Laboratory Manual for BSC 197
Molecular and Cellular Basis for Life

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LAB-REPORT FORMAT and POLICIES
Biological Sciences 197

The laboratories in this course will emphasize the quantitative aspects of data collection. The format required for the weekly lab reports resembles the form most biologists use in reporting data to their colleagues in scientific papers and oral presentations. However, we have simplified several elements in this format to make it more compatible with course structure. To get an idea of the structure of a scientific paper, you should browse papers in scientific journals at Milner Library. You can access these through the Milner Library website by searching for articles (http://library.illinoisstate.edu/library-materials/find-materials/). Skim papers in several journals to get an idea of general format, as there are usually style differences from journal to journal.

Each laboratory exercise may earn up to 10 points. There will be 11 exercises total, including 5 lab reports and 6 take home problem sets. The total for this semester attributable to lab exercises therefore will be 10 x 11 = 110 points. In addition, two laboratory exams worth 20 points each will provide an additional 40 points for a course total for the laboratory portion of 150 points.

The laboratory reports must be completed individually even though in most labs you will work in groups of two or three. The data is to be shared but the calculations, lab write-ups and interpretations are to be your own. The data collected ("raw data") should be organized neatly into tables that are clearly labeled and in which each experimental condition and controls are identified. The data usually will require some sort of processing (such as calculation of mean and standard error). These calculations must also be organized neatly into tables or included as part of the "raw data" table. The processed data then should be plotted or assembled into bar graphs that show mean, standard error and are titled and labeled on both axes. The details are presented below and an example lab report is provided.

There may be some temptation to "adjust" data to fit what you may think are the "correct" results. DO NOT CHANGE YOUR DATA but report exactly what you observed. Sometimes there may be several outcomes possible in an exercise and we are quite to detect your modifications. The most important part of the report is how you interpret the data. Regardless of how your data may differ from other lab groups, sharing data with other lab groups is allowable ONLY if the TA has specifically instructed you to do so.
Use this outline to format your lab reports:

***************************************************************

Date: ______________________ Name’ ______________________

TA Name____________________________

Section__________________________

Title

Introduction (includes objectives and hypotheses)

Materials and Methods

Results

Discussion

Conclusion

References

***************************************************************

The following comments should provide you with a general idea of what is expected in each of the sections listed above. See also the example provided at the end of this handout. Please ask your T.A.s for clarification if you have any additional questions about format.

Title:

• A brief sentence that describes the main topic of the experiment.

Introduction

• Briefly indicate the overall objectives of the exercise

• Relate the objectives to course material and give background information that a reader would need to know in order to understand the experiment

• Specifically state the hypothesis or hypotheses to be experimentally tested in this exercise. It is important to make the statement of hypotheses as clearly and as simply as possible (see the example report for what a hypothesis might look like).

  o *Note that it is often useful to break down the experiment to simple clearly stated hypotheses. Do not overlook some of the most obvious ones when writing this section.
Materials and Methods:

• Describe briefly the materials and methods used.

• This should be a summary written in paragraph form. This should not be a list of steps. Be sure that it is repeatable; that is, if someone who is unfamiliar with the lab were to read it, they should be able to repeat the experiment.

• In this section list clearly exactly what controls were done and how the "experimental" conditions differ from the controls.
  
  o *Note: It must be emphasized that control measurements are as important as the experimental conditions in research. Controls show that the changes you are measuring in some system are not "artifacts" (that is not due to incorrect procedures, faulty reagents or the presence of some unexpected uncontrolled variable). Controls are often overlooked by beginning students, but they are absolutely essential for the conduct of rigorous research.

• Include information about how your measures will be repeated.
  
  o *Note: In most experiments conducted in this laboratory you will be required to repeat individual measurements 3 or more times. The reason for this need for replicates is that there are many sources of error that have nothing to do with biology of the process being measured. Every instrument, no matter how refined, has some error in its capability to measure. Thus, if you measure the same process many different times you usually get slightly different numbers. It is important to minimize these errors by careful design of the experiments and by being meticulous in measurement of solution volumes, temperature, etc.

• Also include important formulas/equations.

Results:

• The results describe the outcome of the control and "experimental" portion of your experiments.

• There will be two portions: 1) a numerical portion that includes data tables and/or graphs depicting the data and calculations, and 2) a verbal description of the results that relate to the numerical information.
  
  o Note: “Data" is a plural word and plural adjectives and verbs should be used for grammatical agreement. Therefore we say "these data" or the "data have shown" etc. The singular form of "data" is "datum" and is rarely used since it usually specifies a single measurement or point.

