Supplemental Material

CBE—Life Sciences Education

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The three exercises contained in the supplementary materials illustrate how data can be used in a biology classroom as well as provide examples of how annotations to class materials can be provided to assist other faculty.

These exercises were created by Amy Mulnix for use in first-year and second-year cell biology courses.

Non-Covalent Bonding and Geckos
Practice Reading a Research Article

LEARNING GOALS

1. Comprehend the main ideas in a scientific article.
2. Extracting information from highly technical and complex writing.
3. Apply your understanding of non-covalent bonds to larger biological phenomena.
4. Understand how physics and biology can interface.
5. Generate hypotheses.

Article: Autumn, K., Liang, Y.A., Hsleh, T., Zesch, W., Chan, W. P., Kenny, T. W., Fearling, R. and Full, R.J. 2000. Adhesive force of a single gecko foot-hair. Nature. 405:681-685.

BACKGROUND

This article discusses the evidence for the mechanism by which a gecko is able to climb smooth, vertical surfaces. While the article is dense and contains concepts from physics and engineering, the essential findings are exciting and understandable by introductory level undergraduate students in biology and chemistry. The article is a wonderful example of applying interdisciplinary perspectives to a biological problem.

Before beginning to examine the article, generate at least two hypotheses for how a gecko is able to climb smooth, vertical surfaces.

Because the article is sophisticated, a series of questions are provided here to focus on the major findings and interpretations relating to the adhesive force of geckos. The process of gecko detachment from a surface is not considered. Experimental method is not emphasized but is examined. One goal of this assignment is to learn to read a complicated article in a selective manner, assimilating information that is familiar, deciding which information needs to be investigated further, and ignoring information that is not relevant to the exercise at hand.

A. Read the abstract and answer the following questions.

1. Give a general description of the organization of the hairs (or setae) on a gecko's foot (how many, how long, diameter, shape, etc.). Make a sketch of a seta.
2. What is the definition of a van der Waals attractive force?
3. Before reading any more of the article, write several sentences that explain how van der Waals forces could account for the adhesion (stickiness) of a gecko's foot to a smooth surface.
4. Examine Figure 1 to obtain visual images of the organization, shape and dimensions of the setae.

B. Examine Figure 1a, e, and f.

1. There are two types of measurements being made in this paper. One is the force required to pull when a surface is perpendicular (lift the gecko straight off the

wall). The other is the force that operates in the parallel dimension (the gecko sliding down the wall, due to gravity for instance).

C. On p. 683 in the first column:

"Setal force did, in fact, depend on three-dimensional orientation (spatulae pointing towards or away from the surface) and the extent to which the seta was preloaded (pushed into and pulled along the surface) during the initial contact. Contacting the surface with the seta in a direction other than with spatulae projecting toward the surface resulted in forces of less than 0.3 microNewtons when the seta was pulled away perpendicular to the surface. By contrast, when the active spatular region was projecting toward the surface, force increased enormously. After an initial push toward the surface, a 'perpendicular preload,' the seta was pulled parallel to the surface. Setal adhesive force parallel to the surface increased until the seta begin to slide off the edge of the sensor."

This set of sentences is a complicated description of how the seta was applied to the force sensor. The diagrams at the top of Figure 2 may be helpful in interpreting the information. The graphical representation in Figure 2a should also be helpful. Put these sentences and figures into your own description of how the seta is being applied to the sensor and at what point a strong force is obtained.

D. At the top of the second column on p. 683, the authors begin to make their argument for van der Waals forces being the source of the ability of geckos to climb smooth vertical surfaces. Where does this interpretation of results begin (rather than merely presenting of results)?

E. What are the other two possible explanations for the adhesive forces and what is/are the negative evidence against these mechanisms?

F. What mechanism does the data in this paper best support for the adhesive force?

G. From what you know of van der Waals' forces (particularly that they are additive and must occur over atomic distances), can you provide an explanation for why an orientation step (preload) followed by a sliding motion of the foot might provide the greatest adhesive force in the model begin investigated?

H. Reflect for a moment on how you approached reading this difficult article. What strategies were helpful? Talk to at least one other person in class and find out what strategies they used.

I. How does this article connect to things you already know? Can you think of any practical applications for this work?

ADDITIONAL RESOURCES

1. Video of a man-made material mimic of the gecko foot adhesion process. http://www.youtube.com/watch?v=HTbrsx1zARs&feature=related

2. This site is at Stanford: http://bdml.stanford.edu/twiki/bin/view/Rise/StickyBot and contains various information about StickyBot, a robot able to climb surfaces based on the Gecko's mechanism. There is a link to numerous videos showing
3. The web page for the Autumn lab which did the research for this paper is located at: http://geckolab.lclark.edu/dept/Welcome.html. http://geckolab.lclark.edu/dept/Welcome.htmlhttp://geckolab.lclark.edu/dept/geckostory.html
4. http://bdml.stanford.edu/RiSE/Downloads/Weird_Connections.mov. This does an excellent job at showing the process of science, including the failures, the testing of alternative hypotheses and then application of the way the gecko works to the development of StickyBot. Students could either watch this video either before or after the assignment.

