation of muscle blood flow patterns in diving Weddell seals was conducted in the 1990s with monitoring of muscle myoglobin (Mb) saturation via application of a backpack near-infrared spectrophotometer (Guyton *et al.*, 1995). This again was a remarkable technical achievement of the Zapol group. Myoglobin saturation profiles during dives were quite variable, and, overall, myoglobin desaturated at rates of 5.1% and 2.5% min^{-1} during dives less than and greater than 17 minutes, respectively. The authors concluded that muscle blood flow decreased but continued to some degree in the latissimus dorsi muscle during the mild bradycardias of these diving seals because Mb saturation profiles revealed that (a) the myoglobin desaturation rate was much less than expected and myoglobin never completely desaturated even during long dives; (b) myoglobin desaturation rates leveled off during some dives; (c) on occasion, myoglobin partially resaturated during a dive; and (d) relative blood volume in muscle (as indexed by the absorption profile of 810 nm wavelength light) often increased during ascent from long dives.

Maintenance of some blood flow to muscle during the relatively mild bradycardias in diving Weddell seals is supported by Grinnell *et al.*'s earlier observations of the coupling of muscle blood flow and heart rate during forced submersions, and with Jobsis *et al.*'s later observations of increased muscle blood flow during the higher heart rates of trained versus naïve submersions (Grinnell *et al.*, 1942, Jobsis *et al.*, 2001). It is also consistent with the intermediate declines in muscle blood flow in the mid-back and forelimb regions of Baikal seals during forced submersions (Cherepanova *et al.*, 1993).

However, interpretation of these data and conclusions in regard to the primary locomotory muscle O_2 store is complex for several reasons. First, the actual O_2 consumption rate of muscle during a dive is not known, especially for the latissimus dorsi muscle on which the near-infrared probe was implanted. That muscle extends from the mid-back to the shoulder, and functions in movement of the foreflipper. Both on an anatomical basis (Howell, 1930), and as indicated in the electromyogram studies of Baikal seals (Neshumova *et al.*, 1986), the latissimus dorsi muscle is not the primary propulsive muscle of the phocid seal. The O_2 consumption of the latissimus dorsi during a dive might well be near that of muscle at rest, i.e., 2 ml O_2 kg^{-1} min^{-1} (Blei *et al.*, 1993); at that rate, its myoglobin O_2 store could last 30 min even in the absence of any blood flow. The well-developed longissimus dorsi–iliocostalis complex is considered to provide the primary propulsion movements of the hind flippers (Howell, 1930, Pierard, 1971); it was also the most active muscle in the Neshumova *et al.* electromyogram study. This muscle complex also has the highest myoglobin concentration, and, in Baikal seals it had the most intense vasoconstriction during forced submersions (Cherepanova *et al.*, 1993). The myoglobin desaturation profile and the muscle blood flow profile of this muscle during free dives will probably have the most implications for utilization and management of O_2 stores.

5.1.17 Weddell seals: heart rate and flipper stroke rate

Examination of the relationship of heart rate to flipper stroke rate of diving Weddell seals has provided another indirect assessment of the relation of muscle blood flow to workload in diving Weddell seals (Davis, 2014, Davis and Williams, 2012, Williams *et al.*, 2015). In short-duration dives of Weddell seals, heart rate was low but was found to increase linearly with stroke rate. The authors concluded that the cardiovascular response in these free-diving animals involved a combination of both a dive response and an exercise response. This combined response was proposed to support aerobic muscle metabolism by both depletion of the Mb-bound O_2 store and blood-to-muscle O_2 transfer during the dive. This hypothesis and maintenance of some degree of muscle blood flow during short-duration, aerobic dives were consistent with (a) the above Mb saturation profiles of Guyton *et al.*; (b) absence of locomotory muscle temperature elevation in free-diving seals (Ponganis *et al.*, 1993b); (c) maintenance of muscle blood flow during Jobsis' trained submersions of seals; (d) Grinnel *et al.*'s findings that, even during forced submersions, small increases in heart rate were accompanied by increases in muscle blood flow; and (e) effects of central command and muscle mechanoreceptor feedback during exercise (see Section 5.3.6).

A positive correlation of heart rate with stroke rate has also been reported in shallow dives of bottlenose dolphins (Davis and Williams, 2012, Noren *et al.*, 2012a, Williams *et al.*, 2015). The observed increase in heart rate with stroke rate in seals and dolphins implies a linkage of heart rate and presumably muscle blood flow to muscle workload. However, in contrast to a classic exercise response, the increase in heart rate was small. In addition, dependent on the level of peripheral vasoconstriction prior to such an increase in heart rate, the expected elevation in blood flow would presumably be to all tissues and not just exercising muscle. Rather than a selective, localized increase in blood flow to working muscle as in a classic exercise response, a decrease in peripheral sympathetic tone should increase flow to all tissues due to the distribution of sympathetic nerve fibers on large, proximal arteries supplying multiple tissues in seals (White *et al.*, 1973). In addition, blood flow to splanchnic organs may increase more than that to muscle for a given change in sympathetic nerve discharge. As demonstrated in nutria (*Myocastor coypus*), muscle blood flow was more sensitive than renal blood flow to sympathetic nerve stimulation (i.e., the kidney required a higher nerve stimulation rate for equivalent blood flow reduction) (Folkow *et al.*, 1971). Based on that observation, any small reduction in peripheral sympathetic discharge during a dive would presumably increase blood flow to the kidney more than to muscle. See Sections 5.35–5.3.7 for further discussion.

5.1.18 Free dives: heart rates of other marine and aquatic mammals

Researchers have also investigated heart rate responses during free dives of other marine mammals. In the 1980s ECG telemetry investigations of manatees demonstrated that heart rate usually remained near 40 bpm during spontaneous dives (see Fig. 5.5f), and only transiently reached 50 bpm during eupnea (Gallivan *et al.*, 1986). At least in the manatee, 40 bpm appeared to be the "normal" resting heart rate, leading these authors to suggest that the manatee did not have a diving bradycardia, but rather a respiratory tachycardia. This heart rate profile in the manatee reflects the description of the sinus arrhythmia previously discussed in elephant seals and other animals. And, again, as in the elephant seal during sleep apnea, these moderate heart rates may maintain blood flow to many organs and tissues during routine diving.

It was not until the 1980s–1990s that application of either microprocessor-based heart rate recorders, Holter monitors, or ECG telemetry units allowed documentation of heart rate profiles in pinnipeds diving to depth (Ponganis, 2007). These studies all confirmed that (a) the degree of bradycardia was variable, but, overall, dive heart rate decreased as dive duration increased; (b) diving heart rates were frequently 25–50% of the eupneic level; (c) at times, diving heart rates could approach the levels observed during forced submersions; and (d) heart rate usually increased during the ascent (the so-called ascent or anticipatory tachycardia) (Fig. 5.5). The species examined included Weddell seals, harbor seals, gray seals (*Haliocherus grypus*), ringed seals (*Phoca hispida*), elephant seals (*Mirounga angustirostris*, *M. leonina*), Antarctic fur seals (*Arctocephalus gazella*), Steller sea lions (*Eumetopias jubatus*), and California sea lions (Andrews *et al.*, 1997, Boyd *et al.*, 1999, Elsner *et al.*, 1989, Fedak, 1986, Fedak *et al.*, 1988, Hill *et al.*, 1987, Hindell and Lea, 1998, Hindle *et al.*, 2010, Ponganis *et al.*, 1997d, Thompson and Fedak, 1993). The Antarctic fur seal was a relative exception to low diving heart rates, with its lowest heart rates during dives near 80 bpm, about two-thirds the eupneic level, even during its long dives of three-minute duration (Boyd *et al.*, 1999); see Fig. 5.5e.

5.1.19 Gray seals and elephant seals: heart rates during free dives

The variability in heart rates during dives of pinnipeds were well-demonstrated in gray seals and elephant seals (Fig. 5.5c, a). Mean dive heart rates of gray seals were 40–50 bpm during 1–3 min dives, but decreased to 10 bpm for dives of 20–26 min duration (Thompson and Fedak, 1993). During one 14-minute dive, heart rate during 90% of the dive was less than 4 bpm! In this dive, peripheral vasoconstriction and conservation of blood O_2 presumably were maximized just as during forced submersions. In comparison, during 30 min dives of northern elephant seals, mean heart rates were still 25–30 bpm (Andrews *et al.*, 1997). Although heart rate also declined with dive duration in elephant seals, the relatively high heart rates in 20–30-minute dives of elephant seals probably allowed for much more tissue perfusion and O_2 delivery than

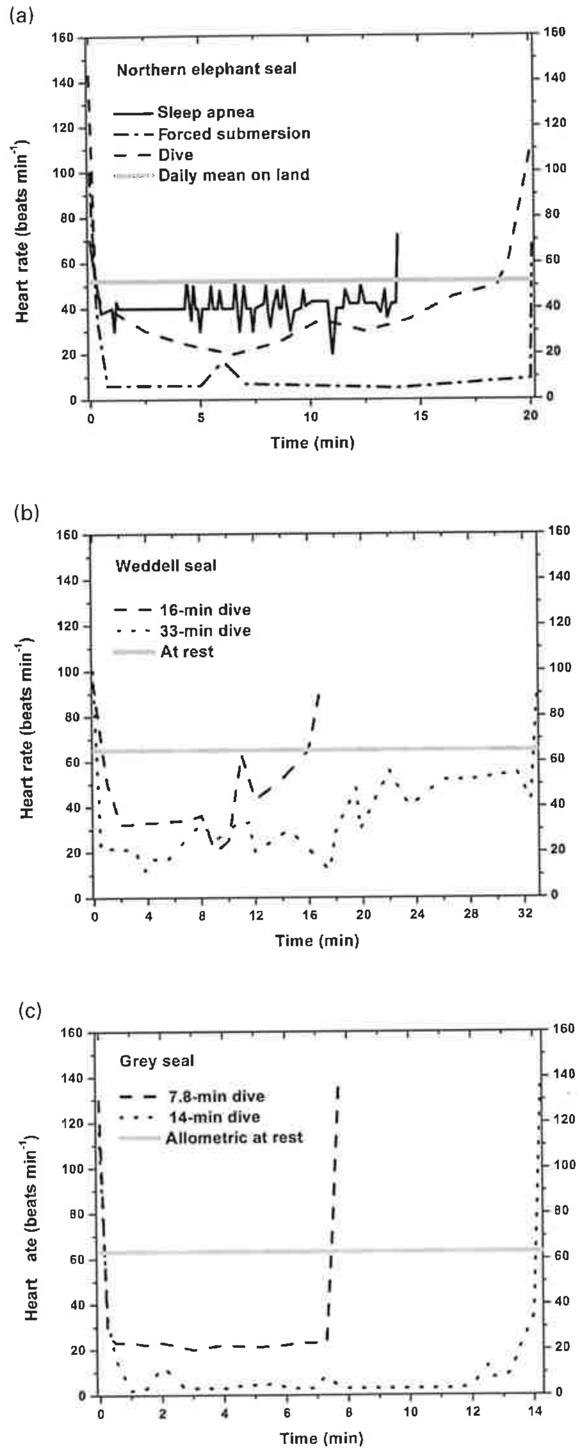

Figure 5.5 Heart rate responses during free dives in relation to heart rate at rest in five pinnipeds and the manatee. Graphs illustrate generalized pattern of heart rate profiles relative to reported heart rate at rest. **(a)** Elephant seal (*Mirounga angustirostris*), free dive, sleep apnea and forced submersion, adapted from Andrews *et al.* (1997) and Elsner *et al.* (1966a). **(b)** Weddell seal (*Leptonychotes weddellii*), adapted from Hill *et al.* (1987). **(c)** Gray seal (*Halichoerus grypus*),

