

A Case of Poisoned Firefighters

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Introduction

This activity was developed for an introductory biology course for science majors. The activity is intended to reinforce the basic concepts of cellular respiration (including the electron transport chain and generation of the proton motive force) and to link O₂ transport explicitly to cellular respiration. Students are initially engaged by considering the cases of firefighters exposed to smoke that could contain carbon monoxide (CO) or hydrogen cyanide (HCN).

Learning Objectives

After completing this activity, students will be able to

- Describe how the proton motive force across the inner mitochondrial membrane is generated
- Apply a description of an inhibitor's activity to predict its effect on cellular respiration (both in terms of O₂ consumption and ATP production)
- Explain the basis for the physiological effects of HCN and CO poisoning
- Analyze patient data to determine whether that patient was exposed to HCN or CO
- Describe some of the factors that influence the O₂ carrying capacity of hemoglobin

Context of this Activity

The course for which this activity was initially designed is a large introductory biology course for science majors. Each week there are two 50-minute lectures (75-135 students in each of two lecture sections) and a 75-minute workshop, led by trained peer facilitators (5-25 students in each of 14 workshop sections). The workshop sessions are intended to reinforce lecture material by having students apply the material to engaging situations.

In our experience, students traditionally struggle to understand cellular respiration, and we wished to provide an opportunity for students to engage with and fully understand this challenging topic. Additionally, as cellular respiration is typically covered early in an introductory course, while the circulatory and respiratory systems are typically covered later in an introductory course, students often fail to remember the significance of O₂ when its transport is discussed later in the course. Thus, we also wished to more explicitly link O₂ consumption in aerobic respiration with O₂ transport in red blood cells.

In order to accomplish these goals (better understanding of cellular respiration and explicit linkage of O₂ transport and aerobic respiration), the following lecture/workshop schedule was followed (see Table 1 for the precise schedule):

- Two full lectures on cellular respiration (aerobic & anaerobic and an introduction to fermentation)
- These lectures were followed by a 75-minute workshop that had an opportunity for a general review of the lecture material (aerobic respiration, anaerobic respiration and fermentation), followed by a microbial bioremediation/anaerobic respiration case
- Then students attended a lecture that finished up fermentation, and introduced O₂ transport in the circulatory system
- Their next workshop used this firefighter case to reinforce aerobic respiration, fermentation and O₂ transport

Table 1: Sequencing of Lecture and Workshop Activities Related to Cellular Respiration, Fermentation and Oxygen Transport. (The activities that directly support cellular respiration and oxygen transport are shown in bold.)

<i>Week</i>	<i>Workshop Topic (Mon./Tues.)</i>	<i>1st Lecture (Wed.)</i>	<i>2nd Lecture (Fri.)</i>
3	Fad Diets & Media Assessment	Digestive System & Digestive Enzymes	Cellular Respiration-1
4	Glucose Homeostasis & Diabetes	First Exam	Cellular Respiration-2
5	Microbial Bioremediation (anaerobic respiration)	Cellular Respiration-3 & O₂ transport	Photosynthesis-1
6	Firefighters (aerobic respiration, fermentation & O₂ transport)	Photosynthesis-2	Transport in Plants-1

Management Notes

A. Preparatory Assignment

As we wished students to be fully prepared to productively work through the workshop activity, we assigned a pre-workshop preparatory assignment. We posted this assignment on the course website approximately one week before it was due in the workshop session. The preparatory assignment involved some reading from the course textbook (Sadava et al., Life 8E), and some questions to answer about the reading. In order to ensure maximum completion rates and effective participation in the workshop activities we decided to assign workshop points based on completion of the preparatory assignment. However, it is certainly possible to assign the prep assignment without having any formal points tied to it.

B. Workshop Activity

We prepared a handout for the students to work through during the workshop session. The handout reiterates some of the material from lecture, but also adds in more specific information regarding the specific “problem” used to engage the students (CO and HCN poisoning). While this handout is somewhat lengthy, it is also intended to provide students with a take-home “study guide” related to this material.

The students are initially introduced to the problem of distinguishing between CO and HCN poisoning from smoke inhalation associated with fires. They then carry out a “think-pair-share” activity to review some of the key features of oxygen transport by hemoglobin.

Students continue to work through the handout, and are introduced to different aspects of the foundational and background material, with opportunities for reflection as they work through the material. This stage of the workshop is intended to ensure that students have a solid understanding of the foundational material related to this process.

In order to provide a more active learning experience, students get up and use whiteboards (or different spaces on the chalkboard, or paper easels) to draw out the electron transport chain, the proton motive force and ATP synthase. By having students draw out diagrams on a large space, it is easier for facilitators &/or instructors to walk around the room and review students’ work. In our workshops, we have up to 5 groups of 4-5 students each, and 5 large whiteboards spread throughout the room. In a larger class, students could work in groups of four on pieces of paper that facilitators can see as they move throughout the room. Large pieces of easel paper could be taped to the walls throughout the room to provide work spaces for different groups.

Once students have drawn the normal functioning of the electron transport chain, the proton motive force and ATP synthase, they are given a description of an inhibitor of cellular respiration. They are then asked to re-draw their diagram to show what would happen after application of the inhibitor. We used three inhibitors, representing two mechanisms of inhibition. This creates some overlap within the class, so that different groups can compare answers, and also makes it easier for the facilitator to quickly check each group’s work. We made several copies of the description of each inhibitor, and handed out one description to each group in the class. Again, as they work on their diagrams, the facilitator circulates throughout the room and makes suggestions as necessary. As we wished to have full participation in this particular activity, we decided to assign points strictly for participation in/engagement with the group activity.