Discussion:

• Discuss the meaning of your results.

• State whether your hypotheses were supported or not supported by your data.
• If experimental problems developed you may discuss them here.
• Relate your data back to the general principles.

Conclusions:
• In a few sentences, succinctly states your conclusions. This should be short and to the point concentrating on important findings only.

References: Any sources used must be cited. This may include the lab manual, the textbook, or any other reputable source. FAILURE TO CITE SOURCES BOTH IN TEXT AND IN A REFERENCES SECTION IS PLAGIARISM. See the Academic Integrity portion of the Undergraduate Catalog or ask your T.A. for more information if needed.
Title: Effect of Temperature on the Respiration Rate of Mealworms

Introduction:

The respiratory rate of animals as measured by oxygen consumption is a general measure of the rate of aerobic metabolism. Aerobic metabolism is the sum of all the cellular chemical reactions that convert certain substrates to energy that may be used for other cellular activities. The principal substrate utilized in most cells is glucose. The conversion of glucose to CO2 and water via the glycolytic pathway, the citric acid cycle and oxidative phosphorylation yields a considerable amount of chemical energy, part of which is converted to ATP. The overall rate of metabolism in any metabolic pathway including those responsible for aerobic metabolism is determined by its slowest step. That is, the slowest enzyme reaction in a series of reactions determines the rate of the whole process. The reduction of oxygen to water, which occurs at the last step of the electron transport chain, should be a good indicator of overall metabolic rate.

The metabolic rate of ectothermic organisms depends on environmental temperature. The higher the temperature, the higher the metabolic rate and therefore the higher the oxygen consumption. In this experiment we will be measuring the effect of temperature on the rate of oxygen consumption by the larvae of the beetle, *Tenebrio molitor* (the mealworm). We predict that as temperature increases oxygen consumption should increase. In general it is known that for every 10°C increase in temperature the rate of an enzyme reaction doubles (Q10 = 2). Since the overall rate of a metabolic pathway is determined by the slowest enzyme in the pathway, we could predict that increasing temperature 10°C should approximately double the rate of oxygen consumption by these larvae.

To understand how temperature affects metabolic rate, we performed an experiment which tested two hypotheses. The first is that as temperature increases, the metabolic rate of mealworm tissues as measured by oxygen uptake should also increase. The second hypothesis is that since respiration in mealworms is enzyme mediated, and it is known that most enzyme reactions double in rate for each 10°C increase in temperature, it is predicted that the rate of oxygen uptake will double for each 10°C increase in temperature.

Materials and Methods:

The rate of oxygen consumption was measured using a Gilson respirometer. Each respirometer flask contained a single mealworm. The weight of each larva was measured before the experiment began and ultimately the values for oxygen uptake were corrected for animal weight. The sidearm of the flask was filled with a CO2 absorbent. The flasks containing the
worms were equilibrated in the water bath at 10°C, 20°C and 30°C before oxygen measurements
at these temperatures were begun. Measurements of the volume of oxygen consumed were made
on 5 mealworms at each temperature for a period of 30 min. Controls consisted of the same
flasks used above without mealworms and measurements of apparent oxygen uptake at each
temperature were made for these empty flasks for 30 min prior to adding the worms. The values
obtained for the empty flasks (Control) at each temperature were subtracted from the values for
the flasks containing mealworms (Exp) to obtain the net oxygen consumption due to the animals
alone. The data were also corrected for time and the weight of each animal so that the final units
are (ml O₂/g* hr). The following formula summarizes the corrections made for each point:

\[
\text{Net O}_2 \text{ Consumption} = \frac{(\mu l \text{ O}_2 \text{ consumed (Exp)}) - (\mu l \text{ O}_2 \text{ consumed (Control)})}{(1000 \mu l/ml) \times (g \text{ weight}) \times (0.5 \text{ hr})}
\]

Results:

Table 1 shows the raw data for this experiment. The oxygen consumption (Exp) and weight
of each individual worm (µl /30 min) is shown. The apparent oxygen consumption (µl/30 min)
by the empty control flasks (Control) for each flask at each temperature is also shown. The net
oxygen consumption by each animal at each temperature after the Control values were subtracted
from the Exp value for each worm at each temperature with corrections made for weight and
incubation time (according to the formula above) is shown in Table 2.

Table 1: Raw Data - Oxygen Consumption (µl O₂/30 min) by Mealworms at 3 Temperatures.

