Cellular Respiration and Fermentation

This lab topic gives you another opportunity to practice the scientific process. Be prepared to use this information to design an experiment in fermentation or cellular respiration.

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Describe alcoholic fermentation, naming reactants and products.
2. Describe cellular respiration, naming reactants and products.
3. Explain oxidation/reduction reactions in cellular respiration.
4. Name and describe environmental factors that influence enzymatic activity.
5. Explain spectrophotometry and describe how this process can be used to measure aerobic respiration.
6. Propose hypotheses and make predictions based on them.
7. Design and execute an experiment testing factors that influence fermentation or cellular respiration.
8. Practice scientific persuasion and communication by analyzing and interpreting experimental results.

Introduction

You have been investigating cells and their activities: enzymatic activities, cellular structure and evolution, and movement across cell membranes. This lab topic investigates energy transformations in cells. Photosynthesis is the process of transferring the sun's radiant energy to organic molecules, namely, glucose (Figure 1). This lab topic investigates **fermentation** and **cellular respiration**, cellular processes that transfer the energy in glucose bonds to bonds in **adenosine triphosphate** (ATP). The energy in ATP can then be used to perform cellular work. Fermentation is an anaerobic (without oxygen) process; cellular respiration is aerobic (utilizing oxygen). *All living organisms, including bacteria, protists, plants, and animals, produce ATP in fermentation or cellular respiration and then use ATP in their metabolism.*

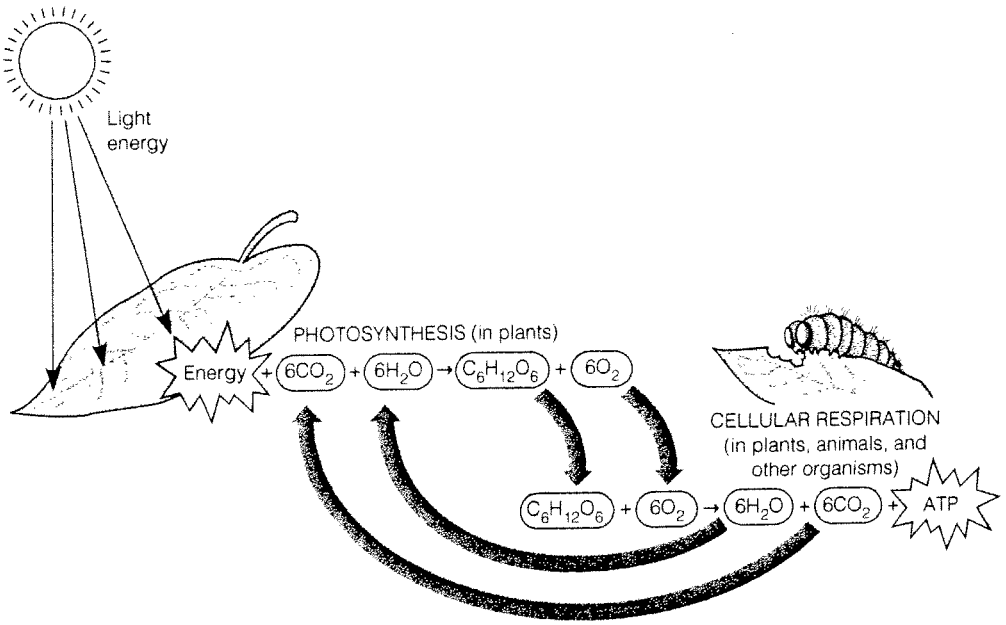

Figure 1.
Energy flow through photosynthesis and cellular respiration. Light energy from the sun is transformed to chemical energy in photosynthesis. Carbon dioxide and water are converted to glucose and oxygen. The energy stored in plant organic molecules—glucose, for example—can be utilized by plants or by consumers. The energy in organic molecules is released during cellular respiration in plants, animals, and other organisms.