The final activity of this workshop is to hand out paired patient data to each group of four students. The students will use this data and provided normal reference values to determine what substance each patient was exposed to. We provide three pairs of patient data, again giving some overlap and making it easier for the facilitator to provide guidance. Within each pair of patients, one has been exposed to HCN, and one has been exposed to CO. Students are given the results of several tests and observations, and asked to determine which patient has the HCN exposure and which patient has the

CO exposure. After each group has made that determination, the groups are rearranged, such that half the members of each group take the HCN patient and half the students in each group take the CO patient. Then all the HCN students get together and all the CO students get together (in a large class you could assign this such that four HCN students get together and four CO students get together to create multiple HCN and CO groups). In these exposure-specific groups, students compare notes and try to determine what the specific features of each exposure are. They are given a one-page worksheet to carry out this final activity.

C. Grading

We implemented the following grading scheme:

- Preparatory assignment (5 points for completion, with problematic answers highlighted so that students can follow-up on these with the course instructor at a later date)
- Diagrams of the electron transport chain, proton motive force and ATP synthase activity pre- and post-exposure to an inhibitor (5 points for participation)
- Final worksheet (12 points for the key features of HCN vs. CO poisoning; 3 points for reflection about what is still confusing about cellular respiration at this point)

Total: 25 points

(In our course, there are between 13 and 15 workshops, each worth between 15 and 25 points, with the workshops worth between 20 and 30% of the final overall grade, depending on the semester.)

Supplemental Materials

Each supplemental handout is provided below (each has a title and is separated by hard page breaks)

- Preparatory assignment (1 page, which will need to be edited for the specific textbook used in the class)
- Student workshop handout (5 pages, with some embedded suggestions for modifications. This will need to be edited before using)
- Individual inhibitor descriptions (1 page that can be copied and cut into thirds to generate individual inhibitor handouts)
- Paired patient data (1 page that can be copied and cut into thirds to generate paired patient handouts)
- Final student worksheet (1 page)
- Student handout answer key with references (6 pages)
- Inhibitor answer keys (3 pages)
- Patient answer keys (2 pages)
- Worksheet answer key (2 pages)

Prep Assignment

This prep assignment will be **handed in** at the **start** of your workshop, and will contribute **5 points** towards your score for this week's workshop. So please be sure to start your assignment early, to leave yourself enough time to complete your assignment (and ask any questions that may come up as you work on your assignment).

Readings

(the textbook readings here are from Sadava et al. Life, the Science of Biology, 8E. Similar readings can likely be found in any introductory biology textbook)

From your textbook:

- Chapter 7 (cellular respiration)
- Pages 1025-1026 (diffusion is driven by concentration differences)
- Section 48.4 (how does blood transport respiratory gases?)

An animation showing the activity of the ETC, available at the URL below.

<http://vcell.ndsu.nodak.edu/animations/etc/movie.htm>

The questions (your answers are to be handed in)

1. What is the function of cytochrome oxidase?
2. How do we measure/express the concentration of a gas (such as O₂)?
3. Which part of a hemoglobin molecule actually binds O₂, and what metal atom is contained within this group?
4. a. When oxygenated hemoglobin reaches a capillary bed in a tissue, what happens to the amount of O₂ bound by hemoglobin?

b. If this tissue has a somewhat low pH, is there any difference in the amount of O₂ bound by hemoglobin compared to in a tissue with a slightly higher pH? (Hint: look at the pH 7.2 human hemoglobin and pH 7.4 human hemoglobin curves in figure 48.13- look at the % O₂ binding for each at the P_{O₂} of a tissue)
5. In a very active tissue (e.g. a muscle during an anaerobic sprint) what cellular process have we talked about that contributes to a reduction in pH?

A Case of Poisoned Firefighters

Rhode Island has had several devastating fires that have attracted attention in the past several years. The Station nightclub fire in West Warwick killed 96 people on the night of February 20, 2003. Another four people later died of their injuries, bringing the death toll to 100, making this the fourth deadliest nightclub fire in U.S. history. In March of 2006, 28 Providence firefighters were admitted to hospitals after fighting three fires in local residences and businesses. None had suffered burns. Instead, they had a variety of symptoms consistent with problems with the nervous system and cardiac muscle, including headache, dizziness, heart attack and apparent confusion.

These fires highlight the danger of smoke inhalation as a substantial risk of exposure to fires. While carbon monoxide (CO) has long been known to be produced during combustion of a variety of materials, the burning of modern plastics (as well as several natural products) produces hydrogen cyanide (HCN). Thus household and business fires can expose unprotected firefighters to both CO and HCN, and it is imperative to distinguish between these exposures in order to provide adequate treatment.

One of the challenges in making the correct diagnosis is that many of the symptoms of each are somewhat nonspecific and overlapping (see Table 1).

Table 1: Selected Symptoms of CO & HCN Poisoning

<i>Selected Symptoms of CO Poisoning</i>	<i>Selected Symptoms of HCN Poisoning</i>
Headache	Headache
Shortness of breath	Respiratory depression
Dizziness	Vertigo
Agitation	Anxiety

Mechanisms-Hemoglobin

CO exerts its primary effects by binding very tightly (with high affinity) to hemoglobin. Hemoglobin is a protein that carries O₂ in red blood cells. Hemoglobin (Hb) binds O₂ reversibly, and releases O₂ (e.g. at active tissues) depending on (among other factors) the P_{O₂}. One of the other factors that influences the ability of Hb to release O₂ is pH. Hydrogen ions can bind to Hb and cause a decrease in the affinity of Hb for O₂. Thus, at low pH, the “unloading” of O₂ by Hb is facilitated.

