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SIMULATED HUMAN DIVING AND HEART RATE: MAKING THE MOST OF THE DIVING RESPONSE AS A LABORATORY EXERCISE

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Laboratory exercises in which students examine the human diving response are widely used in high school and college biology courses despite the experience of some instructors that the response is unreliably produced in the classroom. Our experience with this exercise demonstrates that the bradycardia associated with the diving response is a robust effect that can easily be measured by students without any sophisticated measurement technology. We discuss measures that maximize the success of the exercise by reducing individual variation, designing experiments that are minimally affected by change in the response over time, collecting data in appropriate time increments, and applying the most powerful statistical analysis. Emphasis is placed on pedagogical opportunities for using this exercise to teach general principles of physiology, experimental design, and data analysis. Data collected by students, background information for instructors, a discussion of the relevance of the diving reflex to humans, suggestions for additional experiments, and thought questions with sample answers are included.

When mammals dive, they must cope with the problem of being denied an external source of oxygen. Consummate divers such as the remarkable Weddell seal may remain submerged for up to two hours. To do so, they rely on anatomical features and physiological responses that increase oxygen storage while reducing the use of oxygen for nonessential activities during a dive. Blood vessels supplying nonessential organs are constricted, redirecting blood to the oxygen-requiring brain and heart. Because it is supplying fewer organs with blood, the heart can beat more slowly (a condition known as bradycardia) while maintaining adequate blood pressure to the brain, the most metabolically sensitive organ; a further benefit of bradycardia is that the heart requires less oxygen as

well. Diving bradycardia is an easily measured component of a group of reflexes that also include holding the breath (apnea) and peripheral vasoconstriction. Together these reflexes constitute the "diving response."

In comparison with diving mammals, humans are poorly adapted to life in the water. In 2002, free-diving champion Mandy-Rae Cruikshank set a women's world record for static apnea of 6 minutes 13 seconds (the men's record, set in 2001 by Scott Campbell, is 6 minutes 45 seconds), but most of us are comfortable holding our breaths for less than a minute. The first part of this laboratory exercise is designed to demonstrate that, despite our terrestrial

nature, humans experience bradycardia when simulating a dive by holding the breath and immersing the face in cold water. The second part of this investigatory exercise asks students to consider the cues that stimulate the bradycardia reflex. In common parlance, this question might be phrased "how does the body 'know' that it is diving?" As biologists, we might restate this question as "what are the proximate signals that trigger diving bradycardia?" The simulated dive holds several potential proximate cues: *1*) apnea and *2*) exposure of the face to cold water, which may be further broken down into several components including cold, wetness, and pressure. The goal of the second part of the exercise is to determine which of these cues triggers diving bradycardia.

Human diving experiments have been used widely in the classroom (5, 6) and have gained popularity as an investigative laboratory activity, although some instructors have found that the diving response is unreliably replicated in the classroom. On the basis of eight years of experience with this exercise, we suggest ways to improve student success and elaborate on ways of expanding the pedagogical potential of this exercise, which can be used successfully at a variety of levels of biology instruction. Instructors may wish to use it primarily as a means of teaching the scientific method of inquiry, with relatively little attention to the details of diving physiology or the intricacies of statistical analysis. The exercise can also be rendered suitable for more advanced students by incorporating more physiological information and using formal statistical analysis. In all contexts, we recommend that the instructor demonstrate the simulated dive to the class before beginning the exercise. In our experience, a dripping instructor is the single best introduction to this activity, inviting students to participate and letting them know that everyone in the classroom is engaged in discovery together.

METHODS

Equipment and Supplies

1 plastic basin per student
1 towel per student
tap water
ice
1 thermometer per group
face masks

1 snorkel per student (can use large diameter plastic tubing)
1-pint plastic storage bags or gel packs that have been kept in the freezer
bleach for sterilizing basins between uses by different students
statistical analysis software
If measuring pulse manually:
1 stopwatch per group
If using computer-aided heart rate monitoring system:
1 monitoring device per group
software set up to collect average heart rates over intervals of 15 s

Procedures

Basic protocol for all tests. All tests are conducted in the same posture: leaning over the lab bench with elbows resting on the lab bench and the head down.

Students should work in groups of three. Each student takes a turn being the experimental subject, taking the pulse (or operating the data collection software if using an electronic heart rate monitor), and watching the time.

To measure the radial pulse manually: with the subject's palm facing upward, follow the thumb to its base and continue 2.5 cm (1 in.) beyond the wrist. Use the index and middle fingers to locate the groove between the radial bone (the long bone on the same side as the thumb) and the tendon that runs parallel to the radial bone. Press the fingers gently, sliding slightly up or down in the groove to locate the pulse in the radial artery. Fat deposits in the wrist may make it more difficult to find the pulse in some individuals.

Each test lasts 30 s. Most students will be able to hold their breath this long without too much trouble but should always stop anytime they experience discomfort. Test subjects will appreciate being tapped on the back every 10 s by the timer, particularly when being asked to hold their breath, because it is easy for subjects to lose track of time during the test. In our classroom, tapping helped students to remain calm during the test because they had a better idea of when the 30-s test period would end.

Table 1
Experiments for testing the effect of major components of a simulated dive on HR

Test (Independent) Variable	Exp. no.	Compare These Test Conditions[a]	Difference in HR Between These Two Test Conditions Indicates	Comparisons Between Experiments
Simulated dive	1	Breathing in air vs. holding breath in cold water[b]	Effect of all components of simulated dive on HR	
Apnea (holding breath)	2	Breathing in air vs. holding breath in air	Effect of apnea alone on HR	Apnea in presence of cold water should result in lower HR than apnea alone. Difference in HR reduction between *experiments 2* and *3* indicates additive effect of cold water on HR reduction by apnea.
	3	Breathing in water with snorkel vs. holding breath in cold water[b]	Effect of apnea in presence of cold water on HR	
Facial immersion	4	Breathing through snorkel in air vs. breathing through snorkel in water[b]	Effect of cold water submersion on HR	Cold water submersion in presence of apnea should result in lower HR than cold water submersion while breathing. Difference in HR reduction between *experiments 4* and *5* indicates additive effect of apnea on HR reduction by cold water submersion.
	5	Holding breath in air vs. holding breath in cold water[b]	Effect of cold water submersion in presence of apnea on HR	
Temperature	6	Warm pack on forehead[c] vs. cold pack on forehead[c]	Effect of temperature on HR. Note that this set of treatments fails to remove any additive effects that pressure might have on HR reduction by exposure to cold	Difference in HR reduction between *experiments 6* and *7* indicates additive effect of wetness on HR reduction by cold.
	7	Breathing in warm[d] water with snorkel vs. breathing in cold[d] water with snorkel	Effect of temperature on HR in presence of water (wetness and pressure)	Difference in HR reduction between *experiments 7* and *8* indicates additive effect of apnea on HR reduction by cold.
	8	Holding breath in warm[d] water vs. holding breath in cold[d] water	Effect of temperatue on HR in presence of both water (wetness and pressure) and apnea	

HR, heart rate. [a]Each pair of conditions constitutes a separate experiment. [b]Water at 15°C. [c]See ADDITIONAL EXPERIMENTS for other ideas on testing the role of the forehead. [d]Water at temperature students wish to test. Note that, in very warm water, students may find difficulty holding their breath.