For Faculty

What does this exercise do?
Note, for an alternative use of this exercise that would span an entire hour or part of a laboratory session and would emphasize how science is done, see below.

This is indeed a difficult article for introductory level students to read, but the concepts are relatively simple. One major benefit is that the article emphasizes the importance of non-covalent transient interactions to organismal behavior and function. The questions are intended to guide the students through the portions of the article that are most relevant to a discussion of non-covalent forces. The questions are highly directive given that this is likely to be among the first time students will attempt to read a research article of this type beyond the title and/or abstract. This exercise models how an expert scientist might approach an article of interest that is not directly in her field.

Principles Emphasized

1. Structure determines function at an organismal level.
2. Connections between molecule (even nanoscale) phenomenon and organisms.
3. If the additional resources include an introduction to the StickyBot, then this exercise also emphasizes the practical applications of science.

Opportunities for Transfer and Connection to Previous Knowledge
Students are asked to use their previous experiences to hypothesize about the forces that might be responsible for the gecko's ability.

Students should connect this article to a previous discussion of van der Waals forces.

A specific question is included to help students connect the ideas in this article to other things they know or have experienced.

Skill Development
Students are guided through extracting information from a difficult research paper.

They are also asked to examine diagrams, data and statements in the text and attempt to coordinate these.

Metacognition
The exercise explicitly asks students to consider the approaches they (and a classmate) took to the exercise that were successful.

How to use this exercise.
Small groups of two - four students could be used in a discussion format. Students could be assigned the article and given the questions prior to class. If students arrive with some familiarity of the article, this exercise could take 20 – 30 minutes, including the debriefing.

The content debriefing could be done as an oral one with full class participation. Alternatively, a written set of answers could be distributed at the end of the exercise and then discussed by the small groups. This approach is preferable to posting the answers for students to view later as it would allow an in-class discussion on items that were still unclear.

An oral debriefing on *process* is highly encouraged, even if it is just the faculty member reflecting on his or her own approach to the article. Acknowledgement of the difficulty of the reading is key. Depending on the faculty member's goals, this exercise could be used to emphasize the approach to reading an article more than the content of the article. In this case, the teacher should collect comments from the students about how the process went. Some specific questions to ask are:

- Write down two or three words that describe how you felt at the outset of the exercise.
- Write down two or three words that describe how you felt while doing the questions.
- Write down two or three words that describe how you are feeling now.
- Do you need to understand all of the content of the paper to understand the broad message?
- Did discussing the material with other students help?

Also see below for an alternative to expanding this exercise to illustrate the scientific process and not just the specific content.

Guidance/Support

Support should be provided for students doing this exercise to minimize their anxiety and confusion. In many cases, explicit verbal support and acknowledgement that the article and assignment are difficult but valuable may be necessary. Working in small discussion groups should alleviate some of the anxiety felt by students. This exercise could also be given in a recitation or small laboratory setting (see below).

Another way to provide support for students and to keep them moving forward with the assignment during class is to answer some of the questions posed at intervals during the discussion. For instance, after giving the students a chance to struggle with the text associated with Figure 2, a brief explanation to the entire class would be helpful.

Options for grabbing student attention and/or creating multiple sensory cues around this subject are:

- Showing a video section of the StickyBot (see Additional Resources). Illustrate the 3M® Command strips for hanging pictures available at most hardware stores. The key here is to focus on the surface that attaches to the wall (not the one that holds the picture). You attach the strip by pushing in a perpendicular direction; you remove the strip by pulling in a parallel direction. Ask students if it is possible that a similar mechanism as seen with the geckos is at work.

Alternative Use of this Exercise to Illustrate the Scientific Process

This exercise in combination with the Weird Connections video http://bdml.stanford.edu/RiSE/Downloads/Weird_Connections.mov would provide an excellent example of how science works, as well as provide support in understanding the article. The video explicitly shows development of the idea from an observation in nature through development of a new technology and then application of that technology for saving lives. The program acknowledges the difficulties scientists had in figuring out the mechanism and the scientists themselves talk about how for months they could not figure out how to get a single seta to stably affix to a surface. It also illustrates the cooperative nature of the scientific process and how one idea builds on the next.