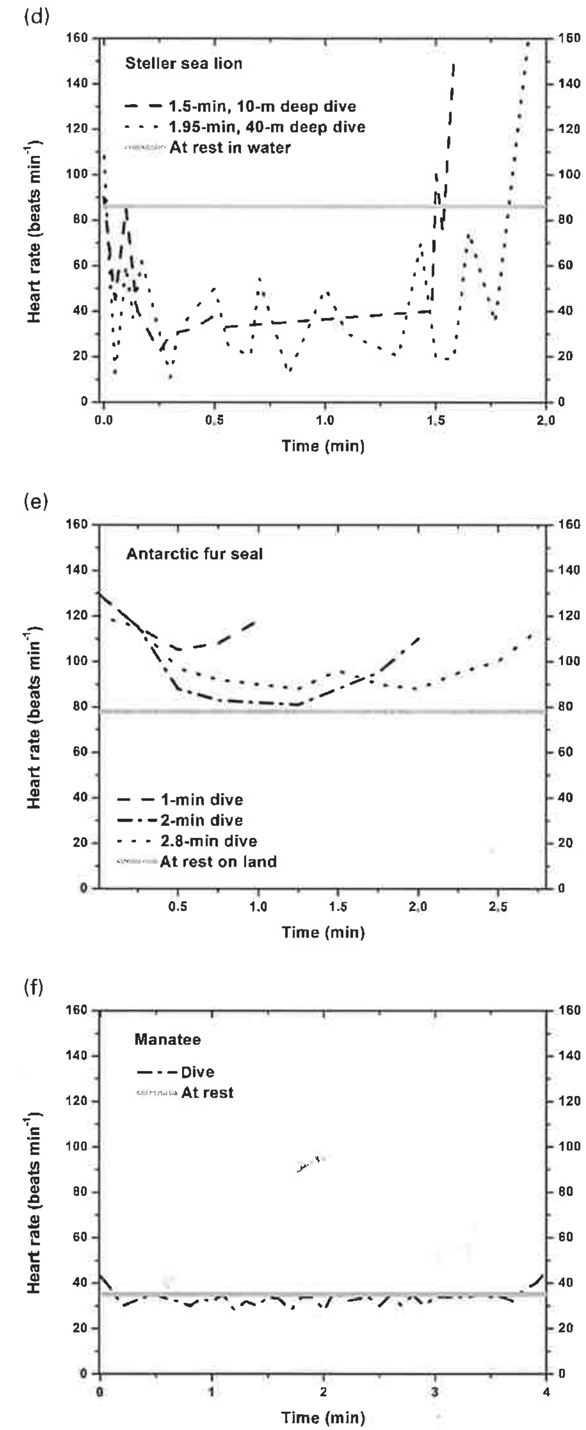

Figure 5.5 (*cont.*) adapted from Thompson and Fedak (1993); heart rate at rest from allometric equation in Stahl (1967). **(d)** Steller sea lion (*Eumetopias jubatus*), adapted from Hindle *et al.* (2010). **(e)** Antarctic fur seal (*Arctocephalus gazella*), adapted from Boyd *et al.* (1999). **(f)** Manatee (*Trichechus manutus*), adapted from Gallivan *et al.* (1986).

during that 14-min dive of the gray seal. However, as reviewed in Chapter 1, the elephant seal is capable of 40–60-minute and even 120-minute dives. During much of the time course of these extreme dives, one would expect that heart rate would be similar to the 4 bpm rate exhibited by the gray seal in the 14-min dive reported by Thompson and Fedak.

5.1.20 Elephant seals: venous oxygen profiles and blood flow implications

In addition to heart rate and blood flow measurements, blood P_{O2} and Hb saturation profiles can also provide insight into possible blood flow patterns during dives. Venous profiles are especially significant because blood O_2 extraction by perfused organs and muscle decreases venous P_{O2} and Hb saturation. In particular, increased or even constant P_{O2} values during a dive are not consistent with extraction of O_2 by working muscle. Yet, increased P_{O2}s and Hb saturations occur early during dives in both the hepatic sinus and extradural vein of elephant seals (Meir *et al.*, 2009). These profiles suggest that working muscle is ischemic during early descent, and, in fact, these observations are consistent with the rapid onset of iliac artery constriction reported by Van Citters *et al.* during forced submersions of elephant seals. Furthermore, in the initial portions of some dives, venous blood actually became arterialized (P_{O2} and saturations equivalent to arterial values). Such high values are not only consistent with muscle ischemia, but they also suggest A-V shunting. Otherwise, such high arterial values could not be achieved in venous blood.

At this point, it is worthwhile to remember that (a) A-V shunting was suggested in the forced submersion microsphere studies of Zapol and Blix; and (b) A-V shunting would be consistent with a constant blood pressure in the presence of a constant cardiac output and a 75% reduction in iliac artery blood flow at the start of a forced submersion as reported by the study by Van Citters *et al.* Thus, studies spanning almost 50 years and ranging from forced submersions to free dives all suggest the possible utilization of A-V shunts during dives.

In contrast to the maintenance or increase in venous O_2 during early descent, blood O_2 profiles decreased during the ascent phase of elephant seal dives (Meir *et al.*, 2009). Such patterns are consistent with increased tissue perfusion, and blood O_2 extraction by muscle and other organs during the increase in heart rate commonly observed during ascent. It has been proposed that such blood O_2 depletion serves to increase the lung to blood P_{O2} gradient and enhance O_2 uptake during the subsequent surface interval (Thompson and Fedak, 1993).

5.1.21 Steller sea lions and California sea lions: heart rate during free dives

In Steller sea lions, a moderate bradycardia occurred and the relationship of heart rate to stroke rate appeared variable and dependent on the nature of different dives. Such variation was reported in a paper which used ODBA (overall dynamic body

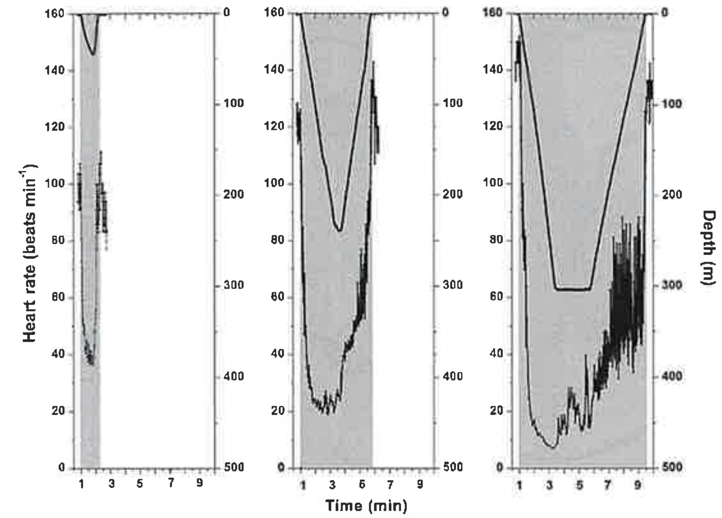

Figure 5.6 Beat-to-beat heart rate profiles of California sea lions (*Zalophus californianus*) during shallow, intermediate, and deep dives. Heart rate at rest on the beach was 52 bpm. Shaded area indicates dive interval, solid line is depth, and dotted line is heart rate. Adapted from McDonald and Ponganis (2014).

acceleration) as an index of stroke rate and muscular effort in dives of Steller sea lions (Hindle *et al.*, 2010). In shallow dives (10 m) of Steller sea lions, heart rate and ODBA correlated whereas, in deeper dives (40 m), there was no correlation between heart rate and ODBA.

Heart rate data in California sea lions during maternal foraging trips to sea revealed U-shaped profiles with higher initial heart rates, lower minimum heart rates, and lower overall dive heart rates as depth of dive and dive duration increased (McDonald and Ponganis, 2014). Dive heart rates were near or above resting heart rate in the typical short-duration (<3 min) dives of sea lions, but were less than resting rates in long-duration, deep (300–400 m) dives (Fig. 5.6). Heart rates were as low as 10 bpm during late descent and the bottom phase of the deep dives.

The U-shaped heart rate profiles during deep dives of sea lions should contribute to greater lung O_2 utilization at shallower depths during the higher heart rates of both early descent and late ascent. However, during the deeper and bottom phases of the dive, the extreme bradycardias should minimize lung N_2 and O_2 uptake even before "lung collapse" at depth. This would allow the sea lion to take advantage of its large respiratory O_2 store at shallower depths, but minimize N_2 absorption even prior to lung collapse during the deepest portions of the dive. Besides limiting pulmonary gas exchange at depth, the severe bradycardias during late descent and the bottom phase of deepest dives should also conserve blood O_2 stores and help maintain arterial oxygenation during periods of lung collapse. In contrast, higher heart rates during shallow dives would provide for greater utilization of the lung O_2 store as well as potential delivery of O_2 to muscle.

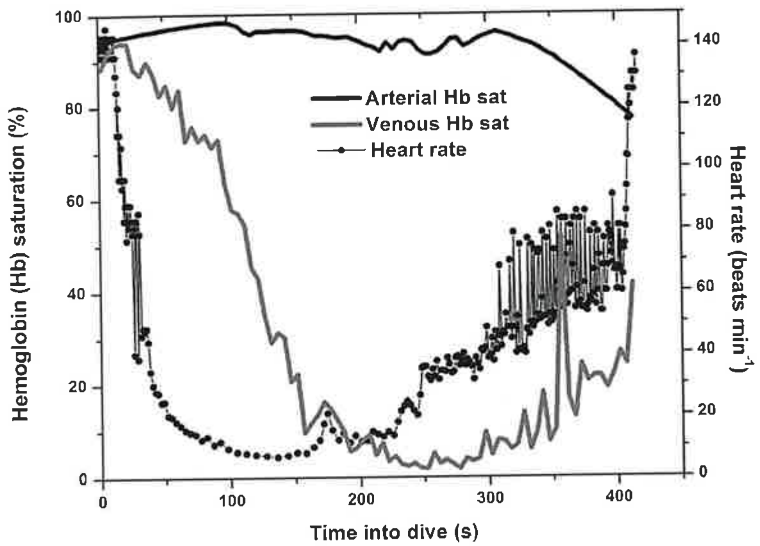

Figure 5.7 Arterial and venous hemoglobin (Hb) saturations relative to the heart rate profile in three 7-min dives by different sea lions (*Zalophus californianus*) to depths of 310–350 m. Although not simultaneous records from one animal, these profiles demonstrate characteristic hemoglobin saturation (S_{O2}) and heart rate profiles during such dives. Adapted from McDonald and Ponganis (2014).