Think-Pair-Share

Try to answer each of the following questions on your own. Then compare your answers to those of a neighbor, then compare with the entire group.

1. Think back to what you know about hemoglobin.
 - a. What type of protein structure does hemoglobin have? (be sure you can explain your answer)

 - b. Which energy-conversion pathway contributes to a reduction of pH in active tissues? Be sure that you can explain why this pathway contributes to a reduction of pH at active tissues.

c. Why would an iron-deficient diet result in a reduced capacity for O₂ transport in red blood cells? (Hint: think about the structure of hemoglobin and how it binds O₂)

Mechanisms-CO

There are many possible sources of exposure to CO, including cigarette smoke. CO is able to bind hemoglobin tightly enough to displace O₂ and reduce the O₂-carrying capacity of hemoglobin. This means that insufficient O₂ can be delivered to cells.

Note: If desired, a diagram of a heme group with CO versus O₂ bound could be inserted here, or displayed in the classroom.

Think-Pair-Share

2. In the absence of O₂, can cells continue to carry out aerobic respiration?
 3. What pathway can be carried out (in humans) in the absence of O₂?
 4. Based on what you have learned so far, what is one possible reason that heavy smokers are more likely to have headaches and are less able to carry out vigorous exercise?
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Mechanisms- HCN

HCN exerts its effects by acting as an inhibitor of the final subunit of the electron transport chain in the inner mitochondrial membrane (*i.e.* it is an inhibitor of cytochrome oxidase). Thus, HCN does not directly interfere with the ability of hemoglobin to carry O₂ in the blood, but does block electrons from being transferred to the final electron acceptor of our ETC (O₂). This means that O₂ is not being reduced to H₂O, and remains in its oxidized form, even in active body tissues. In fact, because cells are not “consuming” O₂, the concentration gradient (P_{O₂}) between cells and arterial blood is essentially eliminated, such that hemoglobin retains its bound O₂.

While it is possible to carry out blood tests to measure the level of cyanide, many hospitals are not able to carry out this test in their own laboratories, which means that physicians must be able to make a probable diagnosis in the absence of a specific test for cyanide.

Note: If desired, a diagram of the electron transport chain (showing cytochrome oxidase and its position) may be inserted here, or shown in the classroom. We used Figure 7.13 from Sadava et al. Life, The Science of Biology 8E (Sinauer/WH Freeman Publishers)

Activity

Before moving on to look at how HCN & CO lead to somewhat different effects on cellular respiration and O₂ transport, let's briefly review the ETC (including the generation of the PMF, chemiosmosis and the role of the final electron acceptor).

5. On your table's whiteboard, draw a representation of the inner mitochondrial membrane. Label the space above the membrane as the intermembrane space (IMS), and the space below the membrane as the mitochondrial matrix.

a. Work as a group to show the flow of electrons (indicate the donor and acceptor) along the ETC. Also show the movement of protons, and the PMF.

b. Now get a handout from your instructor. The handout will give you a brief description of a particular inhibitor. You will need to use the information provided to re-draw your diagram on your whiteboard (*i.e.* Is there an impact on the PMF? Will O₂ continue to be reduced?), and to predict the effect on ATP synthesis. Be prepared to share the information about your inhibitor and your predictions with the entire group.

Diagnosis

Physicians can carry out several rapid tests and observations in the hospital, the results of which can be used to distinguish between CO and HCN poisoning. Some of these tests include

- carboxyhemoglobin levels (the proportion of hemoglobin bound to CO)
 - oxyhemoglobin levels in arterial blood and venous blood (the proportion of hemoglobin bound to O₂)
 - skin color (cherry red, normal or pale)
 - lactic acid levels and blood pH
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Think-Pair-Share

6. What do you predict the effect of CO poisoning to be on aerobic cellular respiration? Explain your answer.

7. What is your prediction about the effect of HCN poisoning on aerobic cellular respiration? Explain your answer.

8. Why would elevated lactic acid levels be an indicator that there is a problem with aerobic cellular respiration?

Treatments

CO poisoning can be treated with hyperbaric O₂ (exposing patients to high levels of O₂ at elevated pressures).

HCN poisoning can be treated with a family of chemicals (nitrites) that bind to hemoglobin, creating a form of hemoglobin known as methemoglobin. Methemoglobin binds to HCN even tighter than cytochrome oxidase. In the presence of methemoglobin, HCN will preferentially bind to methemoglobin, rather than cytochrome oxidase. One factor to keep in mind however, is that methemoglobin cannot bind to O₂.

Think-Pair-Share

9. If a firefighter comes in to the ER with a headache, confusion and other neurological symptoms (following smoke exposure), what are some considerations in choosing a treatment?

a. Review the two possible exposures you have learned about so far, and how each interferes with cellular respiration.

b. Why does hyperbaric oxygen have the potential to treat CO poisoning?

c. Do you predict hyperbaric oxygen to be helpful, harmful or “neutral” in cases of HCN poisoning? (explain your answer)

d. What do you predict to happen if a patient with CO poisoning was given nitrites (to induce methemoglobin formation)? Explain your answer.

Patient Data

Your table will be given two cases: one of CO poisoning and one of HCN poisoning. You will work as a team to distinguish between the two cases, based on what you have learned so far. After you have assigned a diagnosis to your two cases, the groups will re-form, so that all the HCN cases will be grouped together and all the CO cases will be grouped together. You will work in these groups to try and determine what each form of poisoning has in common.