At the beginning of a test that requires holding the breath, students should take a deep but not maximal breath and then hold it. Students should not hyperventilate before holding their breath.

Before conducting any test using water, students should measure the temperature of the water and adjust it by adding warm tap water or ice. Temperature should be 15°C for the initial simulated dive.

For immersion tests, subjects should immerse the face up to the temples.

Allow several minutes for heart rate to return to normal between tests.

Student-designed experiments: pairs of test conditions for evaluating proximate cues. Having demonstrated that diving bradycardia exists in humans, students are asked to discover what proximate

cues stimulate the decrease in heart rate. Potential proximate cues can be evaluated with a series of paired experiments (Table 1). Note that some variables can be investigated with more than one set of test conditions; each of these tests yields slightly different information, and consideration of several experiments together can yield further information not revealed by tests considered separately.

Large diameter flexible plastic tubing may be used as an inexpensive snorkel. For temperature tests in air, use a frozen gel pack or plastic freezer bag filled with water of $\geq 5°C$ [Below this temperature, cold on the face may cause an *increase* in heart rate (1); gel packs are usually coated with cloth, so they don't feel as cold even if they have been stored in the freezer]. Between use by different students, basins and snorkels should be disinfected with a mild bleach solution (5 ml bleach/liter of water) to prevent the spread of infection. In a 3-h laboratory session that includes time for discussion and experiment design, college students are typically able to carry out a total of four experiments—the initial demonstration that the bradycardia reflex exists plus three student-designed tests of proximate cues.

General considerations for conducting maximally effective experiments. The basic testing procedure (above) and the additional recommendations below maximize the success of the exercise by controlling for *1*) individual variation in basal heart rate, *2*) learning or habituation during the exercise, and *3*) other changes that might take place in an individual's heart rate over the course of several hours [or between days, if the exercise occupies more than one laboratory period (21)].

The exercise should consist of a series of experiments, each comprising two test conditions (Table 1). Identical tests in different experiments (e.g., apnea with immersion in *experiments 1, 3, 5,* and *8* or apnea in air in *experiments 2, 5,* and *6*) may yield variable results, even within the same individual. Several studies have shown that the response of heart rate to simulated diving can change over time as the subject becomes habituated to the procedure (21, 24, 26). This is one of the reasons that pairs of tests, conducted close to each other in time and in randomized

order, are the best controls and yield the strongest and most easily interpreted results. Although comparing several test conditions with a single control may appear to save time in the classroom, this approach would require a more complex statistical tool (analysis of variance, or ANOVA) and would require that the order of *all* tests, including the control, be randomized among subjects to control for time-dependent effects such as habituation.

A single paired *t*-test should be used to compare heart rates from the two test conditions in the 15- to 30-s measurement period (Fig. 1). Data from *experiments 2–8* (see Table 2) illustrate the importance of this point. Making more than one comparison within an experiment requires more advanced statistical tools—ANOVA or Bonferroni-corrected *t*-tests—which are inappropriate for the beginning student. In the event that the distribution of the data is significantly nonnormal, the nonparametric Wilcoxon matched pairs test, which makes no assumptions about the distribution of the data, should be substituted for the paired *t*-test.

To make use of paired *t*-tests, each student must complete both test conditions in each experiment, and data must be compiled so that pairs of results from each student are kept together (for instructions on how to format data, see instructions accompanying your statistical analysis software).

Order effects should be controlled for within each experiment by instructing one-half of the groups in the class to perform the test conditions in one order and the remaining groups in the reverse order.

Throughout, remind students that they should remain as quiet as possible. Published studies are usually conducted on subjects who have been resting quietly for up to 30 min, often in a prone position (e.g., Refs. 21 and 24). Additional studies have shown that distraction may reduce or eliminate diving bradycardia (reviewed in Ref. 14). In our experience, boisterous students tend to generate data that fail to show some of the expected features of the diving response.
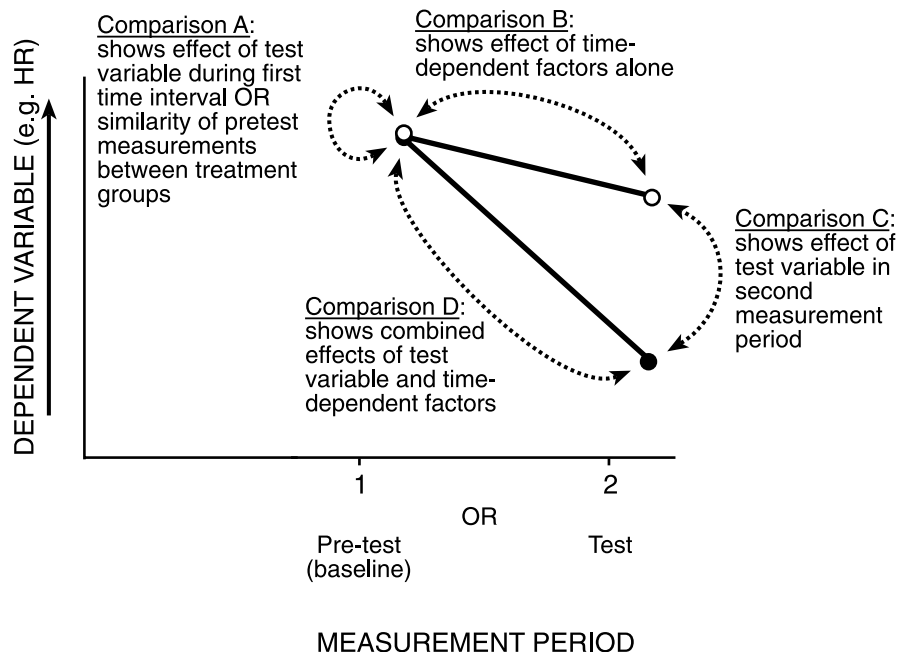
FIG. 1.
Use of controls and statistical tests. Schematic representation of what each statistical comparison can tell us about the effect of an independent variable (e.g., apnea, facial immersion, or cold on the face) on the dependent variable, heart rate (HR). The same principles apply to measurements taken in successive time periods or taken before and after a treatment (sometimes referred to as "baseline" and "experimental" measurements, respectively). Control and test groups of measurements must be compared at the same time period in the experiment, because the control may change over time (because of learning, habituation, circadian rhythms, or other time-dependent effects). The purpose of the control is to allow us to subtract these purely time-dependent effects from the total change to reveal the effect of the independent variable itself. Thus only *comparison C* answers the question of whether the independent variable influences heart rate in the second measurement period.