Fermentation and cellular respiration involve oxidation-reduction reactions (redox reactions). Redox reactions are always defined in terms of electron transfers, oxidation being the *loss* of electrons and reduction the *gain* of electrons. In cellular respiration, two hydrogen atoms are removed from glucose (oxidation) and transferred to a coenzyme called nicotinamide adenine dinucleotide (**NAD^+**), reducing this compound to **NADH.** Think of these two hydrogen atoms as 2 electrons and 2 protons. NAD^+ is the oxidizing agent that is reduced to NADH by the addition of 2 electrons and one proton. The other proton (H^+) is released into the cell solution. NADH transfers electrons to the electron transport chain. The transfer of electrons from one molecule to another releases energy, and this energy can be used to synthesize ATP.

Cellular respiration is a sequence of three metabolic stages: **glycolysis** in the cytoplasm, and the **Krebs cycle** and the **electron transport chain** in mitochondria (Figure 2). Fermentation involves glycolysis but does not involve the Krebs cycle and the electron transport chain, which are inhibited at low oxygen levels. Two common types of fermentation are **alcoholic fermentation** and **lactic acid fermentation.** Animals, certain fungi, and some bacteria convert pyruvate produced in glycolysis to lactate. Plants and some fungi, yeast in particular, convert pyruvate to ethanol and carbon dioxide. Cellular respiration is much more efficient than fermentation in producing ATP. Cellular respiration can produce a maximum of 38 ATP molecules; fermentation produces only 2 ATP molecules.

Before you begin today's lab topic, refer to the preceding paragraph and Figure 2 as you review major pathways, reactants, and products of fermentation and cellular respiration by answering the following questions:

1. Which processes are anaerobic?

2. Which processes are aerobic?

3. Which processes take place in the cytoplasm of the cell?

4. Which processes take place in mitochondria?

5. What is the initial reactant in cellular respiration?

6. What is (are) the product(s) of the anaerobic processes?

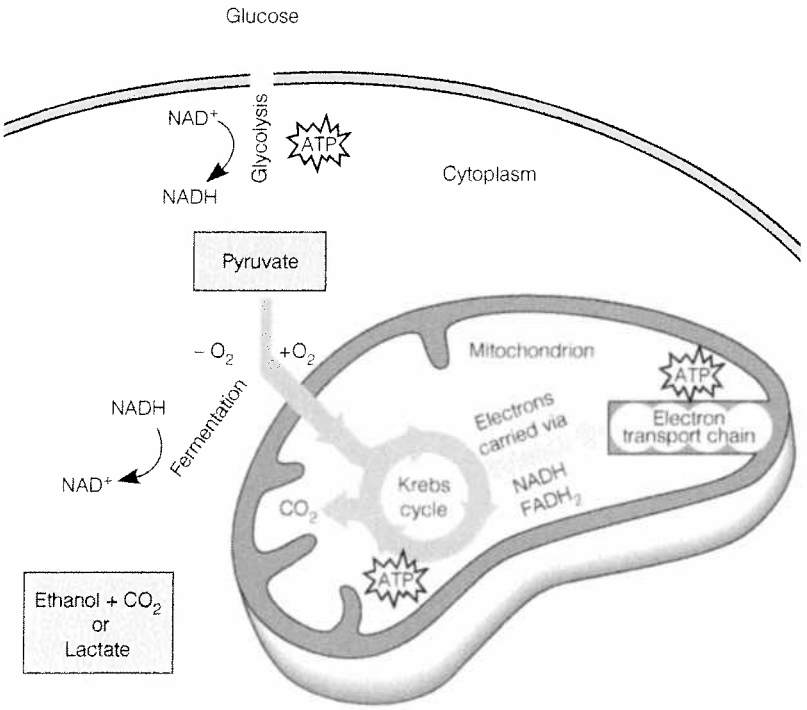

Figure 2.
Stages of cellular respiration and fermentation. Cellular respiration consists of glycolysis, the Krebs cycle, and the electron transport chain. Glycolysis is also a stage in fermentation.

7. What is (are) the product(s) of the aerobic processes?

8. Which gives the greater yield of ATP, alcoholic fermentation or cellular respiration?

In this lab topic you will investigate alcoholic fermentation first and then cellular respiration. Working in teams of two to four students, you will first perform two introductory lab studies (Lab Study A of each exercise). Lab Study B in each exercise provides questions and background to help you propose one or more testable hypotheses based on questions from the lab studies or your prior knowledge. Your team will then design and carry out an independent investigation based on your hypotheses, completing your observations and recording your results in this laboratory period. After discussing the results, your team will prepare an oral presentation in which you will persuade the class that your experimental design is sound and that your results support your conclusions. If required to do so by the lab instructor, *each of you* independently will submit Results and Discussion sections describing the results of your experiment.