One way approach for this lesson is to:

1. Give students 10-20 minutes to begin work on the exercise. They should at least begin the questions in parts A-C. They are likely to find the material difficult and are likely to be a bit confused.
2. Watch the video through the first approximately 9 minutes (to the point where the narrator says: "And the question is: How?"
3. Then let the students return to parts A and B and finish those questions.
4. Give the students another 10-15 minutes to work on parts C-G. Then show the next approximately 2 minutes in which the notion of van der Waals forces is discussed. Stop the video at the image of the dog.
5. Ask students to spend 5 minutes discussing part H. Then move them onto discussing part I.
6. Watch the rest of the video, pointing out how the curling of the toes on the StickyBot is how the force is released (from the perpendicular rather than the parallel direction).
7. Finally, pose the question to the students: what do the article and video tell you about how science is done and how it moves forward?
8. Provide the students with a Command strip for picture hanging and let them experiment with attaching and detaching it. Asking them to make observations about both the picture hanging surfaces and the surfaces that stick the strip to the wall. How are these phenomena like (or not like) the gecko mechanism?

Cystic Fibrosis and Protein Sorting

The majority of mutations associated with cystic fibrosis occur in a protein called the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This protein is found in the plasma membrane of many cell types, including those of the lung, sweat glands, and gastrointestinal tract. The function of the CFTR protein is as a chloride ion (Cl^-) transporter.

Many patients with cystic fibrosis have a *reduced* amount of CFTR in their plasma membrane and, as a consequence, a decreased Cl^- conductance across the membrane. You are researching the intracellular sorting pathway of one type of mutant CFTR protein in relation to cystic fibrosis. You perform an analysis to determine the amount of CFTR present in various organelles from lung cells of normal persons and those of persons suffering from cystic fibrosis. The following table is a summary of those results. A "-" means no protein was found in the organelle, "+" means that some protein was found in the organelle and "+++" means a lot of protein was found in that location.

	NORMAL cells	CYSTIC FIBROSIS cells
cytoplasm	-	-
ER	+	+++
cis GOLGI	-	-
Trans Golgi network	+	+
Plasma membrane	+++	+

In one or two sentences, state the location of the protein in cells expressing the normal CFTR protein.

In one or two sentences, state the location of the protein in cells expressing the CFTR mutant.

From your understanding of protein sorting within the endomembrane pathway, suggest one or more reason(s) why the mutant protein might have this distribution. In other words, generate a hypothesis about how the mutation in the CFTR protein of CF patients disrupts its sorting. There are numerous possible answers to this question; be sure to supply the logic for your suggested mechanism.

For Faculty

What does this exercise do?
This exercises focuses on protein trafficking in the endomembrane pathway. Students are presented with a set of idealized data and asked to interpret the results. Their interpretation is dependent on their understanding of the various factors operating along the endomembrane pathway. Because the exact dysfunction that leads to accumulation of the mutant CFTR protein within the ER remains controversial, the question is truly an open-ended one.

How to use this exercise.
This exercise should follow coverage of sorting along the endomembrane pathway, including the concepts that: 1) protein sorting to the ER is dependent on an ER signal sequence; and 2) retention/retrieval mechanisms exist for maintaining compartment identity. If faculty elect to mention/discuss the ER-misfolded protein pathway (also known as ER quality control checkpoint) by which proteins that do not fold properly are degraded by the cytoplasmic ubiquitin-proteasome machinery, students will have another aspect of the mutant CFTR to consider. Faculty may elect to use this exercise as an introduction to a discussion of this latter pathway.

Students should be asked to work in groups of two to four for this exercise. Although the answers to the first two questions seem obvious (students are asked simply to describe the results), some students have difficulty with this step. Faculty may need to provide some reassurance that there is not a single correct answer to the third question and that if a hypothesis can be supported by logic, the suggested mechanism by which CFTR accumulates in the nucleus is a reasonable one.

Guidance/comments for the exercise.
The data has been idealized and summarized. Nevertheless, it is representative of the situation with the ΔF508 mutant of CFTR: the mutant protein accumulates in the ER whereas a reduced amount reaches the plasma membrane; little other protein is seen along the secretory pathway. In addition, the mutant protein that does reach the surface of the cell is rapidly degraded via the endocytic pathway. The focus of this exercise is on protein sorting through the ER/Golgi/TGN/plasma membrane. Endocytosis has not been considered for this situation.

Answers
Reasons that the mutant protein might accumulate in the ER include:

1) loss of a signal that directs the protein to the next compartment (Golgi network). Depending on how in-depth vesicular trafficking has been discussed, students may be able to specifically suggest that a component of the COP-coated machinery has lost the ability to recognize the region of CFTR that directs CFTR to the next compartment.

2) the mutation exposes or creates a signal that actively retains the CFTR in the ER compartment.

3) the mutation disrupts the ability of the protein to assume a final, stable configuration. This results in the accumulation of the protein in the ER because it does not move forward in the endomembrane pathway. Eventually, these misfolded proteins are targeted for degradation via the ER-misfolded protein pathway.

