Chapter 5

MITOCHONDRIA AND RESPIRATION

All organisms must transform energy. This energy is required to maintain a dynamic steady state, homeostasis, and to insure continued survival. As will be discussed in the following chapter, during photosynthesis radiant energy from the sun is converted to chemical bond energy and stored as potential energy reserves in the form of organic molecules.

In the processes of cellular respiration part of this energy is used for the production of ATP molecules, which have readily available, high-energy bonds. These bonds are the principle source of energy for cellular activity. ATP, adenosine triphosphate, is a nucleotide - one of the simple molecules which comprise macromolecules of nucleic acids. The ATP molecule is itself a combination of five smaller molecules: adenine (a nitrogen base), ribose (a five carbon sugar), and three phosphate molecules. Figure 12-1 depicts a molecule of ATP. It is the high energy bond between the second and third phosphates which contains the most readily available energy. Breaking this bond releases energy to power the work of the cell, leaving adenosine diphosphate, ADP, and inorganic phosphate, P_i, as byproducts.

Cellular respiration occurs in two distinct phases: glycolysis, the breakdown of glucose to yield two molecules of pyruvate, with the production of two molecules of ATP; and oxidative respiration, the breakdown of pyruvate into carbon dioxide and water with the production of up to an additional 36 molecules of ATP. In the presence of oxygen, which serves as the final electron (hydrogen) acceptor, the complete conversion of glucose to carbon dioxide and water will produce 36 - 38 ATPs in a multiple step sequence of enzyme mediated reactions.

In the absence of oxygen, glycolysis serves as the cell's only ATP source because pyruvate cannot be further oxidized in an anaerobic environment. Thus, pyruvate accumulates in the cell. The NADH produced in the glycolytic process also accumulates in the absence of oxygen. Some organisms have overcome this problem by utilizing pyruvate as a hydrogen acceptor in a fermentation process. In the simplest form of fermentation, which may occur in human muscle cells under stress, NADH releases its hydrogen to pyruvate to produce NAD^+ and lactic acid. The NAD^+ may return to accept additional hydrogens in the ongoing glycolytic process while lactic acid accumulates as a byproduct.
Yeast cells accomplish fermentation in a similar but somewhat more complex manner, producing molecules of carbon dioxide, ethanol, and NAD$^+$ from each pyruvate and NADH. The glycolytic pathway and fermentation pathways are diagramed in most general biology textbooks. 

Oxidative respiration in eukaryotic cells occurs only in mitochondria. The “matrix” of these organelles contains the necessary enzymes for the sequential conversion of pyruvate to carbon dioxide, a process that involves the oxidative decarboxylation of pyruvate and the Krebs Cycle. High energy electrons together with hydrogen atoms, which are released during these processes, are transferred to NAD$^+$, producing NADH; pyruvate is completely oxidized to CO$_2$, while NAD$^+$ is reduced (Redox reactions). The NADH molecules in turn transfer their electrons and protons to an electron transport chain located in the inner mitochondrial membrane, where the electrons and protons ultimately combine with oxygen to form water. Three steps along the electron transport system release enough energy to produce a proton gradient that is used to synthesize molecules of ATP from ADP and P$_i$ (chemiosmotic theory). The aerobic respiration pathway, including the electron transport chain, is also diagramed in most biology textbooks.

Laboratory activities today will demonstrate fermentation and aerobic respiration. Two separate sets of procedures and equipment will be utilized. Work in groups of four and begin the yeast fermentation experiment (Part I) and the corn seed respiration experiment (Part II) at the same time. Each student is responsible for all data and conclusions from both parts of the lab, even though they may not have actually set up the experiment.

I. YEAST FERMENTATION

PROCEDURE

1. Obtain six fermentation tubes and label them one through six. Handle the fermentation tubes carefully; they are fragile and expensive.

2. Place the following solutions into the six fermentation tubes:
   - Tube 1 - 20 ml of 5% glucose solution with yeast.
   - Tube 2 - 20 ml of 5% sucrose solution with yeast.
   - Tube 3 - 20 ml of 5% lactose solution with yeast.
   - Tube 4 - 20 ml of 5% glucose solution.
   - Tube 5 - 15 ml of 5% glucose solution with yeast, add 5 ml of NaF solution.
   - Tube 6 - 20 ml of yeast solution.