5.1.22 California sea lions: venous oxygen profiles and blood flow implications

Increases in venous P_{O2} and Hb saturation during early descent of diving sea lions were not consistent with maintenance of muscle blood flow during that segment of the dive (McDonald and Ponganis, 2013). This is analogous to the observations and arguments made for elephant seal venous oxygen profiles in Section 5.1.20. During the mid-dive and latter segments of shallow dives of California sea lions, venous P_{O2} and Hb saturation in the posterior vena cava declined, suggesting that peripheral tissue perfusion and depletion of the lung and blood O_2 stores continued during the moderate bradycardias observed during these dives. However, the venous O_2 depletion patterns were highly variable, and the distribution pattern of blood flow to different tissues, muscle, or even A-V shunts is still unknown.

During the latter descent and early bottom phases of deep (>250 m) dives, venous O_2 declined rapidly to near-zero levels and then, during ascent, gradually increased (Fig. 5.7). In light of the maintenance of arterial Hb saturation and also a severe bradycardia during late descent and the early bottom phase of these deep dives, the rapid and almost complete decline in venous O_2 was interpreted to be secondary to extreme tissue ischemia and almost complete tissue extraction of O_2 from any perfused blood (i.e., severe vasoconstriction in those tissues draining into the posterior vena cava). In contrast, during ascent, the increase in venous O_2 content was postulated to be due to an increase in peripheral blood flow secondary to the observed increase in heart rate during ascent.

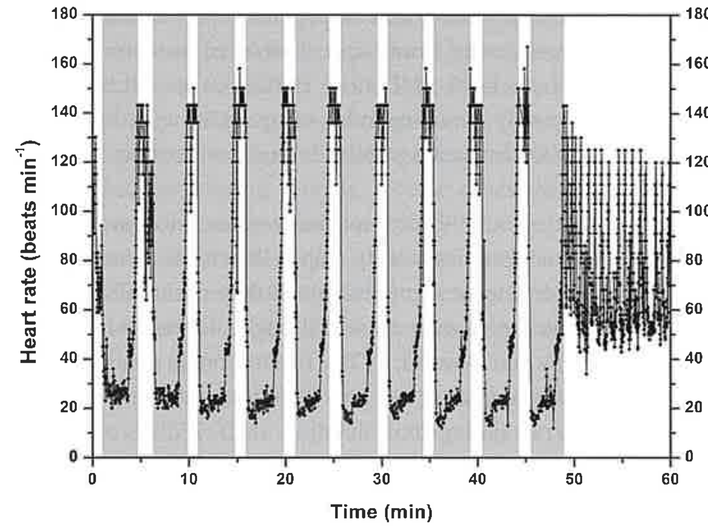

Figure 5.8 Heart rate profiles of a bottlenose dolphin (*Tursiops truncatus*) trained to station at depth and perform ten serial 100-m deep dives. Shaded areas represent dive time. During the recovery period after the session, heart rate returned into a typical sinus arrhythmia pattern. Adapted from Houser et al. (2010).

5.1.23 Dolphins: heart rates during free dives

In diving cetaceans, heart rate research has been focused on the bottlenose dolphin (Houser et al., 2010, Noren *et al.*, 2004, Williams et al., 1999). Beat-to-beat heart rate profiles of dolphins trained to perform serial dives to 100 m are illustrated in Fig. 5.8. The findings in the paper by Williams *et al.* documented the heart rate response of dolphins and, for the first time, provided insight into the relationship of diving heart rate to muscular work effort. In free-dives to 60-m and 210-m depth, heart rates declined from pre-dive average rates of 101–111 bpm to 20–30 bpm within 1 min of submergence; during the bottom phases of these dives, heart rates averaged 37 and 30 bpm for the 60-m and 210-m dives, respectively (Williams *et al.*, 1999). As in pinnipeds, the dolphins had an increase in heart rate during ascent. The authors reported that the bradycardias and heart rate patterns of these diving dolphins were similar to those reported previously for a sedentary dolphin stationing at 2-m depth (Elsner *et al.*, 1966b) despite the fact that their dolphins were actively swimming during the dives (fluke stroke rate was also monitored). They concluded that the heart rate response in diving dolphins was dominated by the dive response and not by an exercise response (in which heart rate would increase in relation to work effort (stroke rate)). Indeed, during the higher heart rates during the final ascent, stroke rates were actually decreased due to periods of prolonged gliding in this phase of the dive. As has already been mentioned, the issue of a linkage between heart rate (i.e., muscle blood flow) and muscular work in diving animals and the question of whether exercise modification of the dive reflex predominates in these animals have become central to the understanding and management of O_2 stores during diving. This paper was probably the first that examined heart rate in relation to stroke rate. Prior papers had relied on swim speed as an index of stroke effort (Guyton *et al.*, 1995, Hill *et al.*, 1987), but as will be seen in Chapter 7, swim speed is not necessarily a reliable index of muscular work because these animals can glide effortlessly at swim speeds similar to those when they are actively stroking (Williams *et al.*, 2000).

Although Williams *et al.* (1999) did not find evidence for an exercise response in their first study, this does not necessarily imply that there is no muscle blood flow during diving. Indeed, persistence of muscle blood flow during dives was suggested by documentation of elevated post-dive muscle nitrogen levels in deep-diving dolphins 20 years earlier (Ridgway and Howard, 1979). In addition, in contrast to those heart rate findings in deep open-ocean dives, it has now been found that there is some correlation of heart rate with stroke rate during short-duration, shallow dives of bottlenose dolphins and in trained, shallow dives of dolphins at sea (Davis and Williams, 2012, Noren *et al.*, 2012a, Williams *et al.*, 2015). Better understanding of the tentative linkage of heart rate and muscle workload awaits further study of the cardiovascular response of dolphins diving to different depths and durations.

5.1.24 Other mammals: heart rates during free dives

Free-diving heart rates have also been recorded in the hippopotamus and in several aquatic, small mammals including the mink, muskrat, and platypus (Elsner, 1966, Evans *et al.*, 1994, MacArthur and Karpan, 1989, Stephenson *et al.*, 1988). Similar to marine mammals, heart rates decrease significantly on submersion, and in some circumstances may be quite low. In the diving muskrat swimming against higher water current flows, submerged heart rates are greater than those at lower swim speeds, again suggesting that, at least in this species, increased muscle workload is accompanied by increased muscle blood flow (i.e., an exercise-modified response).

5.1.25 Marine mammal cardiovascular response during free dives: summary

In summary, heart rate responses in free-diving marine mammals can vary considerably, dependent not only on the species but also on the nature of a given dive. In general, except for perhaps the manatee, heart rates during most dives decrease to below "resting" levels. Species with higher Mb concentrations can exhibit more severe bradycardias, but such slow heart rates are not always utilized. The regulation of peripheral blood flow distribution appears primarily controlled by sympathetic tone via a central vascular "throttle" created by the innervation pattern of sympathetic nerve fibers to large, proximal arteries.

At least in some situations, especially in short-duration dives, heart rate and stroke rate appear to correlate, suggesting that cardiac output and muscle blood flow are, at times, coupled with muscle workload, as in terrestrial exercise. Heart rates, however, are not elevated above resting rates, and the distribution of such increased blood flow is not necessarily selective to exercising muscle. In longer duration and usually deeper dives, heart rates are even slower, but still moderate in range, and the linkage with stroke rate is less evident. In addition, extreme bradycardias (the classic, forced submersion dive reflex) can occur, such as in the long-duration dives of gray seals and during the late descent/early bottom phase of deep dives of California sea lions.

As examined in Weddell seals, gastrointestinal, hepatic, and renal function appear to be maintained during routine, aerobic dives. Maintenance of digestive, hepatic, and renal function during dives is probably more important in "surfacers" such as elephant seals and beaked whales than in "divers" such as sea lions. Hence, the intensity of their cardiovascular responses to diving may vary. However, the exact partitioning of cardiac output into regional blood flow to various organs, although frequently modeled, remains unknown.

5.2 Cardiovascular physiology in seabirds

5.2.1 Forced submersions of birds: bradycardia and peripheral vasoconstriction

Although the heart rate response of ducks to submersion or asphyxia had continued to be investigated by a series of researchers after Bert's original observations in 1870 (Andersen, 1966), it was not until Scholander's monograph in 1940 that a bradycardic response was documented in macaroni (*Eudyptes chrysolophus*) and gentoo (*Pygoscelis papua*) penguins during forced submersions (Scholander, 1940). Although heart rate profiles of complete submersions were not reported, heart rate decreased gradually from pre-submersion rates of 160–200 bpm to rates of 40–60 bpm over the course of 1 min, blood and muscle oxygen decreased to near zero by the end of 5-min submersions, and a wash-out of lactate into the blood occurred after the submersions (Fig. 5.9) (Millard *et al.*, 1973, Scholander, 1940).

However, significant elevations in blood lactate concentration also occurred during the forced submersions of penguins (Fig. 5.9), suggesting that peripheral vasoconstriction in the penguins may not have been as complete as in the seals (Scholander, 1940). In addition, muscle lactate concentrations of penguins did not consistently increase during the submersion. In later studies, femoral artery blood flow, an index of both peripheral blood flow and the degree of vasoconstriction, was reduced to about 25% of the pre-submersion rate in restrained submersions of gentoo penguins (Millard *et al.*, 1973). Complete heart rate profiles during submersions of penguins in this latter study demonstrated considerable variation in heart rate, dependent on the degree of struggling (Millard *et al.*, 1973). It is possible that minor variation in heart rate and vasoconstriction may have also occurred in Scholander's studies and contributed to the elevation in blood lactate during the submersion. Scholander also noted that muscle did not immediately re-oxygenate after the submersion, and that muscle lactate concentration also continued to rise during the early post-submersion period (Fig. 5.9).

During forced submersions of ducks, Scholander again found a moderate decrease in heart rate, near complete depletion of blood oxygen, an elevation in muscle lactate concentration during the submersion, and a wash-out of lactate into the blood after

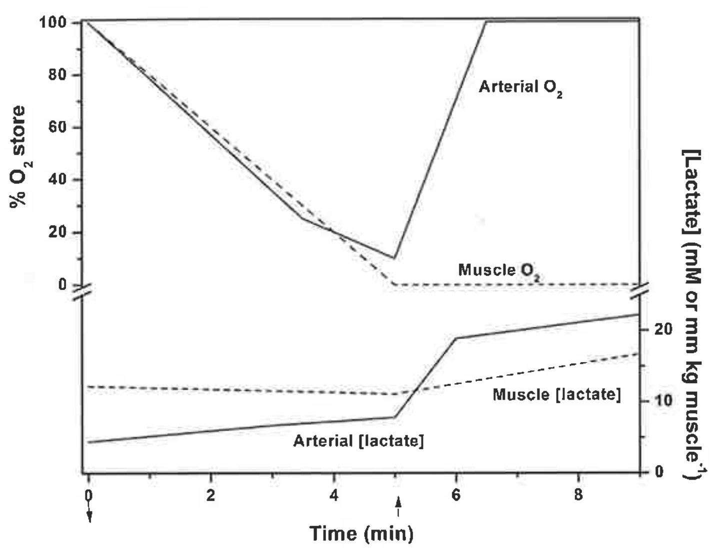

Figure 5.9 Arterial and muscle oxygen contents and lactate concentrations during a forced submersion of a gentoo penguin (*Pygoscelis papua*). The rise in lactate concentration after the submersion provided evidence for peripheral vasoconstriction even during the relatively moderate heart rates of 40–60 bpm reported for the penguin. Notably, muscle oxygen content remained near zero and muscle lactate continued to increase during the early post-submersion period, suggesting that return of muscle blood flow was incomplete or delayed. Arrows indicate start and end of submergence. Adapted from Scholander (1940).

submersion (Scholander, 1940). The decline in heart rate was not as rapid as in seals. Nor were the absolute values of the lowest heart rates as low as in seals (Andersen, 1966). For example, heart rate in "domestic" ducks gradually decreased from a pre-dive rate of 110 bpm to 20 bpm by three minutes of submersion (Andersen, 1959). In mallard or pekin ducks (*Anas platyrhnchos*), forced submersion elicited a gradual decrease from pre-submersion rates of 100 to 150 bpm to a rate of 30–40 bpm over the course of about a minute (Fig. 5.10) (Butler and Jones, 1968, Hudson and Jones, 1986). Such heart rate patterns typified the response of "dabbling ducks" to forced submersion (Furilla and Jones, 1987b). In contrast to dabblers, "diving ducks" such as the pochard, tufted duck, and redhead duck (*Aythya ferina*, *A. fuligula,* and *A. americana*, respectively) had more rapid decreases in heart rate during forced submersions (Fig. 5.10). As an example, heart rate declined from 100 bpm to 40 bpm within two seconds of submersion in the redhead duck (Furilla and Jones, 1986). Similar rapid declines in initial heart rate were originally noted in spontaneous dives of tufted ducks and pochards (Butler and Woakes, 1979).

