Laboratory 5: Cell Respiration

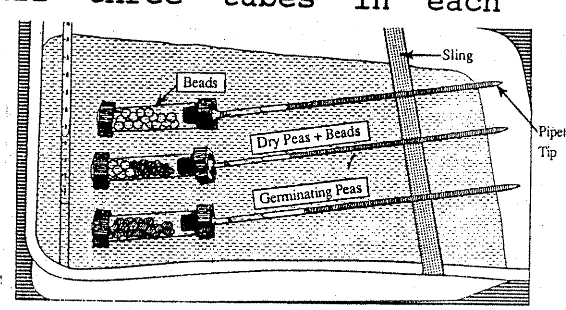
*discuss the gas laws as they apply to the function of a respirometer
*interpret data related to the effects of temperature on cell respiration
*interpret data related to the effects of germination or nongermination on cell respiration
*explain or determine the significance of a control
*explain the relationship between dependent and independent variables
*calculate a rate of cell respiration by utilizing graphed data
*design an experiment to use a respirometer to measure cellular respiration

This experiment will examine the difference between rates of respiration of germinating peas and those still in "suspended animation" (dry peas). It is possible to measure many factors of respiration: consumption of glucose, production of CO_2, of the use of O_2 -- the later will be used here. Since there are two gases in the equation, use of soda lime to absorb CO_2 produced will make it possible to check on level of oxygen only. [Note: to be able to figure actual amounts of O_2, gas used must be adjusted if any changes in barometric pressure occurred -- the purpose of the third tube with glass beads or gravel! The gas law that counts is PV = nRT or the pressure X volume = number of molecules X gas constant X temperature: if you change any of the parts of the equation, you must change something on the other side!]

This lab will also explore how temperature affects the rate of respiration; therefore, two identical setups will be run -- one at room temperature (25°C), one at approximately 10°C.

Twenty-five germinating peas are placed in a large test tube with soda lime to absorb the CO_2 and a weight to keep it underwater. A stopper is inserted with a graduated pipette to make a water-tight seal. The same is done with a tube of 25 dry peas (plus gravel to give an equal volume to that of the germinating ones -- remember: only one variable in the experiment, and a difference in volume could be a significant variation!) and with a third tube containing an equal volume of gravel only (will serve as the control and thermobarometer).

All tubes are submerged after equilibrating and as the O_2 is used in the respiration process, a loss in gas volume should cause more water to enter the tube, which can be measured. Measurements are taken at 0 time and then every 5 minutes for 20 minutes for all three tubes in each temperature situation.



