Student responses to a hands-on kinesthetic lecture activity for learning about the oxygen carrying capacity of blood

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Breckler J, Yu JR. Student responses to a hands-on kinesthetic lecture activity for learning about the oxygen carrying capacity of blood. Adv Physiol Educ 35: 39–47, 2011; doi:10.1152/advan.00090.2010.—This article describes a new hands-on, or “kinesthetic,” activity for use in a physiology lecture hall to help students comprehend an important concept in cardiopulmonary physiology known as oxygen carrying capacity. One impetus for designing this activity was to address the needs of students who have a preference for kinesthetic learning and to help increase their understanding and engagement during lecture. This activity uses simple inexpensive materials, provides an effective model for demonstrating related pathophysiology, and helps promote active learning. The activity protocol and its implementation are described here in detail. We also report data obtained from student surveys and assessment tools to determine the effectiveness of the activity on student conceptual learning and perceptions. A brief multiple-choice pretest showed that although students had already been introduced to the relevant concepts in lecture, they had not yet mastered these concepts before performing the activity. Two postactivity assessments showed that student performance was significantly improved on the posttest compared with the pretest and that information was largely retained at the end of the course. Survey data showed that one-half of the students stated kinesthetic learning as among their learning preferences, yet nearly all students enjoyed and were engaged in this hands-on kinesthetic activity regardless of their preferences. Most students would recommend it to their peers and expressed a desire for more kinesthetic learning opportunities in the lecture curriculum.

THE VERTEBRATE BODY obtains oxygen from the external environment in the lungs or other anatomic structures. Oxygen is then carried via the bloodstream to the peripheral tissues, where it is used by cells to help produce the chemical energy essential to life. Blood oxygen transport is therefore key to understanding homeostasis and other principles that physiology students probably need to learn (11–14).

In the lecture hall, physiology students are typically introduced to oxygen uptake, transport, and delivery within the context of the cardiovascular and respiratory systems. During lecture or study, students usually encounter the term “oxygen carrying capacity,” defined as the total oxygen carried by the blood (18, 22, 23). In technical terms, the oxygen carrying capacity is the sum of dissolved oxygen plus the oxygen bound to hemoglobin. Students learn that the dissolved oxygen content of the blood is inadequate for metabolic needs due to the low solubility of oxygen in plasma. After having taught this area of human physiology for many years, we have noticed three distinct but related concepts concerning oxygen carrying capacity that students inevitably struggle to understand: 1) oxygen molecules are present in the blood in a dissolved form, and their concentration is usually expressed as partial pressure (i.e., P02) in units of mmHg; 2) the concentration of dissolved oxygen directly determines the amount of oxygen bound to hemoglobin; and 3) hemoglobin releases some oxygen in the tissues as a direct result of a decrease in the amount of dissolved oxygen.

One approach to teaching difficult abstract concepts in physiology is to use physical models, which are generally used in the laboratory setting or via demonstration during lecture (1, 4, 8, 15, 16). We designed a new hands-on activity on oxygen carrying capacity to be performed by the students themselves during the lecture period. Pairs of students directly handled representational objects of red blood cells suspended in plasma made from simple, nontoxic, and inexpensive materials, such as tubes, beads, and water.

Part of our rationale for designing this hands-on activity rose from the claim that a match between teaching and learning styles may affect physiology course performance (5). Thus, students favoring kinesthetic (i.e., hands-on) learning might be more engaged in their learning of oxygen carrying capacity concepts by performing our hands-on kinesthetic activity. With that in mind, we gathered data on student learning style preferences, performed in-class assessments, and administered surveys to determine student perceptions.

To facilitate the dissemination and implementation of this new activity on oxygen carrying capacity in the lecture hall, we included a detailed protocol in the appendices, including materials needed, pedagogical approaches, and topics for advanced discussions.

METHODS

Design

A new hands-on activity called the oxygen carrying capacity activity was performed with students enrolled in a human physiology course at San Francisco State University (SFSU). The SFSU campus is a large, urban, multicultural undergraduate public institution that is part of the 23-campus California State University system, the largest university system in the country. At the SFSU campus, the current enrollment is ~25,000 undergraduate students and ~5,500 postbaccalaureate and graduate students in various master degree programs. The activity was carried out with students enrolled in the one-semester lecture course in Human Physiology (Biology 612) during fall 2009 and spring 2010 semesters. Biology 612 is an upper-division course intended for undergraduate biology majors, especially those pursuing a B.S. Biology degree with a physiology concentration. Students have completed prerequisite courses of introductory biology (1 yr), physics (1 semester), and chemistry (3 semesters, including 1 semester of organic chemistry). Students in the course are usually of junior, senior, or postbaccalaureate standing. Many students enroll because...
the course is required for their major and/or they are pursuing health professions careers.

