

I. TEACHER NOTES & GUIDELINES

TITLE OF LAB: Investigating Aerobic Cellular Respiration

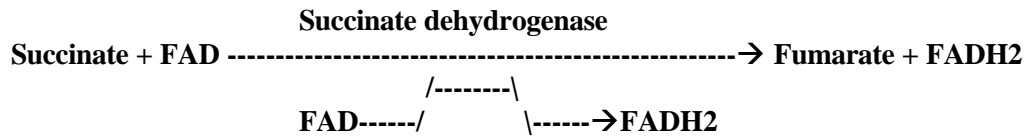
DEVELOPERS OF LAB: Christina Selles JD742, Maureen Clayton JD711, Linda Scarfava JD753, Drena S. Paulson JD425, Janine Guadagno JD524, James Prockup JD575

OVERVIEW OF LAB

DESCRIPTION:

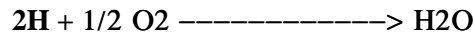
Cellular Respiration:

The electron transport chain is a final step in cellular respiration. Small amounts of energy are released as electrons are passed down the chain. This energy is used to phosphorylate ADP to ATP. Electron transport and phosphorylation both occur within the inner membrane of the mitochondria. By isolating the mitochondria from the other cell organelles, and by comparing the absorbency @ 600nm of DPIP as it is reduced by the activity of the enzyme succinate dehydrogenase, this lab demonstrates that cellular respiration occurs in the mitochondria.



The Electron Transport Chain:

- Ubiquinone (Q)
- Cytochrome b
- Fe S
- Cytochrome c1
- Cytochrome c
- Cytochrome a
- Cytochrome Q3



(See attached Citric Acid Cycle on next page)

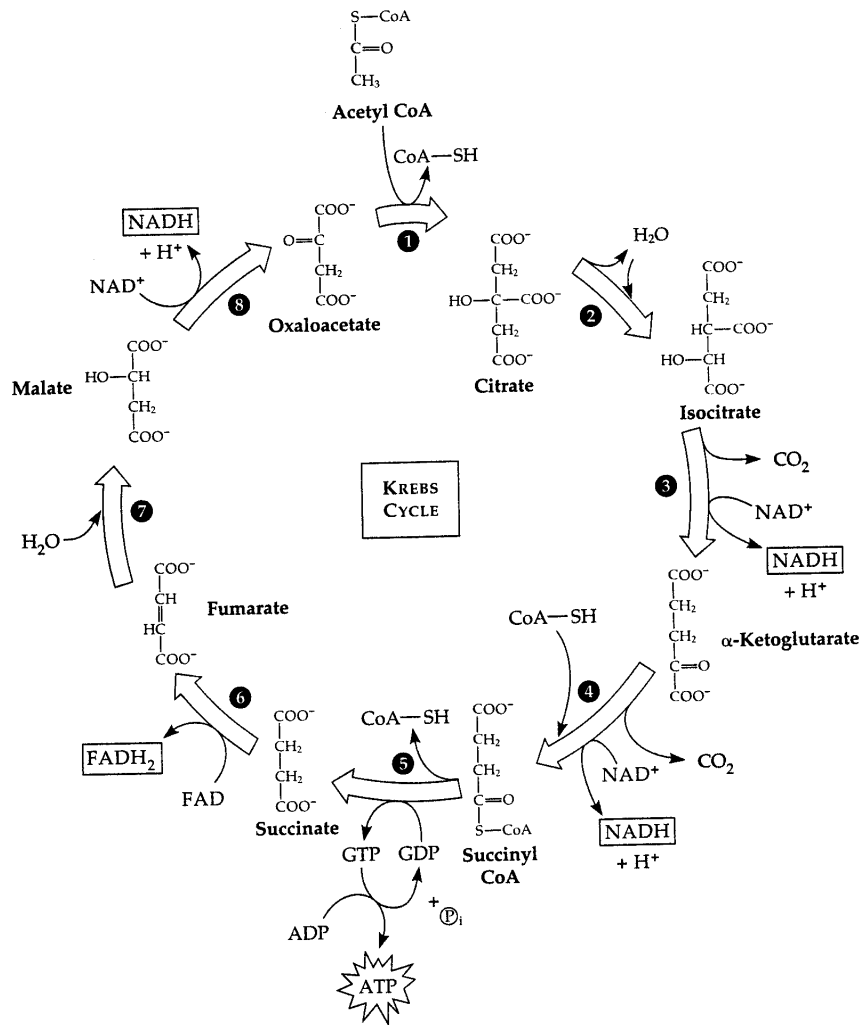
CURRICULUM CONSIDERATIONS:

This lab is to be used in a Regents biology curriculum during study of cellular respiration. This lab will require 2 to 3 lab periods depending on amount of teacher preparation desired.

REAL WORLD APPLICATIONS

1. Knowing inhibitors of food utilization is important to the farming industry.
2. Cellular respiration is important in the understanding of the Citric Acid and Krebs cycles, exercise and lactic acid toxicity.
3. Knowing how to interfere with the citric acid cycle can be important in microbial growth and growth retardation, which is important to the food industry.

Citric Acid Cycle:



SAFETY CONSIDERATIONS

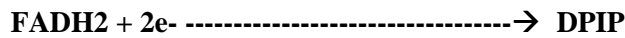
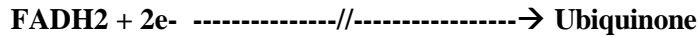
Students should wear gloves and goggles.

BACKGROUND INFORMATION

A. SCIENTIFIC VIEWPOINT:

This lab replaces ubiquinone with 2,6 dichlorophenolindophenol (DPIP). DPIP is a blue dye. When DPIP is reduced (accepts electrons), it becomes colorless. DPIP has a greater affinity for electrons than ubiquinone. However, to ensure that the electrons are used to reduce DPIP, a “poison” (sodium azide) will be added to block FADH₂ from passing electrons to ubiquinone. (Note: This dye is variously called DPIP, DCIP, and DCPIP)

Sodium Azide



B. COMMON MISCONCEPTIONS:

Listed below are some common misconceptions about cellular respiration:

1. Since plants carry out photosynthesis, they do not carry on cellular respiration, only animals do.
2. Cellular Respiration is often confused with breathing.

OBJECTIVES

The students will be able to perform the following tasks:

1. Use a Spec-20.
2. Prepare a standard curve.
3. Make dilutions.
4. Identify the site of cellular respiration.

EQUIPMENT/MATERIALS

PROVIDED BY SOTM:

Spec 20's	Latex Gloves
Pipettes and Pipettors 1ml and 10 ml	Microcentrifuge
Electronic Balance	Mortar and Pestle
Cheesecloth	1.5 ml Microcentrifuge Tubes
Blender	Parafilm
Cuvettes	Glass Rods
Kim Wipes	Lima Beans
Wash Bottles	

Solutions prepared by SOTM: Sucrose/phosphate buffer, Na Azide (0.04M), DPIP, Succinic Acid

PROVIDED LOCALLY:

Source of mitochondria – lima beans	
Test tube holders	Ice
Beakers	small test tubes
Mortar and Pestle	
Funnel	
Scissor	

ADVANCE PREPARATION

Teacher may want to prepare solutions in advance. The beans must be soaked overnight. The blending can be done the day before and filtered, then refrigerated. The standard curve solutions also may be prepared in advance. All solutions must be refrigerated if not used immediately but it is not necessary to keep solutions on ice during the individual lab sessions. If this lab is used for multiple sessions, solutions should be refrigerated in-between them.