3. After you have added the solutions, place your thumb over the open end of each fermentation tube and invert the tube so the solution flows into the closed end of the tube. Return the tubes to the upright position. Make sure there is a complete column of solution, without air bubbles, in the closed end of the fermentation tube.

4. Place each of the tubes in a fermentation rack and place the rack in a warming oven for 90 minutes at 37°C.
5. Remove the rack at 15 minute intervals. Observe the tubes and record the distance the fluid is displaced in millimeters during each observation in the table below. Replace your rack in the oven when you are done with your observations.

6. In the space provided, graph the results from your data table for each experimental fermentation tube. What conclusions can you draw from your graph about the fermentation rates?

<table>
<thead>
<tr>
<th>Table 5-1. Production of CO₂ in mm displacement of solution</th>
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<tbody>
<tr>
<td>t / min</td>
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<tr>
<td>0</td>
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To demonstrate the production of carbon dioxide in the fermentation tubes we will use a pH indicator dye, phenol red. This indicator dye turns red, when the solution is alkaline (basic). However, the color of phenol red is yellow in acidic solutions.

PROCEDURE

1. Add several drops of phenol red to the fermentation tubes after incubation. Invert the tubes to mix the dye and the solution. When the phenol red is added to tubes that have been fermenting, the solution turns yellow. Why?

2. Add 5 % NaOH solution drop by drop until each solution just turns red. The solution is now alkaline. Over the next 5-10 minutes observe the tubes closely to detect any color changes. What would cause certain tubes to turn yellow again?

II. AEROBIC RESPIRATION IN GERMINATING CORN SEEDS

Many students never fully realize that plants as well as animals respire. That is, plant cells also contain mitochondria. In this exercise you will demonstrate aerobic respiration in germinating corn seedlings with a respirometer similar to the one shown in Figure 12-2.

Figure 5-2. Respirometer
A small volume of potassium hydroxide solution (KOH) in the respirometer will remove the carbon dioxide as it is being generated during respiration. The KOH absorbs atmospheric CO₂, producing a potassium carbonate precipitate. Thus, the total volume of gas in the closed system will decrease as will the pressure on the manometer fluid (dye), causing it to move. This movement of the manometer fluid will represent the amount of oxygen consumed during aerobic respiration by the mitochondria, since for each mole of oxygen one mole of CO₂ is generated. Since all gases occupy the same volume at constant pressure and temperature, we can directly measure oxygen consumption by reading the volume from the 1ml pipette.

Before you begin the procedure, answer the following questions.

1. In which direction do you expect the manometer fluid to travel? Explain

2. How will the presence of KOH affect the experiment?

PROCEDURE

1. Each group obtains 2 - 4 germinating corn seedlings and places them in the DRY respirometer tube. Note that there should be cotton in the bottom of the tube.

2. Add a small amount of non-absorbent cotton to the tube, above the seedlings. This cotton should not touch the seedlings.

3. Add a small amount of absorbent cotton to the tube and saturate with KOH solution. Make sure that you do not allow KOH to drip onto the corn seedlings – avoid contact between KOH and the plant material!

4. Add a small amount of dye to the 1ml pipette using the fine Pasteur pipette. Use the syringe to adjust the position of the dye to the middle of the 1ml pipette (exact position is not critical now). Arrange the respirometer so the 1ml pipette is horizontal and the syringe is accessible.

5. Allow the respirometer to sit undisturbed for 5 minutes to equilibrate. Temperature fluctuations (caused by touching the tube) or changes in the position of the 1ml pipette can cause inaccurate results.

6. Avoid touching the glass tube, and use the syringe to set the dye to the zero position (Which scale should you use??) Use the ml markings on the pipette to record the position of the manometer fluid at appropriate time intervals. Terminate a complete run when the manometer fluid has traveled almost the entire distance of the pipette. Repeat the experiment. Make a table to record the results from each run. Average your results and plot the average values on a graph in the space below.
QUESTIONS

1. Briefly discuss the relationships between aerobic respiration, glycolysis, and fermentation.

2. Is fermentation an oxidative or reductive process? Why?

3. Describe the influence of NaF on the fermentation process.

4. Write a generalized formula for aerobic respiration.

5. Where are most of the mitochondria located in the germinating corn seedlings?

6. What effects would temperature have on the rate of respiration?

7. Why is aerobic respiration more efficient in producing ATP than fermentation?