An empirical, observational study was conducted on the impact of the oxygen carrying capacity activity using pre-/postassessments and survey data. Since the course involved was taught as a single large section, all enrolled students in the fall and spring were invited to participate during class, resulting in a single group assignment. Although not designed as a robust controlled study, we wanted to assess student learning and level of engagement using this new activity. All data were collected anonymously, and code numbers were used to track individual performance on assessments. All experimental protocols, including informed consent forms, were submitted and approved by the university’s Institutional Review Board.

Procedures

Preassessment and the activity. The hands-on oxygen carrying capacity activity was presented to students by a researcher (J. Breckler) who is a physiologist and has taught the course in previous years. Neither of the two current course instructors had observed the activity before its presentation in the classroom by the researcher. This was done to prevent the instructors from introducing a potential bias or undue emphasis toward the activity topic that might affect the pretest assessment. However, instructors were told in advance that the activity involved oxygen carrying capacity and hemoglobin-oxygen dissociation curves; both instructors stated that they had already covered this material in their lectures, although the students had not yet been tested on this material. Both instructors felt that their students should know the concepts and physiology involved.

Students were first given a three-question written preactivity test (i.e., pretest) to assess their understanding of the physiological concepts presented in the earlier lecture material. The oxygen carrying capacity activity was then carried out and lasted ~10 min. The activity procedure consisted of a few basic steps, as shown in Fig. 1 and described below.

To summarize the activity, each pair of students was first given a tube of water representing plasma (Fig. 1A) and asked about the dissolved oxygen content. Students were then given red beads to place in the tube (Fig. 1B) to learn the role of red blood cells on oxygen carrying capacity. Students then compared their tube with those of other students (Fig. 1, C–E) to discover the influence of hematocrit. Students were led through the activity by the researcher asking a series of questions, via a problem-based learning type of format. Students were given feedback and acknowledgement of correct answers (see the detailed protocol with questions and answers in APPENDIX A).

Postactivity assessment and survey. After the activity, students were given the same written questions as the pretest (i.e., posttest 1). Attached to posttest 1 was a survey form requesting student demographic information such as class standing, self-reported grade point average (GPA), and reason for taking the course. There were 10 additional questions uniquely created for this activity that were designed to assess student perceptions and attitudes toward the activity.

On the survey, students were also asked to select their preferred learning preference(s) using the following statement: I prefer to take in new information or my ‘learning style’ is (circle all that apply):
A. visual (I like to learn by viewing graphs, drawings, or schematic diagrams)
B. auditory (I like to learn by speaking, listening, or discussing material)
C. read-write (I like to learn by reading the text or writing notes)
D. kinesthetic (I like to learn by touching objects or doing hands-on activities)

Answers to the above questions were later coded as being visual (V), auditory (A), read-write (R), or kinesthetic (K). These data were also grouped according to whether students declared a single or unimodal preference (i.e., 1 learning style), bimodal preferences (i.e., 2 learning styles), trimodal preferences (i.e., 3 learning styles), or quadmodal preferences (i.e., all 4 learning styles).

A final assessment was done to determine if the concepts highlighted during the activity were still retained 10–12 days later (posttest 2), which was included as part of the final exam. Posttest 2 consisted of two of the three questions from posttest 1 except that the answer choice “I don’t know” was not included. Students were tracked for all three assessments and surveys using an anonymous number coding system.

Analysis

We wanted to know which students had stated a K learning preference, as we predicted that these students would respond differently to a hands-on kinesthetic activity than those who did not. Therefore, for our analysis, student data were placed into two groups based on whether a student reported a K preference among their learning preferences (i.e., K group) or did not report a K preference (i.e., non-K group). Percentages were obtained by dividing the number of students by the total number of respondents. We also provide change (Δ) values to show the difference between correct response rates from pretest to posttest 1. In all cases, results are reported for all 149 respondents except when assessments were incomplete and considered as invalid (pretest and posttest 1, n = 4; posttest 2, n = 9).