As in seals, an increase in peripheral vasoconstriction maintained blood pressure despite the decline in heart rate in forcibly submerged ducks (Folkow *et al.*, 1967, Johansen and Aakhus, 1963, Johansen and Krog, 1959). Decreased muscle blood flow due to widespread vasoconstriction was inferred from experiments with use of a hot

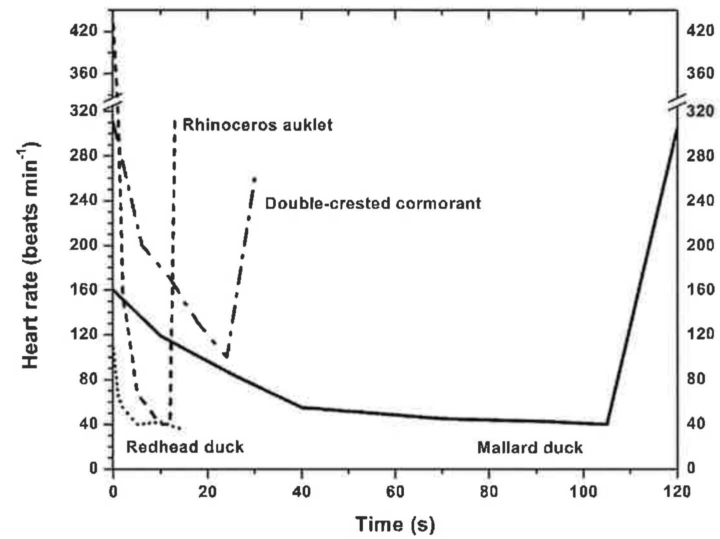

Figure 5.10 Heart rate responses during forced submersions of a mallard duck (*Anas platyrhnchos*), redhead duck (*Aythya americana*), double-crested cormorant (*Phalacrocorax auritus*), and rhinoceros auklet (*Cerorhinca monocerata*). Graphs illustrate generalized pattern of heart rate profiles. Submersions end with the onset of the tachycardias. Heart rates at rest were reported as 90 (restrained), 100 (restrained), 143, and 132–168 bpm in mallards, redhead, cormorants, and auklets, respectively. The gradual decline in heart rate of the mallard, a dabbling duck, is distinct from the more rapid decline in diving ducks and other diving birds. Adapted from Butler and Jones (1968), Enstipp et al. (1999), Furilla and Jones (1986), Stephenson et al. (1992).

wire anemometer as well as with measurements of venous effluent from muscle (Andersen, 1959, Djojosugito *et al.*, 1969).

A bradycardia during forced submersion was also later observed in other diving birds, including cormorants, guillemots, puffins, and rhinoceros auklets (Eliassen, 1960, Stephenson *et al.*, 1992). In these animals, the decline in heart rate after submersion was rapid. In rhinoceros auklets (*Cerorhinca monocerata*), for example (Fig. 5.10), heart rate declined from pre-dive rates of 450 bpm to 80 bpm within 5 sec of submersion (Stephenson *et al.*, 1992). Historical surveys of early cardiovascular research in birds are available in two excellent reviews (Andersen, 1966, Jones and Furilla, 1987).

The pattern of blood flow distribution to organs during the bradycardia and peripheral vasoconstriction of forced submersions of ducks has been examined with both radioisotope tracers and radio-labeled microspheres (Johansen, 1964, Jones and Furilla, 1987, Jones *et al.*, 1979, Stephenson and Jones, 1992a). Similar to findings in seals, these studies confirmed the redistribution of blood flow to essentially the brain, heart, and adrenal glands during forced submersions. Maintenance of blood flow to the adrenal glands was consistent with documented elevations in blood catecholamine levels (Lacombe and Jones, 1990, 1991a, 1991b).

As also already discussed for seals, differences in the sympathetic innervation patterns of major arteries probably contributed to the organ blood flow distribution pattern during forced submersions of birds. For example, sympathetic innervation of the duck's femoral artery, which supplies the propulsive leg muscles, was much more extensive than that in either the turkey or cat (Folkow *et al.*, 1966). This again supported the concept that the distribution of sympathetic fibers to the large, extramuscular arteries of the duck allowed for the maintenance of vasoconstriction even in the presence of muscle hypoxia and accumulation of metabolic vasodilators around intramuscular blood vessels (Folkow *et al.*, 1966).

Interestingly, there are two other exceptions to the widespread vasoconstriction in forcibly submerged ducks. Blood flow to muscles of the head did not decline, presumably because these muscles are supplied by branches of the carotid artery. Blood flow through the carotid artery to the brain was maintained during forced submersion, and, presumably, its arterial branches also did not constrict (Johansen, 1964). The second exception is that of the web of the foot (Djojosugito *et al.*, 1969). Provided the ducks were not alarmed or excited during the forced submersion, blood flow through the A-V anastomoses of the web was maintained. The authors speculated that flow through such A-V shunts allowed blood with high O_2 content to enter the venous pool and return to the heart. However, unlike in seals, increased microsphere deposition in the lungs has not been reported during forced submersions of ducks.

5.2.2 Simulated dives: birds

Simulated dives of birds in a pressure chamber have only been conducted in two studies, one of Adélie and gentoo penguins, and the other of king penguins (*Aptenodytes patagonicus*) (Kooyman *et al.*, 1973c, Ponganis *et al.*, 1999a). In simulated dives of king penguins, heart rate averaged 30 bpm, about 20% of pre- and post-submersion values (Ponganis *et al.*, 1999a). Although not published, similar decreases in heart rate occurred in the other two species.

5.2.3 Surface swimming: birds

Cardiovascular responses during surface swimming in birds have been examined in most detail in tufted ducks (Bevan and Butler, 1992a, Butler *et al.*, 1988, Woakes and Butler, 1983). In tufted ducks, heart rate increased to two-fold above the resting value when the duck was swimming at a metabolic rate almost four times the resting value. Cardiac output and heart rate increased in parallel, and the increase in blood flow was primarily distributed to the working hind limb muscles. Blood flow to the flight muscles, gastrointestinal tract, and kidneys declined while that to the heart increased slightly. Hepatic blood flow was unchanged. At a swimming metabolic rate twice the pre-exercise rate, hind limb muscle blood flow increased three-fold. Such a classic exercise response should be expected in a duck breathing continuously while swimming at the surface.

In graded swimming exercise of emperor penguins to a maximum metabolic rate eight times the value at rest, both submerged and surface heart rates increased with work load (Kooyman and Ponganis, 1994). Surface heart rates were consistently greater than submerged values. Submerged heart rates even at the lowest workloads were above values at rest, and maximum heart rates at the surface were about three times greater than values at rest. Thus, these heart rate responses to exercise were similar to those seen in flume-swimming sea lions. Again, presumably in this situation, even during submergence, there was an increase in blood flow to the primary underwater locomotory muscles. Similar heart rate patterns have also been reported in other surface-swimming penguins. In perhaps the earliest investigation of cardiovascular responses of an unrestrained penguin, average heart rate in a gentoo penguin slowly swimming at the sea surface was 227 bpm, about twice the value while standing upright (Millard *et al.*, 1973). Both carotid and femoral artery blood flow were similarly increased. In gentoo penguins actively swimming in a flume, heart rate was also elevated, near 180 bpm (Bevan *et al.*, 1995b). In Adélie penguins swimming spontaneously in a flume, submerged heart rates were also almost twice the value at rest, and surface heart rates were even higher (Culik, 1992). Therefore, during surface swimming of three species of penguin, an exercise response predominated during the submerged phase. And, of course, submergence durations during surface swimming were quite short.

5.2.4 Free dives: birds

Investigations of cardiovascular function in free-diving birds have primarily focused on heart rate regulation. As will be seen in most avian species, although heart rate during a free dive decreases from the pre-dive level, it usually does not go below the heart rate level at rest. Usually, heart rate only declines to levels observed in birds swimming at the surface or in birds at rest in the water. Technically, therefore, the heart rates of most free-diving birds are not bradycardias, although they are often described as such in the literature (especially when referenced to heart rate at rest in the water or during surface swimming).

5.2.5 Free dives: ducks

One of the earliest papers to describe heart rate patterns in free-diving ducks examined heart rate responses in pochards and tufted ducks during spontaneous and feeding dives of 3–24 sec duration (Butler and Woakes, 1979). Heart rates at rest and while swimming at the surface were near 110 bpm, and 160–220 bpm, respectively. Pre- and post-dive heart rates were very high, ranging between 280 and 473 bpm. During dives, heart rates decreased to about 100 bpm and then progressively increased throughout the dive to final heart rates of 181–249 bpm (Fig. 5.11). Thus, in contrast to seals, heart rates were well above resting rates throughout most of the dive. Similar heart rate responses were also seen in redhead ducks and even in tufted ducks diving as deep as 5.5 m (de Leeuw, 1996, Furilla and Jones, 1986, 1987b). Heart rates below resting levels were only later observed in tufted ducks making extended dives (35-sec mean duration) to obtain food; at 27 seconds into such dives, heart rates were approximately 95 bpm (Stephenson *et al.*, 1986). In even longer dives when surfacing through an exit hole was blocked, heart rate at 27 seconds was 45 bpm, similar to that during forced submersion

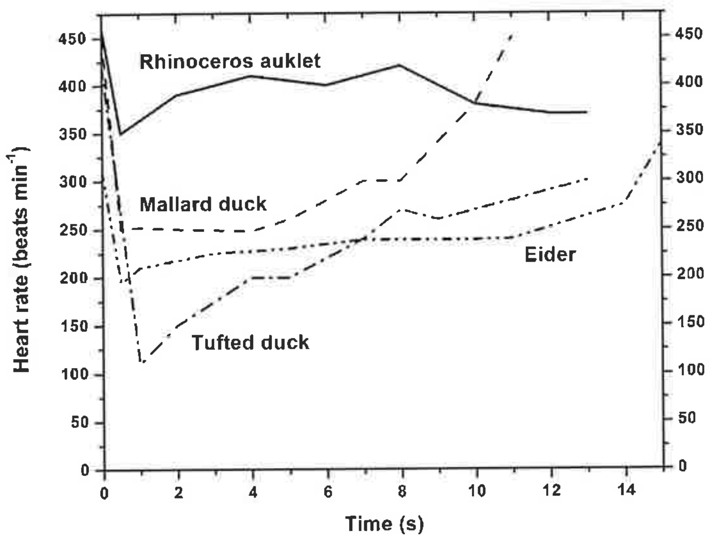

Figure 5.11 Heart rate responses during free dives of a mallard duck (*Anas platyrhnchos*), tufted duck (*Aythya fuligula*), common eider (*Somateria mollissima*), and a wing-propelled, short-duration diver, the rhinoceros auklet (*Cerorhinca monocerata*). Graphs illustrate generalized pattern of heart rate profiles. In each case, dive heart rate is above the reported heart rate at rest (110, 132, and 177 bpm in the tufted duck, eider, and auklet, respectively). Adapted from Butler and Woakes (1979), Furilla and Jones (1987a), Hawkins et al. (2000), Stephenson et al. (1992).