There may be others mechanisms that can be supported by logic.

A recent research article [Tsigelny, I et al., 2005. Identification of molecular determinants that modulate trafficking of Δ508 CFTR, the mutant ABC transporter associated with cystic fibrosis. Cell Biochem. Biophys. 42(1): 41-53.] provides evidence relating to these possibilities. The abstract of this article is relatively easy to read and presents the possibilities in a succinct manner. This abstract may be used to supplement the exercise and to provide guidance for or validation of student thinking. The abstract of this article is a good introduction to reading scientific literature.

Fates of Pyruvate

Learning Goals

At the end of this exercise you will

1. better understand the pathways for the breakdown of glucose in environments with and without oxygen.
2. appreciate that cells have options for how they metabolize compounds depending on the conditions.
3. have practiced generating hypotheses and interpreting data.

Preliminary Exercise

What do the following have in common?

- Tumor cells
- Bacteria at the bottom of a pond
- Muscles in the middle of a sprint
- Production of beer, wine, cider, sake or soy sauce
- Making kimchi, yogurt or sauerkraut
- Extended diving in emperor penguins

Background

Under *aerobic* conditions, pyruvate can enter the reactions of pyruvate dehydrogenase (PDH) and the citric acid cycle. These generate additional ATP (in the form of GTP) and NADH, as well as $FADH_2$. The NADH and $FADH_2$ ultimately transfer their electrons to oxygen; as a result of this electron transfer, even more ATP is synthesized. These latter reactions are absolutely dependent on the presence of oxygen. Thus, the PDH and citric acid cycle reactions occur *only* in the presence of oxygen. This set of pathways is often called aerobic respiration.

In contrast, glycolysis can occur under either aerobic or anaerobic conditions. Although the amount of ATP synthesized is small (only 2), there are numerous anaerobic circumstances in which glycolysis is the major pathway by which ATP is generated. For instance, in a short, sudden burst of muscle activity there is a demand for ATP but oxygen is not yet being delivered via the circulatory system in sufficient quantities to allow aerobic respiration. Glycolysis under anaerobic conditions produces the ATP necessary to sustain muscle activity for a short period of time. Another example: yeast living under anaerobic conditions such as those found in the production of alcohol (beer, wine, liquor).

Glycolysis that occurs under *anaerobic* conditions is called *fermentation*. The ATP produced in this process is used to sustain the cells. Recall, however, that glycolysis also involves the oxidation of glyceraldehyde 3-phosphate to 3-phosphoglycerate, with the electrons being transferred to NAD^+ to produce NADH. This NADH poses a metabolic challenge to the cells. In order for fermentation to continue, NAD^+ must be regenerated from the NADH. If NAD+ is not regenerated, then glycolysis will come to a stop. In aerobic metabolism the NAD+ would be regenerated from the electrons being transferred from NADH to oxygen. In anaerobic conditions a different pathway for transferring the electrons be used to re-oxidize the NADH.

Several metabolic pathways have evolved to accomplish the oxidization of NADH in anaerobic environments. These are diagrammed on the last page of this exercise.

EXERCISE

This portion of the exercise explicitly works to connect with the chemistry you have been learning. Examine the attached diagram and complete the following set of questions. The pathway shown on the far left occurs under *aerobic* conditions. For this pathway:

1. Is pyruvate oxidized or reduced in this reaction? _____
2. What is the fate of the acetyl-CoA produced in this reaction? _____
3. Does this reaction oxidize NADH and regenerate NAD^+? _____
4. How will NADH be oxidized under aerobic conditions? _____

There are two remaining pathways shown in the center and on the right of the diagram. Look first at the pathway in the center of the diagram. In this reaction set, which is performed by yeast under anaerobic conditions, pyruvate is converted to acetaldehyde and then to ethanol. Examine the first reaction: the conversion of pyruvate to acetaldehyde. Fill in the blank to the right of the first arrow with the appropriate compound. Examine the reaction that converts acetaldehyde to ethanol. Fill in the blank with the appropriate compound.

Answer the following:

6. Conversion of pyruvate to acetaldehyde is what general type of reaction?

   A. Dehydrogenation
   B. Condensation
   C. Decarboxylation
   D. Hydrolysis

7. Circle the group within acetaldehyde that is altered in the reaction that converts acetaldehyde to ethanol.

8. Is acetaldehyde OXIDIZED or REDUCED to generate ethanol?
9. Is NAD^+ regenerated in this reaction set? YES NO

Examine the pathway at the far right of the diagram. This reaction occurs in muscles under anaerobic conditions. Fill in the blank next to the arrow with the appropriate compound.

