

A Collection of In-Class Activities in Microbiology

Resource Type: Curriculum: Classroom

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Abstract

The benefits of active learning have been repeatedly demonstrated in educational research from the last few decades. One way to bring active learning into classrooms is to have students work on challenging problems during the class period. We present a collection of microbiology problems on various topics (including evolution, cell structure, metabolism, genetics, ecology, and pathogenesis) that we have used as in-class activities in a large microbiology lecture. We present this as a tool for teachers to encourage the use of active learning. These activities will give students a better understanding of the concepts and will help them to enjoy the lecture experience.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

PROCEDURE

Some of the activities were developed by a very talented group of graduate students in our "Teaching Experience in Microbiology" class. ASM members also contributed ideas at the 2001 ASM Undergraduate Microbiology Education Conference.

Materials.

Teachers can copy figures from the material provided or use digital images on a PowerPoint presentation.

Student version.

Each activity comes with a separate set of instructions:

[Cell Structure and Function, History, and Taxonomy](#)
[Microbial Ecology](#)
[Catabolism](#)
[Genetics](#)
[Cell Growth and Biosynthesis](#)
[Immunology and Medical Microbiology](#)

Instructor version.

There are many ways to use these questions. I typically talk about the concepts for 5 to 15 minutes, then stop talking, hand out sheets with a question pertaining to the topic (or show them using a projector system), and give students 5 minutes or so to answer the question.

I encourage students to talk to their neighbors. Other instructors have formed formal groups in which students work during each class. I do not usually collect and grade students' answers, but I go over them in class immediately. Others collect the answers and post the correct answer on a website after class. Regardless of how the questions are used, the point is to get students to THINK about what you lecture about. Thinking (not listening) leads to understanding.

One way to use in-class problems is to talk about the material before giving students a chance to work through the problem. For example, I may give a 10-minute lecture on photosynthesis, then have students work through a problem on electron transport in photosystems.

Safety Issues. None.

ASSESSMENT and OUTCOMES

Suggestions for assessment.

My goals for using in-class activities are to help students learn the material better and to help them pay attention during class. As mentioned previously, I do not grade these activities.

Field Testing.

I have used this technique, and many of these questions, for over 4 years in a large lecture class of about 150 students. During some semesters, I have team taught with instructors that did not use in-class problems. Students prefer lecture class

with the in-class activities. Comments include:

"I've enjoyed all the activities you guys do. It's a nice change of pace over my other classes."

"This is by far the best science course I've taken. The entire course staff is very supportive of students in general, but learning styles in particular."

"I like that this course is organized so that all types of learning backgrounds benefit."

"It's great to see a large class that is so organized."

"This is the best science class! I love the fact that we have evaluations other than tests."

We have had extensive student evaluations asking if the in-class activities helped their understanding. For example, in the last two semesters, even though working on the problems was entirely voluntary in my class, over 80% of my students did the problems. Students overwhelmingly liked them, and 70% thought they helped them learn the material better.

It would be great if someone could use these methods in one class, but not another (all things being equal). Because I do not teach two simultaneous lectures, I have not been able to do such a controlled experiment.

SUPPLEMENTARY MATERIALS

References.

1. **Bonwell, C., and J. Eison.** 1991. Active learning: creating excitement in the classroom. ASHE-ERIC higher education report **20**(1).
2. **Campbell, W. E., and K. A. Smith.** 1995. New paradigms for college teaching. Jossey-Bass Publishers, San Francisco, Calif.
3. **Cooper, J. L., and P. Robinson.** 2000. Getting started: informal small-group strategies in large classes. *New Directions for Teaching Learning* **81**:17-24. [Online]
4. **Cooper, J. L.** 2000. The argument for making large classes seem small. *New Directions for Teaching Learning* **81**:5-15. [Online]
5. **Springer, L., M. E. Stanne, and S. S. Donovan.** 1999. Effects of small group learning on undergraduates in science, mathematics, engineering, and technology: a meta-analysis. *Rev. Educ. Res.* **69**:21-51.
6. **Sutherland, T., and C. Bonwell (ed.).** 1996. Using active learning in college classes: a range of options for faculty. *New Directions for Teaching Learning* **67**.

Appendices and Answer Keys.

Appendices and answer keys are included in the packets.

Curriculum Resources

A Collection of In-Class Activities in Microbiology: Cell Structure and Function, History, and Taxonomy

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Chronological Events

Put these events in chronological order (1=earliest event)

- ___ Bacteria Help Plants Grow
- ___ Bacteria to Blame for Tuberculosis!
- ___ Delightful Drinks Developed from Fermented Bread
- ___ Genetically Engineered Yeast Crank Out Insulin for Human Use
- ___ Minute "Animacules" Found In Drinking Water-- Partygoers Shocked
- ___ New Kind of Bacteria "Discovered"- Archaea Rule!
- ___ Scientist Crack Universal Code of Life-- DNA Structure Revealed
- ___ Spontaneous Generation Theory-- Gone with the Wind
- ___ "Magic Bullet" Medicine Used on Burn Victims

ANSWERS

- 5 "Magic" Bacteria Help Plants Grow (1890)
- 4 Bacteria Blamed for Tuberculosis! (1882)
- 1 Delightful Drinks from Fermented Bread (4000 BC)
- 9 Genetically Engineered Yeast Crank Out Insulin for Human Use (1982)
- 2 Minute "Animacules" Found In Water--Partygoers Shocked (1684)
- 8 New Kind of Bacteria "Discovered"- Archaea Rule! (1977)
- 7 Scientist Crack Universal Code of Life -DNA Structure Revealed (1953)
- 3 Spontaneous Generation Theory - Gone with the Wind (1864)
- 6 "Magic Bullet" Medicine Used on Burn Victims (1942)

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People and Historical Events

1. Why is it that people didn't know about bacteria before Antoine van Leeuwenhoek first described them in the 1680s?
2. Why did it take over 200 years after van Leeuwenhoek's initial observations to understand the true nature and importance of microorganisms?
3. In the 1860s, Pasteur developed a new flask for his experiments on spontaneous generation. How did this help him disprove the opposing view at the time?
4. What is a pure culture? Why was the invention of solid culture material so important to proving Koch's postulates?

ANSWERS

1. *We cannot see bacteria with our naked eyes, he was the first one to actually see them using a magnifying glass.*
 2. *There were no techniques to study bacteria such as the ability to obtain pure cultures and maintain pure cultures in broth. People didn't understand what "sterile" meant or how to make solutions sterile. These needed to be developed. Also, more powerful microscopes were developed.*
- Reviewer's note: *in addition, disease was often equated with superstition and religious disobedience. The connection between microorganisms and disease was still not openly documented. The role of microorganisms economically for food production (especially beer and wine) was unresolved. And no one was trained to continue van Leeuwenhoek's work.*
3. *He needed a flask that was open to air but would not allow airborne contaminants to come in. The opposing view was that life arose from spontaneous generation; yet he was able to show that you could keep liquids sterile indefinitely if you protected them from contaminants.*
 4. *A pure culture is a population of bacteria grown up from one cell; therefore all cells are identical. Koch needed pure cultures to prove that a given bacterium was causing a disease. He infected animals with a pure culture, and the animals got the disease showing that the bacteria must be the cause of the disease. Solid culture material is so important because individual colonies must be visible to separate them out.*

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Phylogenetic Tree

Find a copy of a three-domain phylogenetic tree constructed from small subunit (SSU) rRNA sequence data.

1. Which organisms are more closely related?
Humans and mold (fungi) OR methanogens (Archaea) and *E. coli* (proteobacteria)
2. Which of these entities are more closely related?
Mitochondria and animal cells OR Mitochondria and proteobacteria
3. Does this refute or support the endosymbiotic theory? Why or why not?

ANSWERS

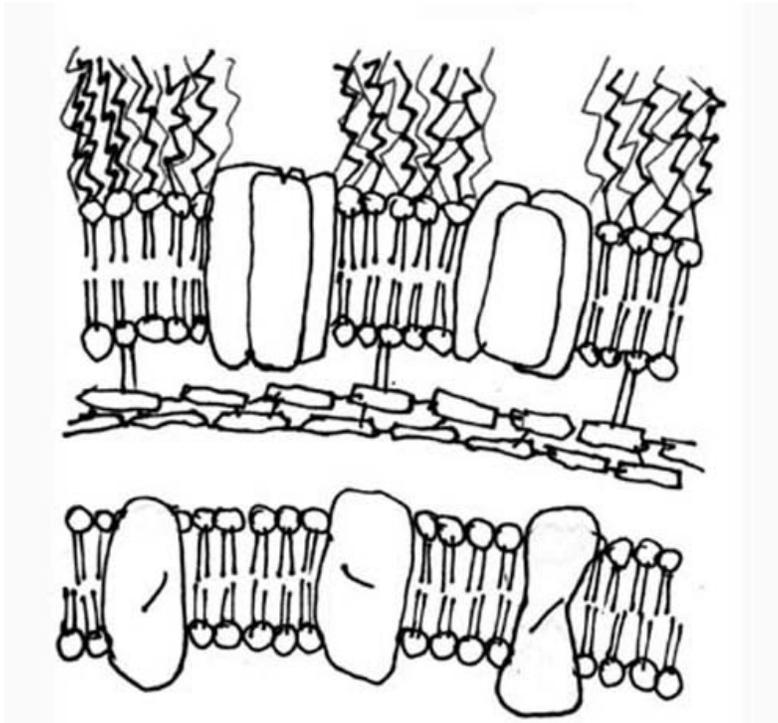
1. *Humans and mold (fungi)*
2. *Mitochondria and proteobacteria*
3. *It supports the endosymbiotic theory, which states that mitochondria evolved from aerobically-respiring bacteria (such as proteobacteria), not from animal or plant cells.*

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Microscopy

What type of microscopy would be appropriate for each of the following? You have isolated a new bacterium from your compost pile, and you...