Pretest and posttest 1 mean scores were compared using a paired Student’s t-test. Survey Likert scale results were compared using a nonparametric test (Mann-Whitney U-test). Pearson’s χ²-test was also performed on the Likert scale data. Analysis was performed using a statistical software package (SPSS version 17). Statistical significance was assigned a P value of <0.05. Physiology students enrolled in the two semesters showed very similar results; thus, all student data were pooled for the entire analysis.

RESULTS

Demographics

During the one-semester Human Physiology course offered in fall 2009 and spring 2010, a total of 184 students was enrolled. Since virtually no students enrolled during the fall were repeating the course in spring, this represented ~184 unique individuals. Of the 184 students enrolled, 149 students were present in class and participated in this activity (i.e., 63 students in the fall and 86 students in the spring), giving an overall response rate of 81%. The majority of enrolled students
were of senior standing (74%) and had a self-reported GPA in the range of 3.0–4.0 (65%).

Learning Preference(s)

The results of the physiology students’ self-reported learning preferences are shown in Fig. 2. The data revealed that 81% of the students selected two or more preferences (i.e., multimodal preferences), whereas the remaining 19% selected only one preference (i.e., unimodal preference).

We wanted to see whether the impact of a kinesthetic activity on students favoring the K learning style differed from the impact on other students, so we separated all students according to the K preference for our analysis. About one-half of the 149 students in the class selected the K learning preference, and these 76 students (i.e., 51%) were placed in the K group, whereas the remaining 73 students (i.e., 49%) did not indicate a K preference and were placed in the non-K group for comparison. These data are shown in Table 1.

As shown in Table 1, a total of 378 learning preferences was selected by the entire group of 149 students. Students in both the K and non-K groups were quite similar in their learning preferences beyond the K preference, as evidenced by similar percentages of V, A, and R preferences for both groups. For instance, V preferences were selected nearly identically, and V was also the most popular preference selected by both K and non-K groups of students (91% and 82%, respectively). In addition, the most common unimodal preference among all students was also V (10% of all students). One notable difference between the groups was that students in the K group slightly favored A learning compared with their non-K colleagues. In summary, the K and non-K groups were similar in size, and the students also had similar V/A/R learning profiles. This similarity between the groups reduces the possibility of skewed results due to sample size or V/A/R preferences.

Student Academic Level

Students in the K and non-K groups had similar class standing and self-reported GPA, as shown in Table 2.

Thus, the two groups were comparable in terms of class level and/or maturity as college students and declared similar college grades. We could therefore reasonably expect that the performance of these two groups on our pre- and posttest assessments might be similar, or at least not have strong biases due to gross differences in academic standing.

Assessment

Preactivity test. Most of the students in the class did not appear to have a firm grasp of the topics to be presented during the activity. For all three questions, students answered only half of the questions correctly (i.e., 51.5%), or an average of 1.5 correct of 3 questions. Both K and non-K groups showed weak performance with similar mean scores (K group: 52.3% correct answers and non-K group: 50.7% correct answers). There were, however, some differences noted between the two groups. For instance, the K group had approximately twice as many students who gave all three correct responses compared with the non-K group (i.e., 17 vs. 10 students). Also, the majority of the non-K group had two correct responses (39.4% of the students) compared with the K group, where the majority of students answered only one question correctly. The same number of students (i.e., 11) in each group did not select any correct responses.

The breakdown of results on individual questions was also informative, as shown in Fig. 3.

Table 2. Number of students reporting class standing and grade point average

<table>
<thead>
<tr>
<th>Class standing</th>
<th>K Group</th>
<th>Non-K Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshman</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sophomore</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Junior</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Senior</td>
<td>56</td>
<td>55</td>
</tr>
<tr>
<td>Postbaccalaureate</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade point average</th>
<th>K Group</th>
<th>Non-K Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0–2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.0–3.0</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>3.0–4.0</td>
<td>51</td>
<td>46</td>
</tr>
</tbody>
</table>
correctly answered “yes.” The most common incorrect answer was “yes, but only if red blood cells are also present.” The second question asked what determines the amount of oxygen bound to a normal hemoglobin molecule inside of red blood cells, and only 44 students (30%) correctly answered “dissolved oxygen.” The most common incorrect answer was that the amount of oxygen bound to hemoglobin is determined by the amount of hemoglobin in the blood, whereas some selected carbon dioxide as the determining factor. This latter answer shows that some students were confused about the role of carbon dioxide in the Haldane effect and the general unloading of oxygen. In response to the third question, 90 students (62%) answered correctly that oxygen exists both dissolved and bound to hemoglobin. Most incorrect answers were that blood oxygen only exists bound to hemoglobin. Overall, the total number of correct responses ranged from zero to three correct responses, with the mode being three correct responses.