10. Circle the group in pyruvate that is altered in the reaction that converts pyruvate to lactate.
11. Is pyruvate OXIDIZED or REDUCED in this reaction?
12. Is NAD+ regenerated by this reaction? YES NO

13. Is the following statement accurate? Explain.

"Under anaerobic conditions, pyruvate can accept electrons, which results in the oxidation of NADH to regenerate NAD^+."

The challenge questions ask you to apply what you have been learning about glycolysis and fermentation. Each of these questions also asks you to generate a hypothesis and then use data extracted from research papers to determine whether your hypothesis is supported or not. Note that the data does not necessarily answer the questions directly; you will have to do some analysis.

Challenge Question 1

Emperor penguins are among the deepest diving mammals known. They typically dive to 100-200 meters; the record depth is over 500 meters. The lengths of most dives are 5-7 minutes, but some penguins have been observed to stay under for as long as 20 minutes! Remember that penguins are birds and that birds have lungs not gills. Penguins are not 'breathing' underwater. Assume you are a researcher studying the physiology of penguin diving. Generate a hypothesis about which of the two alternative pathways for anaerobic respiration is used by penguins. How might you test this hypothesis?

The data is from:

Ponganis PJ, Kooyman GL, Starke LN, Kooyman CA, and TG Kooyman.1997. Post-dive blood lactate concentrations in Emperor penguins, *Aptendodytes foresteri*. J Exp Biol. 200:1623-1626.

The authors report that the mean value of blood lactate concentration in resting penguins (those that had not been diving) was 1.7±0.71mmoll−1 (mmol per liter).

The data in the graph below show lactate levels for individual penguins (and two seals indicated by the WS symbols) beginning at the point that the blood lactate levels peaked after swimming. This means that the first value shown is the highest lactate concentration reached after a dive for a given penguin.

Note that the authors of this paper are asking a more sophisticated question about the limits of diving in the penguins and so they are presenting their data to best show the answers to their questions. But, you can use the data to decide if your hypothesis is or is not supported.

Does the data presented in the paper support your hypothesis?

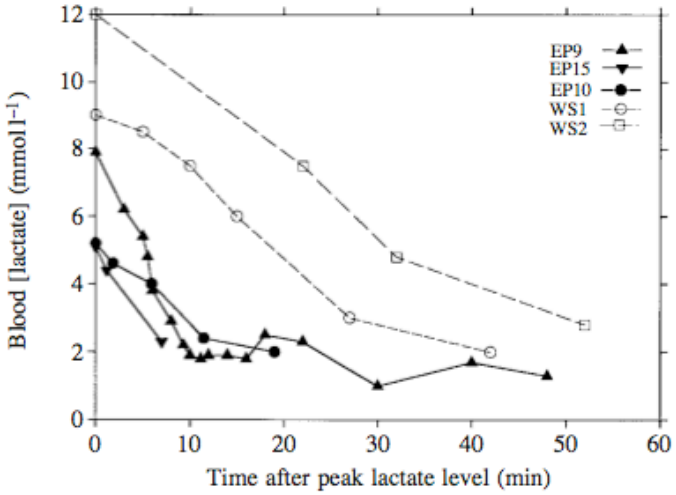


Fig. 1. Decline in blood lactate concentration after the peak post-dive value in three emperor penguins (EP9, EP10, EP15), an adult Weddell seal (WS1) and a juvenile Weddell seal (WS2). The peak value occurred within 5 min post-dive in the seals EP10 and EP15. Although elevated above 5 mmol l^{-1} at 5 and 7 min post-dive, the lactate concentration peaked at 12 min post-dive in EP9. Values were recorded after dive durations of 6.5, 8.1 and 7.7 min of EP9, EP10 and EP15, respectively, after a 43 min dive of WS1 (Kooyman *et al*. 1980) and after a 25 min dive of WS2 (Castellini *et al*. 1988). During these sampling periods, WS1 rested at the surface, WS2 made short dives and the emperor penguins stood on the sea ice. The abscissa represents sample collection time with reference to the time (0 min) at which the peak lactate sample was collected.

Challenge Question 2

As a tumor grows in size, the cells that are most interior in the mass become starved for oxygen – a situation known as hypoxia. This has a limiting effect on the growth of the tumor. One way that a tumor progresses to a more aggressive state is to produce angiogenic factors. These cause blood vessels to infiltrate the tumor and connect it to the blood stream. This not only allows for greater tumor growth (the tumor cells can make more ATP in the presence of oxygen delivered by the red blood cells), it also provides a route for the tumor cells to migrate around the body (a process known as metastasis).

Generate a hypothesis about which of the two anaerobic pathways are used by tumor cells when oxygen is limiting (under hypoxic conditions). How could you test this hypothesis?

Attached is a table taken from S. Walenta, A. Salameh, H. Lyng, J. F. Evensen, M. Mitze, E. K. Rofstad, and W. Mueller-Klieser. 1997. Correlation of high lactate levels in head and neck tumors with incidence of metastasis. Am J Pathol. 150(2): 409–415.