1. wish to see if it is motile.
2. want to know if this specific strain is present in other compost.
3. wonder if it has any internal sub-structures.
4. want to see if it grows attached to particle surfaces.
 - Transmission electron microscopy
 - Scanning electron microscopy



1. Does this show a gram positive or gram negative cell envelope? How can you tell?
2. Describe the process a small molecule like a sugar has to go through to get from the outside to the inside of the cell.
 - a. Which layers are permeable to it?
 - b. Which ones require proteins?
 - c. Are the proteins specific or general?
3. Where would you expect to find the following proteins (point them out on the diagram)?
 - a. Enzymes that break huge polymers like cellulose into smaller units like sugars.
 - b. Enzymes that synthesize crosslinks between tetrapeptides.
 - c. Enzymes that allow specific nutrients into the cell.
 - d. Enzymes that bind to DNA during replication.
4. How would you have to change this diagram to make it look like a gram positive cell envelope?

ANSWERS

1. *Gram negative cell envelope because it has an outer membrane.*
- 2a. *It can get through the porins in the outer membrane and the thin peptidoglycan cell wall.*
- 2b. *It cannot get through the cytoplasmic membrane without a protein transporter.*
- 2c. *Porins are general channels open to molecules that are the correct size; transporters are usually very specific to one type of molecule.*
- 3a. *Outside the outer membrane because huge molecules like cellulose cannot get through the porins in the outer membrane.*
- 3b. *In the periplasmic space, where the cell wall is built.*
- 3c. *In the cytoplasmic membrane, which is the only real semi-permeable barrier.*
- 3d. *In the cell cytoplasm, where the DNA is.*

4. Take away the outer membrane, make a thicker cell wall, and add teichoic acids and lipo-teichoic acids

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Archaea - Comparisons to Other Groups

List at least one trait that Archaea have in common with the following groups:

	All cells	Bacteria	Eukaryotes	Archaea
Archaea				

ANSWERS

	All cells	Bacteria	Eukaryotes	Archaea
Archaea	<i>DNA genetic material</i> <i>Proteins as catalysts</i> <i>Lipid bilayer membrane</i>	<i>No nucleus</i> <i>Single celled</i>	<i>Functionally similar RNAP</i> <i>Functionally similar translational machinery</i> <i>Do not have peptidoglycan cell walls</i>	<i>Ether-linked lipids</i>

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Cell Structure and Function

Some thought questions.

1. What do you think are the essential parts of any living cell?
2. Which macromolecule do you think is the most important in the cell; protein, carbohydrate, nucleic acid or lipid ?
(Note: there is not one correct answer. Pick one macromolecule and justify your choice with facts about the cell).
3. How could one compare the cell to life in an apartment house? Or a city? Or a modern-day building?

4. You are hired to design new antibiotics to kill only bacterial cells. Given that you want to do no harm to the host, what target sites would you choose and why?
5. Why do antibiotics inhibit bacterial and not mammalian cells?
6. Why do some antibiotics work on gram positive but not gram negative cells?
7. What cell structure causes some bacteria to stain gram positive while others stain gram negative?
8. Do you think gram negative or gram positive bacteria would be more sensitive to organic solvents? Why?
9. Structurally, what determines a bacterial cell's shape? Functionally, why do you think cells have different shapes (like cocci, bacilli, spirochete, spirilla, etc.)?
10. Look at a phylogenetic tree of the bacterial domain. Which cell envelope type evolved first: gram negative or gram positive? Why do you think this was so?
11. You have an unknown microorganism. Could you tell if it is a prokaryotic or eukaryotic without a microscope?
12. What keeps a cell from bursting when in low osmotic conditions?
13. Bacterial cells are found in many extreme environments. How do the cell membranes of thermophiles differ from those of mesophiles? How do the cell membranes of psychrophiles differ from those of mesophiles?

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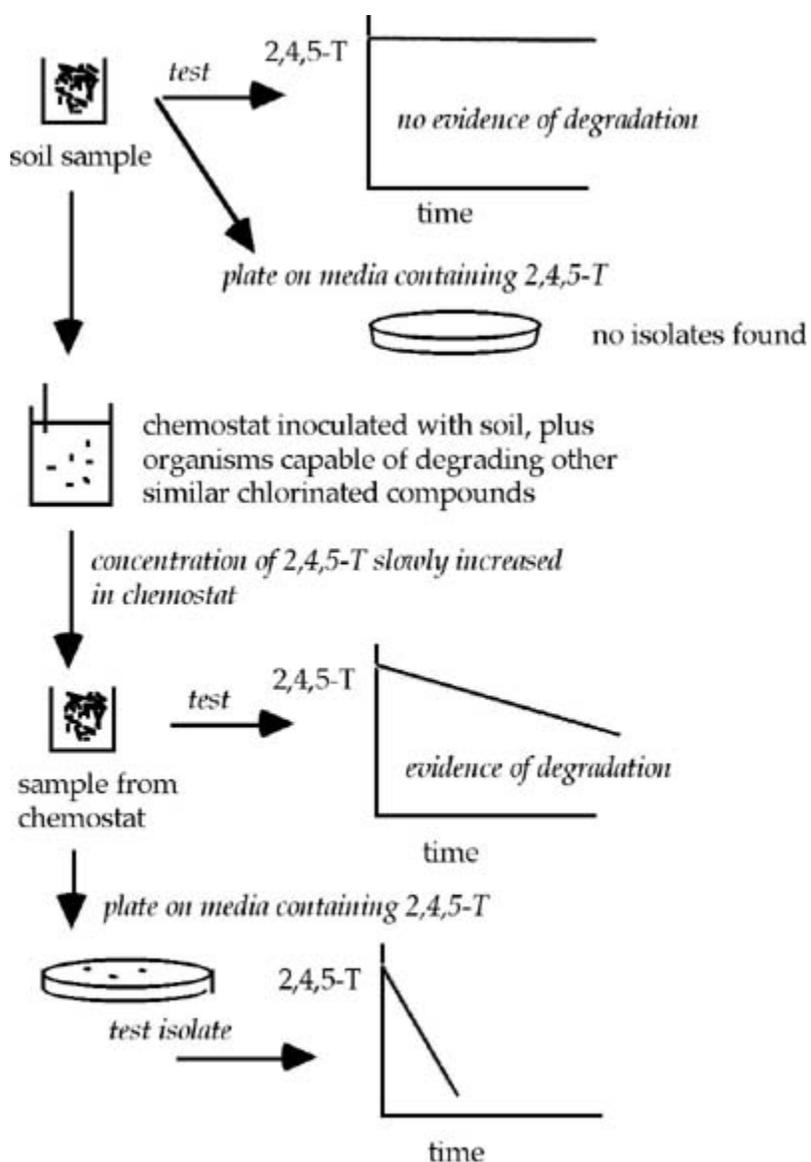
[Heterotroph versus Nitrifier](#)

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Isolating Bacteria

You are trying to isolate a bacterium that can degrade 2,4,5-T.



new isolate carries a plasmid containing genes
obtained from others in community

1. In the first step, you find "no evidence of degradation." What evidence are you looking for, both on

- the plate and by measuring the concentration of 2,4,5-T? What would degradation look like?
- Why did you add organisms capable of degrading other similar chlorinated compounds to the soil? How would that help?
 - What results did you get after adding the other bacteria? What do you think happened in the soil to get those results?
 - Would you aerate the soils during your experiments? Or let them sit and go anoxic? Why?

ANSWERS

- The plate would show colonies growing that could use 2,4,5-T. The concentration of 2,4,5-T should drop over time.
- Sometimes genetic exchange or mutations can occur that allow bacteria to transform new, but similar compounds. The more similar the compounds, the more likely that existing enzymes may recognize the new substrate.
- Some bacteria could have mutated so enzymes could use similar substrates, or they could have exchanged genes to make new pathways.
- 2,4,5-T is chlorinated, so I would try anaerobic decomposition first, to see if I could get reductive dechlorination.

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Adventure of a Nitrogen Atom

Here is a story about the adventures of a nitrogen atom, with a few key words missing. Fill in the blanks from the list below.

At the start of this story, you found yourself a happy nitrogen atom, the "amino" part of an amino acid within a plant root. You felt useful as part of a RuBisCo team that carried out carbon fixation. Unfortunately, a frost came and your host cell burst, spewing you out as a vulnerable organic-N in the soil. Soon a _____ came along and picked you up or so you thought. Instead it stripped you of your carbon backbone and left you as an ammonium ion, all alone. Almost immediately, you were transported into a _____ and stripped of your precious electrons. The cell got energy all you got was oxidized and discarded. You were soon snatched up again, and this time by _____, robbed of your last remaining electrons, and discarded. Is there no justice in this world? Wasted, you became caught in ground water and flushed into a place with no molecular oxygen. You had never been anoxic before and were surprised how active this new world was. Cells were fighting left and right for you. You were transported into a _____ and placed at the end of an electron transport chain until electrons rolled your way. You were reduced to di-nitrogen gas. Tired of all these exhausting transformations, you escaped into the atmosphere and lived a life of leisure for the next few thousand years, vowing never to return again to that land where the nitrogen cycles.