First complete Lab Study A in each exercise. Then discuss possible questions for investigation with your research team. Be certain you can pose an interesting question from which to develop a testable hypothesis. Design and perform the experiment today. Prepare to report your results in oral and/or written form.

EXERCISE 1
Alcoholic Fermentation

For centuries, humans have taken advantage of yeast fermentation to produce alcoholic beverages and bread. Consider the products of fermentation and their roles in making these economically and culturally important foods and beverages. Alcoholic fermentation begins with glycolysis, a series of reactions breaking glucose into two molecules of **pyruvate** with a net yield of 2 ATP and 2 NADH molecules. In anaerobic environments, in two steps the pyruvate (a 3-carbon molecule) is converted to ethyl alcohol (ethanol, a 2-carbon molecule) and CO_2. In this process the 2 NADH molecules are oxidized, replenishing the NAD^+ used in glycolysis (Figure 2).

Lab Study A. Alcoholic Fermentation in Yeast

Materials

4 respirometers:
  test tubes, 1-mL graduated pipettes, aquarium tubing, flasks, binder clips
pipette pump
3 5-mL graduated pipettes, labeled "DI water," "yeast," and "glucose"
3-inch donut-shaped metal weights
yeast solution
glucose solution
DI water
water bath
wax pencil

Introduction

In this lab study, you will investigate alcoholic fermentation in a yeast (a single-celled fungus), *Saccharomyces cerevisiae,* or "baker's yeast." When oxygen is low, some fungi, including yeast and most plants, switch from cellular respiration to alcoholic fermentation. In bread making, starch in the flour is converted to glucose and fructose, which then serve as the starting compounds for fermentation. The resulting carbon dioxide is trapped in the dough, causing it to rise. Ethanol is also produced in bread making but evaporates during baking.

In this laboratory experiment, the carbon dioxide (CO_2) produced, being a gas, bubbles out of the solution and can be used as an indication of the relative rate of fermentation taking place. Figure 3 shows the respirometers you will use to collect CO_2. The rate of fermentation, a series of enzymatic reactions, can be affected by several factors, for example, concentration of yeast, concentration of glucose, or temperature. In this lab study you will

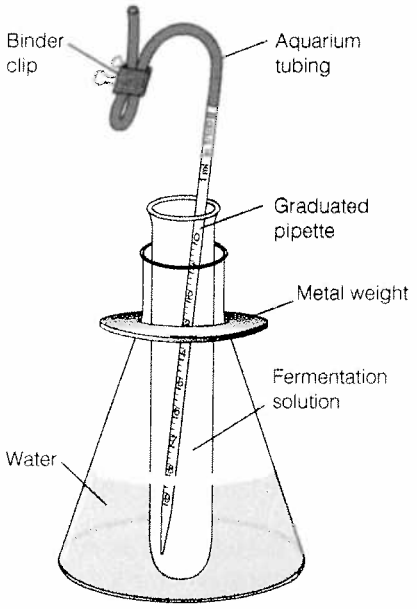

Figure 3.
Respirometer used for yeast fermentation.

investigate *the effects of yeast concentration.* In your independent study you may choose to investigate other independent variables.

Hypothesis

Hypothesize about the effect of different concentrations of yeast on the rate of fermentation.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Obtain four flasks and add enough tap water to keep them from floating in a water bath (fill to about 5 cm from the top of the flask). Label the flasks 1, 2, 3, and 4. To stabilize the flasks, place a 3-inch donut-shaped metal weight over the neck of the flasks.
2. Obtain four test tubes (fermentation tubes) and label them 1, 2, 3, and 4. Add solutions as in Table 1 to the appropriate tubes. Rotate each tube to distribute the yeast evenly in the tube. Place tubes in the corresponding numbered flasks.
3. To each tube, add a 1-mL graduated pipette to which a piece of plastic aquarium tubing has been attached.
4. Place the flasks with the test tubes and graduated pipettes in the water bath at 30°C. Allow them to equilibrate for about 5 minutes.