Students in the two groups performed better on different questions. Students in the K group had more correct responses on question 2, whereas the non-K group performed better on questions 1 and 3.

Postactivity test. Performance on posttest 1 showed significant improvement by the class overall, as shown in Fig. 3. There were 90.6% mean correct responses compared with only 51.5% mean correct responses on the pretest \( (P < 0.0001 \text{ by a paired } t\text{-test}) \). Moreover, the total number of correct responses ranged from two to three correct responses, with the mode being three correct responses.

To determine whether students with a K learning preference benefitted more from the hands-on activity than other students, we compared assessment results for the K and non-K groups. Both groups performed well, with a mean score of 90.5% and 90.6% correct answers for K and non-K groups, respectively. Both groups also had very similar numbers of correct responses among individuals. This can be seen by the percentage of students with three (K group: 77% and non-K group: 77%), two (K group: 18% and non-K group: 17%), and one (K group: 5% and non-K group: 6%) total correct response(s). All students in both groups answered at least one question correctly.

We also compared the difference in correct responses from the pretest to posttest 1, a value we termed “Δ.” On specific questions, the performance by K and non-K groups varied.
slightly but showed significant improvements for all three questions, as shown by the positive Δ-values (see Fig. 3). Thus, both groups appear to have similarly benefitted from the activity, as exhibited by the similar improvements from pretest to posttest 1 (K group: \( P < 0.0001 \) and non-K group: \( P < 0.0001 \); by paired \( t \)-tests).

Knowledge retention at the final exam. Assessment questions 2 and 3 were included on the final exam (termed posttest 2), which occurred 10–12 days after posttest 1. The overall class result was 81.1% mean correct responses, a considerable improvement over pretest results. Since posttest 2 had only two questions, we were unable to do a paired \( t \)-test with posttests 1 and 2. In general, students had mostly retained the concepts and knowledge at the time of the final exam.

Students in both K and non-K groups performed well (80.7% and 81.4% correct answers, respectively). Next, we compared Δ-values from the pretest to posttest 2. For question 2, the Δ-values showed an improvement of 30.8% and 47.5% for the K and non-K groups, respectively. For question 3, Δ-values were 31.9% and 29.5% for the K and non-K groups, respectively. Thus, students improved their performance at the time of the final exam, whether or not they had stated a K preference.

We also compared Δ-values from posttest 1 to posttest 2 to see how well students retained the information during the intervening period. Generally, both groups exhibited lower posttest 2 scores compared with posttest 1 scores. On question 2, more students answered incorrectly that “the amount of carbon dioxide in the blood” determines the amount of oxygen that is bound to a hemoglobin molecule (\( n = 22 \), or 15.7% of posttest 2 responses, compared with \( n = 8 \), or 5.5% of posttest 1 responses). Again, this shows the strength of the misconception about the Haldane effect among students in the physiology course. There were also differences in posttest 2 performance observed between K and non-K groups. On question 3, the K group showed a 3.2% decrease in correct responses from posttest 1 to posttest 2, whereas the non-K group showed a 9.9% increase.

Survey Results

The oxygen carrying capacity activity was well received by students, as shown by the survey responses in Table 3.

Students reported a high level of participation during the activity, and we also observed this directly in the classroom. The two strongest survey responses were that students fully participated by touching the objects and that the lecture activity was unique compared with other classes of this type. A small number of students felt that they fully participated even though they did not touch the objects, a statement expected to receive a low “agreement” score. The weakest response was that the activity did not robustly enhance interest in the topic for either group. Interestingly, students felt that the activity reinforced what they already knew, despite our pretest assessment results showing otherwise (see Fig. 3).

We further grouped the survey answers on a Likert scale according to whether the student generally agreed (4 and 5), neither agreed nor disagreed (3), or disagreed (1 and 2) with a particular statement, and these results are shown in Fig. 4. Student data were separated into K and non-K groups.