II. PRE-LAB

PRE-LAB EXERCISE TO ELICIT STUDENTS' PRIOR KNOWLEDGE AND MISCONCEPTIONS

1. How do organisms get energy?
2. Can the energy that is stored in glucose be used directly by the cell?
3. What has to happen to this energy contained in glucose in order for the cell to use it?
4. What is the useable form of energy for living organisms?
5. Do plants carry out cellular respiration?
6. What are the two kinds of cellular respiration? What is required for both?
7. What are the products for each type of respiration named?
8. Where does anaerobic respiration occur? Aerobic respiration occur?
9. Could you measure the amount of cellular respiration occurring? How?
10. What kinds of activities do you use energy for? Do plants use energy for?

DISCUSSION OF PRECONCEPTIONS

Teacher might put the answers on the blackboard for general discussion during the Prelab sessions.

III. EXPLORATION OF SCIENTIFIC PRINCIPLE & INTRODUCTION OF EXPERIMENTAL PROTOCOL

PROBLEM

Where does aerobic respiration occur? How can we prove it?

EXPERIMENT AND TECHNICAL OPERATION OF EQUIPMENT

Procedure:

If this lab is done in one session, warm up the Spec-20's before grinding the beans. If this is done in multiple sessions, the spec-20 should be turned on at the start of the lab period of that day. The Spec-20's take 20 minutes to warm up.

Mitochondria isolation-If you use lima beans, soak the beans in plenty of water overnight. If the lab will be done over two or more days, soak new beans overnight before each session.

For the whole class:

1. Grind soaked lima beans (9-12 beans, 10g dry weight or 20 gms soaked weight) with mortar and pestle. Add 25 ml of sucrose/phosphate buffer and then blend in the blender for 2 minutes
2. Pour through 4 layers of cheesecloth and collect filtrate in a beaker. Add 10ml additional buffer to rinse the blender and filter this as well.

3. Chill the filtrate over ice for 10 min.
4. For each lab team: Fill a microtube with 1.5 ml of filtrate. Close the lid of the microtube and place it in the microcentrifuge.
5. Be sure the sample is balanced with another full tube opposite it in the microcentrifuge.
6. Centrifuge the samples for at least 5 min at 2000 rpm. (Check centrifuge itself for exact settings).

These tubes can now be placed in the refrigerator and the lab resumed the next day. (Steps 1-6 may be done as a teacher prep).

Use of the Spec-20 – Instructions

1. Turn on the Spec-20 and allow it to warm up for 20 minutes.
2. Set the wavelength for 600nm and set the bottom left filter lever to the right.
3. Set the mode to transmittance using the mode button.
4. Turn the transmittance knob, the left one, until you have a reading of zero. This is done **without** a tube in the Spec-20.
5. **DO NOT TOUCH THIS KNOB AGAIN OR YOU WILL HAVE TO RECALIBRATE IT. You may want to put a piece of masking tape over this knob to prevent its accidental movement.**
6. Now set the mode to absorbance, and place an appropriate blank tube into the chamber. Remember to wipe off the tube with a Kimwipe before placing it into the Spec-20. Be sure the white line on the cuvette is lined up with the arrow on the Spec-20.
7. Close the lid and turn the front **RIGHT KNOB** to obtain a zero reading. Each time you zero the Spec-20, you will use the appropriate blank and will adjust only the right knob.

Prepare a standard curve for DPIP.

The standard curve tells you what range absorbance to work within.

Tube Number	Conc. of DPIP	Amount of DPIP	Amount of Buffer
1	0%	0	5ml
2	2%	0.1ml	4.9ml
3	4%	0.2ml	4.8ml
4	8%	0.4ml	4.6ml
5	12%	0.6ml	4.4ml
6	16%	0.8ml	4.2ml

Preparing standard curve solutions (Two Teams Cooperating):

Have two teams cooperate with each team doing 3 tubes. For this activity allocate to each pair of teams:

1. 1 beaker with about 30ml of sucrose/phosphate buffer
2. 1 test tube with 5 ml of DPIP

Procedure:

1. Label 6 cuvettes # 1-6.
2. Add to each tube the DPIP and Sucrose/phosphate buffer as in the above table.
3. Using Tube number 1 as your blank, zero the Spec-20 for absorbance.
4. Take the absorbance @600nm for each concentration of DPIP.
5. Record the Data in a Data Table.
6. Graph absorbance vs. concentration. This gives you your standard curve for DPIP

Tube #		Amount of DPIP	Amount of buffer	Absorbance at 600 nm

1		0ml		5 ml		
2		0.1ml		4.9 ml		
3		0.2ml		4.8 ml		
4		0.4ml		4.6 ml		
5		0.6ml		4.4 ml		
6		0.8ml		4.2 ml		

Cellular Respiration

Tube Number	Buffer	Azide	Succinate	DPIP	Supernate
1	3.5ml	0.5ml	0.5ml	0	0.5ml
2	3.0ml	0.5ml	0.5ml	0.5ml	0.5ml
3	4.0ml	0.5ml	0.5ml	0	0
4	3.5ml	0.5ml	0.5ml	0.5ml	0

Allocate to each individual team:

- 1 beaker with 15ml sucrose/phosphate buffer
- 1 tube with 2ml DPIP
- 1 tube with 3ml succinate
- 1 tube with 3ml sodium azide

Preparing Cellular Respiration Tubes, using the supernatant liquid in the previously prepared tubes of lima bean extract.

1. Label 4 cuvettes #1-4
2. Add the solutions to each tube in order. You may add all solutions except DPIP and supernate and refrigerate overnight to be run the next day. **Once you add the supernate you must immediately begin taking readings on the Spec-20. You will need a 0 time reading.**
3. *Review data table below. Tubes number 1 and 3 are zero absorbance tubes. You will zero the machine with tube 1 and then read and record tube 2. You will then again zero the machine with tube 3 and read and record tube 4. This will be done every 2 minutes for a total of 20 minutes. Tube number one is with supernate and without DPIP. Tube number 3 is without supernate and without DPIP. These are the control tubes.*
4. Record your data in the table below.
5. Make a graph of the absorbance vs. time.
6. Using the standard curve for DPIP make some inferences about the enzyme activity for the tubes 2 and 4 at each time point.

		ABSORBANCE (%)									
Tube #	0 min	2 min	4 min	6 min	8 min	10 min	12 min	14 min	16 min	18 min	20 min
1	0	0	0	0	0	0	0	0	0	0	0
2											
3	0	0	0	0	0	0	0	0	0	0	0
4											

Discussion

What does your data tell you about the contents of the supernatant? What cell components were removed by the grinding, blending and centrifugation? How could you visually check to see what is in the supernatant?

IV. ELABORATION OF SCIENTIFIC PRINCIPLE: INQUIRY-BASED STUDENT INVESTIGATION

PROBLEM

Different cells have different numbers of mitochondria present.

Does the number of mitochondria present effect the amount of cellular respiration occurring? If so, what can you say about the cell's activities based on the number of mitochondria present?

At this point, what conclusions if any can you make concerning cell organelles, which carry out cellular respiration?

How can you obtain a cell extract, which has only mitochondria-size organelles in it?

Note: The process of obtaining the supernate removed structures larger than the mitochondria. Equal sized particles and smaller particles are still in the supernate. Discuss how you might isolate only mitochondria-sized particles from the smaller ones. If time and filtration equipment are available, Part IV could involve further isolation processes.

Would you then be able to show that only mitochondria carry out cellular respiration? What other difficulties might be encountered?

State a problem related to the above questions, which you think you, could investigate.

HYPOTHESIS OR PREDICTION

Make an If-Then statement about the problem you just wrote in the previous section.

EXPERIMENTAL DESIGN

Given the same equipment, plus any other lab equipment available, and the basic procedure of the first lab investigation, design an experiment that will test your hypothesis. If the effect of concentration of mitochondria on the rate is the topic, stress the idea of dilution and relative amounts. If isolation of a cell extract containing only mitochondria is involved, research possible methods of isolation and plan your procedure.