Just from the title of the article, is your hypothesis supported?

It turns out that advanced tumors produce lactate regardless of whether oxygen is abundant or not. The reasons behind this are unclear and are still being investigated. This feature is so unique that lactate levels can be used as a diagnostic feature in staging a tumor. That is to say that normal tissues do not produce lactate when oxygen is present and if tumor cells are producing lactate when oxygen is present then the tumor is at an advanced stage. In this article, the authors make a comparison of tumors of the head and neck that are metastatic with those that are not metastatic.

Note: The data that is pertinent to this question is in the last column of the table.

Summarize the general results shown in the table. Your answer can be in everyday vocabulary and should be only a few sentences long.

The authors indicate in their results section that the difference between the metastatic samples and the nonmetastatic samples is *statistically significant*. From this data, what can you conclude about the nature of glycolysis in metastatic tumors as compared to nonmetastatic tumors?

Table 1. *Patient Statistics*

	Patient	Sex	Age (years)	KI	Tumor site	Regrowth	Survival (months)	Lactate (μmol/g)
Metastatic								mean ± SD
	1	F	70	100	Oral tongue	Yes	D (27)	8.4 ± 3.1
	2	F	58	100	Supraglottis	No	A (28)	10.8 ± 4.3
	3*	M	69	100	Floor of mouth	Yes	D (18)	14.2 ± 5.4
	4	F	63	90	Soft palate	Yes	D (22)	17.7 ± 8.5
	5*	M	54	100	Tongue base	No	A (34)	10.8 ± 6.7
	6	M	67	100	Nasopharynx	Yes	A (30)	12.1 ± 5.7
Total (mean ± SD)								12.3† ± 3.3
Nonmetastatic								
	7*	M	60	90	Floor of mouth	No	D (38)	2.4 ± 2.0
	8	M	58	100	Tongue base	No	A (39)	6.2 ± 3.8
	9	M	72	100	Tongue base	No	A (39)	4.5 ± 2.6
	10	M	68	100	Supraglottis	No	A (41)	6.2 ± 3.7
	11	F	65	90	Tongue base	No	A (42)	5.3 ± 3.9
	12*	M	51	100	Nasopharynx	No	A (40)	3.6 ± 2.5
Total (mean ± SD)								4.7† ± 1.5

M, male; F, female; KI, Karnofsky index; A (n), alive after n months; D (n), died after n months. For regrowth, a partial response is scored as yes and a complete response is no.

*Alcoholic disease.

†$P < 0.005$; Mann-Whitney-test.

For Faculty

What does this exercise do?

Principles Emphasized

1. In order for catabolic reactions to continue, a supply of NAD+ must be available.
2. Even universal pathways can have different outcomes, depending on the environment in which the organism evolved.
3. Pathways within organisms are responsive to environmental stimuli.

Connection with Previous Knowledge.

Students are extremely likely to be familiar with the process of fermentation from their life experiences. If the video is shown (see below), the connection of fermentation and food is even further emphasized. Students are also likely to recognize other anaerobic conditions (bottom of a pond and extended diving in mammals). Many students are attracted to penguins and will be able to relate to the questions relating to their metabolism during dives. Similarly, some students will have an interest in or experience with tumor biology.

Transfer to New Contexts.

Depending on content covered previously in the course, students will have encountered the ideas of oxidation and reduction. This is an opportunity to use those concepts in a new context. This exercise, if used between discussing glycolysis and the citric acid cycle, will also introduce students to decarboxylation reactions.

Skills Development

Students have the chance to practice interpreting diagrams of chemical reactions. If the option is taken (see below), students are also asked to apply their knowledge to a real-life scenario, generate a hypothesis and determine if their hypothesis is supported by a real data set.

How to use this exercise

Students can be given the first portion of the exercise either in-class or as a homework assignment. If given as an in-class assignment, 10-12 minutes should be allowed for students to complete the exercise working alone. If an emphasis has been placed on recognizing the chemistry of glycolysis and the citric acid cycle, students should be able to complete this assignment on their own. Indeed, this assignment can be used as a formative assessment tool; if students have trouble completing the exercise, they need to continue working with the concepts. It is important to debrief students at the end of this first section before doing the challenge exercises (if that option is taken). This immediate feedback is necessary to correct any misunderstandings before moving to the next portion.

The second portion of the exercise uses the challenge questions. The intent is to allow students: 1) to connect with previous knowledge (about penguins and tumors respectively); and to practice hypothesis generation, interpretation of data and drawing conclusions. These could be given as homework or done in class.