(Stephenson *et al.*, 1986). In the eider, during voluntary dives of up to 26-sec duration, dive heart rate, although declining below pre-dive rates, averaged 239 bpm, well above the rate of 132 bpm while resting in water (Hawkins *et al.*, 2000). Thus, only in rare long dives of ducks were heart rates below resting levels.

In 15-sec dives of tufted ducks, ischiadic artery blood flow to the leg musculature increased five-fold, and constituted 57% of cardiac output by the end of a dive (Bevan and Butler, 1992a). At the same time, brachial artery flow to the inactive wing muscles decreased by about 40%, and carotid artery blood flow to the brain and head/neck muscles more than doubled. Thus, during the high heart rates of free-diving tufted ducks, blood flow to the active leg muscles and to the head/neck increased, while flow to the inactive chest musculature and most other organs of the body was restricted by vasoconstriction. More than 99% of cardiac output during diving was directed toward the leg muscles and the head and neck. In contrast to the situation during forced submersion of ducks, perfusion to leg muscles was increased in free-diving ducks. The cardiovascular response of free-diving ducks does not isolate working muscle from the circulation. It is only during extreme dives that the dive response of forced submersions is invoked in ducks.

5.2.6 Free dives: other flighted birds

In free-diving shags/cormorants, heart rate was high prior to the dive, declined at the start of the dive, usually stabilized at depth, and then did not increase until the start of

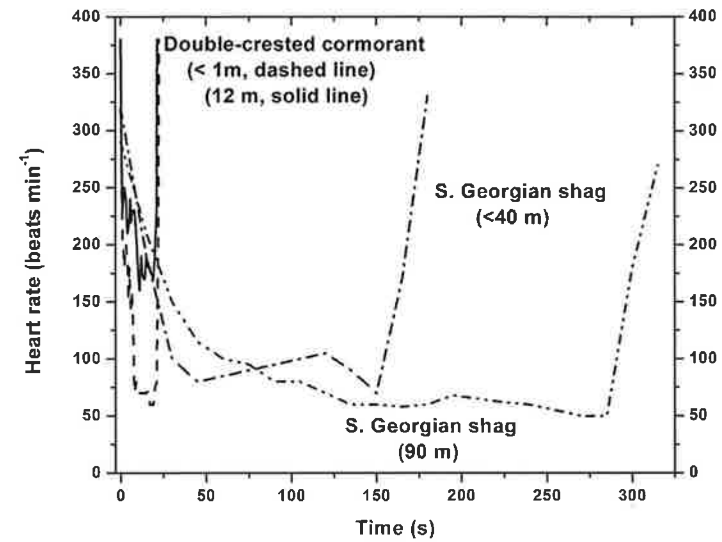

Figure 5.12 Heart rate responses of a double-crested cormorant (*Phalacrocorax auritus*) and South Georgian shags (*Phalacrocorax atriceps georgianus*) during free dives to different depth ranges. Graphs illustrate generalized pattern of heart rate profiles. Heart rate at rest in the double-crested cormorant was 138 bpm, while those of the shallow- and deep-diving shags were 85 and 95 bpm, respectively. Adapted from Bevan et al. (1997, Enstipp et al. (2001).

ascent (Fig. 5.12) (Bevan *et al.*, 1997, Enstipp *et al.*, 1999, 2001, Kanwisher *et al.*, 1981). However, mean dive heart rates were not significantly different from values at rest. The magnitude of the decrease in heart rate and, presumably, the increase in vasoconstriction varied among species. In deep-diving South Georgian shags (*Phalacrocorax atriceps georgianus*), mean minimum heart rates were 65 bpm, about 40 bpm less than the average resting heart rate (Bevan *et al.*, 1997), while in double-crested cormorants (*Phalacrocorax auritus*), heart rate did not decline below 100 bpm in dives up to 12 m in depth (Enstipp *et al.*, 2001). Despite low minimum heart rates, mean dive heart rates were still equivalent to or greater than those at rest, regardless of species.

In free-diving rhinoceros auklets (*Cerorhinca monocerata*), heart rate did not even decrease from pre-dive levels (Stephenson *et al.*, 1992). Thus, during unrestrained dives, the auklet does not exhibit a dive response; rather, it has heart rates in the 350–450 bpm range, approximately twice the 177 bpm rate at rest (Fig. 5.11). However, as demonstrated in "trapped" dives, the auklet can exhibit a dramatic dive response with heart rates as low as 20 bpm.

5.2.7 Free dives: penguins

Heart rates of diving penguins were first reported in tethered 1-min dives of gentoo penguins, in which heart rate declined to about 75 bpm during dives and rose to 180 bpm during surface intervals (Millard *et al.*, 1973). During 30-sec dives of

Humboldt penguins (*Spheniscus humboldti*) (Butler and Woakes, 1984), heart rate declined from elevated pre-dive values, but diving heart rate remained about 120 bpm, which was above or near resting values (Fig. 5.13). It was not until nine years later that diving heart rates of penguins were reported for dives of longer duration. In a 10-min dive of an emperor penguin (Kooyman *et al.*, 1992b), minimum heart rate was 30 bpm, about 50% of the resting value, and the overall dive heart rate was about 15% less than the resting value. This was the first report of an overall dive heart rate in a free-diving bird that was less than the resting value.

In contrast to the bradycardia observed in the emperor penguin, later studies of gentoo, king and macaroni penguins at sea revealed that overall dive heart rates in these species were not below resting values, even for their longest duration dives (Fig. 5.13) (Butler, 2000, Froget *et al.*, 2004, Green *et al.*, 2003). More recently, heart rate profiles in emperor penguins were documented in further detail with use of a digital ECG recorder (Meir *et al.*, 2008). In short dives of less than 6-min duration, diving heart rate, although less than at the surface, was greater than or equivalent to values at rest. However, a "true bradycardia" occurred in longer dives. In an 18-min dive of an emperor penguin, overall heart rate was 23 bpm, minimum heart rate was 3 bpm, and heart rate was 6 bpm for over 5 min (Fig. 5.13).

In summary, for routine dives among avian divers, a true bradycardia (diving heart rate less than resting value) has only been demonstrated in longer dives of emperor penguins. Minimum heart rates of diving South Georgian shags also decreased below rest values, but overall dive heart rate was not significantly different from that at rest. Reviews of avian diving heart rates have primarily focused on implications for peripheral blood flow, especially muscle blood flow, and whether an exercise response or dive response predominates during dives of most birds (Butler, 1991, Butler and Jones, 1997). If similar to the duck, blood flow during these higher heart rates of diving birds would be primarily directed toward the underwater locomotory muscles, with less flow to non-swimming muscles, splanchnic organs, and the kidneys. As suggested by Bevan and co-workers for the South Georgian shag, only when heart rate declines below resting values in the latter segments of dives would blood flow to all tissues, including the primary locomotory muscle, presumably also decrease.

In this model proposed for the South Georgian shag, an exercise response (selective increase in primary swim muscle blood flow) in the initial portion of a long dive is followed by a classic dive response (decreased muscle and organ blood flow) later in the dive. Under such conditions in the early part of the dive, myoglobin desaturation rates would be minimized, while venous O_2 content should decline due to blood O_2 extraction by exercising muscle. Such a cardiovascular response during dives of flighted avian divers is consistent with the large respiratory O_2 store and relatively small muscle O_2 store in these birds (see Chapter 4: respiratory system – about 50% – and muscle – less than 11% of total body O_2 stores). But what of organ and muscle blood flow in birds such as king and emperor penguins, in which respiratory O_2 comprises only one-third of the total body O_2 store and muscle myoglobin concentrations are 4–6 times the concentration in flighted divers?

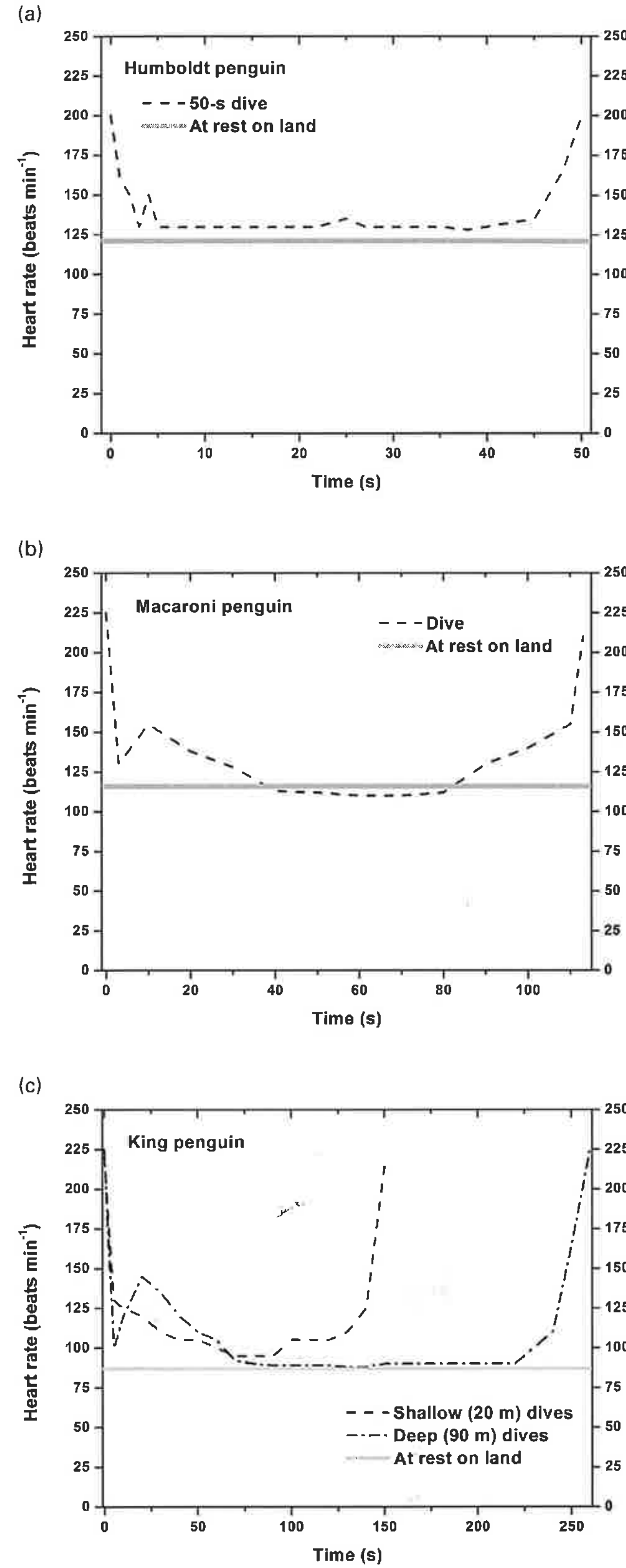

Figure 5.13 Heart rate responses during free dives of four penguin species to different depth ranges. Graphs illustrate generalized pattern of heart rate profiles in the Humboldt penguin