Table 2: Normal reference values for indicators of aerobic respiration

	Normal Range
Blood pH	7.35-7.45
Blood (plasma) lactate levels	0.5-2.2mM
Carboxyhemoglobin (non-smokers)	0-2.3%
Carboxyhemoglobin (smokers)	2-15%
Oxyhemoglobin (Arterial)	84-97%
Oxyhemoglobin (Venous)	55-75%
Skin tone (face and lips)	Uniform “flesh” color

Inhibitor Information

1. CCCP (carbonyl cyanide *m*-chlorophenylhydrazine)

This inhibitor (which is known as an “uncoupler”) acts by providing an alternate (& effective) passageway for protons to move through the inner mitochondrial membrane (down their concentration gradient), thereby circumventing their passage through ATP synthase.

Use the information provided to draw the “after” version of your diagram of the PMF, and to predict the impact on ATP production. You will need to indicate the following on your “after” diagram:

- what the PMF will look like (draw the distribution of H⁺ predicted after exposure of cells to this inhibitor)
- the relative amount of ATP production (compared to your diagram in the absence of inhibitor)
- where the inhibitor is acting (e.g. draw an arrow)

2. HCN (hydrogen cyanide)

HCN acts to prevent the transfer of electrons from cytochrome oxidase (in the inner mitochondrial membrane) to O₂.

Use the information provided to draw the “after” version of your diagram of the PMF, and to predict the impact on ATP production. You will need to indicate the following on your “after” diagram:

- what the PMF will look like (draw the distribution of H⁺ predicted after exposure of cells to this inhibitor)
- the relative amount of ATP production (compared to your diagram in the absence of inhibitor)
- where the inhibitor is acting (e.g. draw an arrow)

3. DNP (2,4-p-dinitrophenol)

DNP acts as an “uncoupler”. It provides an alternate (& effective) passageway for protons to move through the inner mitochondrial membrane (down their concentration gradient), thereby circumventing their passage through ATP synthase.

Use the information provided to draw the “after” version of your diagram of the PMF, and to predict the impact on ATP production. You will need to indicate the following on your “after” diagram:

- what the PMF will look like (draw the distribution of H⁺ predicted after exposure of cells to this inhibitor)
- the relative amount of ATP production (compared to your diagram in the absence of inhibitor)
- where the inhibitor is acting (e.g. draw an arrow)

Patient Data

Patients A & B

One of these patients (a firefighter) was exposed to high levels of HCN in an industrial fire, while the other was exposed to high levels of CO in a residential fire. Neither of these patients is a smoker.

	Patient A	Patient B	Normal Range
Blood pH	7.1	7.25	7.35-7.45
Blood (plasma) lactate levels	8 mM	2.7 mM	0.5-2.2mM
Carboxyhemoglobin	1.4%	9%	0-2.3%
Oxyhemoglobin (Arterial)	99%	45%	84-97%
Oxyhemoglobin (Venous)	89%	37%	55-75%
Skin tone (face and lips)	Bright cherry red	Normal appearing	Uniform "flesh" color

Patients C & D

One of these patients (a non-smoking college student) was exposed to high levels of CO due to a defective gas furnace in their home. The other patient (also a non-smoker) was exposed to high levels of HCN in a suicide-attempt "inspired" by the 1978 Jonestown incident (in which nearly 1000 people died in a murder-suicide attempt based on consumption of a cyanide-laced beverage).

	Patient C	Patient D	Normal Range
Blood pH	7.1	7.25	7.35-7.45
Blood (plasma) lactate levels	7.5 mM	2.5 mM	0.5-2.2mM
Carboxyhemoglobin	2.1%	8%	0-2.3%
Oxyhemoglobin (Arterial)	98%	52%	84-97%
Oxyhemoglobin (Venous)	89%	44%	55-75%
Skin tone (face and lips)	Slightly pink (in lips)	Pale	Uniform "flesh" color

Patients E & F

One of these patients had an involuntary intake of HCN, through the consumption of cassava and an unproven cancer treatment (laetrile). The other patient attempted suicide by sitting in their running car in an enclosed garage (thereby exposing themselves to elevated levels of CO). Neither patient has a history of smoking.

	Patient E	Patient F	Normal Range
Blood pH	7.0	7.3	7.35-7.45
Blood (plasma) lactate levels	5 mM	5 mM	0.5-2.2mM
Carboxyhemoglobin	2.2%	9%	0-2.3%
Oxyhemoglobin (Arterial)	98%	45%	84-97%
Oxyhemoglobin (Venous)	91%	35%	55-75%
Skin tone (face and lips)	Normal appearance	Normal appearance	Uniform "flesh" color

Worksheet: A Case of Poisoned Firefighters

Name: _____

1. Based on comparing other patients with the same exposure (e.g. other HCN patients or other CO patients), what generalizations can you make about each test/observation? In the table below, don't put in specific numbers, instead draw a general conclusion, e.g. "all cases had a slightly basic pH" or "there was not a clear pattern of abnormal pH with this exposure". (12 points)

Circle the exposure you looked at with new people:

	HCN	CO
Test/Observation	General Conclusion	
Blood pH		
Blood (plasma) lactate levels		
Carboxyhemoglobin		
Oxyhemoglobin (Arterial)		
Oxyhemoglobin (Venous)		
Skin tone (face & lips)		

2. You have now spent quite a bit of time in lecture and workshop thinking about cellular respiration. What is your "muddiest point" about cellular respiration, and what are you going to do to get this point cleared up? (3 points)

A Case of Poisoned Firefighters ***ANSWER KEY FOR THE STUDENT HANDOUT***

Rhode Island has had several devastating fires that have attracted attention in the past several years. The Station nightclub fire in West Warwick killed 96 people on the night of February 20, 2003. Another four people later died of their injuries, bringing the death toll to 100, making this the fourth deadliest nightclub fire in U.S. history. In March of 2006, 28 Providence firefighters were admitted to hospitals after fighting three fires in local residences and businesses. None had suffered burns. Instead, they had a variety of symptoms consistent with problems with the nervous system and cardiac muscle, including headache, dizziness, heart attack and apparent confusion.