Table 1
Contents of Fermentation Solutions (volumes in mL)

Tube	DI Water	Yeast Suspension	Glucose Solution
1	4	0	3
2	6	1	0
3	3	1	3
4	1	3	3

5. Attach the pipette pump to the free end of the tubing on the first pipette. Use the pipette pump to draw the fermentation solution up into the pipette. Fill it past the calibrated portion of the tube, but do not draw the solution into the tubing. Fold the tubing over and clamp it shut with the binder clip so the solution does not run out. Open the clip slightly, and allow the solution to drain down to the 0-mL calibration line (or slightly below). If the level is below the zero mark, open the clamp slightly while another student adjusts the level using the pipette pump. Be patient.

This may require a couple attempts! Quickly do the same for the other three pipettes.

6. In Table 2, quickly record your initial readings for each pipette in the "Initial reading" row in each "Actual (A)" column. This will be the *initial* time (I).
7. Two minutes after the initial readings for each pipette, record the actual readings (A) in mL for each pipette in the "Actual (A)" column. Subtract I from A to determine the total amount of CO_2 evolved (A – I). Record this value in the "CO_2 Evolved (A – I)" column. *From now on, you will subtract the initial reading from each actual reading to determine the total amount of CO_2 evolved.*
8. Continue taking readings every 2 minutes for each of the solutions for 20 minutes. Remember, take the actual reading from the pipette and subtract the initial reading to get the total amount of CO_2 evolved in each test tube.
9. Record your results in Table 2.

Results

1. Complete Table 2.

Table 2
Total CO_2 Evolved by Different Concentrations of Yeast. Actual values are the graduated pipette readings. For CO_2 evolved values, subtract the initial reading from the actual reading. This is the amount of CO_2 accumulated over time.

Time (min)	Tube 1		Tube 2		Tube 3		Tube 4	
	Actual (A)	CO_2 Evolved (A – I)	Actual (A)	CO_2 Evolved (A – I)	Actual (A)	CO_2 Evolved (A – I)	Actual (A)	CO_2 Evolved (A – I)
Initial reading (I)								
2								
4								
6								
8								
10								
12								
14								
16								
18								
20								

2. Using Figure 4, construct a graph to illustrate your results.
   a. What is (are) the independent variable(s)? Which is the appropriate axis for this variable?

   b. What is the dependent variable? Which is the appropriate axis for this variable?

   c. Choose an appropriate scale and label the *x* and *y* axes.
   d. Should you use a legend? If so, what would this include?

   e. Compose a figure title.

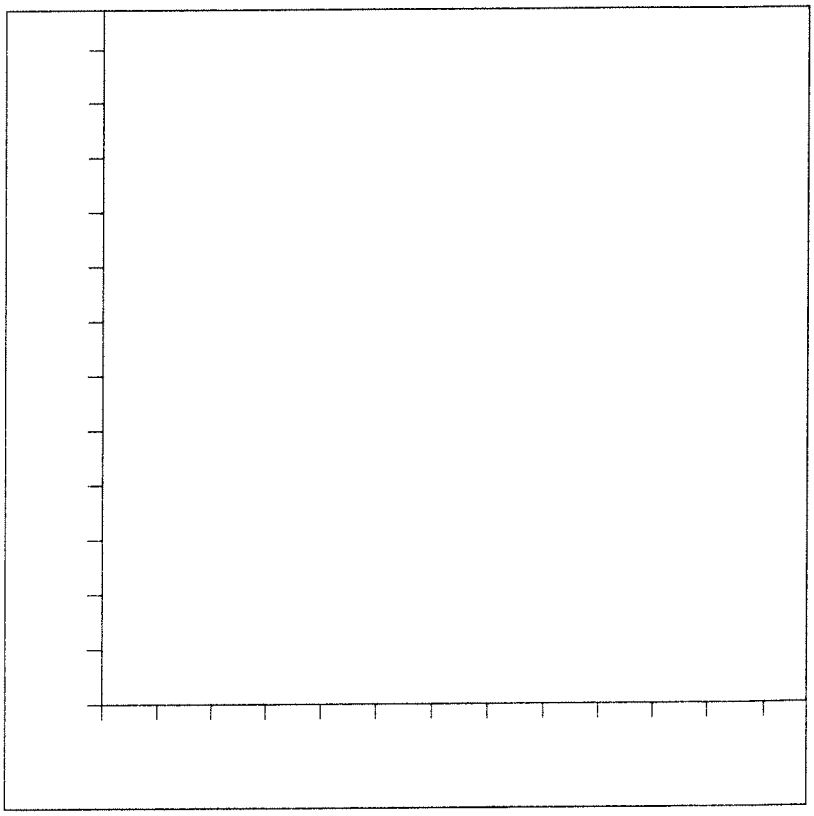

Figure 4.