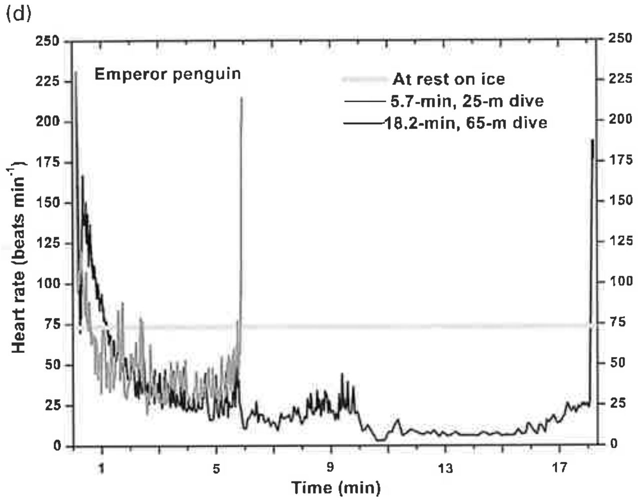

Figure 5.13 (*cont.*) (*Spheniscus humboldti*), macaroni penguin (*Eudyptes chrysolophus*), king penguin (*Aptenodytes patagonicus*), and beat-to-beat profiles in the emperor penguin (*A. forsteri*). Data for the king and macarconi penguins were collected at sea, for the Humboldt penguin in a captive tank, and for the emperor penguin at an experimental dive hole in McMurdo Sound, Antarctica. Adapted from Butler and Woakes (1984), Froget et al. (2004), Green et al. (2003), Meir et al. (2008).

5.2.8 Free dives: recent studies of emperor penguins at an isolated dive hole

Although Millard and co-workers found that femoral artery flow decreased by 75% from surface values in tethered dives of gentoo penguins (Millard *et al.*, 1973), direct measurements of organ and muscle blood flows have not been feasible in other free-diving penguins. Insight into the potential cardiovascular responses of diving penguins can be gained, however, from recent studies of heart rate patterns, blood O_2 profiles, and Mb saturation profiles of free-diving emperor penguins making relatively shallow (<100 m) dives at an isolated dive hole (Meir and Ponganis, 2009, Meir *et al.*, 2008, Williams *et al.*, 2011a).

5.2.9 Heart rate and blood O_2 profiles of emperor penguins: implications for blood flow

Heart rate profiles during dives of emperor penguins reveal that initial heart rates, though far less than pre-dive heart rates, are still relatively high (100–140 bpm) and may not reach resting values until 2–3 min into the dive (see Fig. 5.13). Once at resting levels, heart rate then gradually declines further throughout the remainder of the dive, with ever lower heart rate values in the latter portions of dives as dive duration increases. These low heart rates continue until the ascent, at which time heart rate begins to increase. The maintenance of high initial heart rates also occurs in king and macaroni penguins (Fig. 5.13), and, to some extent, even in South Georgian shags (Bevan *et al.*, 1997, Froget *et al.*, 2004, Green *et al.*, 2003). At least one function of these relatively high initial heart rates is optimization of gas exchange early in the dive.

The significance of this lung-to-blood O_2 transfer was demonstrated by increases in arterial P_{O2} and maintenance of arterial Hb saturations near 100% during much of the dive of the emperor penguin (Meir and Ponganis, 2009). Such gas exchange would also be optimized by increased movement of air through the lung secondary to differential air-sac pressures generated by the high wing stroke rates documented at the start of dives (Boggs *et al.*, 2001, van Dam *et al.*, 2002, Williams *et al.*, 2012).

In addition to O_2 uptake from the lung, another important function of such high heart rates early in the dives of emperor penguins is delivery of O_2 to tissues. If muscle blood flow increases and blood O_2 is extracted by working muscle, one would predict that venous O_2 content would decline or at most remain the same. However, venous P_{O2} and Hb saturation often increased early in the dive; in fact, venous blood could even become arterialized (Meir and Ponganis, 2009, Ponganis *et al.*, 2009). Such high values were not consistent with muscle blood flow and muscle O_2 extraction in these situations. Muscle temperature profiles in diving penguins were also not consistent with muscle blood flow (Ponganis *et al.*, 2003b, Schmidt *et al.*, 2006). In addition, overall dive heart rate and dive stroke frequency did not correlate in emperor penguins, suggesting that heart rate and muscle blood flow were not linked with muscle workload (Meir *et al.*, 2008).

Rather, the observed arterialization of venous blood early in the dive would argue for the occurrence of arterio-venous shunting during these periods. This might occur through A-V anastomoses in the extremities, as observed by the Folkow team in forced submersions studies of ducks, or, as Ponganis and associates suggested, through the wing vasculature (Djojosugito *et al.*, 1969, Meir and Ponganis, 2009, Ponganis *et al.*, 2009). Further support for the maintenance of blood flow through the wing early in the dive was also found in Kooyman's report that a nicked, bleeding wing of an emperor penguin continued to bleed underwater during the initial portion of a dive (Kooyman *et al.*, 1971a). Because of these observations, it has been suggested that one role of the early high heart rates during dives of emperor penguins is to transfer O_2 from the respiratory O_2 store via the arterial system into the venous O_2 store.

However, venous P_{O2} and Hb saturation profiles are highly variable in emperor penguins. The postulated A-V shunting may not always occur. At times, venous P_{O2} and Hb saturation progressively decline, and/or they intermittently increase during the dive (Meir and Ponganis, 2009, Ponganis *et al.*, 2009). Such patterns suggest a very plastic peripheral vascular response with periods of continuous versus intermittent blood flow and/or A-V shunting. The question of muscle blood flow during dives of emperor penguins was further addressed with near-infrared spectroscopy determinations of myoglobin (Mb) saturation in the pectoralis muscle, the primary underwater propulsive muscle of the penguin (Williams *et al.*, 2011a).

5.2.10 Muscle O_2 profiles in emperor penguins: implications for muscle blood flow

Myoglobin saturation profiles of diving emperor penguins revealed two patterns of desaturation. As illustrated in Fig. 5.14, one pattern was a monotonic decline to near 0%

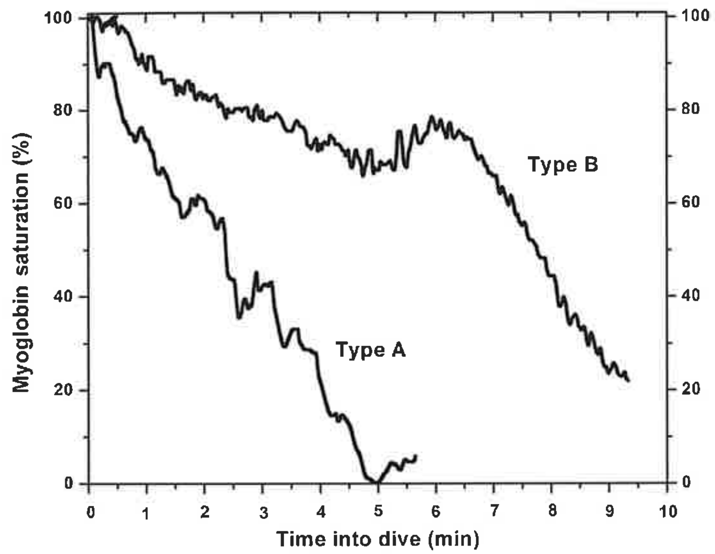

Figure 5.14 Myoglobin desaturation patterns in diving emperor penguins (*Aptenodytes forsteri*). During dives with relatively constant stroke rates, the two distinct patterns are consistent with different muscle blood flow patterns. There is probable complete ischemia in Type A, whereas muscle blood flow is probably intermittent in Type B, where myoglobin saturation is maintained throughout the mid-segment of the dive. Adapted from Williams et al. (2011a).

at about 6-min dive duration, while the other pattern was remarkable for a mid-dive plateau in desaturation followed by resumption of a monotonic decline toward 0% in the final segment of the dive. The first profile, reminiscent of the monotonic declines in Mb saturation in Scholander's forced submersion experiments in seals, was considered consistent with complete muscle ischemia (lack of blood flow) during the dive. In contrast, the plateau in the second pattern was interpreted as evidence for temporary resumption of muscle blood flow with blood-to-muscle O_2 transfer supporting aerobic metabolism in muscle during that period.

The existence of two patterns of Mb desaturation and muscle blood flow responses during dives of emperor penguins was also consistent with the highly variable venous P_{O2} and Hb saturation profiles during dives. Thus, although direct muscle blood flow measurements during dives have not been made, it appears that the peripheral vascular response during dives of emperor penguins can be highly variable both between dives and within a dive – hence, the lack of correlation between heart rate and stroke rate patterns reported in dives of emperor penguins by Meir *et al.* Cardiovascular management during a dive is not a typical exercise response in which heart rate and muscle blood flow are linked with muscle workload. There is certainly little muscle blood flow when heart rate is only 5 bpm in the latter portions of an 18-minute dive. However, when heart rate is high (100–140 bpm) in the early portions of a dive, it appears that emperor penguins have the option to perfuse muscle and supplement muscle metabolism with O_2 or to completely stop muscle blood flow, isolating muscle from the

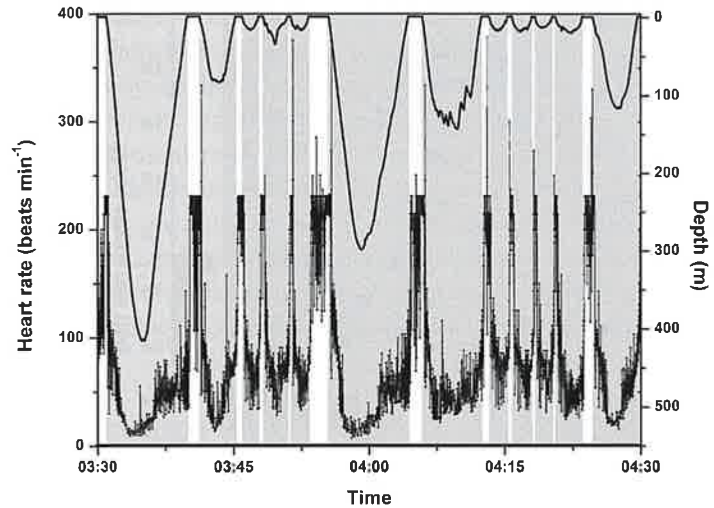

Figure 5.15 Beat-to-beat heart rate profiles of dives of an emperor penguin (*Aptenodytes forsteri*) at sea. Dives ranged from two to nine minutes and were as deep as 415 m. Mean heart rate at rest in this study was 56 bpm. Adapted from data of Wright et al. (2014).

circulation. In the latter situation during complete muscle ischemia, blood flow may be shunted through A-V shunts to increase the magnitude of the blood O_2 store for use later in the dive.