These fires highlight the danger of smoke inhalation as a substantial risk of exposure to fires. While carbon monoxide (CO) has long been known to be produced during combustion of a variety of materials, the burning of modern plastics (as well as several natural products) produces hydrogen cyanide (HCN). Thus household and business fires can expose unprotected firefighters to both CO and HCN, and it is imperative to distinguish between these exposures in order to provide adequate treatment.

One of the challenges in making the correct diagnosis is that many of the symptoms of each are somewhat nonspecific and overlapping (see Table 1).

Table 1: Selected Symptoms of CO & HCN Poisoning

<i>Selected Symptoms of CO Poisoning</i>	<i>Selected Symptoms of HCN Poisoning</i>
Headache	Headache
Shortness of breath	Respiratory depression
Dizziness	Vertigo
Agitation	Anxiety

Mechanisms-Hemoglobin

CO exerts its primary effects by binding very tightly (with high affinity) to hemoglobin. Hemoglobin is a protein that carries O₂ in red blood cells. Hemoglobin (Hb) binds O₂ reversibly, and releases O₂ (e.g. at active tissues) depending on (among other factors) the P_{O₂}. One of the other factors that influences the ability of Hb to release O₂ is pH. Hydrogen ions can bind to Hb and cause a decrease in the affinity of Hb for O₂. Thus, at low pH, the “unloading” of O₂ by Hb is facilitated.

Think-Pair-Share (10 minutes)

Try to answer each of the following questions on your own. Then compare your answers to those of a neighbor, then compare with the entire group.

1. Think back to what you know about hemoglobin.
 - a. What type of protein structure does hemoglobin have? (be sure you can explain your answer)

Quaternary (it has four chains)

b. Which energy-conversion pathway contributes to a reduction of pH in active tissues? Be sure that you can explain why this pathway contributes to a reduction of pH at active tissues.

Lactic acid fermentation (lactic acid is produced by the reduction of pyruvate, and lactic acid is an acid, therefore reduces the pH)

c. Why would an iron-deficient diet result in a reduced capacity for O₂ transport in red blood cells? (Hint: think about the structure of hemoglobin and how it binds O₂)

Heme groups on Hb bind O₂, and these heme groups contain an iron atom. So lack of iron would lead to an inability to make hemoglobin, and therefore an inability to transport O₂. (this is known as iron-deficient anemia)

Mechanisms-CO

There are many possible sources of exposure to CO, including cigarette smoke. CO is able to bind hemoglobin tightly enough to displace O₂ and reduce the O₂-carrying capacity of hemoglobin. This means that insufficient O₂ can be delivered to cells.

Note: If desired, a diagram of a heme group with CO versus O₂ bound could be inserted here, or displayed in the classroom.

Think-Pair-Share

2. In the absence of O₂, can cells continue to carry out aerobic respiration?

No, because there is no final electron acceptor.

3. What pathway can be carried out (in humans) in the absence of O₂?

Lactic acid fermentation

4. Based on what you have learned so far, what is one possible reason that heavy smokers are more likely to have headaches and are less able to carry out vigorous exercise?

Heavy smokers may have higher exposures to CO, and CO exposure can cause headaches (see Table 1), and with a reduction in the O₂-carrying capacity of blood, it is less likely that a heavy smoker will be able to deliver enough oxygen to muscles during exercise. (There are, of course, other aspects of smoking that contribute to both of these observations!)

Mechanisms- HCN

HCN exerts its effects by acting as an inhibitor of the final subunit of the electron transport chain in the inner mitochondrial membrane (*i.e.* it is an inhibitor of cytochrome oxidase). Thus, HCN does not directly interfere with the ability of hemoglobin to carry O₂ in the blood, but does block electrons from being transferred to the final electron acceptor of our ETC (O₂). This means that

O₂ is not being reduced to H₂O, and remains in its oxidized form, even in active body tissues. In fact, because cells are not “consuming” O₂, the concentration gradient (P_{O₂}) between cells and arterial blood is essentially eliminated, such that hemoglobin retains its bound O₂.

While it is possible to carry out blood tests to measure the level of cyanide, many hospitals are not able to carry out this test in their own laboratories, which means that physicians must be able to make a probable diagnosis in the absence of a specific test for cyanide.

Activity (15-20 minutes) (Depending on your grading scheme, you may need to make a note of who is present for this activity, if you choose to award points for being present and participating at this point)

Before moving on to look at how HCN & CO lead to somewhat different effects on cellular respiration and O₂ transport, let’s briefly review the ETC (including the generation of the PMF, chemiosmosis and the role of the final electron acceptor).

5. On your table’s whiteboard, draw a representation of the inner mitochondrial membrane. Label the space above the membrane as the intermembrane space (IMS), and the space below the membrane as the mitochondrial matrix.

a. Work as a group to show the flow of electrons (indicate the donor and acceptor) along the ETC. Also show the movement of protons, and the PMF.

b. Now get a handout from your instructor. The handout will give you a brief description of a particular inhibitor. You will need to use the information provided to re-draw your diagram on your whiteboard (*i.e.* is there an impact on the PMF? Is there an impact on O₂ reduction?), and to predict the effect on ATP synthesis. Be prepared to share the information about your inhibitor and your predictions with the entire group.