Discussion

1. Explain the experimental design. What is the purpose of each test tube? Which is (are) the control tube(s)?

2. Which test tube had the highest rate of fermentation? Explain why.

3. Which test tube had the lowest rate of fermentation? Explain why.

4. Why were different amounts of water added to each fermentation solution?

Lab Study B. Additional Investigations of Alcoholic Fermentation

Materials

all materials from Lab Study A
beakers
graduated pipettes of various sizes
different substrates: sucrose, saccharin, Nutrasweet™, Splenda™, fructose, starch, glycogen, honey, corn syrup, pyruvate
different types of yeast: dry active, quick rise, Pasteur champagne (for wine making)
various fermentation inhibitors: sodium fluoride, ethyl alcohol, Na benzoate
various salt solutions
various pH buffers
spices: ground cinnamon, cloves, caraway, ginger, cardamom, nutmeg, mace, thyme, dry mustard, chili powder, cayenne pepper
disposable gloves
additional glassware

Introduction

If your team chooses to study alcoholic fermentation for your independent investigation and report, design a simple experiment to investigate some factor that affects alcoholic fermentation. Use the available materials or ask your instructor about the availability of additional materials.

Procedure

1. Collaborating with your research team, read the following potential questions, and choose a question to investigate using this list or an idea from your prior knowledge. You may want to check your text and other sources for supporting information. You should be able to explain the rationale behind your choice of question. For example, if you choose to investigate *starch* as a substrate, you should be able to explain that the yeast must first digest starch before the glucose can be used in alcoholic fermentation and the impact this might have on the experiment.
   a. Would other substrates be as effective as glucose in alcoholic fermentation? Possible substrates:
      sucrose (table sugar—glucose and fructose disaccharide)
      honey (mainly glucose and fructose)
      corn syrup (fructose and sucrose)
      starch (glucose polymer in plants)
      saccharin, Equal™, Splenda™
      fructose
      pyruvate
   b. Would fermentation rates change with different types of yeasts?
   c. What environmental conditions are optimum for alcoholic fermentation?
      What temperature ranges?
      What pH ranges?
   d. What is the maximum amount of ethyl alcohol that can be tolerated by yeast cells?

If you select toxins or fermentation inhibitors for your investigation, ask the instructor about safety procedures. Post safety precautions and follow safety protocol, including wearing gloves and protective eyewear. Notify the instructor of any spills.

   e. Sodium fluoride, commonly used to prevent tooth decay, inhibits an enzyme in glycolysis. At what concentration is it most effective?
   f. Would adding $MgSO_4$ enhance glycolysis? $MgSO_4$ provides Mg^{++}, a cofactor necessary to activate some enzymes in glycolysis.
   g. Does a high concentration of sucrose inhibit fermentation?
   h. An old German baker's wisdom says, "A pinch of ginger will make your yeast work better." Some spices enhance yeast activity while others inhibit it (Corriher, 1997). What effect do spices have on yeast activity? Try ginger, ground cardamom, caraway, cinnamon, mace, nutmeg, thyme, dry mustard, cayenne pepper or others.
   i. Salt is often used as a food preservative to prevent bacterial and fungal growth (for example, in country ham). But salt is also important to enhance the flavor of bread when added in small amounts. At what concentration does salt begin to inhibit yeast fermentation?
   j. Does the food preservative Na benzoate inhibit cellular respiration?

2. Design your experiment, proposing hypotheses, making predictions, and determining procedures as instructed in Exercise 3.