Guidance/comments for the exercise

Many but not all introductory cellular/molecular texts include a discussion of fermentation. The introductory comments for this exercise should be sufficient to allow completion of this exercise even if the text does not cover these concepts.

This first portion of the exercise is intended to be used when an emphasis has been placed on understanding and recognizing the chemistry of glycolysis and the citric acid cycle (e.g., decarboxylation, phosphorylation, oxidation, etc.). Students that have not previously been asked to recognize a decarboxylation or oxidation reaction are likely to have difficulty with this assignment.

The second portion of the exercise could be used on its own if the proper content has been presented previously. Students could complete this in class or as homework. This may be an appropriate approach in an upper level course.

During the time that students are working, faculty and/or teaching assistants should circulate. A major goal is to see where students are getting stuck. If there is a common misunderstanding, this can then be addressed immediately. Circulating in this manner also helps faculty better understand the students' strategies for completing the assignment. When helping students, the goal should be to direct their attention to a feature (such as how many carbons are in each compound or what are the differences between the reactant and product?) rather than providing an answer directly.

The entire exercise could be used as an alternative to a lecture on this material. An outline for how that might be done is:

- Ask students what the list of items has in common.
- Watch the video on fermentation in food (link is below).
- Complete the portion of the worksheet on the possible fates for pyruvate. Faculty may wish to model how to approach the exercise by talking through their approach of the pyruvate to acetyl-CoA series. Students would then work the two pathways to the left.
- Model for students how to generate a hypothesis and look for evidence by asking them which of the two anaerobic processes they believe is happening in the baking of bread and the fermentation of ginger ale (examples given in video). Then ask for one piece of data from the video that supports their hypothesis (answer = giving off CO_2 gas causing the bread to rise and the pressure to increase in the bottle of ginger ale). You could also extend this to beer and wine making with the piece of evidence being that alcoholic fermentation is happening because the product is ethanol.
- Let students work the challenge questions. Students could chose which topic they want to work with (both the tumor and penguin examples deal with production of lactate).
- When the students have finished working their exercise they should turn to their neighbor and discuss their work (it won't matter if their neighbor did the same or different topic).
- Debrief both the penguin and tumor scenarios with the entire class. Place an emphasis on data interpretation. Note on the graph provided in the penguin example that the WP points are not for Emperor penguins. Be sure to identify the axes on the graph and discuss the quick decrease in lactate in the first 10 minute followed by a leveling off in the remaining time. You could also discuss the individual variability in the data.

- End class with a brief discussion of the facts that production of lactate is only a temporary solution to anaerobic conditions in mammals since its production disrupts the acid/base balance in tissues. You may want to incorporate the following facts: 1) humans too produce lactate during exercise; the lactate is sent to the blood stream and this must be metabolized by the liver (this is the Cori cycle); 2) part of the way that tumors are located is by providing a radioactive sugar compound and asking where the radioactivity shows up in a PET scan – tumors are one of the major tissues using the sugar and so they light up readily.

Options for grabbing student attention and/or providing multiple sensory cues.
This video explains fermentation in the context of food. The first half of the video is sufficient for the explanation. The second half shows how to make ginger ale. It is a bit kitschy but fun. http://www.youtube.com/watch?v=tNqfPsVAdYk. Of course you can serve ginger ale as students come to class.

The introduction to this second video provides some facts on Emperor penguin diving (the remainder of the video discusses the speaker's unusual path to research in biology). http://www.youtube.com/watch?v=VPUetsnURVk A few pictures of diving penguins are at the Cool Antartica website. http://www.coolantarctica.com/Antarctica%20fact%20file/wildlife/Emperor_penguin_pictures.htm.

Answers.
The following modifications should be made to the diagram. A CO_2 should be placed in the blank to the right of the arrow from pyruvate to acetylaldehyde. NADH to NAD^+ should be placed in the blanks to the right of the arrow from acetylaldehyde to ethanol. An NADH to NAD^+ should also be place to the right of the arrow from pyruvate to lactate.

1. Pyruvate dehydrogenase

2. Oxidized

3. Goes to citric acid cycle

4. No

5. NADH will be oxidized by passing its electrons into the electron transfer chain and finally onto oxygen.

6. C. Decarboxylation

7. The ketone group is reduced to a hydroxyl group

8. Reduced

9. Yes

10. The ketone group is reduced to form a hydroxyl

11. Reduced

12. Yes

13. NAD^+ is regenerated under aerobic conditions by passing electrons in to the electron transport chain and then to oxygen.

14. Ethanol, carbon dioxide, lactate

15. The statement is accurate but incomplete. Pyruvate directly accepts electrons (is reduced) only in the case of its conversion to lactate.