- a denitrifying *Pseudomonas*
- a heterotrophic, ammonifying decomposer
- a nitrogen-fixing *Azotobacter* (a cyanobacterium)
- an autotrophic eukaryotic cell
- Nitrosomonas* (a nitrosifying bacterium)
- Nitrobacter* (a nitrifying bacterium)

ANSWERS

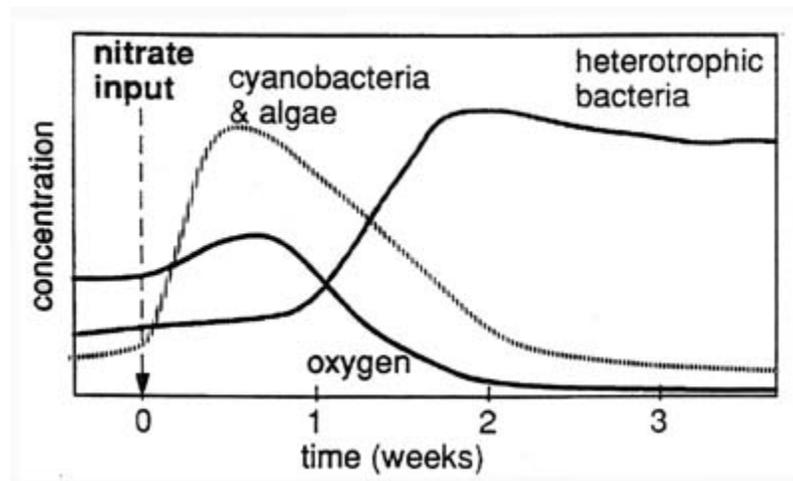
At the start of this story, you found yourself a happy nitrogen atom, the "amino" part of an amino acid within a plant root. You felt useful as part of a RuBisCo team that carried out carbon fixation.

Unfortunately, a frost came and your host cell burst, spewing you out as a vulnerable organic-N in the soil. Soon **B)** a heterotrophic, ammonifying decomposer came along and picked you up or so you thought. Instead it stripped you of your carbon backbone and left you as an ammonium ion, all alone. Almost immediately, you were transported into **E)** *Nitrosomonas* (a nitrosifying bacterium) and stripped of your precious electrons. The cell got energy, all you got was oxidized and discarded. You were soon snatched up again and, this time by **F)** *Nitrobacter* (a nitrifying bacterium), robbed of your last remaining electrons and discarded. Is there no justice in this world? Wasted, you became caught in ground water and flushed into a place with no molecular oxygen. You had never been anoxic before and were surprised how active this new world was. Cells were fighting left and right for you. You were transported into **A)** a denitrifying *Pseudomonas* and placed at the end of an electron transport chain until electrons rolled your way. You were reduced to di-nitrogen gas. Tired of all these exhausting transformations, you escaped into the atmosphere and lived a life of leisure for the next few thousand years, vowing never to return again to that land where the nitrogen cycles.

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Nutrient Dumping

Here is a graph that shows what happens when nutrients such as nitrogen are dumped into a pond.



1. Why did nitrate stimulate growth of the cyanobacteria rather than the heterotrophs?
2. Is nitrate usually considered to be a component of "BOD"? Did it indirectly stimulate the production of BOD?
3. Why did the oxygen level decline after 1 week (and not immediately)?
4. How would the curve be different if you added organic carbon waste instead of nitrogen? What kinds of microorganisms would be stimulated?

ANSWERS

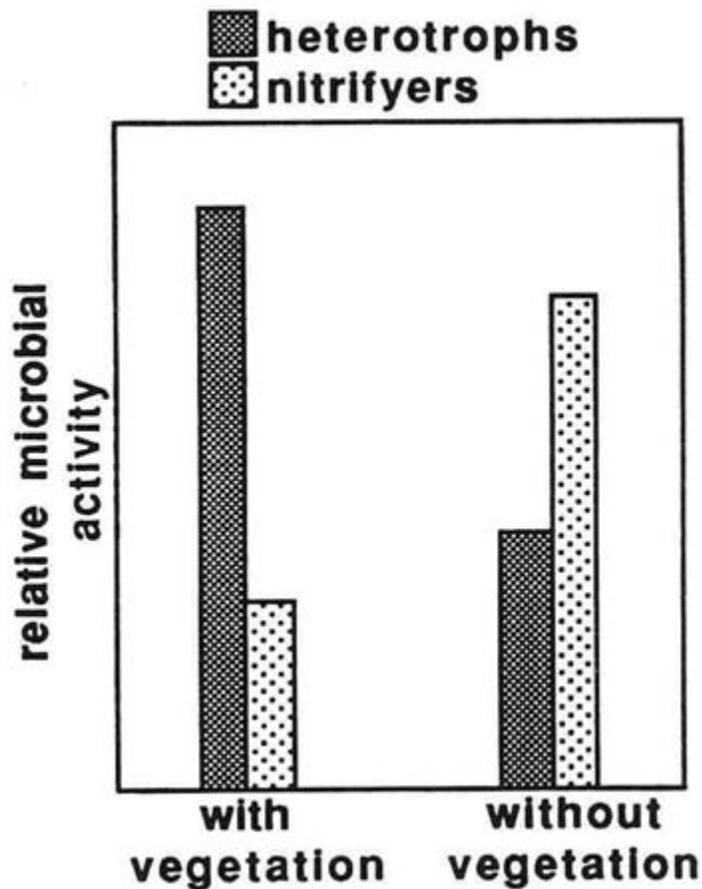
1. Photo-autotrophs are often limited for nitrogen (since they have plenty of carbon, and nitrogen is the next most needed nutrient). Heterotrophs are often limited for carbon and nitrogen, so adding just nitrogen doesn't stimulate their growth as much.
2. No.
Yes, it caused cells to grow and make more biomass (organic carbon), which is definitely a respirable material.

3. As the cyanobacteria died off, their biomass (cellular material) became available for heterotrophs to consume. As they consumed organic carbon, they also consumed dissolved oxygen.
4. Organic carbon and nitrogen would stimulate the growth of heterotrophs first. Then, after they peaked, nitrogen would be released as the heterotrophs died, and some phototrophs may be stimulated.

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Heterotroph versus Nitrifier

Agricultural field studies reveal that heterotrophic bacteria prevail over nitrifying bacteria when leftover vegetation is plowed into the field at the end of the growing season. Yet, nitrifying bacteria (ammonia oxidizers) prevail over heterotrophic bacteria if the vegetation is removed from the field.



1. What are these heterotrophic bacteria using for their carbon and energy sources?
2. What are these nitrifying bacteria using for their carbon and energy sources?
3. Explain why heterotrophs predominate when vegetation is left, while nitrifiers predominate when vegetation is removed.

ANSWERS

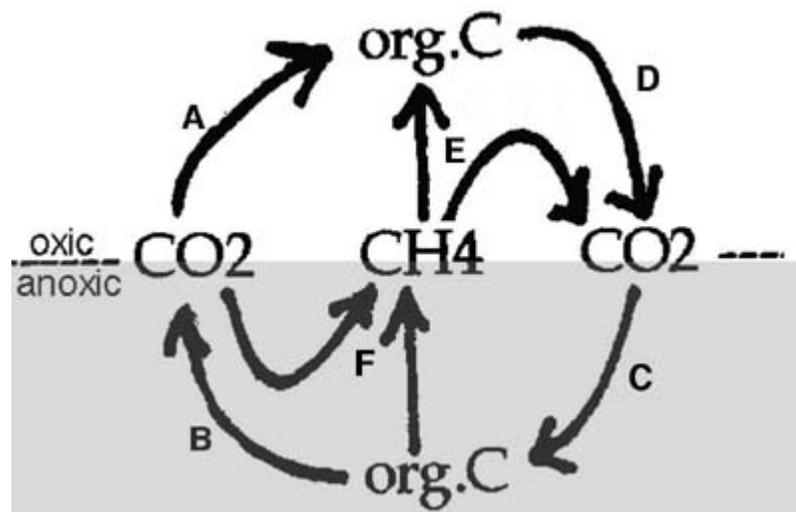
1. The left-over organic carbon from the vegetation.

2. Energy comes from any NH_4^+ that may reside in the soils (and oxygen); carbon comes from CO_2 fixation (these bacteria are auto-lithotrophs).
3. With lots of organic carbon around, heterotrophs have everything they need to grow. They outcompete the nitrifiers because there is far more organic carbon than NH_4^+ . Also, the heterotrophs may consume so much oxygen that the soil could become anoxic in regions, thus nitrification could not occur but heterotrophic growth could, i.e., fermentation and anaerobic respiration. Without vegetation, the heterotrophs are more limited for carbon. Because the nitrifiers use CO_2 as their carbon source, they are not limited for carbon.

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Carbon Cycle

For the carbon cycle, decide which bacteria could carry out each conversion (labeled with letters A



through F) using the list below.

- an aerobic heterotroph, like *Pseudomonas*
- an NH_4^+ oxidizing lithoautotroph, like *Nitrosomonas*
- a purple sulfur bacteria, like *Chromatium*
- a fermentative heterotroph, like *Clostridium*
- a methanogen, like *Methanobacterium*
- a methane-oxidizing bacterium