<table>
<thead>
<tr>
<th>Larva #</th>
<th>10°C Control</th>
<th>10°C Exp</th>
<th>20°C Control</th>
<th>20°C Exp</th>
<th>30°C Control</th>
<th>30°C Exp</th>
<th>Mealworm Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>4.29</td>
<td>3.5</td>
<td>10.04</td>
<td>6.5</td>
<td>18.50</td>
<td>0.053</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>4.53</td>
<td>5.5</td>
<td>10.84</td>
<td>9.0</td>
<td>20.54</td>
<td>0.046</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>5.36</td>
<td>7.0</td>
<td>12.84</td>
<td>4.5</td>
<td>18.57</td>
<td>0.059</td>
</tr>
<tr>
<td>4</td>
<td>3.5</td>
<td>5.58</td>
<td>4.5</td>
<td>9.64</td>
<td>6.0</td>
<td>15.68</td>
<td>0.042</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>6.13</td>
<td>4.0</td>
<td>10.98</td>
<td>7.5</td>
<td>21.20</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Table 2: Corrected Oxygen Consumption (ml O₂/g* hr) by Mealworms at 3 Temperatures.
These data show that the rate of oxygen consumption rapidly increases as temperature increases. Using a Student's t-test the differences between the rate at 10°C and the other two temperatures used in this experiment were statistically significant (p < 0.001). This same level of significance also applies to a comparison of the 20°C and the 30°C mean values. For each 10°C increase in temperature the rate approximately doubles. The ratio of oxygen consumption at 20°C to 10°C (0.230/0.117) is 1.96. The ratio of oxygen consumption at 30°C to 20°C (0.468/0.230) is 2.03. These data indicate that the Q_{10} (the rate of increase per 10°C increase in temperature) is about two for the range of 10°C to 30°C. These data were also plotted in Fig 1. Although only 3 temperature conditions were used these data appear to conform an exponential function that might be expected for temperature dependent enzyme processes.

<table>
<thead>
<tr>
<th>Larva #</th>
<th>10°C Exp</th>
<th>20°C Exp</th>
<th>30°C Exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.124</td>
<td>0.247</td>
<td>0.453</td>
</tr>
<tr>
<td>2</td>
<td>0.110</td>
<td>0.232</td>
<td>0.502</td>
</tr>
<tr>
<td>3</td>
<td>0.131</td>
<td>0.198</td>
<td>0.477</td>
</tr>
<tr>
<td>4</td>
<td>0.099</td>
<td>0.245</td>
<td>0.461</td>
</tr>
<tr>
<td>5</td>
<td>0.119</td>
<td>0.229</td>
<td>0.449</td>
</tr>
</tbody>
</table>

Mean ± S.E. 0.117 ± 0.006 0.230 ± 0.009 0.468 ± 0.010

Figure 1: Oxygen consumption measurements were made on individual mealworms in a Gilson respirometer. The points and error bars shown are the means ± S.E. (n = 5).

Discussion:
The data show that the rate of oxygen consumption by mealworms is rapidly increased by increasing temperature. The calculated Q10 was about 2 for the range from 10°C to 30°C. These data strongly support both hypotheses I and 2 proposed above. The value of 2 for Q10 is typical of most thermochemical and enzyme reactions. The rate of oxygen uptake is determined by the overall rate of the glycolytic pathway, the citric acid cycle and oxidative phosphorylation. The value of 2 for the Q10 for oxygen uptake is consistent with the possibility that the overall rate of these metabolic pathways is determined by some rate limiting step (presumably a "slow" enzyme step) although these data do not prove this unequivocally. These data also demonstrate that ectothermic organisms are likely to experience rather drastic changes in metabolic rate depending on environmental temperature. This suggests that special physiological adaptations may be necessary for those organisms living in extreme environments.

**Conclusion:**

Oxygen consumption by the larvae of the mealworm, *Tenebrio niolitor*, rapidly increases with increasing temperature. In the range 10°C to 30°C the rate of oxygen consumption increased from $0.117 + 0.006 \text{ ml/g*hr}$ to $0.468 + 0.010 \text{ ml/g*hr}$ ($n = 5$). The calculated Q10 for this process was 2. These data are consistent with possibility that one or more enzymatic rate limiting steps with a Q10 of 2 determines the overall rate of oxidative metabolism.

**References:** Any information that you got from an outside source (whether that be the lab manual, the textbook or elsewhere) must be cited properly.

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**Data Presentation**
When presenting your data in a lab report, you should present your raw data and calculations, as well as graphs.