EXERCISE 2

Cellular Respiration

Most organisms produce ATP using cellular respiration, a process that involves glycolysis, the Krebs cycle, and the electron transport chain. In cellular respiration, many more ATP molecules are produced than were produced in alcoholic fermentation (potentially 38 compared to 2), and water, unlike ethanol, is not toxic to the cells. After the series of reactions in the cytoplasm (glycolysis), pyruvate enters the mitochondria, where enzymes for the Krebs cycle and the electron transport chain are located. The Krebs cycle is a series of eight steps, each catalyzed by a specific enzyme. As one compound is converted to another, CO_2 is given off and hydrogen ions and electrons are removed. The electrons and hydrogen ions are passed to NAD^+ and another electron carrier, FAD (flavin adenine dinucleotide). NADH and $FADH_2$ carry the electrons to the electron transport chain, where the electrons pass along the chain to the final electron acceptor, oxygen. In the process, ATP molecules are produced (Figure 2).

Lab Study A. Oxidation-Reduction Reactions in a Mitochondrial Suspension

Materials

mitochondrial suspension
succinate
buffer
DPIP solution
1-mL graduated pipette
pipette pump
4 cuvettes or small test tubes
Parafilm® squares
Kimwipes®
spectrophotometer
wax pencil

Introduction

In this lab study, you will investigate cellular respiration in isolated mitochondria. Your instructor has prepared a mitochondrial suspension from pulverized lima beans. The suspension has been kept on ice to prevent enzyme degradation, and the Krebs cycle will continue in the mitochondria as in intact cells. Sucrose has been added to the mitochondrial suspension to maintain an osmotic balance.

One step in the Krebs cycle is the enzyme-catalyzed conversion of succinate to fumarate in a redox reaction. In intact cells, succinate loses hydrogen ions and electrons to FAD, and, in the process, fumarate is formed (Figure 5).

We will utilize this step in the Krebs cycle to study the rate of cellular respiration under different conditions. To perform this study, we will add a substance called DPIP (di-chlorophenol-indophenol), an electron acceptor that

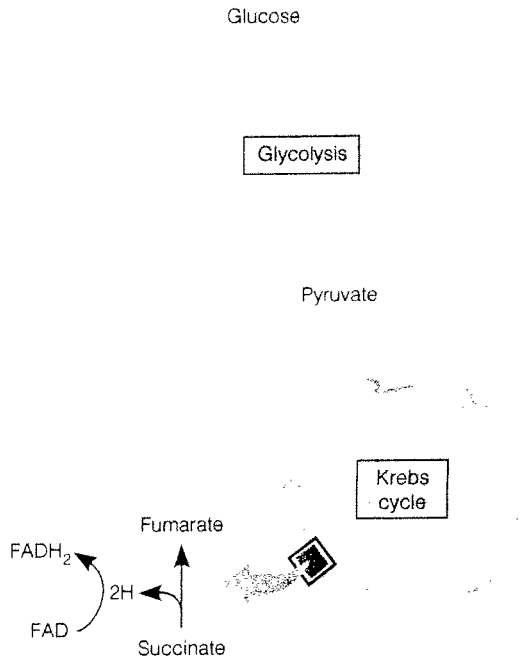

Figure 5.
At one point in the Krebs cycle, succinate is converted to fumarate. Hydrogens from succinate pass to FAD, reducing it to $FADH_2$.

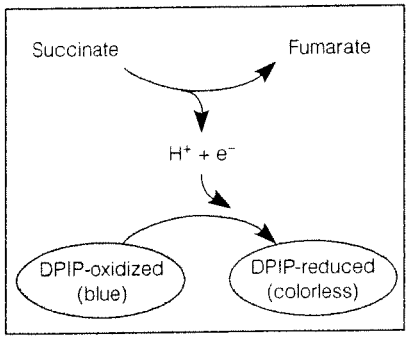

Figure 6.
DPIP intercepts the hydrogen ions and electrons as succinate is converted to fumarate. DPIP changes from blue to colorless.

intercepts the hydrogen ions and electrons released from succinate, changing the DPIP from an oxidized to a reduced state. DPIP is *blue* in its oxidized state but changes from blue to *colorless* as it is reduced (Figure 6).