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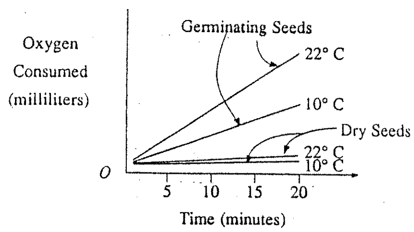
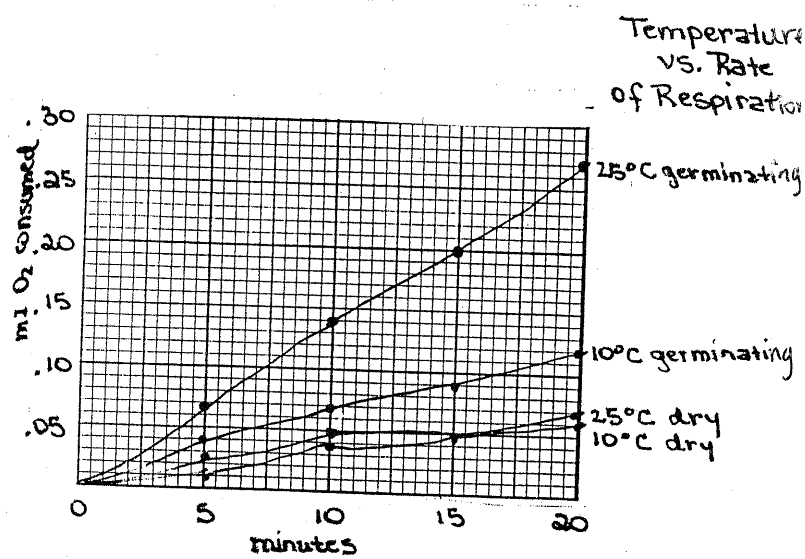
Temp. (°C)	Time (min)	Beads Alone: Reading at time X	Beads Alone: Diff.*	Germinating Peas: Reading at time X	Germinating Peas: Diff.*	Germinating Peas: Corrected diff.^Δ	Dry Peas and Beads: Reading at time X	Dry Peas and Beads: Diff.*	Dry Peas and Beads: Corrected diff.^Δ
25	Initial - 0	0.93		0.91			0.92		
	0-5	0.91	0.02	0.84	0.07	0.05	0.89	0.03	0.01
	0-10	0.90	0.03	0.77	0.14	0.11	0.87	0.05	0.02
	0-15	0.90	0.03	0.71	0.20	0.17	0.87	0.05	0.02
	0-20	0.90	0.03	0.64	0.27	0.24	0.85	0.07	0.04
10	Initial - 0	0.95		0.92			0.91		
	0-5	0.94	0.01	0.88	0.04	0.03	0.90	0.01	0.00
	0-10	0.92	0.03	0.85	0.07	0.04	0.87	0.04	0.01
	0-15	0.93	0.02	0.83	0.09	0.07	0.86	0.05	0.03
	0-20	0.93	0.02	0.80	0.12	0.10	0.85	0.06	0.04

* Difference = (initial reading at time 0) − (reading at time X)

Δ Corrected difference = (initial pea seed reading at time 0 − pea seed reading at time X) − (initial bead reading at time 0 − bead reading at time X)





Obviously, the rate of reaction and the graph indicate that germinating peas respire at a much higher rate, no matter what the temperature: this makes sense because they are actively growing, while dry peas are in "arrested development" (NOT the rap group). Cold inhibits chemical reactions in any system, and it does also here!

Lab 5

BIOLOGY

SECTION II

Time—1 hour and 30 minutes

Answer all questions. Number your answer as the question is numbered below.

Answers must be in essay form. Outline form is NOT acceptable. Labeled diagrams may be used to supplement discussion, but in no case will a diagram alone suffice. It is important that you read each question completely before you begin to write.

1. The results below are measurements of cumulative oxygen consumption by germinating and dry seeds. Gas volume measurements were corrected for changes in temperature and pressure.

Cumulative Oxygen Consumed (mL)

Time (minutes)	0	10	20	30	40
22° C Germinating Seeds	0.0	8.8	16.0	23.7	32.0
Dry Seeds	0.0	0.2	0.1	0.0	0.1
10° C Germinating Seeds	0.0	2.9	6.2	9.4	12.5
Dry Seeds	0.0	0.0	0.2	0.1	0.2

a. Using the graph paper provided, plot the results for the germinating seeds at 22° C and at 10° C.
b. Calculate the rate of oxygen consumption for the germinating seeds at 22° C, using the time interval between 10 and 20 minutes.
c. Account for the differences in oxygen consumption observed between:
   (1) germinating seeds at 22° C and at 10° C;
   (2) germinating seeds and dry seeds.
d. Describe the essential features of an experimental apparatus that could be used to measure oxygen consumption by a small organism. Explain why each of these features is necessary.

2. Describe the steps of protein synthesis, beginning with the attachment of a messenger RNA molecule to the small subunit of a ribosome and ending with the release of the polypeptide from the ribosome. Include in your answer a discussion of how the different types of RNA function in this process.