5.2.11 Heart rate profiles of emperor penguins at sea

In order to evaluate heart rate responses during deep dives of emperor penguins, heart rates have been evaluated from the dives of their foraging trips to sea during the chick-rearing period. Heart rate profiles of dives of emperor penguins at sea were characterized by (a) an initial decline from pre-dive rates; (b) a plateau at relatively high rates early in descent; (c) a further decline to lower levels during latter descent and the bottom phase of the dive; and (d) a gradual increase in heart rate during ascent (Wright *et al.*, 2014) (Fig. 5.15). As dives became deeper and longer, it was notable that (a) initial heart rates were higher; (b) the plateau heart rates were higher; and (c) the minimum heart rates were lower. Overall dive heart rate declined as dive duration increased, and was distinctly less than resting heart rate in deeper dives. Heart rates were as low as 10 bpm during the bottom phase of the deepest dives of emperor penguins. Less-than-resting heart rates in dives of emperor penguins at sea again contrasted with heart rates of the longest dives of macaroni and king penguins at sea (which were always near or above resting values). This suggested greater dependence of muscle metabolism on myoglobin-bound O_2 stores in emperor penguins than in other penguin species.

The heart rate profiles of deep dives of emperor penguins were consistent with greater gas exchange at shallow depths early in the dive, and minimization of both pulmonary

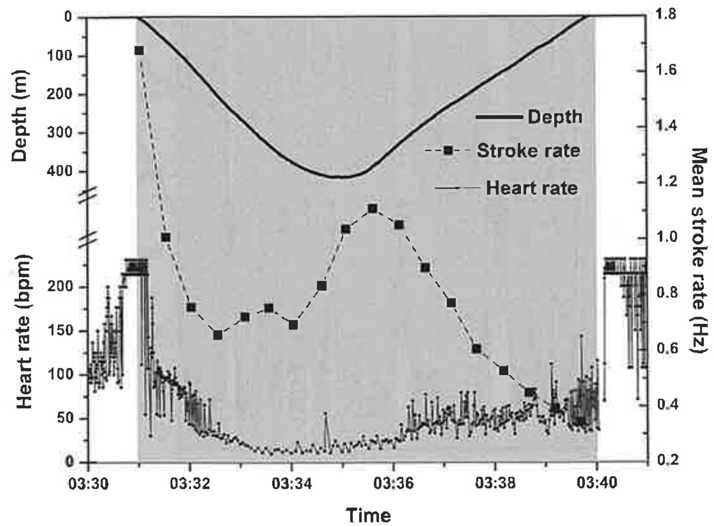

Figure 5.16 Beat-to-beat heart rate profile of a 415-m deep dive of an emperor penguin (*Aptenodytes forsteri*) in comparison to the mean wing stroke rate during dives greater than 400 m. During bottom phase of the dive, the lowest heart rates of the dive do not appear coupled to the typically high stroke rates of such dives. Data are from two different studies. Adapted from Williams et al. (2012), Wright et al. (2014).

gas exchange and peripheral O_2 delivery during the deep phases of the dive. As previously reviewed in this chapter for the similar heart rate profiles of deep-diving sea lions, this heart rate pattern in the emperor penguin should minimize N_2 absorption at depth, and conserve blood O_2 during the deepest portions of the dive. The similarity of heart rate profiles of deep-diving emperor penguins and sea lions suggest that such a heart rate pattern may be characteristic of deep dives of higher vertebrates that dive on inspiration with a relatively large respiratory O_2 store.

Lastly, the heart rate profiles of deep dives of emperor penguins at sea were not consistent with the hypothesis that exercise modifies the dive response and increases heart rate. Although the data have not yet been collected simultaneously, heart rate in the bottom phase of deep dives appears to be lowest when stroke rate is highest (Fig. 5.16) (Williams *et al.*, 2011a, Wright *et al.*, 2014). Further research with simultaneous records of heart rate and stroke rate is required to further resolve the potential mechanisms of heart rate regulation in emperor penguins.

5.2.12 Avian cardiovascular responses during free dives: summary

In summary, cardiovascular responses during free dives of birds are complex. In flighted avian divers with large respiratory O_2 stores but small muscle Mb concentrations, heart rates are usually high during dives, and an exercise response with muscle perfusion is assumed to predominate. Only in more extreme or longer dives does a more classic diving response occur with lower heart rates and presumably more peripheral vasoconstriction.

In contrast, in penguins, birds that have smaller respiratory O_2 stores but much higher Mb concentrations in muscle, heart rates are high initially during dives, but then progressively decline as dive duration increases. Such high initial heart rates early in the dive would support continued gas exchange with the respiratory O_2 reservoir. Peripheral vascular responses in emperor penguins appear to be quite variable early in the dive. Muscle may or may not be perfused during these periods of high cardiac output, and, in addition, A-V shunts may be utilized to enhance venous blood O_2 storage. During deep dives of emperor penguins, extremely low heart rates at the bottom phase of the dive should limit N_2 absorption, conserve blood O_2, and increase dependence of aerobic muscle metabolism on myoglobin-bound O_2 stores.

5.3 Neuroregulation of the dive response in mammals and birds

The neuroregulatory control of the bradycardia and vasoconstriction of the dive response during forced submersion was the subject of extensive investigation from the 1950s through the 1980s. The findings were thoroughly examined in several excellent reviews, to which interested readers are referred (Andersen, 1966, Blix and Folkow, 1983, Butler, 1982, Butler and Jones, 1997, Jones *et al.*, 1988). In both mammals and diving ducks the dive reflex of forced submersion (apnea, bradycardia, and vasoconstriction) could be elicited by facial immersion, wetting of the nostrils and glottis, or stimulation of trigeminal and glossopharyngeal nerves (Drummond and Jones, 1970, Dykes, 1974, Furilla and Jones, 1986).

5.3.1 Neuroanatomical pathways

Such stimulation of the face and nasopharynx is considered to result in inhibition of the respiratory center in the medulla with subsequent increased parasympathetic activity via the vagus nerve to slow the heart, and also with increased sympathetic nerve activity to increase peripheral vascular resistance. Paranasal stimulation results in activation of the anterior ethmoidal nerve (Panneton, 2013). As early as 1965, it was known that stimulation of the periventricular region of seals resulted in a reflex bradycardia (Van Citters et al., 1965). Trigeminal stimulation of medullary centers in the medulla of the muskrat also resulted in bradycardias (Panneton and Yavari, 1995).

In the rat, the anterior ethmoidal nerve has extensive input into the medullary dorsal horn of the spinal trigeminal nucleus (Panneton, 2013). Such linkage of naso-trigeminal stimulation with apnea, bradycardias, and elevated blood pressure in the rat has been shown to be mediated via the neurotransmitter N-methyl-D-aspartate in the pontine neurotaxic center (Dutschmann and Herbert, 1998), and via glutamate, another neurotransmitter, in the spinal trigeminal nucleus interpolaris (McCulloch *et al.*, 1995). Yet another neurotransmitter, serotonin, appears to mediate transmission from trigeminal afferent fibers to cardiac vagal neurons in the nucleus ambiguus (Gorini *et al.*, 2009). Increased sympathetic discharge during the dive reflex is associated with activation of the rostral ventrolateral medulla (Panneton, 2013). It has been proposed that the laboratory rat is an excellent model for further investigation of the central neural regulation of the dive response (McCulloch, 2012, Panneton *et al.*, 2010). A detailed review of these neural pathways in the rat is highly recommended to readers (Panneton, 2013).

5.3.2 Chemoreceptors in dabbling ducks

In dabbling ducks, which have a slow decline in heart rate in contrast to the rapid decrease of diving ducks during forced submersion, the development of the bradycardia was unaffected by nasal or upper airway stimulation, and appeared dependent on a decline in blood oxygen and subsequent carotid body chemoreceptor stimulation (Furilla and Jones, 1986, Jones and Purves, 1970, Jones *et al.*, 1982). The arterial chemoreceptor stimulation was, in turn, associated with increased vagal activity.

5.3.3 Higher cortical input

Despite the demonstrations of the reflex response to forced submersion that occurred even in decerebrate and brain-transected animals (Andersen, 1963, Drummond and Jones, 1970, Gabbott and Jones, 1991), it has always been noted and emphasized that cortical or suprabulbar input can influence the response. The variability in heart rate profiles during trained breath holds and free dives already reviewed in this chapter attest to the ability of these animals to modify their cardiovascular responses during diving. And, as reviewed in Section 5.1.11, seals are capable of altering their cardiovascular responses even during forced or trained submersions (Grinnell *et al.*, 1942, Jobsis *et al.*, 2001).

5.3.4 Reflex pathways in seals and diving ducks

Contributions from other reflex pathways to the dive response have been investigated in both seals and ducks. In seals, Elsner, Angell-James, and de Burgh Daly examined the roles of carotid chemoreceptors, lung inflation, and baroreceptors in a series of studies. Stimulation of chemoreceptors of the carotid bodies by hypoxic blood was required for maintenance of the bradycardia during submersion; isolated perfusion of the carotid bodies with oxygenated blood resulted in a tachycardia which could be reversed by reperfusion with hypoxic blood (de Burgh Daly *et al.*, 1977, Elsner *et al.*, 1977). It was also found that there was an increase in the gain of the baroreceptor reflex toward bradycardias; in other words, for a given change in blood pressure, there was a larger increase in heart beat interval (i.e., a slower heart rate) (Angell-James *et al.*, 1978). The effect of lung inflation on pulmonary stretch receptors and the Herring–Breuer reflex (inflation – tachycardia, deflation – bradycardias) was as expected as in other mammals (Angell-James *et al.*, 1981). Inflation of the lungs interrupted the bradycardia of the experimental submersion. Thus, ambient depth and lung compression have potential input into the intensity of bradycardia just as has been noted for human breath-hold divers (Ferrigno and Lundgren, 2003). For other potential effects of pressure, see Section 2.1.8. In conclusion from all these experiments, reflex input into the dive response is significant from (a) the trigeminal nerve afferents into the respiratory center; (b) carotid body stimulation by hypoxic blood; (c) lung deflation; and (d) adjustment of the gain of the baroreceptor reflex.

In tufted ducks (i.e. a diver, not a dabbling duck), chemoreceptor stimulation did not appear to play a role in the bradycardia of forced submersion (Butler and Woakes, 1982). The role of baroreceptors in regulation of the bradycardia of forced submersion has been debated (Butler and Jones, 1997). Acute baroreceptor denervation did not play a role in the forced submersion response in ducks (Jones *et al.*, 1983). Baroreceptor denervation also had no effect on the heart rate response during free dives of redhead ducks(*Aythya americana*, divers), but it abolished the free dive response in mallards (*Anas platyrhynchos*, dabblers) (Furilla and Jones, 1987a, 1987b).