Table 3. Survey results on the oxygen carrying capacity activity

<table>
<thead>
<tr>
<th>Question</th>
<th>Score</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>1. I felt this activity was engaging.</td>
<td>3.99</td>
<td>0.93</td>
</tr>
<tr>
<td>2. I felt adequately prepared for this activity.</td>
<td>3.73</td>
<td>0.87</td>
</tr>
<tr>
<td>3. I fully participated in the activity by touching the objects.</td>
<td>4.43</td>
<td>0.89</td>
</tr>
<tr>
<td>4. I fully participated in this activity but I did not touch the objects.</td>
<td>1.91</td>
<td>1.22</td>
</tr>
<tr>
<td>5. I would like to have more hands-on activities in this type of lecture class.</td>
<td>3.77</td>
<td>1.11</td>
</tr>
<tr>
<td>6. I would recommend this hands-on experience to my peers.</td>
<td>3.77</td>
<td>1.01</td>
</tr>
<tr>
<td>7. This hands-on activity helped me learn something new about this physiology topic.</td>
<td>3.74</td>
<td>1.06</td>
</tr>
<tr>
<td>8. This activity mostly reinforced what I already knew about this topic.</td>
<td>4.02</td>
<td>0.90</td>
</tr>
<tr>
<td>9. This activity enhanced my interest in this topic.</td>
<td>3.54</td>
<td>0.94</td>
</tr>
<tr>
<td>10. This activity was unique compared with other classes of this type that I took.</td>
<td>4.16</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Values are means ± SD. Students responded to the following directions: For the next set of questions, circle one answer using the following scale. The scale was as follows: 1, strongly disagree; 2, disagree; 3, neither agree nor disagree; 4, agree; and 5, strongly agree.

The grouped analysis showed that students with a K preference were slightly more enthusiastic in their responses than their colleagues. In particular, students in the K group showed significantly (by Mann-Whitney \( U \)-test) stronger support in the areas of wanting more hands-on activities during lecture, recommending the experience to peers, and feeling that the activity was engaging and unique. The Pearson’s \( \chi^2 \)-test showed that K and non-K groups were significantly different only for questions 5 and 6, and these two questions more specifically addressed the hands-on aspect, suggesting that there may be a very slight preference for the activity among students with a K preference.

DISCUSSION

In this article, we present a new kinesthetic activity for learning about the concept of blood oxygen carrying capacity and related physiology. To our knowledge, this is the first attempt to create a hands-on experience on this topic that can be performed in a lecture setting using simple materials and instructor-guided step-by-step exploration. After completing this exercise, prepost data showed that students comprehended the relationship between dissolved oxygen and hemoglobin.

In the course of performing the activity with students, we also discovered a misconception that students have about the role of carbon dioxide and the unloading of oxygen by hemoglobin. One student suggested that carbon dioxide was responsible for the unloading of oxygen by hemoglobin, and many students agreed. This confusion about the Haldane effect was also represented by the data in our assessments. We do not know the overall impact of misconceptions on this topic since we did not set out to study them, although perhaps our activity can help dispel or at least illuminate these misconceptions for class discussion.

One area of difficulty in teaching about oxygen carrying capacity is the direct relationship between dissolved and bound oxygen, which is represented by the oxygen-hemoglobin dissociation curve. Yet, despite their familiarity with
the dissociation curve, we have noticed that many students have a lack of understanding of the underlying concepts. Indeed, when queried, many students cannot even recall what is being measured on the x-axis (i.e., Po2) nor what Po2 represents, whereas others will simply focus on the binding “cooperativity” aspects of the curve. An earlier attempt has been made to improve the teaching of this important topic (19). The activity described in our article directly addresses the foundation of the dissociation curve itself and can lead to an indepth discussion of the relationship between oxygen and hemoglobin. For advanced discussion, we also suggest ways in our protocol to elaborate by introducing anemia, high altitude, and hyperbaric oxygen.

Performing simple activities such as the oxygen carrying capacity activity likely increases student motivation since it can be considered a form of active learning strategy (3, 6, 10). Active learning, currently a popular method for teaching biology, typically involves the use of hand-held clickers in many lecture classrooms. While the efficacy of clickers as a motivating tool has been well documented (6), we suggest that hands-on models might also be beneficial. Our activity adds to the growing list of material objects being created to help improve student understanding of physiological concepts (1, 4, 8, 15, 16). The use of simple, inexpensive materials and quick setup for our activity enables many students to actually touch the objects, perhaps increasing the likelihood of a greater impact on student learning and motivation.