3. Discuss the adaptations that have enabled flowering plants to overcome the following problems associated with life on land.

a. The absence of an aquatic environment for reproduction
b. The absence of an aquatic environment to support the plant body
c. Dehydration of the plant

GO ON TO THE NEXT PAGE

Question Rubrics, Sample Responses, and Commentary

Free-response questions from the 1990 AP Biology Examination, scoring standards, sample answers, and guides to applying the scoring standards are presented below. Free-response samples are reprinted, with the students' permission, as they appeared in the students' free-response booklets. Because the exam is given under strict time limits and requires that students respond quickly, perfect spelling and grammar are not expected. For the purpose of this publication, mistakes are reproduced as given by students.

Sample essays have been chosen to represent the following categories of scoring: Excellent - 10, 9; Very good - 8, 7; and; Fair - 4, 3.

Question 1 (Scale 0-10 total points)

1. The results below are measurements of cumulative oxygen consumption by germinating and dry seeds. Gas volume measurements were corrected for changes in temperature and pressure.

Cumulative Oxygen Consumed (mL)

Time (minutes)		0	10	20	30	40
22° C	Germinating Seeds	0.0	8.8	16.0	23.7	32.0
	Dry Seeds	0.0	0.2	0.1	0.0	0.1
10° C	Germinating Seeds	0.0	2.9	6.2	9.4	12.5
	Dry Seeds	0.0	0.0	0.2	0.1	0.2

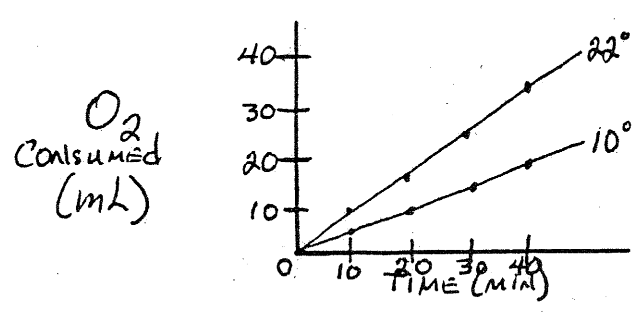
a. Using the graph paper provided, plot the results for the germinating seeds at 22° C and at 10° C.
b. Calculate the rate of oxygen consumption for the germinating seeds at 22° C, using the time interval between 10 and 20 minutes.
c. Account for the differences in oxygen consumption observed between:
   (1) germinating seeds at 22° C and at 10° C;
   (2) germinating seeds and dry seeds.
d. Describe the essential features of an experimental apparatus that could be used to measure oxygen consumption by a small organism. Explain why each of these features is necessary.

Scoring Standards for Question 1

a. Graph(s)

1 pt. correct orientation of x (independent) and y (dependent) axes
1 pt. scale and label axes
1 pt. curves plotted (both lines drawn and identified as 10°/22°); histograms accepted if correctly drawn
1 pt. title of graph

3 pts. maximum



b. Rate calculation

1 pt. setup (16-8.8)/(20-10) or number 7.2 or 0.72
1 pt. rate concept - units (volume/time)
7.2mL/10 min or 0.72mL/min

c. Explanations

1. temperature variation
   1 pt. seeds show no temperature regulation (at environmental temperature); do not increase 0_2 consumption to maintain preset temperature
   1 pt. temperature increase causes increased activity (or increased respiration or metabolism)
   1 pt. extended explanation of respiratory enzyme reaction rate, rate increases (to limit) with increased temperature (Enzymes generally have Q_{10} about 2.)
   *1 pt. 22° vs. 10° rates *reversed* in cold hardiness (genetically determined) seeds

2. germinating seeds vs. dry seeds
   1 pt. dry seeds dormant and/or germinating seeds metabolically active
   1 pt. extended explanation of dormancy and/or metabolism
   1 pt. explanation of water-based chemistry of respiration enzyme reactions

Parts a, b, and c together: 8 pts. maximum

d. Experimental apparatus

1 pt. method to separate 0_2 consumption vs. CO_2 release, something (KOH, etc.) to remove CO_2 (gas → solid)
1 pt. closed system
1 pt. method to measure pressure/volume change — graduated tube/pipet, containing bubble/liquid
1 pt. method to control temperature — water bath
1 pt. method to control volume — glass beads or some other inert material vs. seeds
1 pt. timing device
1 pt. equal numbers of organisms in experimental and control groups