When individual classes are small or there is considerable variability among the responses of students, pooling data from several classes or laboratory sections can provide sufficient data to show the effects that are described in this report and in the literature.

If possible, practice the immersion procedure in advance of the diving exercise to reduce anxiety and the consequent tachycardia that can obscure the bradycardia reflex (10, 26).

SAMPLE RESULTS

For sample results, see Table 2.

DISCUSSION: INTERPRETING THE SAMPLE RESULTS

What the Results Tell Us About the Bradycardia Reflex

All numerical analyses refer to data shown in Table 2 for the 15- to 30-s measurement period unless otherwise noted.

***Experiment 1*: simulated dive.** *Experiment 1* demonstrates that the full bradycardia reflex exists in humans, with an average heart rate reduction of 20 beats/min (76 beats/min at rest to 56 beats/min dur-

Table 2
HRs pooled from diving reflex experiments conducted by 5 laboratory sections of introductory college biology students measuring radial pulse manually. Students had had no prior experience with the lab procedure before beginning *experiment 1*

Test (Independent) Variable		Exp. no.	0–15 s			15–30 s			Averaged over 0–30 s			n
			Avg HR	Avg decr in HR	Significance/ type of t-test	Avg HR	Avg decr in HR	Significance/ type of t-test	Avg HR	Avg decr in HR	Significance/ type of t-test	
Simulated dive (breathing in air vs. apnea in cold water)		1	79.8 74.0	5.8	‡2-samp. ‡paired	76.0 56.1	19.9	§2-samp. §paired	77.9 65.0	12.9	§2-samp. §paired	101
Apnea	In air (breathing in air vs. apnea in air)	2	73.0 71.3	1.6	NS	70.1 65.2	4.9	*2-samp. ‡paired	71.6 68.3	3.3	†paired	60
	In water (snorkel in water vs. apnea in cold water)	3	68.5 66.0	2.5	NS	65.3 54.9	10.3	*2-samp. §paired	66.9 60.5	6.4	*2-samp ‡paired	34
Facial immersion	Breathing (snorkel in air vs. snorkel in cold water)	4	74.8 71.1	4.2	‡paired	71.2 66.9	4.9	*paired	73.0 68.0	4.6	‡paired	46
	With apnea (apnea in air vs. apnea in cold water)	5	71.7 69.0	2.7	*paired	69.6 61.5	8.2	§2-samp. §paired	70.7 65.2	5.4	‡2-samp. §paired	64
	With apnea (apnea in air vs. apnea in RT water)	6	71.7 68.6	3.1	NS	70.5 60.6	9.8	*paired	71.1 64.6	6.5	NS	13
Temperature	In air (RT pack on forehead vs. cold pack on forehead)	7	71.8 69.3	2.4	NS	69.7 67.0	2.8	*paired	70.7 68.1	2.6	*paired	44
	In water (apnea in warm water vs. apnea in cold water)	8	70.1 69.2	0.9	NS	65.5 58.5	7.1	†2-samp. §paired	67.8 63.8	4.0	§paired	39

Average HRs (beats/min) are shown for the 2 treatments described for each experiment in *column 2*; n = no. of students participating in each experiment. RT, room temperature, 23°C. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.005$; §$P \leq 0.0001$. Only tests with statistically significant results are shown.

ing the dive; Fig. 2*B*). This result matches that of Reyners et al. (21) for apneic subjects with a cold pack pressed against the face, which suggests that apnea and cold facial stimulation are sufficient to elicit the full diving response. This experiment also shows that diving bradycardia does not develop immediately, but becomes more pronounced with time (10, 20, 24) (Fig. 2, *A* and *B*).
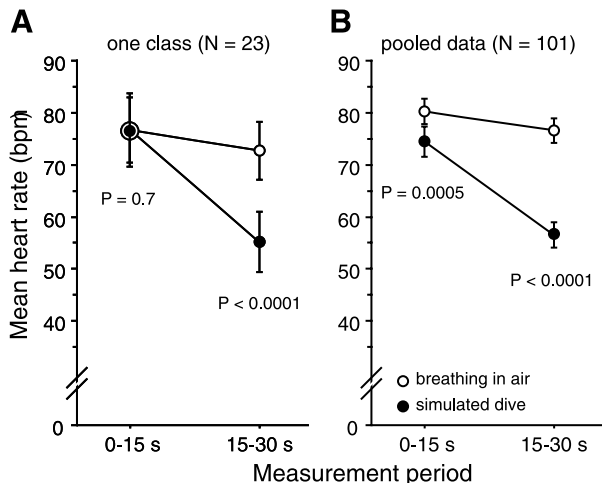
FIG. 2.
Mean HRs in beats/min [bpm; ±95% confidence interval (CI)] in college students of both sexes during a simulated dive (apnea with facial immersion in 15°C water) compared with control condition of breathing in air. *A*: data from 1 laboratory section, showing that significant differences were present only in 15- to 30-s measurement. *B*: pooled data from 5 laboratory sections showing that, when sample size is increased, smaller but nevertheless significant differences may also be present in 0- to 15-s measurement. CI indicates 95% certainty that population mean lies within this range; thus, if two 95% CIs do not overlap, a 2-sample *t*-test would show a significant difference, with *P* < 0.05 (similarly, if two 99% CIs do not overlap, there is a significant difference, with *P* < 0.01). This type of graph is an effective visual representation of how the 2-sample *t*-test works. Note, however, that *P* values shown here are for a paired, rather than 2-sample, *t*-test, because the paired *t*-test is better suited for the paired data the students collected. This graphic presentation is therefore good for showing mean HRs and how they change during the experiment, but students should be aware that it does not correspond conceptually with the paired *t*-test. See Fig. 3 for graphic representation of how the paired *t*-test works.