Although we expected students with a K preference to be the most engaged in the oxygen carrying capacity activity, we were surprised to find in our survey that virtually all students responded very positively to the hands-on experience. Indeed, more than half of the physiology students selected the K learning style among their preferences (i.e., 51%), which is comparable with other studies (i.e., 69.3% using VARK in Ref. 2 and 87.8% using VARK in Ref. 9). A small number of students had a strong sole preference for K learning (i.e., 4–5% in Ref. 2, 18% in Ref. 9, and 2% in our study). Together, students with unimodal and multimodal preference(s) for the K learning style represent at least half of the class.

We expected that students with a K preference would be more likely to perform better on our written assessments. Again, we found very little difference between students who stated a K preference and those who did not.

The laboratory setting has been the classical venue for exploring hands-on learning and designing new kinesthetic activities, although the goals may be somewhat different than in lecture. New laboratory sessions or case discussions to improve conceptual learning in cardiovascular physiology have been created (17, 20). Our study takes a slightly different approach because it does not require any data collection to explore the basic concepts. Also, our hands-on activity is inexpensive, safe, and “low tech,” a distinct advantage for high-enrollment physiology courses, and it can be carried out under the guidance of the professor in a large group setting. We agree that large-scale in-class demonstrations using instructor-held objects are effective (16), although we take this one step further by allowing students to hold the objects themselves. The levels of engagement and enthusiasm in our study show that this type of hands-on learning is both effective and desirable.

Limitations

This one-semester physiology course was taught by two different instructors who also adopted two different textbooks in fall versus spring (7, 23), making it possible that the coverage and level of the lecture material on this topic may have been somewhat different between the two classes and

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**Fig. 4.** Likert survey results grouped according to whether the student agreed (A), neither agreed nor disagreed (N), or disagreed (D). Responses were grouped for students stating a K preference (K group, n = 76) or not (non-K group, n = 73). *P < 0.05; **P < 0.01 (by Pearson’s χ²-test). (See Table 3 for the actual survey questions.)
might have led to gross differences in the learning of oxygen carrying capacity by students before the activity period. However, the students in both semesters showed low pretest performance and very similar improvements after the activity.

Both instructors in whose classes we performed the study mentioned that their students should do well on the pretest since this material had been covered in the lectures. Instructors were surprised to learn that the students did not perform better on the pretest. The placement of the activity before (or in lieu of) the lectures may have had a different result. We also do not know whether the activity would be equally effective as an instructor demonstration or in a laboratory setting.

Learning preference information was collected via self-reporting rather than by validated instruments, although our data were similar to other published data, including our own (2).

APPENDIX A: OXYGEN CARRYING CAPACITY

ACTIVITY PROTOCOL

Introduction

In this exercise, students handle simple tubes containing water and beads, which represent plasma and blood cells, respectively. The activity protocol is written as three procedural steps, each followed by a series of questions to stimulate learning. Answers may be provided, or students can discuss their own answers to stimulate learning.

Student Learning Outcomes

After performing this exercise, the student will learn that:

1. The amount of dissolved oxygen in liquid is expressed as PO2.
2. The binding of oxygen to hemoglobin is determined by the amount of dissolved oxygen in the solution, and this relationship is expressed by the hemoglobin-oxygen dissociation curve.
3. The blood’s oxygen carrying capacity is also known as the blood’s total oxygen content.
4. Oxygen is carried by blood in both the dissolved form and bound to hemoglobin.
5. Normal blood carries 60–70 times more oxygen bound to hemoglobin than in the dissolved form.

Items Needed to Perform This Exercise

The following items are needed to perform this exercise:

- Plastic or glass test tubes, uncapped (note: 1 tube for every pair of students).
- Tap water
- Small red objects that fit easily inside the tube, such as red beads, buttons, vinyl circles, or other plastic objects; when added to the tube, they will occupy about half the tube volume. Red candy is not desirable since the dye changes the color of the water.

Procedure Step 1

Take an empty tube and fill it with water to roughly one-half to three-quarters full. (The instructor may do this ahead of time.)

Question 1. Is there any oxygen in the tube of water? If so, in what form is the oxygen?