Diagnosis

Physicians can carry out several rapid tests and observations in the hospital, the results of which can be used to distinguish between CO and HCN poisoning. Some of these tests include

- carboxyhemoglobin levels (the proportion of hemoglobin bound to CO)
- oxyhemoglobin levels in arterial blood and venous blood (the proportion of hemoglobin bound to O₂)
- skin color (cherry red, normal or pale)
- lactic acid levels and blood pH

Think-Pair-Share (10 minutes)

6. What do you predict the effect of CO poisoning to be on aerobic cellular respiration? Explain your answer.

It will stop aerobic respiration b/c no O₂ will be delivered to cells, so there is no final electron acceptor.

7. What is your prediction about the effect of HCN poisoning on aerobic cellular respiration? Explain your answer.

It will also inhibit aerobic respiration, as there is no way (in the presence of HCN) to deliver the electrons to O_2 (so O_2 is present, but its not having electrons passed to it). The electron transport chain will “back up” and cease to function.

8. Why would elevated lactic acid levels be an indicator that there is a problem with aerobic cellular respiration?

Because lactic acid fermentation is carried out in the absence of O_2 . So a build up of lactic acid suggests an anaerobic environment and a cessation of aerobic respiration.

Treatments

CO poisoning can be treated with hyperbaric O_2 (exposing patients to high levels of O_2 at elevated pressures).

HCN poisoning can be treated with a family of chemicals (nitrites) that bind to hemoglobin, creating a form of hemoglobin known as methemoglobin. Methemoglobin binds to HCN even tighter than cytochrome oxidase. In the presence of methemoglobin, HCN will preferentially bind to methemoglobin, rather than cytochrome oxidase. One factor to keep in mind however, is that methemoglobin cannot bind to O_2 .

Think-Pair-Share (15 minutes)

9. If a firefighter comes in to the ER with a headache, confusion and other neurological symptoms (following smoke exposure), what are some considerations in choosing a treatment?
a. Review the two possible exposures you have learned about so far, and how each interferes with cellular respiration.

Again: CO prevents O_2 delivery to cells, HCN prevents passing electrons to O_2 at the bottom of the electron transport chain.

b. Why does hyperbaric oxygen have the potential to treat CO poisoning?

As CO is binding with high affinity to the O_2 binding sites on Hb, increasing the partial pressure of O_2 may help to increase the probability of O_2 binding to Hb, rather than CO. (The medical literature mentions that both 100% O_2 and hyperbaric O_2 reduce the half-life of carboxyhemoglobin. 100% O_2 is reported to reduce its half life to 1-1.5 hours, while hyperbaric O_2 is reported to reduce its half life to 15-30 minutes. The normal half-life of carboxyhemoglobin is between 4 and 6 hours)

c. Do you predict hyperbaric oxygen to be helpful, harmful or “neutral” in cases of HCN poisoning? (explain your answer)

Theoretically, it won't help, b/c in cases of HCN poisoning, there is plenty of O_2 bound to Hb in the blood (even more than usual, especially in tissue veins, as O_2 is not being dropped off at cells).

d. What do you predict to happen if a patient with CO poisoning was given nitrites (to induce methemoglobin formation)? Explain your answer.

This would make a bad situation even worse! If they have CO poisoning, they are already compromised in their ability to transport O₂. As methemoglobin can't transport O₂, inducing methemoglobin will even further restrict O₂ carrying capacity of Hb in the blood.

Patient Data (15 minutes)

Your table will be given two cases: one of CO poisoning and one of HCN poisoning. You will work as a team to distinguish between the two cases, based on what you have learned so far. After you have assigned a diagnosis to your two cases, the groups will re-form, so that all the HCN cases will be grouped together and all the CO cases will be grouped together. You will work in these groups to try and determine what each form of poisoning has in common.

Table 2: Normal reference values for indicators of aerobic respiration

	Normal Range
Blood pH	7.35-7.45
Blood (plasma) lactate levels	0.5-2.2mM
Carboxyhemoglobin (non-smokers)	0-2.3%
Carboxyhemoglobin (smokers)	2-15%
Oxyhemoglobin (Arterial)	84-97%
Oxyhemoglobin (Venous)	55-75%
Skin tone (face and lips)	Uniform "flesh" color

Grading for This Activity

- Handing in the completed prep assignment at the start of the workshop/class meeting (5 points)
 - Participation in the drawing of the ETC and PMF in the presence of different inhibitors (5 points) (for simply being present and working on it)
 - Worksheet to hand in
 - compare and contrast HCN and CO poisoning (developed as they do the patient data analysis) (10 points)
 - **brief** explanation for why BOTH HCN and CO result in a reduction of blood pH (3 points)
 - "muddiest point" about cellular respiration and plan to get this point cleared up? (2 points)
-

References (all links tested 9/3/07)

General

Electron Transport Chain Movie (for the prep assignment):

<http://vcell.ndsu.nodak.edu/animations/etc/movie.htm>

Sadava, D. H.C. Heller, G.H. Orians, W.K. Purves and D.M. Hillis. Life, The Science of Biology (Eighth edition). Copyright 2008 by Sinauer Associates, Inc. First Printing December 2006.

Carbon monoxide poisoning:

http://www.emedicinehealth.com/carbon_monoxide_poisoning/article_em.htm

<http://www.emedicine.com/EMERG/topic817.htm>

Ernst, A. and J.D. Zibrak. 1998. Carbon Monoxide Poisoning. *New Engl J Med* 339(22): 1603-1608.