ANSWERS

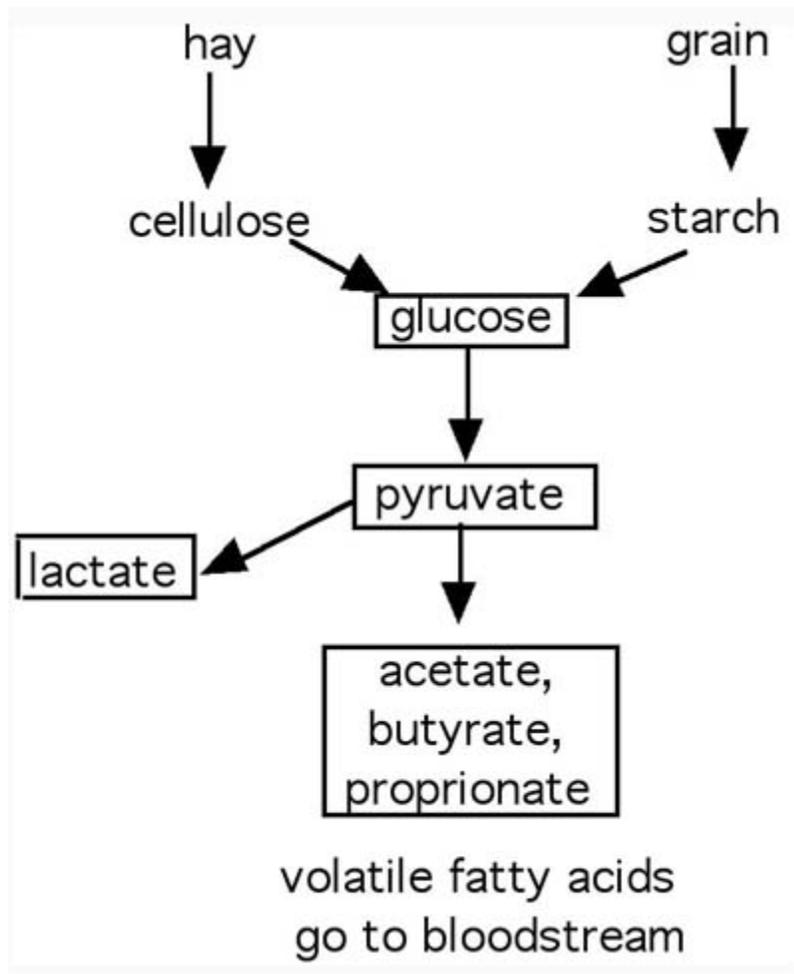
- A) NH_4^+ oxidizing lithoautotroph like Nitrosomonas
 B) fermentative heterotroph like Clostridium
 C) purple sulfur bacteria like Chromatium
 D) aerobic heterotroph like Pseudomonas
 E) a methane-oxidizing bacterium
 F) methanogen like Methanobacterium

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The Reindeer Rumen Blues

A novice reindeer farmer was becoming a bit concerned about the higher costs of hay so he switched to a cheaper brand of feed called Chow Down. After a few days with Chow Down, all the reindeer appeared ill, they were lethargic and had no appetite. Immediately the farmer called a veterinarian and the reindeer were diagnosed with rumen acidosis, a buildup of acid in the rumen. The reindeer had a high concentration of *Streptococcus bovis* in the rumen; 1,010 cells/ml compared to a concentration of 106 to 107 cells/ml for all other bacteria. The veterinarian and the farmer investigated the Chow Down ingredients and to their dismay discovered it consisted primarily of grain. The veterinarian advised that next time the farmer gradually switch to the grain diet.

Use the rumen biochemical pathway to answer the questions.



1. How did a sudden switch to grain lead to the rumen acidosis? Which end product was responsible?
2. What happened to the other organic acids?
3. Why did the veterinarian suggest a gradual change to the grain diet?

ANSWERS

1. *S. bovis* produced lactate as a fermentation product. The buildup of lactate caused the acidosis.
2. Acetate, butyrate, and proprionate went into the reindeer's bloodstream so they didn't build up.

3. *The gradual change to the grain diet would allow the other starch decomposers to grow more in balance with S. bovis.*

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Energy and Electrons

Imagine two redox pairs: XO_2^{-1} / XH_3 and Y^0 / YH_2

$E_o' = -0.35 \text{ V}$ for the redox pair XO_2^{-1} / XH_3

$E_o' = +0.5 \text{ V}$ for the redox pair Y^0 / YH_2

Predict which molecule will be the energy source and which molecule will be the electron acceptor in an energy generating process.

For each 2 redox pair given below, decide which compound is the energy source, which is the electron acceptor and what the waste products are. You will need to refer to an electron tower in your notes or textbook.

- $CO_2/\text{glucose}$ and NO_3^{-1}/NO_2^{-1}
- SO_4^{-2}/H_2S and $1/2 O_2/H_2O$
- $1/2 O_2/H_2O$ and $2H^+/H_2$
- Fe^{+3}/Fe^{+2} and $CO_2/\text{acetate}$
- $CO_2/\text{glucose}$ and $\text{pyruvate}/\text{lactate}$
- $2H^+/H_2$ and Mn^{+4}/Mn^{+2}

ANSWERS

The energy source (electron donor) will be XH_3 .

The electron acceptor will be Y^0 .

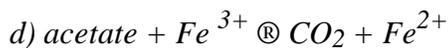
The energy generating reaction is : $XH_3 + Y^0 \rightarrow XO_2^{-1} + YH_2$

electron donor + electron acceptor \rightarrow leftovers (waste products)

a) $\text{glucose} + NO_3^{-1} \rightarrow CO_2 + NO_2^{-1}$

b) $H_2S + O^2 \rightarrow SO_4^{-2} + H_2O$

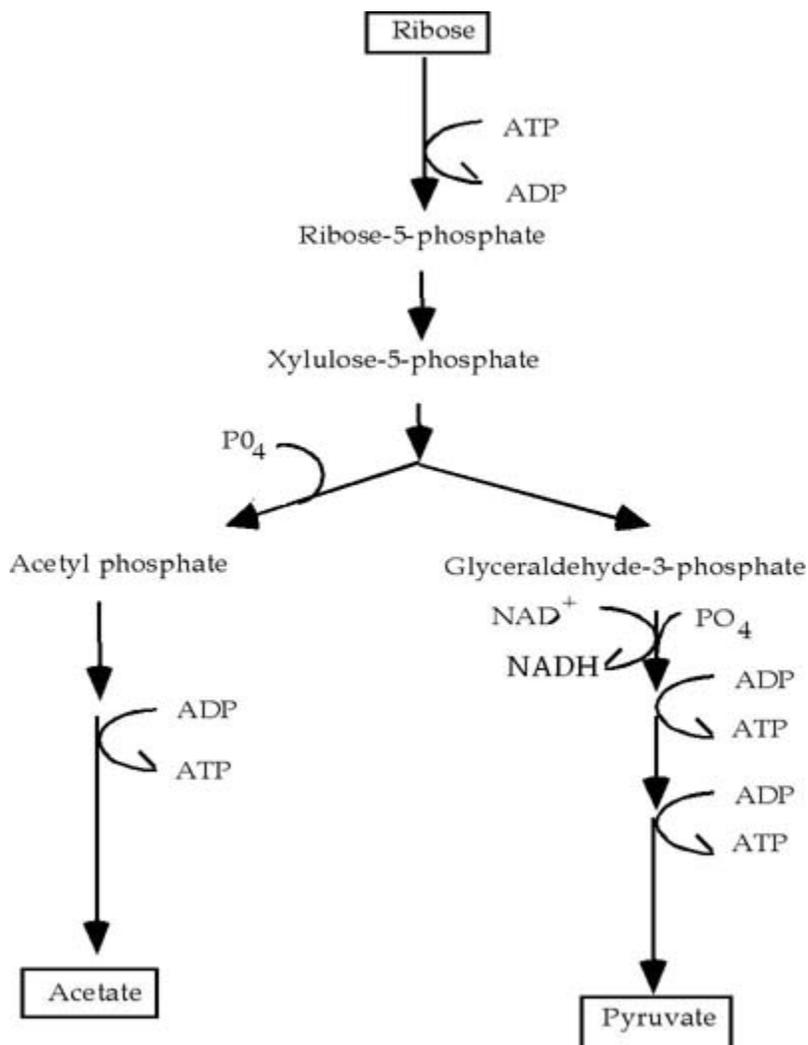
c) $H_2 + O_2 \rightarrow 2H^+ + H_2O$



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Fermentation

Use the following pathway to answer the questions below:



- Which reaction(s) show an electron "fall"? (a reduction/oxidation reaction)
- Where is substrate-level phosphorylation taking place?
- What is the net ATP production per glucose?
- Could a bacterium grow using this pathway as the only pathway for catabolism? Why or why not?

ANSWERS

a). Where NAD^+ is reduced to $NADH$.

b) Where ADP is phosphorylated to ATP .

c) 2 moles ATP per mole glucose.

d) No, $NADH$ is not oxidized to NAD^+ .

It would need an enzyme to reoxidize $NADH$, perhaps by dumping electrons onto pyruvate.

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Lithotroph Respiration

Refer to an electron tower to answer the following questions about lithotrophs.

a) Which electron donor has the most potential energy: H_2 , NH_4^+ , H_2S , or Fe^{+2} when oxygen is the terminal electron acceptor?

b) Many bacteria that can grow using H_2 as an energy source use O_2 , NO_3^{-1} , or SO_4^{-2} as an electron acceptor. Yet bacteria that use NH_4^+ , H_2S , or Fe^{+2} as an energy source use O_2 almost exclusively as a terminal electron acceptor. Can you explain this phenomenon?

c) Why are most lithotrophs also autotrophs? (Hint: think about where organotrophs get their carbon for biosynthesis.)

d) Below are the components used by *Thiobacillus* to make energy from oxygen and iron.

Cytochrome a	+0.80
Cytochrome c	+0.79
Fe^{3+}/Fe^{2+}	+0.77
O_2/H_2O	+0.82
Rustocyanin	+0.78

i) Put them in the correct order in the membrane below and show electron and proton flow.

ii) Why do you think that wherever *Thiobacillus* grows there are HUGE amounts of oxidized iron (Fe^{+3})?