Other techniques/methods for measuring O_2 consumption:

1 pt. Winkler titration to determine O_2 concentration before and after;
Polarographic, oxygen, or Clark-type electrode

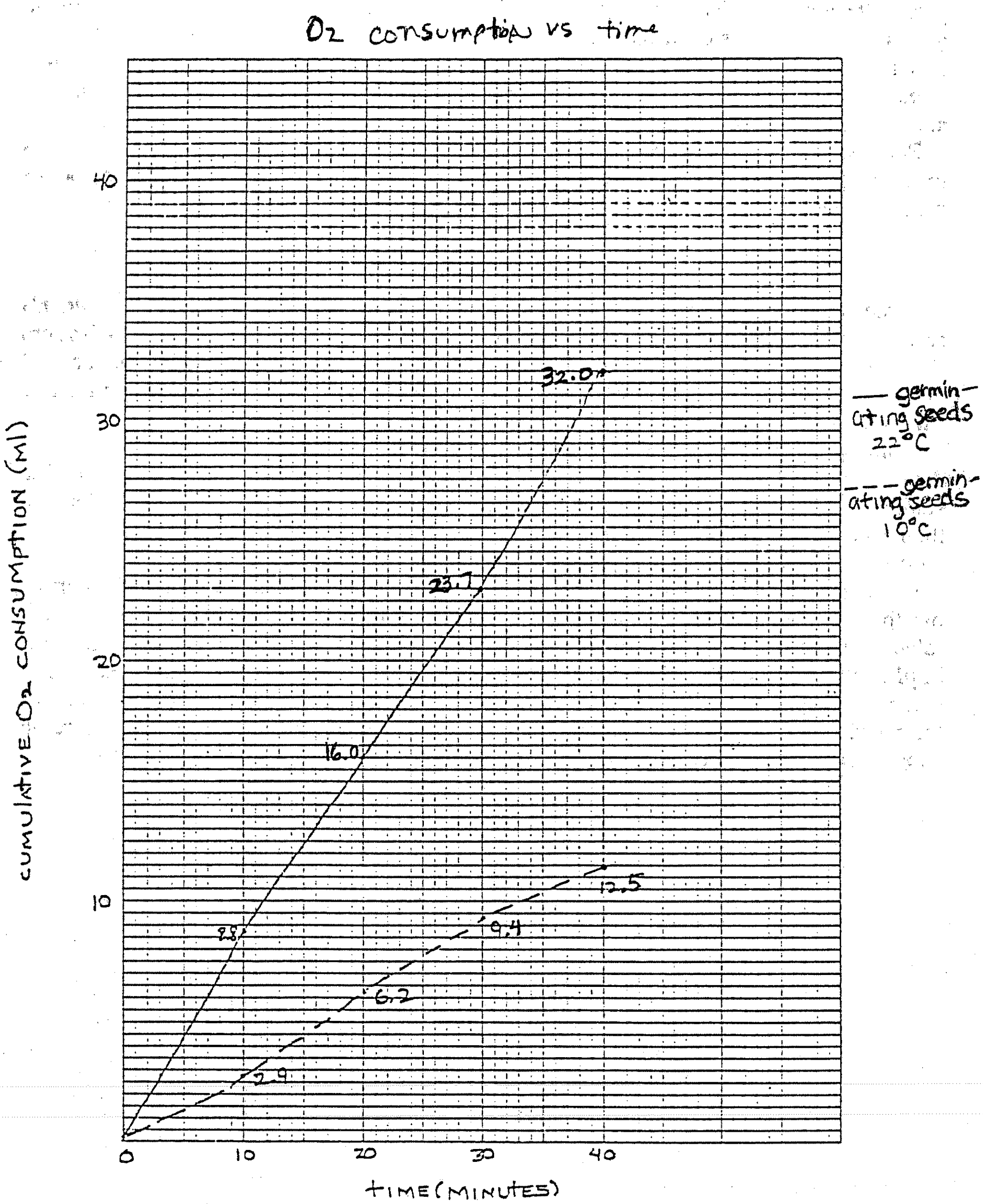
1 pt. detailed explanation of technique, for any of the techniques above