Kales, S.N. and D.C. Christiani. 2004. Acute Chemical Emergencies. *New Engl J Med* 350(8): 800-808.

Cyanide poisoning:

<http://www.bt.cdc.gov/agent/cyanide/basics/facts.asp>

http://www.emedicinehealth.com/cyanide_poisoning/page3_em.htm

<http://www.emedicine.com/EMERG/topic118.htm>

<http://www.emedicine.com/MED/topic487.htm>

http://www.projo.com/ri/providence/content/projo_20060531_cyanide31.17c2bc23.html

Mutlu, G.M., J. B. Leikin, K. Oh and P. Factor. 2002. An Unresponsive Biochemistry Professor in the Bathtub. *Chest* 122; 1073-1076.

D.M.G. Beasley and W.I. Glass. 1998. Cyanide Poisoning: pathophysiology and treatment recommendations. *Occup. Med.* 48(7):427-431.

Baud, FJ. 2007. Cyanide: critical issues in diagnosis and treatment. *Human and Experimental Toxicology* 26:191-201.

Inhibitor Information ANSWER KEY

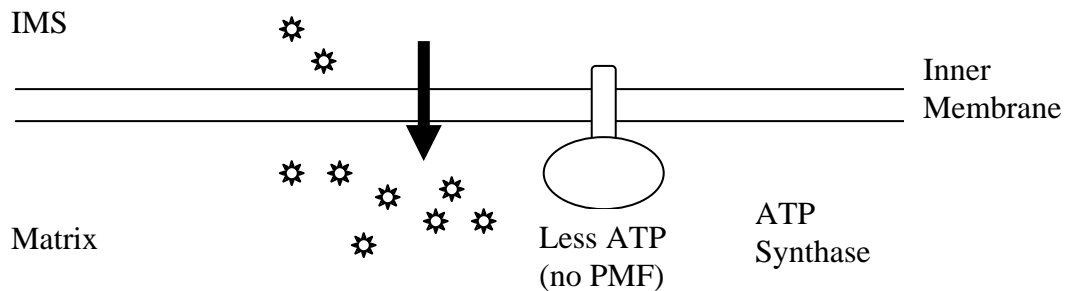
1. CCCP (carbonyl cyanide *m*-chlorophenylhydrazine)

This inhibitor (which is known as an “uncoupler”) acts by providing an alternate (& effective) passageway for protons to move through the inner mitochondrial membrane (down their concentration gradient), thereby circumventing their passage through ATP synthase.

Use the information provided to draw the “after” version of your diagram of the PMF, and to predict the impact on ATP production. You will need to indicate the following on your “after” diagram:

- what the PMF will look like (draw the distribution of H⁺ predicted after exposure of cells to this inhibitor)
- the relative amount of ATP production (compared to your diagram in the absence of inhibitor)
- where the inhibitor is acting (e.g. draw an arrow)

So the inhibitor acts at the level of membrane (see arrow). With a huge “hole” in the membrane through which protons can pass, the gradient will not be maintained, and ATP synthesis will cease.



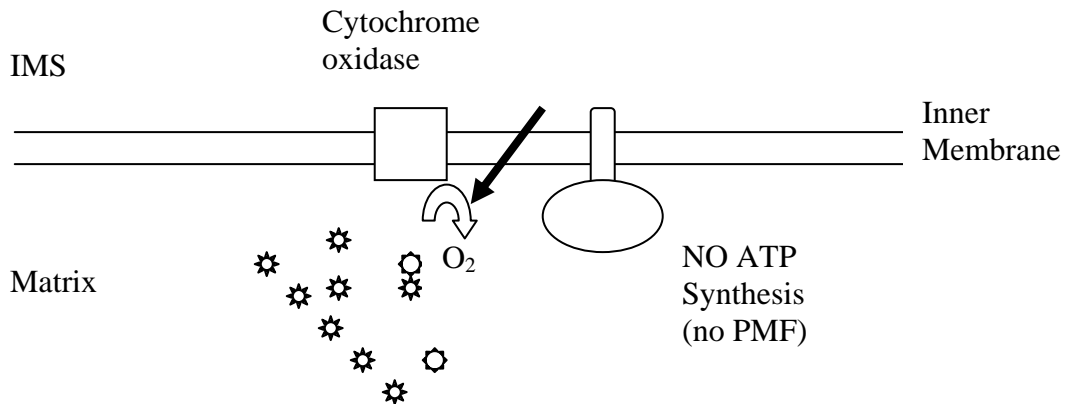
2. HCN (hydrogen cyanide)

HCN acts to prevent the transfer of electrons from cytochrome oxidase (in the inner mitochondrial membrane) to O_2

Use the information provided to draw the “after” version of your diagram of the PMF, and to predict the impact on ATP production. You will need to indicate the following on your “after” diagram:

- what the PMF will look like (draw the distribution of H^+ predicted after exposure of cells to this inhibitor)
- the relative amount of ATP production (compared to your diagram in the absence of inhibitor)
- where the inhibitor is acting (e.g. draw an arrow)

After a short while, the ETC will “back up”, and stop working. The PMF will quickly dissipate through ATP synthase, but without e^- transfer and proton pumping, there will no longer be a PMF maintained. No PMF, no ATP synthesis.