***Experiments 2 and 3*: effect of apnea.** *Experiments 2* and *3* demonstrate that apnea itself can cause bradycardia, even in the absence of immersion (similar results in Ref. 26). *Experiment 3* demonstrates that the degree of bradycardia caused by apnea is approximately doubled when the subject's face is immersed [a similar result was obtained by Gross et al. (15)and Zbrozyna and Westwood (26)]. Note that this experiment isolates the effect of apnea because in both tests the subject is immersed. In this experiment, one

of the test conditions is actually the simulated dive-apnea in cold water, and the result of this test is a heart rate that closely matches the heart rate measured during the simulated dive in *experiment 1*, ~55 beats/min. According to *experiment 3*, approximately one-half of the heart rate reduction during the simulated dive in *experiment 1* is caused by apnea enhanced by the additive effect of immersion in cold water. This result implies that the remainder of the 20 beats/min decrease in heart rate during the simulated dive is due to the effect of being immersed in cold water (a separate effect from the enhancing effect of immersion on apnea); this explains why facial immersion in cold water while breathing through a snorkel reduces heart rate to 65 beats/min, ~10 beats/min below the resting control in *experiment 1*. This conclusion is also addressed by *experiment 5* and discussed in that section.

***Experiments 4, 5,* and 6: effect of immersion in water.** *Experiments 4, 5,* and *6* are designed to test the effect of immersing the face in water. *Experiment 4*, which controls for the effect of apnea by allowing the subject to breathe in both test conditions, shows that facial immersion alone significantly decreases heart rate, by ~5 beats/min. *Experiment 5* also controls for apnea, this time requiring the subject to hold his/her breath both in air and when the face is immersed in cold water. The interesting result of this experiment is that immersion is found to decrease heart rate significantly and, furthermore, that apnea increases the bradycardia due to immersion. Just as immersion has an additive effect on heart rate reduction by apnea (*experiment 3*), so too does apnea have an additive effect on heart rate reduction by immersion in cold water. The decrease in heart rate due to immersion in the presence of apnea is 8 beats/min, which, when added to the 10 beats/min reduction caused by apnea in the presence of immersion, is close to the total reduction of 20 beats/min caused by the simulated dive. To resolve the appearance that effects are being "double counted" in this reckoning, it is important to recognize that, for each experiment, we are primarily interested in the *difference* in heart rate between the two treatments. In *experiment 5*, the difference of 8 beats/min is that caused by immersion in cold water with apnea; the control condition, apnea in air, already shows a lower heart rate (~70 beats/min) than breathing in air (76 beats/min), be-

cause apnea itself depresses heart rate as previously demonstrated in *experiment 2*. The additional decrease of 8 beats/min beyond the reduction in heart rate already shown by the control tells us that this additional effect is due to immersion. The same logic may be applied to *experiment 3*, in which the 10 beats/min reduction in heart rate relative to the control (i.e., the reduction due to apnea) is above and beyond the reduction already shown by the water-immersed control (65.3 beats/min pm) relative to breathing in air (76 beats/min, *experiment 1*).

One laboratory section wished to isolate the effects of water (wetness, greater pressure than air) as separate from the effects of the water's temperature (*experiment 6*). Noting that the temperatures of air and water in *experiment 1* were different, they attempted to control for temperature by comparing apnea in air and in water of the same temperature (23°C). Although their intentions were good, they failed to consider the fact that water and air of the same temperature usually have different thermal effects on an organism. Heat is lost to water at 23°C more rapidly than to air at 23°C, because water has a higher conductance than air (in addition, water colder than the skin ultimately extracts more heat than air because of its higher specific heat). These differences in conductance and specific heat are reflected in the well-known experience that 23°C water feels cooler than 23°C air. *Experiment 6* showed that immersion in 23°C water resulted in about the same reduction in heart rate as immersion in cold water, a result that contradicts the findings of *experiments 7* and *8*. Note, however, that the sample size in this experiment was small; even though the qualitative result that water causes bradycardia is unlikely to change, a larger group of subjects would give us greater confidence in the magnitude of the temperature effect.

Experiments 7 and 8: **effect of temperature.** As with apnea and water, there are several ways of testing for the effect of temperature. *Experiment 7* tests the effect of temperature on the forehead (the location of the ophthalmic branch of the trigeminal nerve) in the absence of the other qualities of water (e.g., wetness, pressure). Although the reduction in heart rate due to an ice pack is not large (3 beats/min), it is weakly significant [similar to results of Allen et al. (1)]. We have noted in past years, however, that this experiment often does not yield significant results except when data from several laboratory sections are pooled. Some studies indicate that forehead cooling may result in a transient (or at very low temperatures constant) tachycardia (6, 10, 26). Like *experiment 3, experiment 8* demonstrates that the effect of temperature is potentiated by the presence of water to result, in this case, in a 7 beats/min reduction in heart rate over a water temperature range of 8°C (15–23°C), consistent with findings summarized by Gooden (14). In light of the discussion regarding *experiment 6*, it might be useful to test 15°C water against water in the thermoneutral zone, 34–35°C (14, 19), to attempt to isolate temperature from wetness and pressure.

ADDITIONAL EXPERIMENTS

Below are listed some additional avenues along which students might wish to investigate diving bradycardia. Or, instead of actually performing the experiments, students might be asked to design experiments, as a thought exercise, to test these ideas.

Water as a cue. What are the critical components of water immersion for the bradycardia reflex, other than temperature (e.g., pressure, wetness)? Although some authors have concluded that temperature is the only relevant component of facial immersion stimulating diving bradycardia, other studies suggest that wetness may be a factor (14). One laboratory section at Swarthmore College designed the following experiment. Heart rate during immersion with apnea was compared with heart rate during immersion with apnea when a plastic-wrap barrier separated the face from the water. The experiment proved too difficult to execute with manual pulse measurement but might be possible with electronic pulse monitors.

Location of skin sensors. Is the forehead critical in sensing the presence of cold water? Students might address this question by comparing immersion with and without a face mask or by comparing full-face immersion with immersion of only the mouth and nose. They might also compare immersion of the face with immersion of some other body part, such as a hand or foot.