ANSWERS

a) H_2 because it has the most negative reduction potential.

b) There is not enough of an electron fall between NH_4^+ , H_2S , or Fe^{+2} and the other electron acceptors to easily generate a proton motive force (PMF).

c) Many of them do not have the appropriate enzymes to incorporate different kinds of organic compounds into their biosynthetic pathways because they do not have those pathways in place for catabolism.

d)

i) *Electrons flow from Fe^{+2} ® Rustocyanin ® Cytochrome c ® Cytochrome a ® oxygen*

ii) *Because the electron fall is so small, it has to oxidize huge amounts of iron to get enough of a PMF to make enough ATP to grow.*

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Antibiotic Effect on Bacterial Respiration

You have just discovered a new antibiotic. This antibiotic is a small organic molecule that dissolves in a cytoplasmic membrane then "ferries" protons (H^+) back and forth across the membrane. The antibiotic releases the H^+ 's on whichever side of the membrane the concentration is lower, until ultimately the proton concentration across the membrane reaches equilibrium.

a) When this antibiotic is added to a culture of aerobically respiring bacteria, which of the following processes will be stopped virtually immediately:

- i. electron transfer reactions from NADH to flavoprotein to non-heme iron protein, etc.?
- ii. the Kreb's (or TCA) cycle?
- iii. utilization of oxygen?
- iv. ATP synthesis by substrate-level phosphorylation?
- v. ATP synthesis by oxidative phosphorylation?
- vi. motility?

b) Which type of bacterium, *Lactobacillus* or *Pseudomonas*, would suffer the effects from the antibiotic most quickly? Explain.

ANSWERS

a) *The cell will not be able to generate a proton motive force, therefore, these steps will be stopped:*

- v. *ATP synthesis by oxidative phosphorylation*
- vi *Motility; flagellar rotation depends on a PMF*

b). *Pseudomonas, because it depends on the PMF to make ATP. Lactic acid bacteria such as Lactobacillus make ATP via substrate-level fermentation, so don't use a PMF to make ATP.*

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Synthesis of ATP via Two Different Processes

Compare and contrast ATP synthesis in oxidative phosphorylation and photo-phosphorylation.

- a) What is the ultimate source of energy in each process?
- b) Where does the "electron fall" happen in each process?

- c) From where do the low potential electrons come in each process?
- d) Where do protons move across a membrane in each process?
- e) What drives ATP synthesis in each?

ANSWERS

- a) *Oxidative phosphorylation: a molecule that is oxidized; photophosphorylation: light*
- b) *Oxidative phosphorylation: from a reduced compound to a terminal electron acceptor; photophosphorylation: from water to a reaction center to another reaction center*
- c) *Oxidative phosphorylation: reduced chemicals; photophosphorylation: in this system, from water (they are excited to a low potential by light energy)*
- d) *Primarily in the quinone pool (or other electron/proton carriers)*
- e) *The electron fall through the electron transport system generates a proton motive force.*

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Bacterial Photosynthesis

Consider the diversity of oxygenic and anoxygenic photosynthesis in bacteria:

- a) Which group(s) of bacteria carry out oxygenic photosynthesis?
- b) Which group(s) of bacteria carry out anoxygenic photosynthesis?
- c) How does electron flow differ in oxygenic and anoxygenic photosynthesis?
- d) What parts of the oxygenic photosynthetic system are similar to the anoxygenic photosynthetic system?
- e) Different bacteria have evolved many different light-absorbing pigments. What advantage is this to bacteria in the environment?

ANSWERS

- a) *Primarily cyanobacteria*
- b) *Purple and green, sulfur and non-sulfur bacteria, as well as Heliobacterium*
- c) *In anoxygenic photosynthesis, electron flow is cyclic; in oxygenic photosynthesis, electrons flow from water ,eventually to NADP+ ® NADPH*
- d) *Photosystem I has components that are similar to the photosystem in some green bacteria and Heliobacterium (e.g., FeS). Electrons can flow cyclically through it. Photosystem II has components that are similar to the photosystem in purple and green non-sulfur bacteria (e.g., Bph).*
- e) *It allows them to compete for different portions of the visible light spectrum, because the different pigments absorb different wavelengths of light.*

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Some thought questions:

1. Many fermentative cells make energy during glycolysis (the conversion of sugars to pyruvate). Why don't cells just release pyruvate as a waste product, rather than make all those reduced organic acids?
2. Why does wine have a 12% alcohol concentration?
3. Why does bread rise? What's the microbial "food" and why does it give the yeast gas?
4. List four products of microbial fermentation that you use in everyday life.
5. What are the possible bacterial origins of H₂S?
6. What are the possible bacterial origins of methane?
7. Eukaryotic cells use mitochondria for respiration. How can prokaryotic cells, which lack these organelles, perform this function? Where would it happen and why here?
8. Why do we only get a 40% efficiency for aerobic respiration?
9. Compare fermentation and respiration: in which process would you have to consume more glucose to get equivalent amounts of ATP? What waste products are produced? How do these waste products affect the overall growth of the bacterium?
10. How does your metabolism compare with that of a fermentative bacteria? A respiring bacteria? On the molecular level, are the processes similar or very different?
11. Compare electron transport in prokaryotes and in eukaryotes? Where does electron transport take place in prokaryotes and eukaryotes? On the molecular level are the processes similar or very different?
12. Why do respiring bacteria "spit out" H⁺ (protons) as a result of catabolic reactions? Why do some fermentative bacteria make H₂ gas instead?

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Genetics

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Gene Expression: Transcription

Shown below is a nucleotide sequence of a fragment of double-stranded DNA from *E. coli*.

5'..TCTACGCATCTAGCGGGCTCTTGACA(17bases)TATAATGCTCAATGTGCGCTAAGGAGGTGCTTGATGATCTGGCGAC

3'..AGATGCGTAGATCGCCCGAGAAGTGT(17bases)ATATTACGAGTTAACAGCGATTCTCCACGAAGTACTAGACCGCTG

Assume that the *E. coli* consensus promoter is:

5'...TTGACA...(any 17 bases)...TATAAT...3'

3'...AACTGT...(any 17 bases)...ATATTA...5'

1. What molecule recognizes and binds to the promoter region?
2. What molecule carries out transcription of DNA?
3. In which direction does transcription take place (left or right on the diagram above)? What determines the direction of transcription?
4. Which DNA strand will be actively transcribed? What determines which DNA strand gets copied?
5. Write out the mRNA that will be transcribed from this gene, include 5' and 3' ends.
6. On your diagram of the mRNA, indicate where the 30s subunit of the ribosome will initially bind.
7. On your diagram of the mRNA, indicate the position of the first translated codon. What is this codon?
8. What is the second amino acid in this polypeptide?
9. What is the sequence of the anti-codon region of this tRNA? Include 5' and 3' ends.

ANSWERS

1. *The function of sigma factor is to recognize and bind (with RNA polymerase) to the promoter region. Different sigma factors recognize different promoter regions.*
2. *RNA polymerase.*
3. *Left to right. The lower DNA strand is the template (or "antisense") strand. Only the template strand is transcribed. Because the newly transcribed strand is antiparallel to its template, the newly made RNA will have its 5' -end on the left and be synthesized by adding new nucleotides to the 3' -end on the right.*
4. *RNAP-sigma can only bind one way to the asymmetric promoter.*
5. *The bottom strand.*
6. *RNAP can only add to the 3'-OH end of the transcript*
7. *5'-AUUGUCGCUAAGGAGGUGCUUGAUGAUCUGGCGAC-----3'*
The middle of the -10 region of the promoter (TATAAT) occurs approximately 10 bases upstream from the actual start of transcription. Count about 10 bases to the right (downstream) to find the

first base that is transcribed.

8. *(see underline) This is the Shine-Dalgarno sequence.*
9. *5'-AUG-3'*
10. *5'-AUC-3' = ile*
11. *3'- UAG-5'*

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Gene Expression: Translation

Shown below is a partial sequence of an RNA molecule.

5' GUACUAAAGGAGGUG¹UGU²GAUGAACCAAGUCUAGGG...

1. Could this be mRNA? Why or why not?
2. What determines whether or not a piece of RNA will be translated?
3. What is meant by "setting the frame"?
4. Would the frame change if you deleted the base marked G¹ on the sequence above? Why or why not?
5. Would the frame change if you deleted the base marked U² on the sequence above? Why or why not?
6. There are at least 61 different kinds of tRNA molecules. What determines which amino acid a given tRNA will carry when it gets acylated?

ANSWERS

1. *Yes, it has a ribosome-binding site (underlined) and an AUG start codon (otherwise translation will not occur).*
2. *It has a ribosome-binding site (underlined) and an AUG start codon (otherwise translation will not occur).*
3. *Determining which three bases make a codon; this is done by the first AUG after the ribosome binding site.*
4. *No, the first AUG and all codons afterwards would be the same*
5. *Yes, by deleting the base in bold all the codons after the deletion change (relative to what you started with).*
6. *One of the loops of the tRNA has a sequence recognized by specific tRNA-acylating enzymes; for example, the enzyme that carries tryptophan only binds with the tRNA with the anticodon for tryptophan.*

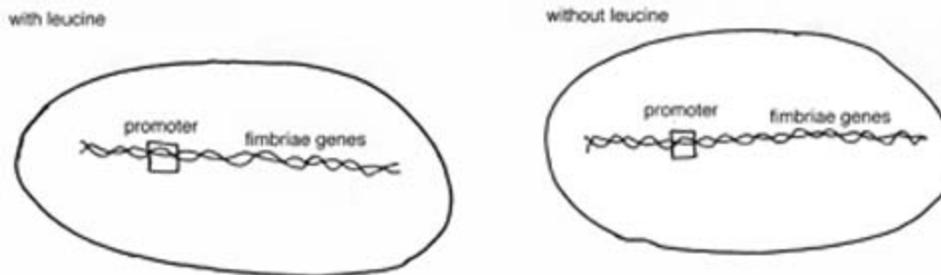
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Regulation of Gene Expression: Induction

When certain pathogenic bacteria find themselves in a high nutrient environment (like an animal's gut),

they begin to make long, thin surface proteins (called fimbriae or adhesins) that they use to attach themselves to the surface of their host (like the wall of a small intestine). Leucine is an amino acid that can trigger such synthesis.