ANSWER. Yes. There is indeed oxygen in the water, and it is present in a dissolved form as individually dissolved oxygen molecules. These molecules are very small, so we cannot visualize them. Bubbles are large collections of air molecules, and their presence is not needed to dissolve oxygen in the blood.¹ (Note: additional oxygen atoms exist in the tube as part of each water molecule.)

Question 2. Where does the oxygen in the water come from?

ANSWER. It comes from the oxygen molecules present in the atmosphere. Oxygen in the air enters solutions and, depending on its solubility, will eventually reach equilibrium with the air.

The concentration of dissolved oxygen molecules in a fluid is not described in units of ‘molarity’ but rather in units of partial pressure. At sea level, atmospheric pressure is 760 mmHg.² Since ~20% of the air molecules are oxygen, the PO2 is roughly 0.2 × 760 mmHg = ~150 mmHg. Thus, when a liquid is exposed to room air and reaches equilibrium (i.e., the same amount of oxygen enters and leaves the liquid), we say that the liquid and room air share the same PO2 value.

Question 3. How can we increase the amount of oxygen dissolved in the water?

ANSWER. We can increase the amount of oxygen dissolved in the water by increasing the amount of oxygen in the air. For instance, we could expose the water to medical oxygen (i.e., 90% oxygen). Conversely, to decrease the amount of oxygen in the water, we could go up to high altitude, where atmospheric pressure is less; therefore, the PO2 value is also less.

Question 4. Let’s pretend the tube is now filled with plasma (the liquid portion of the blood) instead of plain water. Is there oxygen in the plasma?

ANSWER. Yes. Let’s review how plasma obtains oxygen from the air. The PO2 of alveolar air is somewhat less than room air because of the humidity of the airways and the addition of carbon dioxide from the blood, which reduce the PO2 in alveolar air to about PO2 = 100 mmHg. Oxygen from the alveolar air then enters the plasma as follows. Oxygen molecules in the alveolus first interact and dissolve into the thin fluid lining of the alveolus. Oxygen is very permeable to membranes, and so oxygen easily passes through alveolar pneumocytes and capillary endothelial cells to reach the plasma. This movement of oxygen from air to plasma will continue as long as there is a concentration gradient (i.e., PO2 gradient). Finally, the oxygen is at equilibrium when the PO2 of alveolar air and plasma are the same. The blood PO2 value therefore reflects the oxygen that is dissolved in the blood.

Procedure Step 2

Suppose we add some red blood cells to this sample, as represented by some red beads. (Add beads so that each student has slightly different amounts, ranging from 25% to 50% of the final fluid volume.) The tube sample now represents whole blood.

Question 5. How does the addition of red blood cells change the amount of oxygen in the tube?

ANSWER. Hemoglobin in the red blood cells will greatly increase the total amount of oxygen in the tube because hemoglobin can bind oxygen.

Question 6. Where does the oxygen bound to the hemoglobin come from?

ANSWER. Oxygen dissolved in the plasma will first enter the red blood cell. As the dissolved oxygen concentration inside the cell increases, hemoglobin will begin to bind the oxygen. Each hemoglobin molecule is a tetramer consisting of four subunits, and each subunit binds one oxygen molecule. Therefore, each hemoglobin molecule can bind a total of four oxygen molecules. Once the oxygen

¹ Some students may have a misconception that may have arisen from familiarity with “bubblers” in fish tanks. A bubbler simply enhances the

² Regarding the units of mmHg: at sea level, the downward atmospheric pressure of dry air can support a column of mercury fluid about 760 nm (or 30-in.) high. At high altitude, where the air molecules are less dense and the atmospheric pressure is therefore less, the column of mercury supported is shorter (e.g., 600 mmHg).
is bound to hemoglobin, it no longer contributes to PO₂. Thus, hemoglobin is like a "sink" that can take up oxygen until each hemoglobin molecule is fully (100%) saturated with four oxygen molecules.

**Question 7.** Quantitatively, by how much does the total oxygen content increase when we add a normal number of red blood cells?

**Answer.** The total oxygen content of whole blood is ~60–70 times greater than the dissolved oxygen of plasma alone! This is due to the large number of hemoglobin molecules inside each red blood cell and the ability of hemoglobin to bind oxygen. The total oxygen content of the blood is also known as the oxygen carrying capacity of the blood.