Sex differences. Do males and females have differences in heart rate, and do they respond differently to facial immersion?

Training effect. Trained divers report that training extends their breath hold time and deepens the diving response, resulting in greater bradycardia during a dive. Students may wish to compare bradycardia achieved by trained swimmers/divers with that achieved by others in the class in response to simulated diving. Students may also wish to determine whether individuals can achieve greater bradycardia by habituation or "short-term training" [try five 30-s immersions, 2 min apart (24)] or by training over longer periods of time (days or weeks). They may also wish to determine whether repeated immersions have a greater effect on trained athletes than on other students; some authors suggest that the increase in breath-holding ability and bradycardia after short-term training can be used as a general indicator of cardiovascular health (reviewed in Ref. 24). In interpreting the results of this experiment, students should be aware that any changes that occur after repeated immersions may have both physiological and psychological components [e.g., reduced anxiety (26)].

Anticipatory effects. Diving mammals may begin a cardiovascular diving response just before they actually dive (8), and their response while diving may also change depending on when they anticipate resurfacing. Diving bradycardia may begin to disappear as the animal rises toward the surface at the end of a dive. In humans, however, the anticipatory response before a dive begins may be tachycardia rather than bradycardia (24). To examine anticipatory effects, the subject should first rest for some period of time (2 min) without knowing what the next test condition will be (order of test conditions is randomized so that subjects cannot predict what they will experience next). The test condition is then announced and another 30 s allowed to elapse before the subject undergoes the announced test condition. Heart rates during the 30-s anticipation period are then compared between the test conditions.

Psychological control. Diving heart rate in diving mammals is a function of whether or not the subject knows it can resurface (see BACKGROUND INFORMATION FOR INSTRUCTORS). In humans, a sense of control, or lack thereof, can also affect the development of diving bradycardia (12). Students might compare heart rates near the end of a simulated dive between subjects who are given no time cues (never tapped on the back during the dive or told the time) and those who know when the dive will end because they receive continual verbal or tactile cues from a group member.

Lung volume. Does the size of the breath taken before immersion affect *1*) how long the breath can be held during a simulated dive and *2*) bradycardia during a simulated dive? Andersson and Schagatay (2) found that a breath of 100% lung volume (i.e., the deepest breath possible) produced the longest dives but the least diving bradycardia, whereas a breath of 60% lung volume produced the shortest dives but the most pronounced bradycardia. Note: We recommend against student investigations on the effect of hyperventilation because hyperventilation may result in black-out.

Mental effects. Does performing mental arithmetic during a simulated dive reduce bradycardia, as reported in one study (11)?

PRECAUTIONS IN STUDIES USING HUMAN SUBJECTS

Experiments using human subjects should be cleared with your institution's research committee, which may include a separate human subjects section, before using the exercise in your classroom. Students need to know that their participation in the exercise is optional and that the exercise is in no way an endurance contest (6). Students should not try to hold their breath longer than is comfortable, and anyone with a heart arrhythmia disorder should not participate (19). Note that, because some of the experiments do not involve apnea or immersion, students having difficulty with this portion of the exercise can still participate in some experiments and contribute to data collection by the class. In our experience, the percentage of students unwilling or unable to participate is typically low. We have found, however, that students who have little swimming experience may find facial immersion challenging.

GENERAL PRINCIPLES THAT CAN BE TAUGHT WITH THIS LABORATORY EXERCISE

Transferable general principles are arguably the most valuable, and best remembered, content of any laboratory exercise. Instructors are encouraged to draw attention to the following general principles, demonstrated by the diving bradycardia exercise, and to

remind students when they recur in later lectures or laboratory exercises.

A physiological response is the product of both ultimate and proximate factors. Ultimate factors are those that lead to positive natural selection for a trait, whereas proximate factors refer to the mechanism by which the response is brought about within an individual. The difference between these two kinds of causation represents a central concept in biological thought, articulated by an unidentified author when he posed the question "Why is a house?" and recognized that there are two classes of answers: *1)* "because man needs shelter" (ultimate cause) and *2)* "because man laid brick on brick" (proximate cause).

Biological data are inherently messy, even when measurement is precise. Individuals vary in their responses to any given perturbation, as may be shown by asking students to plot the data from individuals in the class (Fig. 3). Statistical analysis was invented so that we can see beyond this individual variation to significant population trends. It is worth noting that this variation, if heritable, is the variation on which natural selection acts; without it, there would be no evolution.

A control for any test condition is the test condition minus the independent variable you are interested in testing. Many students (and many science teachers!) erroneously believe that a control is the subject, in their words, "with nothing" or "doing nothing." On the contrary, the control treatment should match *every feature* of the test condition except for the independent variable being tested. For example, each of the three experiments that test the effect of immersion in water (*experiments 4, 5,* and *6*; Table 2) has two test conditions that are identical except that one includes immersion in water and the other does not. Although breathing in air (which might be considered the subject "doing nothing") is the appropriate control for *experiments 1* and *2* (Table 2), it is not a good control for the other experiments (not even *experiment 4*, which is better controlled by having the subject breathe through a snorkel in air).

Experiments can be properly controlled without having a particular test condition or group of subjects designated as the control. Experiments testing the effect of temperature always simply com-
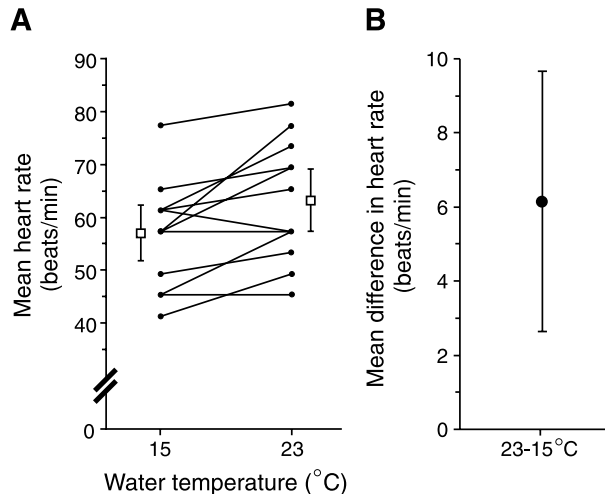

FIG. 3.