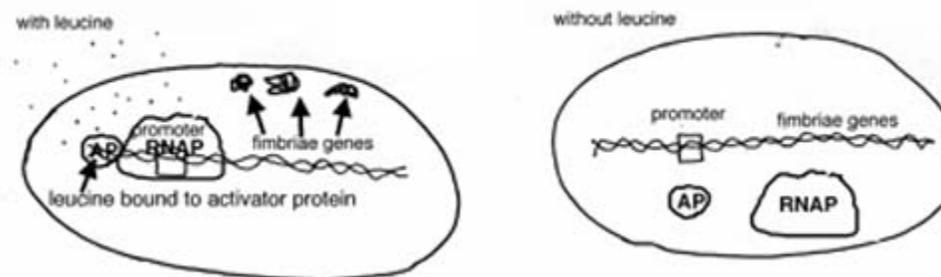
Assume that leucine is the inducer for an activator protein that controls the fimbriae genes. On the diagrams below, draw in the positions of the activator protein and RNA polymerase in the presence and absence of leucine.



ANSWER

In the presence of leucine, leucine binds to the activator protein, changing it to its active conformation. The activator protein can now bind to DNA near the promoter region. Without the activator protein, the promoter is not recognized by RNA polymerase. Now that the activator protein has bound to the DNA, RNAP can bind (with sigma) to the promoter region and transcribe the genes.

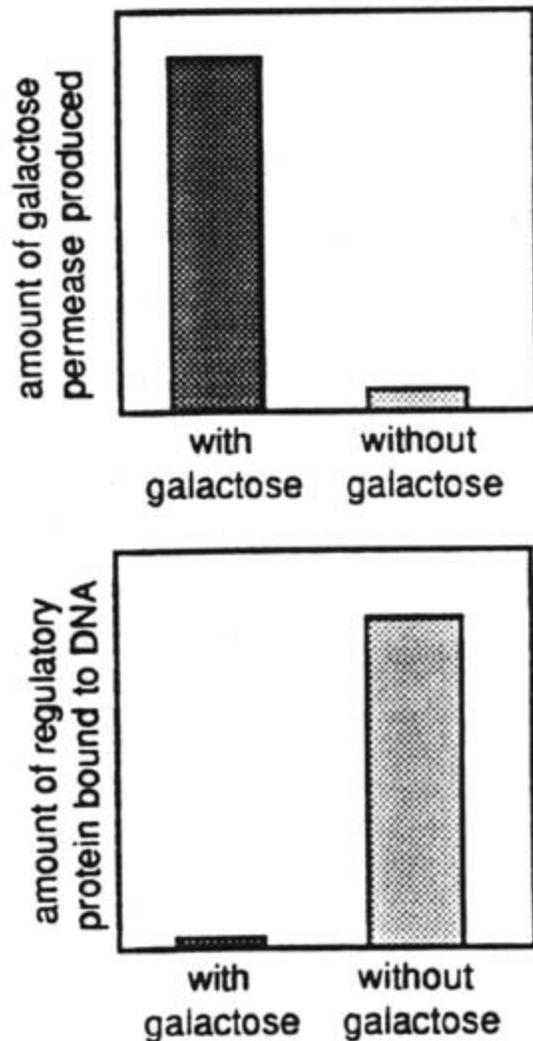
Without leucine, the activator protein does not bind, so RNAP does not bind; therefore the genes are not transcribed.



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Regulation of Gene Expression: Galactose Permease Model

You have found both a regulatory protein that influences galactose uptake and an active transport protein (galactose permease) that is required for *Your favorite yogurt bacterium* to take up galactose. Now, you can do experiments to look at the regulation of galactose permease.



1. Do these data indicate that galactose induces or represses transcription of the galactose permease genes? Why?
2. Do these data indicate that the galactose genes are under positive or negative control? How do you know?
3. Putting together the data from a) and b), explain how this bacterium regulates the production of galactose permease. How does this help the cell?

ANSWERS

1. *Induction, because there is more protein (permease) made in the presence of galactose*
2. *More regulatory protein is bound to DNA when there is no galactose present; therefore, when the regulatory protein binds, protein synthesis decreases. It must be negative control.*
3. *When galactose is present, it binds to the repressor (regulatory) protein, causing the repressor to go into the inactive (non-DNA binding) conformation. The repressor protein cannot bind DNA, therefore, σ^+ RNAP have access to the promoter, and the gene is transcribed. When there is no*

galactose, the repressor is in the active conformation, so it binds DNA near the promoter region and blocks σ^+ RNAP. This way, the genes to use galactose as a carbon and energy source are only transcribed when galactose is present.

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Gene Transfer

You have a strain of kanamycin-resistant *Enterococcus* and a strain of kanamycin sensitive *Streptococcus*. You mix the strains together, leave them overnight, and check them in the morning. Now the *Streptococcus* is resistant to kanamycin. What experiments could you do to determine the mechanism of genetic exchange (transformation, conjugation or transduction)? Assume there was no experimental error, i.e., contamination.

ANSWERS

Transformation is the only process that uses naked (non-protected) DNA. If you added DNase to the cell mixture and did not get an exchange, the process was transformation.

If it is not transformation, try transduction. Transduction can occur without cells, that is, with a cell-free filtrate. If you grew the Enterococcus to stationary then filtered the cells out, you would have a cell-free filtrate. Mix this with the sensitive Streptococcus cells. If they grow up resistant, it was most probably transduction.

If both of the above experiments don't work (i.e., do not produce resistant Streptococcus), it must be conjugation!

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Curriculum Resources

A Collection of In-Class Activities in Microbiology: Cell Growth

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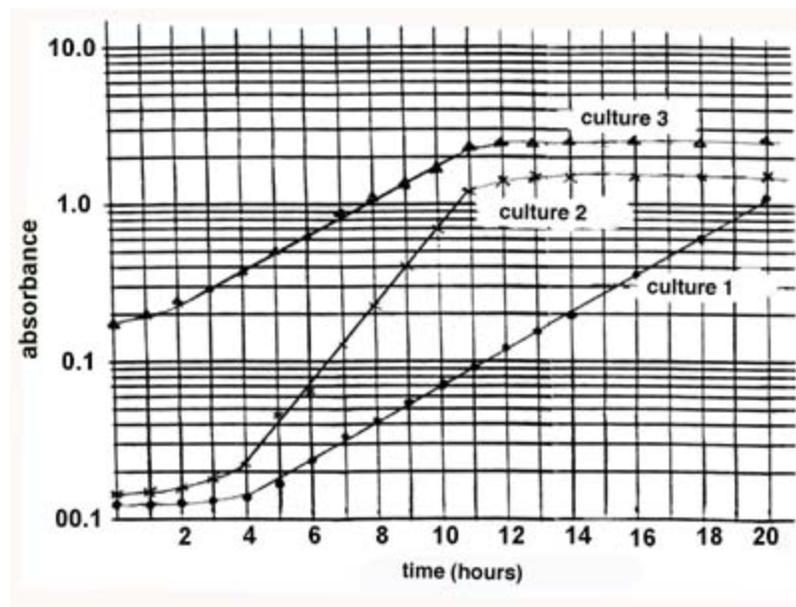
[Nitrogen Utilization](#)

[Respiration and Energy Pathways](#)

[Bacterial Growth in Extreme Environments](#)

Bacterial Growth Curves

Use the growth curve to answer the questions in class.



1. Which culture has the longest lag time?
2. Which culture has the fastest growth rate?
3. Which culture has the shortest doubling time?
4. Which culture has the highest cell density?
5. What is the doubling time of culture 1?
6. What is the growth rate of culture 1?

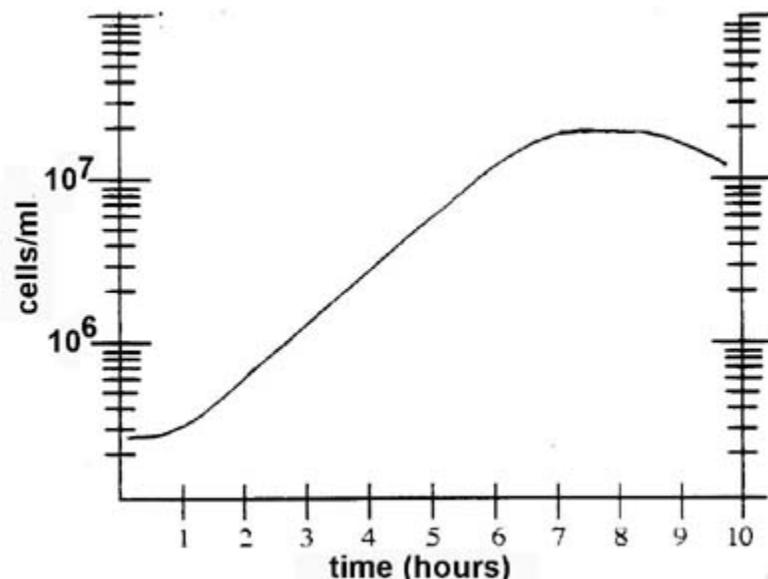
ANSWERS

1. Culture 1
2. Culture 2
3. Culture 2
4. Culture 3
5. About 2.5 hours/generation (5/2 hours/generation)
6. About 0.4 generations/hour (2/5 generations/hour)

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Experimental Growth Curves

To generate the growth curve below, you transferred cells from an old plate in the refrigerator to a flask of sterile, defined, minimal salts medium.



For each experiment, predict how the growth curve would change relative to the curve shown.

1. Make the medium 1 pH unit more acidic than optimal
2. Dilute the growth medium so that all components are half strength
3. Use an inoculum of exponentially growing cells
4. Omit all N from the medium

ANSWERS

1. *Slope decreases*
2. *Cell yield decreases by half*
3. *No lag time*
4. *No growth*

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Bacterial Culture Media

Mannitol sugar agar is often used to distinguish between different species of *Staphylococcus*, a gram positive bacterium that is well adapted to living on dry, salty skin. Disease-causing strains of *Staphylococcus* ferment mannitol; non-pathogenic strains cannot use mannitol.