We can use this color change to measure the respiration rate. To do this, however, we must have some quantitative means of measuring color change. An instrument called a **spectrophotometer** will allow us to do this. A spectrophotometer measures the amount of light absorbed by a pigment. In the spectrophotometer, a specific wavelength of light (chosen by the operator) passes through the pigment solution being tested—in this case, the blue DPIP. The spectrophotometer then measures the proportion of light *transmitted* or, conversely, *absorbed* by the DPIP and shows a reading on a calibrated scale. As the DPIP changes from blue to clear, it will absorb less light and more light will pass through (be transmitted through) the solution. The change in transmittance will be read by the spectrophotometer. As more light passes through the solution, the transmittance reading goes up. As aerobic respiration takes place, what should happen to the percent transmittance of light through the DPIP?

Our experiment will involve using succinate as the substrate and investigating the effect that *changing the amount of succinate will have on the cellular respiration rate.*

Hypothesis

Hypothesize about the effect of an increased amount of substrate on the rate of cellular respiration.

Prediction

Predict the results of the experiment based on your hypothesis (if/then).

Procedure

1. Prepare the spectrophotometer.

   The instructions that follow are for a Bausch & Lomb Spectronic 20 (Figure 7). Turn on the machine (power switch C) at least 5 minutes before beginning.

   a. Using the wavelength control knob (A), select the wavelength: 600 nm. Your instructor has previously determined that this wavelength is absorbed by DPIP.

   b. Zero the instrument by adjusting the control knob (the same as power switch C) so that the meter needle reads 0% transmittance. There should be no cuvette in the instrument, and the sample holder cover must be closed. Once it is set, do not change this setting.

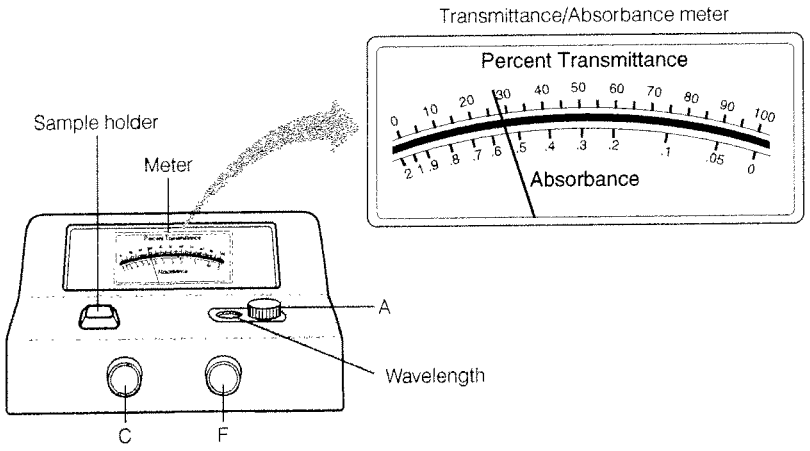

Figure 7.
The Bausch & Lomb Spectronic 20.
A spectrophotometer measures the proportion of light of different wavelengths absorbed and transmitted by a pigment solution. Inside the spectrophotometer, light is separated into its component wavelengths and passed through a sample. Transmittance or absorbance can be read on a calibrated scale.

2. Obtain four cuvettes and label them B, 1, 2, and 3. The B will be the blank.
3. Prepare the blank first by measuring 4.6 mL buffer, 0.3 mL mitochondrial suspension, and 0.1 mL succinate into the B cuvette. Cover the cuvette tightly with Parafilm and invert it to mix the reactants thoroughly.
4. Calibrate the spectrophotometer as follows: Wipe cuvette B with a Kimwipe and insert it into the sample holder. Be sure you align the etched mark on the cuvette with the line on the sample holder. Close the cover. Adjust the light control (F) until the meter reads 100% transmittance, or 0 absorption. Remove cuvette B. You are now ready to prepare the experimental cuvettes. The blank corrects for differences in transmittance due to the mitochondrial solution.
5. Measure the buffer, DPIP, and mitochondrial suspension into cuvettes 1, 2, and 3 as specified in Table 3.

   Do not add the succinate yet!

Table 3
Contents of Experimental Tubes (volumes in mL)

Tube	Buffer	DPIP	Mitochondrial Suspension	Succinate (add last)
1	4.4	0.3	0.3	0
2	4.3	0.3	0.3	0.1
3	4.2	0.3	0.3	0.2

6. Perform the next two steps as *quickly* as possible. First, add the succinate to each cuvette.
7. Cover tube 1 with Parafilm, wipe it with a Kimwipe, insert it into the sample holder, and record the percent transmittance in Table 4 in the Results section. Repeat this step for tubes 2 and 3.