HRs of college students in 1 class (n = 13) performing simulated dives with apnea in water at 2 temperatures. *A*: each line connecting 2 points (●) represents data from 1 subject; □, displaced *right* or *left* for clarity, represent mean HR of all subjects ± 95% CI. A 2-sample *t*-test would compare these 2 means; because there is overlap between the 95% CIs of the 2 means, one could not conclude that there is a significant difference between them with $P < 0.05$. Note, however, that although absolute HRs varied widely among individuals, the HR of most individuals increased from 15 to 23°C. *B*: in this graphic representation of the data as viewed from the perspective of the paired *t*-test, the mean of all the individual differences between the 2 temperatures is shown with its 95% CI. The null hypothesis, that there is no difference in HR between the 2 temperatures, would be represented by a difference of 0. Thus, if the 95% CI does not overlap with 0, then there is a significant difference between HRs at the 2 temperatures, with $P < 0.05$ (likewise, if 99% CI does not overlap with 0, then there is a significant difference, with $P < 0.01$).

pare two or more temperatures, because there is no such condition as "no temperature." As long as the test conditions being compared are identical to one another except for the temperature chosen, the experiment is well controlled. As the experiments in this exercise demonstrate, it is also not necessary for one group of subjects to be the control group; if all subjects undergo all test conditions and the order in which they experience the test conditions is randomized, then the experiment is controlled.

Only by comparison with a control can we demonstrate that a change is due to the independent variable. The importance of this point cannot be

overemphasized. Many published studies in the field of diving physiology do not make the statistical comparisons that address the purpose of the experiment, either because they lack the appropriate controls or because they do not use the controls correctly in their statistical analysis. We therefore strongly urge instructors to take the time to explain this important concept. A useful exercise for advanced students is to read a few primary papers on diving physiology to determine whether the authors correctly designed their controls and statistical analysis.

Consider *experiment 1*, the comparison between the simulated dive and the control of breathing in air. Students will be tempted to plot the change in heart rate from the first 15 s to the second 15 s, apply a paired *t*-test, and interpret the result as showing that a simulated dive causes a decrease in heart rate over time. Drawing such a conclusion from this analysis, however, is completely invalid, because the comparison just described tells us nothing about the relative roles of the dive and of separate time-dependent factors in stimulating bradycardia. It may be, for example, that heart rate simply decreases over time during the test no matter what the test condition. Indeed, we find that heart rate decreases over time even in subjects breathing in air (see *experiments 1* and *2*; Table 2). This tells us that some of the decrease in heart rate over time in any of the test conditions (~4 beats/min in *experiment 1*) is due to some time-dependent factor other than the test variable [such as the subject habituating to the test condition, as shown by Zbrozyna and Westwood (26) and Reyners et al. (21)]. The remainder of the decrease in heart rate, the portion that is due to the simulated dive conditions, is the *difference* in heart rate between breathing in air and the simulated dive *during the same measurement period*. The analysis must compare heart rates between test condition and control (or between two test conditions, if it is not possible to designate one of the conditions as the control) during the same measurement period, rather than comparing heart rates between measurement periods within the same test condition. These concepts are illustrated graphically in Fig. 1.

Paired analysis, which makes use of the fact that each subject was tested in both test conditions, is more powerful than two-sample or unpaired analysis, which simply compares the means of heart rates between the two conditions without taking note of how the data from each individual are paired. When combined with the concept of controls described in the preceding general principle, this concept states that, if each subject undergoes both test conditions, then the power of a paired statistical test can be gained by comparing the subjects' performance in the control and test conditions during the same time period of the test (Fig. 3).

Increased sample size increases statistical significance of differences where they exist. The more individuals we measure in our sample, the more certain we can be about the mean heart rate for the whole population under the conditions tested. This increased certainty is reflected in a lower *P* value. Students can demonstrate this principle by statistically analyzing the data from their own laboratory section and then repeating the analysis on data that have been pooled with one or more other laboratory sections.

BACKGROUND INFORMATION FOR INSTRUCTORS

Physiological Adaptations for Coping with Hypoxia During Diving

Lung-breathing animals face two problems when they dive: a shortage of oxygen (hypoxia) and, if they reach sufficient depth, abnormally high pressures (hyperbaria). Here, we consider the problem of hypoxia, which may be solved in three general ways: by storing oxygen before the dive, by conserving oxygen during the dive, and by replenishing oxygen stores quickly between dives.

The animals best adapted for diving, the seals and sea lions (pinnipeds), are able to remain submerged without resorting to anaerobic metabolism for up to 20 min (7), during which time they rely heavily on oxygen bound to muscle myoglobin, present in such large quantities that the muscles of diving mammals appear nearly black when exposed to air (23, p. 28). Hemoglobin in red blood cells also stores oxygen. During a dive, the spleen of pinnipeds contracts, expelling the oxygen-rich red blood cells it stores into the general circulation and increasing the hematocrit from below 40 to near 70. During periods when seals are hauled out on land, the spleen plays the equally important role of storing these extra blood cells,
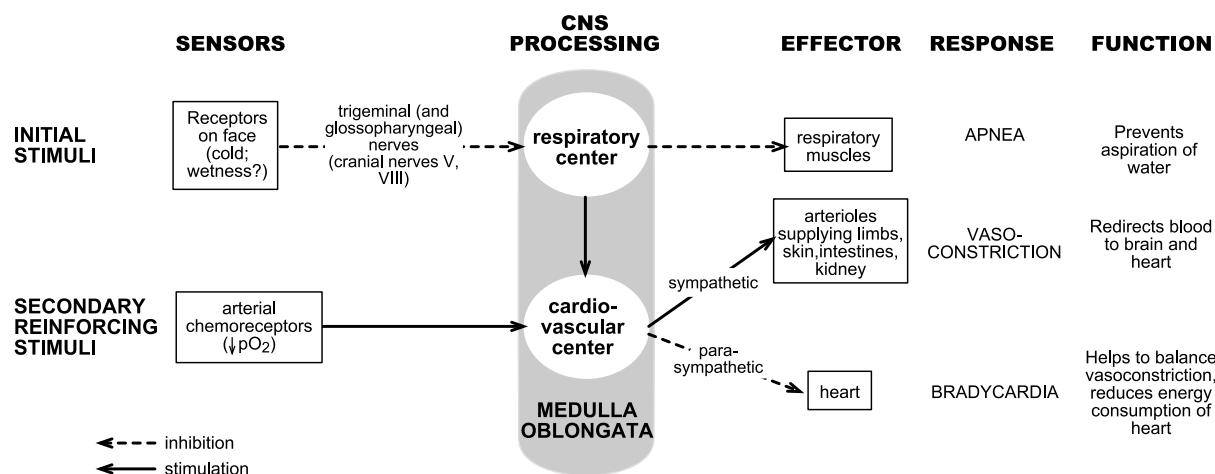
FIG. 4.