1. What variable(s) will you be studying?
2. How will you explore the effects of this variable?
3. What equipment will you be using? Are all the equipment/reagents available?
4. What safety procedures will you use to complete your experiment?

Checkpoint (Teacher checks students' experimental design for feasibility.)

PLAN FOR DATA COLLECTION & ANALYSIS

1. How will you record your results of your variable vs. your reaction rate?
2. Will you use data tables/graphs?
3. How will you design an appropriate data table?
4. What information will be recorded?
5. How will you use your data to confirm your hypothesis?

Checkpoint (Teacher checks students' plan for feasibility.)

CONDUCTING THE EXPERIMENT

Each of the lab teams will experiment by using the written procedures and steps that have been developed and approved. Each group should at least design a table and a graph.

Checkpoint (Teacher monitors students' investigations in progress.)

ANALYSIS OF DATA

Special care should be taken when looking at the students' procedures to make sure that the proper controls are used and that the Spec-20 is zeroed with an appropriate blank.

The conclusions must include a comparison of the sets of data obtained. If concentrations of mitochondria are studied, graphs should be drawn and slopes compared. A class (display) chart should be completed by each team.

Checkpoint (Teacher checks students' analysis.)

DISCUSSION OF RESULTS

COMPARE

Arrive at a consensus about what effect the number and size of mitochondria has on the amount of respiration going on in a cell.

RELATE

Relate that information to the energy requirements of different kinds of cells. (muscle cells, unicellular organisms, etc.)

PERSUADE

Prepare to present the results of your investigation to the class. If students' hypotheses were confirmed, the students are to convince their peers of their experimental findings. Guide students to conclusions concerning points of disagreement. Remember that conclusions may be tentative and may suggest additional questions for investigation.

PREDICT

Predict the energy requirements of various cells given some information about numbers of mitochondria or functions of cells. (Using pictures and or electron micrographs where mitochondria are visible so they can count them; or any other source(s) you have will be helpful here.) Research cell biology sources.

V. EVALUATION

POST-LAB SURVEY OF STUDENTS' CONCEPTIONS

Have students retake the Pre-Lab Exercise. Compare pre-lab and post-lab responses.

TRADITIONAL

1. Have the student's list 5-10 statements, which assess that the objectives were met.
2. Have students create a concept map about cellular respiration or the experiment itself.
3. Class discussion of cellular respiration, this may be mediated by one or a group of students.

ALTERNATIVE

1. Students may make a presentation involving the Internet and research involving either their experimental hypothesis or cellular respiration in general. They must use a minimum of 5 sources found and must make the presentation orally and in writing. Presentation software like PowerPoint may be used.
2. Students may make a video presentation of their experiment, using either themselves and or other members of the class. Students may utilize any and all things learned in this lab to make this video. This may be presented as a commercial selling something, as a sales presentation or as a general education presentation.
3. Students with artistic skills may want to make a drawing or other artistic representation of either the Citric Acid/Krebs Cycle or cellular respiration. All avenues of artistic talent may be investigated; permission from the teacher is required before beginning.

Solutions to be prepared before hand:

.2M Succinate

sodium succinate – 6H₂O 5.4g
Distilled H₂O 90ml
Adjust pH to 7 and bring to 100ml with di H₂O
Store at 4C

.04M Sodium Azide

sodium azide 0.26 g
100 ml distilled H₂O
Dissolve the azide; store at room temperature.

.0048% DCIP

2,6-dichlorophendindophenol 4.8mg
Di H₂O to 100 ml
Prepare just before lab period

Sucrose/Phosphate Buffer

0.2M Na₂HPO₄ 32.4 ml
0.2M NaH₂PO₄ 17.6 ml
Sucrose 17.1 g
Dissolve the sucrose in the two combined phosphates. Add dist H₂O to 100ml total volume

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