If the initial reading is higher than 30%, tell your instructor immediately. **You may need to add another drop of DPIP to each tube and repeat step 7. The reading must be low enough (the solution dark enough) to give readings for 20–30 minutes. If the solution is too light (the transmittance is above 30%), the reactions will go to completion too quickly to detect differences in the tubes.**

8. Before each reading, insert the blank, cuvette B, into the sample holder. Adjust to 100% transmittance if necessary.
9. Continue to take readings at 5-minute intervals for 20–30 minutes. *Each time, before you take a reading, cover the tube with Parafilm and invert it to mix the contents.* Record the results in Table 4.

Results

1. Complete Table 4. Compose a title for the table.

Table 4

Tube	Time (min)						
	0	5	10	15	20	25	30
1							
2							
3							

2. Using Figure 8, construct a graph to illustrate your results.
   a. What is (are) the independent variable(s)? Which is the appropriate axis for this variable?

   b. What is the dependent variable? Which is the appropriate axis for this variable?

   c. Choose an appropriate scale and label the *x* and *y* axes.
   d. Should you use a legend? If so, what would this include?

   e. Compose a figure title.

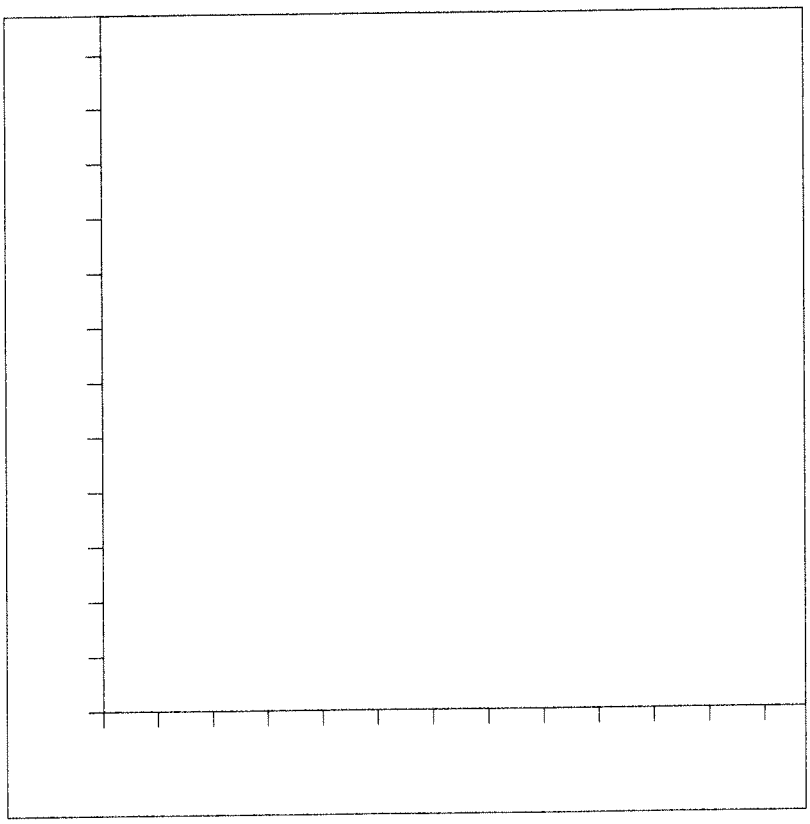

Figure 8.

Discussion

1. Explain the experimental design. What is the role of each of the components of the experimental mixtures?

2. Which experimental tube is the control?

3. In which experimental tube did transmittance increase more rapidly? Explain.

4. Why should the succinate be added to the reaction tubes last?

5. Was your hypothesis falsified or supported by the results? Use your data to support your answer.

6. What are some other independent variables that could be investigated using this technique?

Lab Study B. Additional Investigations in Cellular Respiration

Materials

all materials from Lab Study A
additional substrates: glucose, fructose, maltose, artificial sweeteners, starch, glycogen
inhibitors: rotenone, oligomycin, malonate, antimycin A
different pH buffers
ice bath
water bath
disposable gloves