Principal components of the diving response. Solid arrows indicate stimulation, dashed arrows inhibition. CNS, central nervous system. Inputs primarily from trigeminal receptors on the upper part of the face are integrated by the respiratory and cardiovascular centers in the medulla oblongata (in the brainstem), generating neural signals to the lung, heart, and arterioles that include both sympathetic and parasympathetic divisions of the autonomic nervous system. During longer dives, chemoreceptive inputs from aortic and carotid bodies help maintain these effects. Bradycardia reduces cardiac output and therefore should reduce blood pressure, but in humans this effect is more than compensated for by the increase in blood pressure brought about by peripheral vasoconstriction, with the ultimate result that blood pressure increases during a dive in humans. Although arterial chemoreceptors may respond to pH and P_{CO_2} as well as to P_{O_2}, during diving P_{O_2} seems to be the more important in regulating cardiovascular center outputs (14). Based on figures from Gooden (14) and Hochachka and Somero (16).

which when circulating increase blood viscosity and require the heart to work harder, particularly at the higher heart rates that prevail when the seal is not diving (16). Peripheral vasoconstriction reduces circulation to the muscles and other tissues while maintaining blood flow (and hence oxygen delivery) to the brain and heart. Lowering the oxygen demand of peripheral tissues during a dive is accomplished mainly by metabolic depression, which results when tissues are cooled or become hypoxic. The reduced blood circulation (hypoperfusion) caused by peripheral vasoconstriction seems to have two related functions: *1*) reducing the delivery of precious oxygen to the less needy peripheral tissues, and *2*) decreasing the *need* of peripheral tissues for oxygen by denying them oxygen and allowing them to cool when circulation is restricted, a process that takes place especially quickly when the animal is immersed in cold water (8, 14, 25).

The principal sensory components and integrative pathways involved in the diving response are shown in Fig. 4. Trigeminal receptors on the face (and, to some extent, glossopharyngeal receptors on the tongue and upper airways) provide the first proximate cue that a dive has begun (16). Subsequent signals from arterial chemoreceptors reinforce and deepen the diving response, which takes several minutes to develop fully in a diving mammal; ultimately, heart rate may drop to as little as 5 beats/min (8). In humans, the response is also not immediate but develops more rapidly than in diving mammals, sometimes to equally impressive levels [one human subject had heart rates as low as 6 beats/min (16)]. A principal difference between the diving responses of humans and diving mammals is that, in diving mammals, cardiac output (a product of heart rate and stroke volume) and peripheral vasoconstriction are balanced so that mean blood pressure does not increase during a dive (in humans, blood pressure increases slightly during a dive because the effects of vasoconstriction exceed the effects of reduced cardiac output). During a dive that exceeds the aerobic dive limit (ADL), peripheral vasoconstriction has the added advantage of preventing lactic acid (lactate) produced by muscle cells from entering the general circulation where it would have toxic effects. Blood lactate typically does

not rise until the animal surfaces, at which time it is flushed into the general circulation and detoxified aerobically (16). Postdive tachycardia speeds this process and reloads circulating red blood cells with oxygen before they are returned to the spleen for storage (16, 22). Spleen contraction has also been observed in human divers, suggesting that a similar mechanism might occur in nondiving mammals (18).

In addition to the principal sensory inputs described above, inputs from thoracic muscles as well as cognitive and emotional factors may modify the diving response as measured by the development of bradycardia. As soon as a diving mammal becomes aware that it cannot resurface (e.g., when being chased by a predator or when in a tank over which an investigator has just placed a cover), heart rate decreases further (8). In some cases, there may be anticipatory bradycardia before the start of a dive and/or an anticipatory return to nondiving heart rate as the animal begins to swim toward the surface (8). In humans, fear and facial stimulation with very cold water may stimulate an initial transient tachycardia, which is later replaced by the more conventional bradycardia, either within the same dive (10), or in later dives after the subject has habituated to the test situation (26).

Bradycardia, peripheral vasoconstriction, and metabolic suppression occur in many mammalian taxa, not only in response to diving but also in other hypoxic conditions. These responses occur during hibernation as well as during birth, when uterine contractions temporarily occlude placental blood vessels that supply the fetus with oxygen (22). Many vertebrates, including lizards and snakes, develop bradycardia in a variety of conditions in which oxygen is in limited supply, suggesting that this is an ancient response with a general function that has been put to specific use during diving (16, 22). The fact that fish develop bradycardia when taken *out* of water further supports the idea that hypoxia, rather than immersion per se, is the ultimate cause of bradycardia (22).

Implications of the Diving Response for Humans

Diving bradycardia is a response to immersion. In the event of involuntary immersion, when the subject has not had a chance to hold the breath voluntarily, the apnea reflex stops breathing to prevent aspiration of cold water (see Fig. 4 and Refs. 16 and 19). The momentary inability to breathe that we may experience when a gust of very cold wind blows into our faces on a winter day is one manifestation of the apnea reflex; babies in this situation can be at particular risk because they may be unable to move out of the wind by themselves. Conversely, students may notice that it is more difficult to hold their breath when they immerse their faces in warm water (personal observation), partly because the cue of cold on the face is absent. It is widely believed that the evolutionary benefit of the diving response is that it can improve survival not only in near-drowning (13, 19), but in any situation with reduced oxygen availability (hypoxia) (16).

The professional breath-hold divers of Japan and Korea, known as *ama*, depend on the diving response for increasing the time they can spend on the ocean floor, but even they remain submerged on average for only 30–60 s per dive (17). Clinically, plunging the face into cold water and/or holding the breath are recommended for treating tachycardia or stimulating the vagus nerve (3, 4, 21). Dental examinations and medical procedures that stimulate the glossopharyngeal receptors on the back of the tongue or upper airway can sometimes trigger apnea and bradycardia (19). Some investigators have suggested that the diving response may be responsible for Sudden Infant Death Syndrome (19), but this is only one of many hypotheses under consideration. Others are studying the similarities between the diving response and sleep apnea, a sleep disorder in which hypoxia develops when breathing is disrupted (22)

THOUGHT QUESTIONS

In a diving mammal, what would be the consequence of constricting peripheral blood vessels without making any other circulatory adjustments? Why would this be dangerous? What normally happens to prevent this from occurring? *Peripheral vasoconstriction by itself would reduce the total volume of open blood vessels, thus increasing blood pressure to organs that continue to receive blood, such as the brain. The increase in pressure could cause arteries in the brain to burst. Diving mammals compensate for peripheral vasoconstriction by lowering cardiac*

output (achieved at least partly by decreasing heart rate), thus keeping blood pressure roughly constant.