None of these requires special techniques to distinguish volume of CO_2 from volume of O_2; all are specific for O_2. None of these depends on pressure changes. Other features of procedure (constant temperature, appropriate controls, etc.) remain the same.

3 pts. maximum

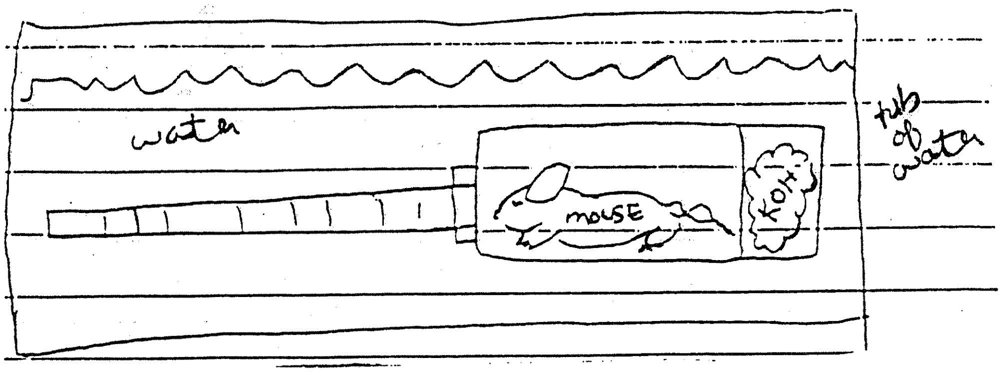
*This is an extra point that is awarded to students who explained how their data were different from the data presented in this example. (See page 38.)

EXCELLENT



At the time interval between 10 and 20 minutes, the germinating seeds at 22°C consume .72 ml of oxygen per minute (.012 ml. per second). The difference in consumption between the germinating seeds at 22°C and 10°C can be attributed to the fact that respiration occurs faster at warmer temperatures (up to a point). The many chemical reactions that make up the pathways of glycolysis, the bridge to the Krebs cycle, the Krebs cycle, and the electron transport chain occur faster at warmer temperatures because heat makes molecules move faster. Substrates interact with enzymes and other substrates more because the molecules are moving around and bumping into each other at a faster rate. Excessive heat or boiling can denature enzymes and slow down the rate of these reactions, but this did not occur in this experiment because the seeds were at only 22°C. Germinating seeds consumed more oxygen than dry seeds because they were activated and beginning life. Dry seeds are still somewhat dormant. Oxygen consumption of mice could be measured by placing

them in submerged respirometers (as shown in figure 1). KOH would be placed on one end of the respirometer to absorb CO_2, and a glass tube with ml. markings would project from the other end.



The K0H would be necessary because CO_2, is a byproduct of respiration and if it went out the glass tube it would mess up the movement of water into the tube that measures O_2 intake. The respirometer is necessary for containing the mouse and the glass tube is needed for measuring O_2 intake. The tub is necessary for containing water.

Comment: This student's graph was neat, correct, and well-labeled with a key included. The student showed an understanding of the rate concept and correctly calculated the rate of oxygen consumption. An explanation for differences in rate at 10°C vs. 22°C was given and the student demonstrated a basic understanding of the fact that molecules move faster at warmer temperatures. An explanation for the difference in oxygen consumption for germinating vs. dry seeds was also presented. The student received two points for the experimental design of a respirometer for measuring oxygen consumption by mice. Overall, the student response was accurate and concise, and addressed all parts of the question. A total of 10 points was given for this answer.