**Question 8.** What determines how much oxygen will bind to the hemoglobin?

**Answer.** The amount of dissolved oxygen surrounding a hemoglobin molecule directly determines the amount of oxygen that binds to hemoglobin. This relationship is illustrated by the hemoglobin-oxygen dissociation curve. In other words, the more dissolved oxygen there is, the more oxygen is bound to hemoglobin. The less dissolved oxygen there is, the less oxygen is bound to hemoglobin.

**Question 9.** Consider if instead of a tube, you are holding a systemic capillary. Why will hemoglobin let go of some oxygen when this blood reaches tissues?

**Answer.** In the capillaries, blood velocity slows down, which enhances the diffusional exchange of dissolved substances among the plasma, interstitial fluid, and tissues. The dissolved oxygen will thus leave the plasma due to an oxygen gradient (as we said before, dissolved oxygen is described in units of partial pressure). This PO₂ gradient exists because oxygen is being constantly "consumed" inside the mitochondria via the process of oxidative phosphorylation. Hence, there is a downhill gradient for the movement of oxygen out of the bloodstream and into tissue cells toward the mitochondria. Therefore, as dissolved oxygen leaves the plasma, the PO₂ of the plasma is lowered, and, in turn, this causes dissolved oxygen to diffuse from the interior of the red blood cells. Once the PO₂ inside the red blood cell is lowered, oxygen will come off the hemoglobin molecule, as predicted by the hemoglobin-oxygen dissociation curve. Since hemoglobin is fully saturated in normal arterial blood at a PO₂ of 100 mmHg, each hemoglobin molecule enters the systemic capillary with all four oxygen binding sites occupied. The average hemoglobin molecule will release only a single oxygen molecule; thus, normal mixed venous blood has hemoglobin with an average of three of four binding sites occupied (i.e., 75% saturation at PO₂ = 40 mmHg).

**Procedure Step 3**

Raise your tube of “blood” and compare it with tubes held by the other students.

**Question 10.** Suppose the tubes in this room contain blood from different patients. Does your patient have anemia or polycythemia?

**Answer.** Examine your tube to judge the approximate “hematocrit” of your sample. The term “hematocrit” refers to the percentage of the blood volume that is occupied by red blood cells. Generally, anemia is a clinical condition that results from a low hematocrit (lack of red blood cells) and/or low hemoglobin. There are many types of anemia, often stemming from low red blood cell production or enhanced red blood cell breakdown. Women normally have lower hematocrits than men (range for women: 38–46% and range for men: 42–54%), so women tend to exhibit anemia more often. Polycythemia refers to an abnormally high hematocrit, which is a rare condition at sea level but often arises in normal individuals residing at high altitude for several months, leading to the overproduction of red blood cells.

**Question 11.** Who is holding the patient’s blood with the highest oxygen carrying capacity?

**Answer.** The person whose sample has the highest hematocrit has the patient with the highest oxygen carrying capacity.

**APPENDIX B: ADVANCED TOPICS FOR DISCUSSION OF PHYSIOLOGY AND PATHOPHYSIOLOGY CONCEPTS**

**Hypoxia**

A deficiency in tissue oxygen is termed “hypoxia.” Hypoxia can be caused by many reasons, such as impaired respiratory or cardiovascular function, metabolic toxic agents, and/or a reduction in oxygen carrying capacity (i.e., low PO₂ or anemia).

**Administration of Oxygen**

Breathing a gas mixture with additional oxygen can increase the amount of dissolved oxygen in the blood. Examples are as follows: neonatal oxygen tents, oxygen therapy for respiratory disease, hyperbaric oxygen chambers, oxygen masks at sporting events, and the recreational use of oxygen in some countries.

**Hemoglobin-Oxygen Dissociation Curves**

There are several types of hemoglobin-oxygen dissociation curves. View a figure in which the PO₂ values on the x-axis are greatly expanded (i.e., above 100 mmHg). This enables one to see how further increases in PO₂ above 100 mmHg do not result in hemoglobin saturation above 100% yet do reflect increased dissolved oxygen.

**Blood Oxygen Content**

View a figure of blood oxygen content, in which the y-axis is expressed in units of ml oxygen/100 ml blood. The two curves represent dissolved oxygen and oxygen bound to hemoglobin. Thus, further increases in PO₂ beyond 100 mmHg can dissolve more oxygen in blood but hardly increase the oxygen carrying capacity (i.e., total oxygen content) since hemoglobin is already fully saturated.

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**REFERENCES**