5.3.5 Autonomic nervous system

In addition to investigations of the role of various reflex pathways in regulation of the dive response, contributions of the sympathetic and parasympathetic nervous system to the dive response have been investigated with the use of various pharmacological blockers (Blix and Folkow, 1983, Butler and Jones, 1997). For readers unfamiliar with the autonomic nervous system and the pharmacology of these drugs, a brief review follows. Effects of the parasympathetic nervous system, mediated by acetylcholine, can be blocked by atropine, which binds to the muscarinic acetylcholine receptor, thereby preventing the physiological response to acetylcholine. Sympathetic responses are mediated by epinephrine and norepinephrine via binding to alpha (α) and beta (β) adrenoreceptors. Alpha receptors are classified into α_1 and α_2 receptors, and it is the α_1 receptors, distributed in the peripheral vasculature, that contribute to vasoconstriction. The primary β receptors involved in cardiovascular regulation are β_1 receptors, the activation of which will increase heart rate and cardiac contractility, and β_2 receptors, which cause smooth muscle relaxation. Alpha receptor blockade can be induced with phentolamine, and selective α_1 blockade with prazocin, while beta blockade can be achieved with use of propranolol or nadolol, and selective β_1 blockade with a drug such as metoprolol.

Atropine has long been known to block the bradycardia of the dive reflex in both ducks and diving mammals (muskrats, seals), thus establishing that it is the parasympathetic nervous system, via its vagal innervation of the heart, that slows the heart rate during diving (Blix and Folkow, 1983, Butler and Jones, 1997). Alpha blockade does not affect the bradycardia of forced submersion in the muskrat (Signore and Jones, 1995), although it does block the reinforcement of the initial bradycardia in forcibly submerged ducks (Blix *et al.*, 1974). Beta blockade with nadolol does not affect the degree of bradycardia in either muskrats or ducks (Furilla and Jones, 1987b, Signore and Jones, 1995). However, consistent with activation of cardiac sympathetic fibers, propranolol does decrease ventricular contractility in the atropinized heart of nutria (*Myocastor coypus*) during forced submersion (Ferrante and Opdyke, 1969). It should also be noted that peripheral vasoconstriction occurs in the absence of bradycardias during forced submersions of atropinized ducks (Blix *et al.*, 1974) and of seals whose hearts were electronically paced at fast rates (Murdaugh *et al.*, 1968). Thus, the development of bradycardia during forced submersion is vagally mediated and not dependent on an increase in peripheral blood pressure. Furthermore, the onset of vasoconstriction is not dependent on the prior development of a bradycardia. In addition, sympathetic nerve fibers to both the heart and the peripheral vasculature are activated during forced submersion. However, the parasympathetic response dominates over activation of the cardiac sympathetic fibers and slows heart rate.

In the 1990s, Elliott *et al.* investigated heart rate responses in free-diving harbor seals which had been selectively blocked either with a muscarinic (acetylcholine) blocker, α_1 blocker, β_1 blocker, or a combination thereof (Elliott *et al.*, 2002). As would be predicted, muscarinic blockade resulted in higher diving heart rates (about twice the control value), but no change in surface heart rates. α_1 blockade resulted in a slight but significant increase in diving heart rate, and no change in surface heart rate. β_1 blockade had no significant effect on diving heart rate, but significantly decreased surface heart rate, supporting a role for sympathetic cardiac activation during the surface interval. During combined muscarinic and β_1 blockade, dive heart rates were lower than in muscarinic blockade alone, suggesting that there was also increased sympathetic cardiac nerve activity during the dive, just as had been documented during forced submersion in nutria. The study also provided evidence to support the role of vagal withdrawal during the pre-surfacing tachycardia of the seals. This increase in heart rate prior to surfacing was unaffected by either alpha or beta blockade, but was decreased by muscarinic blockade. Interestingly, although confirming insights into the autonomic regulation of heart rate in seals, none of the pharmacological blockade regimens had an effect on dive behavior (dive durations) of the freely diving, captive seals.

5.3.6 Neuroregulation of the exercise response

The review of the regulation of the cardiovascular response during diving would not be complete without consideration of neural mechanisms underlying the cardiovascular response to exercise. As already reviewed in this chapter, heart rates are variable during dives and several authors have hypothesized that at least some changes in heart rate during diving are linked to exercise. The coupling of heart rate and stroke rate in a diving mammal or bird could be potentially achieved through the same neural mechanisms responsible for the initiation of locomotion and the cardiorespiratory responses to exercise in non-diving animals. Such processes may primarily occur during the submerged phases of surface swimming or even during short-duration dives. A link between heart rate and activity may especially occur in species that dive on inspiration with a large respiratory O_2 store. These animals include otariids, dolphins, and seabirds, all of which have high heart rates (above resting levels) during sub-surface swimming and short-duration dives. However, during longer dives with heart rates well below resting levels, the neuroregulatory mechanisms that would link heart rate to stroke effort remain unclear.

The exercise response is generally considered secondary to (a) a central command mechanism which leads to the activation of muscle motor units for locomotion and to

the activation of cardiorespiratory centers; (b) peripheral feedback of physical activity to higher centers from contracting muscle (exercise pressor reflex); and (c) the arterial baroreceptor reflex (blood pressure regulation: ↑blood pressure → ↓heart rate) (Kaufman and Forster, 1996, Mitchell *et al.*, 1983, Smith *et al.*, 2006, Waldrop and Iwamoto, 2006, Waldrop *et al.*, 1996). Similar to the dive response, cerebral cortical input can influence and contribute to the response. Stimulation of cell bodies in the hypothalamic locomotory region has been found to initiate both locomotory and cardiorespiratory responses; the neurotransmitter, γ-aminobutyric acid (GABA), inhibited this response (Waldrop *et al.*, 1988). In addition, isolated muscle contractions resulted in stimulation of the cells in this hypothalamic region (Waldrop and Stremel, 1989).

Classically, the increase in heart rate with exercise had been considered to be secondary to a decrease in vagal (parasympathetic) nerve activity to the heart (Rowell and O'Leary, 1990). However, vagal nerve activity does not decrease at the start of exercise in experimental models (Kadowaki *et al.*, 2011, Matsukawa, 2012). Rather, muscle stimulation has been shown to increase cardiac sympathetic nerve activity as well as renal sympathetic nerve activity, thus demonstrating a role of the sympathetic nervous system in increasing heart rate and decreasing blood flow to renal and other regional vascular beds during and at the start of exercise (Matsukawa *et al.*, 1990, 1991, 1992, Tsuchimochi *et al.*, 2002, 2009). Therefore, potential neuroregulatory pathways exist whereby stroking activity in a diving mammal or bird could activate sympathetic cardiac accelerator fibers and elicit an increase in heart rate.

5.3.7 Cardiovascular neuroregulation during dives

As reviewed by Butler (1982) in birds, and as recently suggested by Davis and Williams (2012) in mammals, the heart rate response in a diver would then be dependent on whether the dive response or the exercise response predominated. And, of course, both responses are not isolated reflexes, but can be modified by higher cortical input. Certainly, this potential interaction of neuroregulatory pathways may contribute to the variability in heart rate profiles during dives.

However, it must also be remembered that during the classic dive response of a forced submersion, there is both extreme bradycardia (via the vagus nerve – parasympathetic) and extreme vasoconstriction (via sympathetic nerves) at the same time. Pharmacological blockade studies indicate that both the cardiac accelerator fibers and peripheral vascular fibers of the sympathetic nervous system are activated. In terms of heart rate control during forced submersion, the parasympathetic system predominates over activation of the sympathetic nervous system. In breath holds with less intense bradycardias, muscle blood flow persists presumably because vasoconstriction is less due to decreased sympathetic nerve activity (Jobsis *et al.*, 2001, Ponganis *et al.*, 2006b). Because of this decrease in sympathetic activity, the higher heart rate in these situations should be secondary to decreased vagal afferent activity to the heart and not increased cardiac sympathetic activity. Otherwise, the sympathetic vascular response would have to decrease while the activity of sympathetic cardiac accelerator fibers increased.

A key question is whether differential activation of cardiac and vasomotor sympathetic fibers occurs during the dive response. Although differential activation of cardiac and peripheral vascular sympathetic fibers have been demonstrated in human and experimental animal studies (Lovick, 1987, Morrison, 2001), the previously reviewed pharmacological blockade data from seals and nutria indicated that both cardiac and peripheral vascular sympathetic activities were increased during diving (Elliott *et al.*, 2002, Ferrante and Opdyke, 1969). In addition, increased parasympathetic activity and acetylcholine concentrations are known to override both cardiac sympathetic activity and the effects of elevated concentrations of epinephrine and norepinephrine (Levy, 1971, O'Leary, 1993). Lastly, during the bradycardia induced by nasopharyngeal stimulation in the rabbit, simultaneous activation of both cardiac sympathetic activity and vagal nerve activity occurred (Paton *et al.*, 2005, 2006). Therefore, variation in heart rate during dives is probably most dependent on parasympathetic control.

If increases in heart rate during diving were secondary to a further increase in sympathetic nerve activity, a simultaneous withdrawal of vagal activity would presumably still be required. Furthermore, if increased sympathetic nerve activity beyond that already induced by the dive response were to induce elevations in heart rate during dives, increased activation of the densely distributed sympathetic fibers in the extramuscular, proximal branches of the aorta would constrict those vessels, preclude an increase in blood flow to muscle, and result in an increase in blood pressure. Consequently, if the sympathetic nervous system links muscle workload to heart rate in order to increase muscle blood flow in diving marine mammals, it should primarily involve sole activation of sympathetic cardiac accelerator fibers. Thus, the exact mechanisms by which an exercise response might modify the dive response remain unclear.

Based on the extensive work by Butler and co-workers on birds (see Sections 5.2.5–5.2.6), and the recent observations and suggestions of Hindle *et al.* (2010) and Davis and Willliams (2012) for diving mammals, perhaps a simpler conceptual model for neuroregulation of heart rate during dives of most marine mammals is that an exercise response always occurs during active dives but that it can be reduced and even overwhelmed by activation of the dive response. Partial activation of the dive response would (a) limit the increase in heart rate; (b) only partially constrict the proximal arterial tree (remember the dense proximal sympathetic innervation of those vessels); (c) allow for a limited exercise response with small elevations in heart rate and increased blood flow to active muscle via release of local vasodilators; (d) result in continued or decreased flow to splanchnic organs dependent on the intensity of exercise; and (e) allow for heat dissipation/conservation via peripheral vascular thermoregulatory responses. Complete activation of the dive response would result in the dive reflex of forced submersion, with (a) intense vagal stimulation overwhelming the cardiac sympathetic responses associated with exercise as well as the dive reflex; and (b) intense peripheral sympathetic activation and vasoconstriction in the proximal aortic tree, thus inducing widespread ischemia to most major organs and muscle, even despite the release of local vasodilators (except for possible A-V shunts and thermoregulation). Such a model is consistent with findings during forced submersions, sleep apnea, short-duration dives, and long-duration dives. And, of course, many factors, including

nasopharyngeal reflexes, venous return, atrial stretch receptors, depth, lung inflation/deflation, blood gases and pH, exercise intensity, fright, and suprabulbar control could affect the response, dependent on the species and the nature of a given dive.

More research is clearly needed to further define the mechanisms by which cardiovascular responses are regulated during diving. Although laboratory models can further define anatomical and molecular pathways, well-designed investigations of cardiovascular responses during free diving are also needed because of the many factors that can influence the nature of the response.