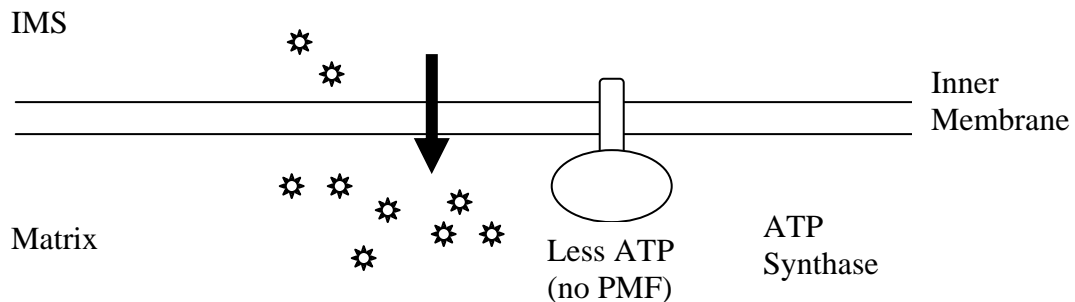
3. DNP (2,4-p-dinitrophenol)

DNP acts as an “uncoupler”. It provides an alternate (& effective) passageway for protons to move through the inner mitochondrial membrane (down their concentration gradient), thereby circumventing their passage through ATP synthase.

Use the information provided to draw the “after” version of your diagram of the PMF, and to predict the impact on ATP production. You will need to indicate the following on your “after” diagram:

- what the PMF will look like (draw the distribution of H^+ predicted after exposure of cells to this inhibitor)
- the relative amount of ATP production (compared to your diagram in the absence of inhibitor)
- where the inhibitor is acting (e.g. draw an arrow)

So the inhibitor acts at the level of membrane (see arrow). With a huge “hole” in the membrane through which protons can pass, the gradient will not be maintained, and ATP synthesis will cease.



Patient Data

Patients A & B

One of these patients (a firefighter) was exposed to high levels of **HCN (PATIENT A)** in an industrial fire, while the other was exposed to high levels of **CO (PATIENT B)** in a residential fire. Neither of these patients is a smoker.

	Patient A	Patient B	Normal Range
Blood pH	7.1	7.25	7.35-7.45
Blood (plasma) lactate levels	8 mM	2.7 mM	0.5-2.2mM
Carboxyhemoglobin	1.4%	9%	0-2.3%
Oxyhemoglobin (Arterial)	99%	45%	84-97%
Oxyhemoglobin (Venous)	89%	37%	55-75%
Skin tone (face and lips)	Bright cherry red	Normal appearing	Uniform "flesh" color

Patients C & D

One of these patients (a non-smoking college student) was exposed to high levels of **CO (PATIENT D)** due to a defective gas furnace in their home. The other patient (also a non-smoker) was exposed to high levels of **HCN (PATIENT C)** in a suicide-attempt "inspired" by the 1978 Jonestown incident (in which nearly 1000 people died in a murder-suicide attempt based on consumption of a cyanide-laced beverage).

	Patient C	Patient D	Normal Range
Blood pH	7.1	7.25	7.35-7.45
Blood (plasma) lactate levels	7.5 mM	2.5 mM	0.5-2.2mM
Carboxyhemoglobin	2.1%	8%	0-2.3%
Oxyhemoglobin (Arterial)	98%	52%	84-97%
Oxyhemoglobin (Venous)	89%	44%	55-75%
Skin tone (face and lips)	Slightly pink (in lips)	Pale	Uniform "flesh" color

Patients E & F

One of these patients had an involuntary intake of **HCN (PATIENT E)**, through the consumption of cassava and an unproven cancer treatment (laetrile). The other patient attempted suicide by sitting in their running car in an enclosed garage (thereby exposing themselves to elevated levels of **CO (PATIENT F)**). Neither patient has a history of smoking.

	Patient E	Patient F	Normal Range
Blood pH	7.0	7.3	7.35-7.45
Blood (plasma) lactate levels	5 mM	5 mM	0.5-2.2mM
Carboxyhemoglobin	2.2%	9%	0-2.3%
Oxyhemoglobin (Arterial)	98%	45%	84-97%
Oxyhemoglobin (Venous)	91%	35%	55-75%
Skin tone (face and lips)	Normal appearance	Normal appearance	Uniform "flesh" color

A Case of Poisoned Firefighters

Name: _____ **ANSWER KEY** _____

1. Based on comparing other patients with the same exposure (e.g. other HCN patients or other CO patients), what generalizations can you make about each test/observation? In the table below, don't put in specific numbers, instead draw a general conclusion, e.g. "all cases had a slightly basic pH" or "there was not a clear pattern of abnormal pH with this exposure". (12 points- 2 points each)

Circle the exposure you looked at with new people:

HCN

Test/Observation	General Conclusion
Blood pH	Slightly acidic
Blood (plasma) lactate levels	Elevated
Carboxyhemoglobin	In the normal range
Oxyhemoglobin (Arterial)	High
Oxyhemoglobin (Venous)	Very high
Skin tone (face & lips)	Normal to pink/red (due to high oxygenation of both arterial and venous Hb)

CO

Test/Observation	General Conclusion
Blood pH	Slightly acidic
Blood (plasma) lactate levels	High
Carboxyhemoglobin	High
Oxyhemoglobin (Arterial)	Low
Oxyhemoglobin (Venous)	Low
Skin tone (face & lips)	Normal to pale <i>(Note: while carboxy Hb is reported to appear red, we encountered several references that consistently indicated that bright red skin is actually quite rare in CO poisoning, and that it is more likely that the patient will be pale. However, retinal veins are almost always red in cases of CO poisoning)</i>

2. You have now spent quite a bit of time in lecture and workshop thinking about cellular respiration. What is your “muddiest point” about cellular respiration, and what are you going to do to get this point cleared up? (3 points)