Ingredient	Function	Amount
------------	----------	--------

Beef extract	soluble components of beef tissue	1 g
Peptone	enzymatic digest of meat proteins	10 g
NaCl	inhibits growth of most salt-intolerant cells	75 g
Mannitol	6-C alcohol	10 g
Phenol red	pH indicator: is red at pH 7; turns yellow at low pH	0.02g
Agar		15 g
Water		1000 ml

1. Is this medium complex or synthetic?
2. Is it selective? Is it differential? Why?
3. Why would this medium be useful in a clinical setting?

ANSWERS

1. *Complex, because it has beef extract and peptone, two ingredients with unknown components.*
2. *Yes, it is selective because it has NaCl to select for salt tolerant bacteria. Yes, it is differential because it has phenol red to differentiate fermenting cells from non-fermenting cells.*
3. *By growing mixed cultures from patients, you could select for Staphylococcus and pick up pathogenic strains by looking for red (fermenting) strains.*

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Bacterial Growth Media

Success in microbiology often depends on being able to differentiate one bacterium from another when a mixture of bacteria are plated on a specific growth media.

The recipe for MacConkey agar is given below. MacConkey agar is often used to differentiate *E. coli* from other gram negative rods.

Peptone	17 g
Proteose peptone	3 g
Lactose	10 g
Bile salts (inhibits growth of gram positive cells)	1.5 g
NaCl	5 g
Neutral red (pH indicator, red at low pH)	0.03 g
Crystal violet (inhibits growth of gram positive cells)	0.001 g
Agar	13.5 g
Water	1 liter

a. Suppose you plated a mixed culture of *E. coli*, *Bacillus*, and *Streptococcus* onto a MacConkey agar plate. Describe what that plate would look like after an appropriate incubation period.

b. It turns out that *E. coli* strain O157:H7 is unique among other strains of *E. coli* because strain O157:H7

cannot ferment sorbitol. (Most strains of *E. coli* have the enzymes to ferment this sugar). Describe how you could change the recipe of MacConkey agar to specifically test for strain O157:H7 and describe the appearance of this strain on both plates. What would other strains of *E. coli* look like? Why would this be useful?

regular MacConkey agar your modified MacConkey agar

ANSWERS

a. Bacillus and Streptococcus would not grow because of the bile salts that inhibit the growth of nonenterics. E.coli would grow and ferment lactose, produce acid, and appear red.

b. Substitute sorbitol for lactose. You would get large red colonies on lactose (cells ferment lactose) and small white colonies on sorbitol (growing only on peptone).

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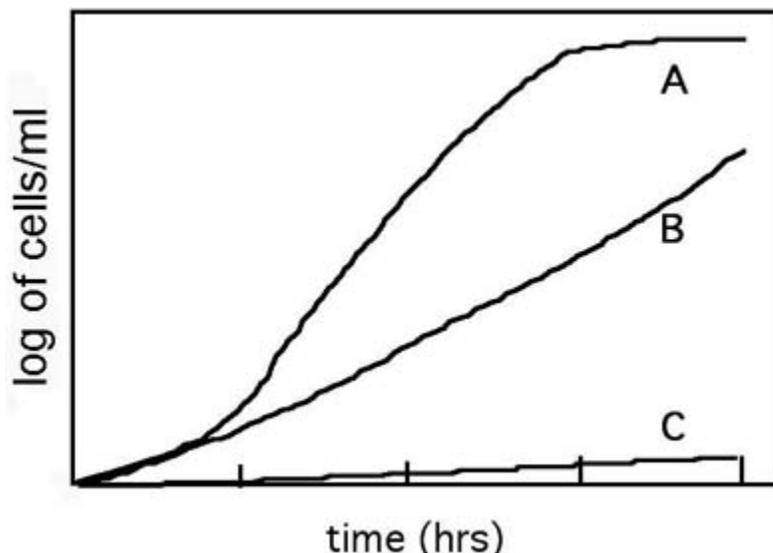
Nitrogen Utilization

Working with a purple bacterium, your next set of experiments investigates nitrogen utilization. You grow the cells in three tubes of the following medium, with no other nitrogen source except:

Acetate	0.2 g
K ₂ HPO ₄	0.5g
MgSO ₄	0.2 g
CaSO ₄	0.1 g
CaCO ₃	5.0 g
Water	1 liter

- Tube 1: add N₂; grow under oxic conditions
- Tube 2: add N₂; grow under anoxic conditions
- Tube 3: add NH⁴⁺; grow under anoxic conditions

You monitor their growth over time, and get the graph below.



Unfortunately, your lab partner forgot to label which tube was which. Looking at the data, predict which graph (A, B, or C) came from which treatment (tube 1, 2, or 3) . Explain your reasoning.

ANSWERS

Graph A came from tube 3 because these cells can grow using the NH_4^+ , with only the cost of transport.

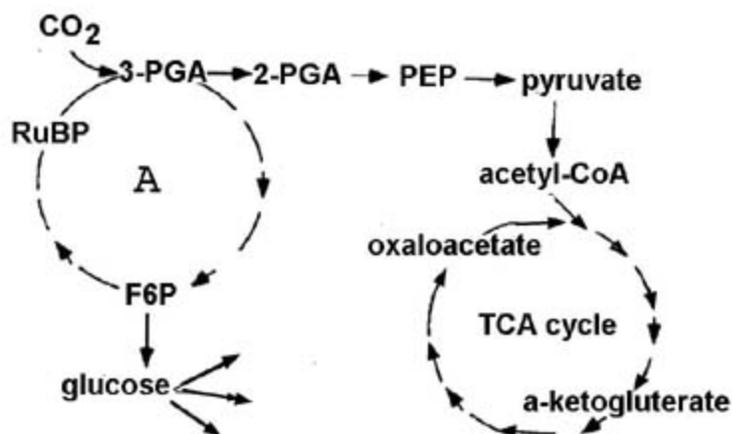
Graph B came from tube 2 because the cells were able to grow by fixing nitrogen, but they had to use a lot of ATP to fix the nitrogen, so could not grow as fast as cells that were just taking up NH_4^+ .

Graph C came from tube 1 because the only nitrogen source is N_2 , and these bacteria cannot fix nitrogen when oxygen is present.

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Respiration and Energy Pathways

You have isolated a new cyanobacterium, and determined that it is an obligate phototroph. Further studies show it has the enzymes for the pathways below.



a) In your new bacterium, what is the purpose of Cycle A?

b) In your new bacterium, what is the purpose of the TCA cycle?

ANSWERS

a) *To fix CO₂ into organic carbon for biosynthesis*

b) *To make precursors for biosynthesis (amino acids, nucleic acids, cell wall, etc.)*

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Bacterial Growth in Extreme Environments

a) Why can't psychrophiles live above 10°C?

Why can't mesophilic cells live below 10°C?

b) Why can't mesophilic cells live above 50°C?

Why can't thermophilic cells live below 50°C?

c) Why is a high pH environment particularly challenging for respiring bacteria? How do some bacteria cope?

d) Strictly anaerobic bacteria are killed by oxygen.

What makes O₂ so toxic to strict anaerobes? How do aerobes overcome this? Why can't strict anaerobes just keep O₂ out? (How does it get into the cell?)

e) Many bacteria have high intracellular concentrations of potassium (K⁺), usually more than 100X the environmental concentration. Some bacteria tolerate fluctuations of Na⁺ levels by accumulating more or less K⁺ as needed. In these bacteria, would you expect to find the most transcription of K⁺ transport proteins in relatively high Aw or low Aw? Explain.

ANSWERS

a) *Proteins denature; membranes get too fluid (melt).*

Enzymes slow; membranes get too solid (gel).

b) *Proteins denature; membranes get too fluid (melt).*

Enzymes slow; membranes get too solid (gel)/

c) *They need a high concentration of H⁺ outside their membranes to generate energy. They have to pump lots of protons per ATP made.*

d) *Reactive oxygen compounds (radicals) alter enzymes and nucleic acids. Aerobes overcome this problem with catalase and peroxidase enzymes that destroy peroxides and other reactive oxygen compounds.*

O₂ diffuses freely through the membrane.

e) *A low Aw means there is a lot of salt in the environment. Under these conditions, cells would have to import K⁺ to counteract the high concentration of Na⁺ and would need to turn-on genes for potassium transporters.*

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Curriculum Resources

A Collection of In-Class Activities in Microbiology: Immunology and Medical Microbiology

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[Immune Response: Antibody Titer and Vaccination](#)

[Viral Diseases: Potential Drug Therapy](#)

Mechanisms of Virulence

1. For each virulence factor below, describe whether it contributes to increased virulence by being invasive or toxic or both, and how it helps the pathogen to overcome a host defense mechanism.

- i) *Neisseria gonorrhoea* produces pili and adhesins specific to the human urogenital epithelium.
- ii) The pilin genes in *Neisseria gonorrhoea* periodically combine.
- iii) Many *Streptococcus* strains coat themselves in a slimy glycocalyx.
- iv) *Staphylococcus aureus* can synthesize hemolysins.
- v) *Chlamydia* infects a host phagocyte and prevents lysosome fusion.
- vi) *Staphylococcus aureus* carriers are very susceptible to wound infection after undergoing surgery.
- vii) *Clostridium perfringens* spores can germinate deep within wounds, where they release a protein that moves along muscle fibers killing all host cells.

Explain how, or if, antibodies help fight each of the virulence factors above.