Penguin Scenario

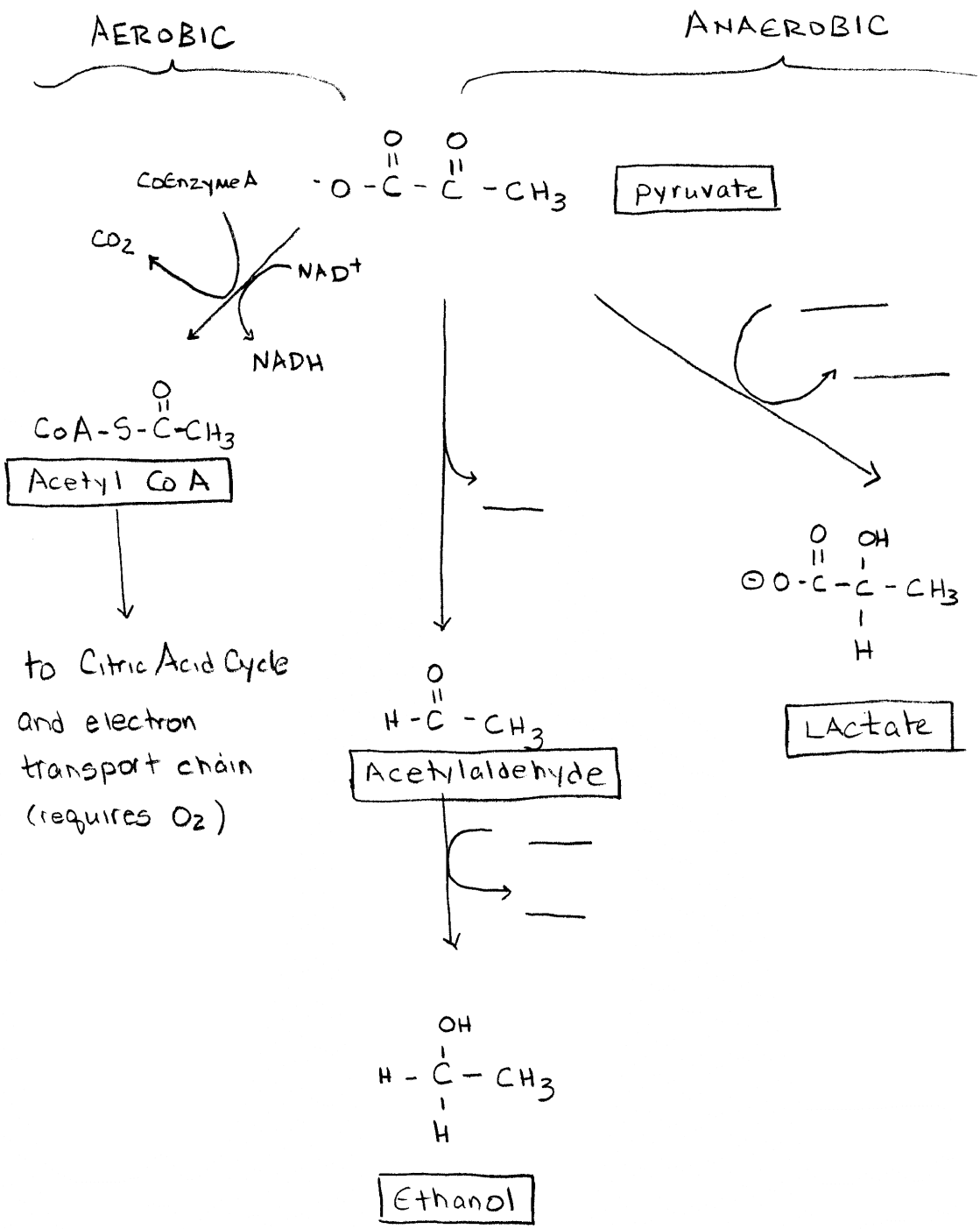
Possible hypothesis: Penguins will convert pyruvate to lactate while they are diving and are not breathing. Testing the hypothesis would involve measuring lactate in their bodies before and after diving.

The data in the statement from the authors and the graph indicate that lactate levels increase in the penguins during their dives. This suggests that glycolysis is following the lactate pathway to regenerate NAD+ when oxygen is not available (has been used up).

Tumor Scenario

Possible hypothesis: Tumors produce lactate under anaerobic conditions. This could be tested by measuring the lactate present in metastatic tumor (or in the blood) and comparing it with the lactate levels in persons nonmetastatic tumors.

It is clear from the last column in the table that lactate is produced by tumors; and the amount produced by metastatic tumors is higher than that produced by nonmetastatic tumors.


AEROBIC
ANAEROBIC
Coenzyme A
CO_2
NAD^+
NADH
pyruvate
$CoA-S-C-CH_3$
Acetyl Co A
to Citric Acid Cycle
and electron
transport chain
(requires O_2)
$H-C-CH_3$
Acetylaldehyde
Ethanol
Lactate