Arrange the following species in order of how well developed you think the bradycardia reflex would be, from least developed to most developed, and explain your reasoning: muskrat, tadpole, alligator, adult frog, human. State the general principles on which you are basing your answers. *General principles: 1) the bradycardia reflex develops in response to hypoxia; 2) the higher the metabolic rate, the faster hypoxia develops; 3) selection should favor development of a more robust diving response in air-breathing animals that spend more time submerged. Answer: A tadpole should not require a bradycardia reflex because it has gills. The adult frog breathes through lungs but it can also absorb oxygen across the skin (in and out of water); furthermore, it has a very low metabolic rate because it is an ectotherm and has reduced needs for oxygen at all times. Alligators are larger and tend to have higher body temperatures as a result, even though they are ectotherms; higher body temperatures result in higher oxygen needs in this animal, which spends much of its life in and near water. Both humans and muskrats are endotherms with high rates of oxygen consumption. Because humans are larger, they have a lower rate of oxygen consumption per gram body mass than do muskrats; the fact that muskrats live near water and regularly dive further supports the conclusion that the diving response of muskrats should be more developed than that of humans.*

Chester (9) writes, "The diving reflex presumably served our amphibian ancestors well as an oxygen conserving technique with submersion, but serves no known useful function now." Do you agree with Chester? Why or why not? *See Background information for instructors. Even if near-drowning events occur infrequently, they should be sufficient to select for a mechanism that improves survival. See also the answer to the previous question, which explains why an amphibian is less likely to show diving bradycardia than lung-breathing vertebrates.*

If the function of bradycardia and the other adjustments in the body that take place during diving is to conserve oxygen use when oxygen supplies are low, why isn't arterial oxygen the only proximate cue that

triggers the diving response? Use your thinking on this question to make a general statement about proximate and ultimate causation in biology and why both may be necessary for survival. What is another example of ultimate and proximate mechanisms that illustrates the general principle that you have identified (you may use an example from any area of biology that you have studied)? *The oxygen sensors in our arteries do not respond immediately when the animal stops breathing because it takes some time for arterial oxygen tension to decrease. A diving mammal, however, benefits from cardiovascular adjustments that take place early in the dive (or even in anticipation of it), because these adjustments reduce the total oxygen requirement of the animal and increase dive time. Thus responding to a cue that is immediate, such as cold on the face, is advantageous. General principle: using a proximate cue (cold) that predicts the ultimate difficulty (lack of oxygen) allows the animal to prepare itself for the coming change. Another example of this principle is the timing of seasonal reproduction. The ultimate goal is to synchronize the birth of young with the time at which food is most abundant, but animals that wait until food is abundant to begin their reproductive effort (i.e., start regrowing their gonads and mating) would be at a disadvantage compared with animals that are ready to give birth when food is abundant. Most animals living in seasonal environments use proximate cues that reliably* predict *the coming of abundant food (e.g., day length), instead of food itself, to trigger the onset of their annual reproductive effort.*

Given what you know from your results about what triggers the diving response, what do you think would be the response of heart rate, blood flow, and metabolic rate in a human if all body parts *except* the head were immersed? *When hypoxia is a limiting factor, the most important need for survival is to protect the brain from injury, which is facilitated by providing as much oxygen as possible to the brain while reducing the brain's need for oxygen by allowing it to cool, an effect thought to be responsible for the survival of some extraordinary cases of near-drowning in cold water (13). When hypoxia is not limiting, the most important need for survival is the prevention of overall hypothermia. The response to*

head-out immersion is a sharp increase in metabolic rate, heart rate and blood flow (13, 19).

After a harbor seal surfaces from a long dive, it experiences tachycardia (very high heart rate). Propose a function for this tachycardia. *As soon as oxygen becomes available, circulation should increase to quickly remove lactic acid from the muscles and reoxygenate hemoglobin and myoglobin (23, p. 29).*

When the forehead is cooled with a cold pack, it is sometimes possible to observe an initial increase in heart rate, which is then followed by the bradycardia that we are accustomed to observing in the diving response (10). Propose an adaptive function for this initial tachycardia. Does your hypothesis predict that the amount of tachycardia would be different at high or low temperatures? *Tachycardia might be a response to prevent the brain from cooling by circulating warm blood to the head; in this case we would expect tachycardia to be more pronounced at lower temperatures.*

Investigators of a published study wished to determine whether the diving response could help children survive near-drowning in cold water. The children who were subjects in this study simulated dives (immersion with apnea) in 29°C water, but fewer than 20% of the children were able to hold their breath to 25 s, which was the average time it took for diving bradycardia to reach its maximum. The investigators concluded that children have a weak bradycardia reflex and that their ability to hold their breath was not sufficient to improve their chances of surviving near-drowning in cold water. Using examples from your data, evaluate this experiment and its conclusions. *At 29°C, which is warmer than most bodies of water in which a person might accidentally become submerged, diving bradycardia should be less pronounced than at lower temperatures. If the diving response reduces oxygen requirements, then increased bradycardia could extend the survival time of a child immersed in cold water.*

APPENDIX

Definitions

Aerobic dive limit (ADL): the amount of time a diving animal can remain submerged while relying on aerobic metabolism

Bradycardia: lower than normal heart rate

Confidence interval: in statistics, an interval calculated from sample data that has a stated percentage probability of containing the population mean; e.g., the 95% confidence interval has a 95% probability of containing the population mean

Hematocrit: the percentage of blood volume consisting of red blood cells (RBCs), expressed as a whole number; e.g., human hematocrit is normally ~45 (45% RBC by volume)

Oxygen tension: a measure of the concentration of oxygen, expressed as the partial pressure of oxygen in a fluid

Tachycardia: higher than normal heart rate

Thermoneutral zone: a range of ambient temperatures in which heat is neither lost to nor gained from the environment; at these temperatures, the medium (e.g., air or water) should feel neither warm nor cool

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