ANSWERS

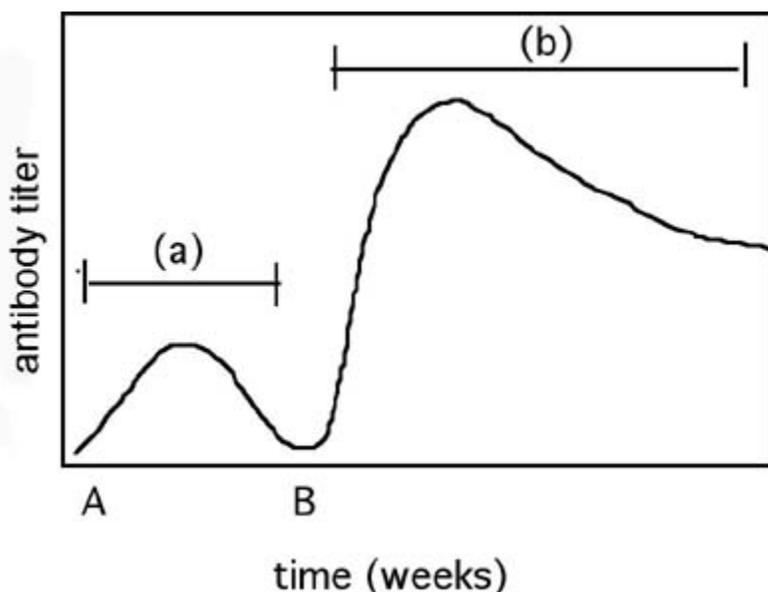
1.
 - i) *Invasive; attachment evades rinsing mechanisms (innate defense).*
 - ii) *Invasive; antigenic shift helps evade the humoral response.*
 - iii) *Invasive; capsules help evade phagocytosis and allows cells more time to spread.*
 - iv) *Invasive and toxic; hemolysins kill host cells (like phagocytic cells) allowing more time to spread, hemolysins could also damage tissue cells.*
 - v) *Invasive; lets pathogen grow and spread inside phagocyte.*
 - vi) *Invasive; resistance to drying and salt allows Staphylococcus to live on the skin surface, human induced breaks in the wound allow Staphylococcus to get past the skin barrier.*
 - vii) *Toxic; the toxin allows Clostridium to cause tissue damage and inhibit white blood cells.*
2.
 - i) *Antibodies that bind to pili or adhesins prevent cells from attaching to host surfaces.*
 - ii) *Antibodies can't do much about antigenic shift, except respond to the new antigens.*
 - iii) *If antibodies could bind to the polysaccharides they would help label the pathogen as "foreign."*
 - iv) *Antibodies to the toxin could interfere with its binding to host cells.*
 - v) *Anti-Chlamydial antibodies could bind to the surface proteins required for preventing lysosome fusion.*
 - vi) *Anti-Staphylococcal antibodies would label the cells as "foreign" and allow phagocytic cells to destroy them more effectively.*
 - vii) *Anti-toxin antibodies could neutralize the toxin.*

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Immune Response: Primary and Secondary

The objective of this exercise is to help you distinguish between a primary and secondary immune response, and to identify the cell and antibodies involved with these events.

The following graph represents a typical primary and secondary immune response.



1. What does the Y-axis (antibody titer) represent?
2. About how long does (a) last
3. What type of white blood cell produces antibodies in (a) and (b)?
4. What type(s) of antibodies are being produced in (a)?
5. What type(s) of antibodies are being produced in (b)?
6. Why is the response in (b) so much higher than the response in (a)?
7. Does the response in (b) occur EVERY TIME you get exposed to the same pathogen twice? For EVERY PATHOGEN? Why or why not?

ANSWERS

1. *It represents the relative amount of antibody in the blood for that pathogen.*
2. *Weeks to months*
3. *Plasma cells*
4. *IgM*

5. IgG

6. *Memory cells are made in (a) that can respond in (b). They make the response faster and stronger.*
7. *The response is not based on the pathogen, but on the antigenic determinants of the pathogen. If you get exposed to a flu virus you have seen before, then you will have memory cells that recognize the antigen and fight the infection. But if you get a new strain of flu with new antigens, you will not have the appropriate memory cells, and you will have to mount a primary response.*

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Immune Response: Humoral

A. Put the following steps in the humoral immune response in order (1=first, 6=last):

- 1 Antibody on surface of B cells binds antigen.
 Antigen breakdown products bind major histocompatibility complex protein and are "displayed" on B cell surface.
 T cell secretes cytokines which stimulate B cell.
 Antigen-antibody complex is internalized and processed.
 Helper T cell binds antigen via its T-cell receptor.
 B cell divides and differentiates into plasma cells and memory cells.

B. Which steps ensure that this particular immune response will be specific for the current pathogen, and not some other pathogen?

ANSWERS

A.

- 1 Antibody on surface of B cells binds antigen.
 3 Antigen breakdown products bind MHC protein and are "displayed" on B cell surface
 5 T cell secretes cytokines which stimulate B cell
 2 Antigen-antibody complex is internalized and processed
 4 Helper T cell binds antigen via its T-cell receptor
 6 B cell divides and differentiates into plasma cells and memory cells.

B. Antibody on surface of B cells binds antigen.
 Helper T cell binds antigen via its T-cell receptor.

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Immune Response: Antibody Titer and Vaccination

The following questions refer to the measles (but they could be altered to fit most any disease):

1. People who recover from the measles have a high anti-measles antibody titer.
- What does "high anti-measles antibody titer" mean?
 - Which immune response generates antibody?
 - How do viruses (which are intracellular pathogens) stimulate antibody production?
 - About how many different kinds of antibodies are produced in a measles infection? (0, 1,

10's, 1000's, millions)? Explain how you came to your estimate.

2. A generation ago, doctors routinely began vaccinating every child against measles.
 - a. About how long does it take a human host to launch a *specific* immune response when first exposed to a pathogen or virus?
 - b. Why does it take so much longer than the *nonspecific* cellular responses? (What prohibits the specific response from being faster?)
3. How does a measles vaccine prevent us from coming down with the measles disease? Does the vaccine prevent us from being infected, or do vaccines prevent infection from progressing to disease? How?
4. The measles vaccine is an *attenuated* strain of the measles virus. What does this mean? Why would an attenuated strain be more effective against a measles infection than a "killed" or nonreplicating virus?
5. Why are the children of women who have been vaccinated *more* at risk for getting measles than the children of women who had the measles disease?
6. Women are able to pass antibodies to their newborns through breast feeding. Is this considered passive or active immunity?
7. The measles virus is spread through respiratory droplets. Would you expect that only a few people would need to be immunized to protect the population or most of the population?
8. Doctors are concerned about too little use of the vaccine among pre-school children. What is not being achieved?
9. The genetic information carried by the measles virus includes genes for:
 - an enzyme involved in particle assembly,
 - protein H (which helps the virus attach to host cells), and
 - protein F (which helps release viral RNA into the host cell).

If you were to develop a synthetic-peptide vaccine, which of these proteins would you choose for a target? Why?

ANSWERS

1.
 - a. *Their blood serum contains a large number of antibodies that recognize and bind to the measles virus (as measured by an enzyme-linked immunosorbent assay or fluorescent antibody test)*
 - b. *Humoral.*
 - c. *Two ways: during the infection, some viral particles circulate in the blood and lymph systems. These are picked up by macrophages, digested, and presented. Also, during the course of infection, viral particles are destroyed and pieces of virus get filtered through the lymph system into the nodes where they are picked up by B cells.*

d. Each measles protein (of which there are a few) could stimulate a few different antibodies because most average-sized proteins have multiple antigenic determinants (many sites to which different antibodies bind). This should stimulate 10 to 100 different B cells, and therefore make 10 to 100 of different antibodies. Viruses have fewer surface proteins than bacteria.

2.

a. 10 to 14 days

b. Many specific interactions must take place: 1 in 10^5 helper T cells must match up with a macrophage that is presenting antigen; 1 in 10^5 B cells must bind free-floating antigen; those rare helper T and B cells that recognize antigen must find each other to further stimulate the B cell to divide and differentiate into a plasma cell.

3. Vaccines prevent infection from developing into disease by stimulating the specific immune responses to make memory cells (helper T, cytotoxic T, and B cells) that recognize antigen on the pathogen. When the pathogen itself invades, the secondary immune response will defeat it.

4. Attenuated means the strain is less-virulent, or in this case, non-virulent. The strain could still be infectious, that is, it could still have the ability to get inside host cells, but does not cause the disease. This would allow stimulation of the cell-mediated response (the primary weapon for fighting intracellular parasites) which killed virus would not do.

5. The goal of vaccine design is to make the vaccine as much like the pathogen as possible without causing damage, but nothing mimics the pathogen like the pathogen itself. Therefore, the best memory response is stimulated by getting and recovering from, the disease. Assuming that we are continually exposed to measles virus (which is probably true if you have kids), women who had the disease would have a stronger response than women who had the vaccine.

6. Passive. During the first few months of life, a baby's immune response is weak that even if they are exposed to antigens (which they are) they cannot mount a strong response. Antibodies passed through mother's milk is one of the only protections a newborn has against disease (a strong argument for breast feeding babies!).

7. Most of the population, because spread doesn't require contact.

8) Herd immunity, don't just vaccinate your kids so they don't get sick, it's important to vaccinate your kids so others don't get sick!

9) Protein H, this is prominently displayed on the surface of the virus, and is most likely to be detected by antibodies. Also, it would help stop the virus from infecting host cells.

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Viral Diseases: Potential Drug Therapy

The goal of this activity is to get you to review the viral diseases we talked about in class and to think about how knowledge of their replication cycle can be useful to humans.

You are a scientist working on drug research at a major pharmaceutical company. Your task is to design a drug that will stop polio, influenza, and HIV. Describe a potential target for each disease and explain why you chose that target.

ANSWERS

Ideas include any component that is unique to the virus and not the host cell, e.g., viral polymerases, surface proteins required for infection and proteases required for viral protein processing.

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