**Raw data/calculation:**
Present this in the form of a chart. An example of this in Table 1 of the example lab report. You can also present calculated numbers in the form of a chart, as in Table 2 of the example lab report. All tables should include:

- A caption, describing the data that the table contains
- Row and column headings
- Units on any numbers

**Graphs:**
Use graphs to show your data in a more visual format. The graphs you may be asked to create are:

- Pie chart
  - Use when describing a part or percentage of a whole.
- Line graph
  - Use when you are looking at a change over time. In these graphs, the x-axis does not need to be evenly numbered, nor does it need to have numerical categories (could be letters/groups). The lines are often representing the different treatments.
- Bar graph (column graph in excel)
  - Use when only plotting a few data points or presenting means. This can have words in the x-axis and the x-axis does not necessarily need to be evenly numbered. Often the x-axis categories are the different treatments.
- XY scatter plot
  - Use this when trying to detect a trend or relationship between points. The x-axis drawn to scale in these plots and the data will be plotted in its exact position. This plot cannot be used if there are letters needed on the x-axis.

Graphs should be able to stand alone so that someone could get all necessary information from the graph itself. To achieve this, all graphs should contain the following:

- X- and Y-axis titles that describe what the axis represents (with units!).
- X- and Y-axis labels that describe what the different groups or parts of the axes represent.
- A descriptive graph/chart title.
- A legend indicating which lines, bars or colors match which treatments.
- Caption describing what the graph is depicting.
- Error bars that represent the standard error (for graphs that depict means) that go both above and below the point.

Continued on next page…

**Data Presentation (continued)**

**Graphs will be made using Microsoft Excel.** If you do not know how to use Excel, ask your TA
for help, and use these Microsoft tutorials and help pages. This software is available for use on ISU computers if you do not have your own copy. It is very important that you learn how to use this software now, as you will be required to use this in subsequent courses. Hand-made graphs will NOT be accepted.

- Charts I: How to create a chart in Excel 2007 (Tutorial)
- Create a Basic Chart (screenshots with descriptions)
- Customize Your Chart (screenshots with descriptions)
- Overview of Available Chart Types
- Quick Reference Card

**ELEMENTARY STATISTICS FOR LAB REPORTS**

This handout describes the statistical calculations you will need to do to process your data:
(a) calculate the \textit{mean}, (b) calculate the \textit{standard error} of the mean, and (c) conduct a \textit{Student's t-test} to test the statistical significance of the difference between two means.

**MEAN**

The mean of a group of measurements is simply the average. The following discussion will use as a specific example an experiment in which the oxygen consumption of mealworms has been measured at two temperatures. Let us assume we have measured the rate of oxygen consumption by 5 individual mealworms at 10°C and 20°C and that the raw data obtained are as follows:

<table>
<thead>
<tr>
<th>Larva #</th>
<th>10°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.124</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>0.110</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td>0.131</td>
<td>0.198</td>
<td></td>
</tr>
<tr>
<td>0.099</td>
<td>0.245</td>
<td></td>
</tr>
<tr>
<td>0.119</td>
<td>0.229</td>
<td></td>
</tr>
</tbody>
</table>

The mean value is just the sum of the numbers for each replicate divided by the number of replicate measurements. Or stated mathematically:

\[
\text{MEAN} = \frac{(X_1 + X_2 + X_3 + \ldots)}{N}
\]

where XI, X2, etc are the individual data points and N = the number of replicates. In this example the means are:

For the 10°C condition
\[
(0.124 + 0.110 + 0.131 + 0.099 + 0.119)/5 = (0.583)/5 = 0.117
\]

For the 20°C condition
\[
(0.247 + 0.232 + 0.198 + 0.245 + 0.229)/5 = (1.155)/5 = 0.230
\]

**NOTE:** The calculated answer for the 10°C condition was actually 0.1166, but mean should be rounded to 3 significant digits. Extra decimals are usually \textit{useless}; DO NOT write long strings of extra digits. Most biological measurements can do no better than a 1% or 2% error. Extra decimals simply are irrelevant and misleading. The only case in which one keeps all these long decimals is when doing certain statistical calculations (see below).

**STANDARD ERROR**

The standard error (or standard error of the mean, abbreviated S.E.) provides an \textit{estimate of}
the reliability of the mean. Since one does not know a "true" value, one can only estimate what the chances are that the mean one has measured is likely to be correct. In other words, you may not know the mean exactly but you may be able to tell whether the mean you measured is likely to be correct most of the time. This reliability measurement can be stated in the following terms:

The odds are about 1:2 that the mean value ± 1 S.E. differs from the "true" value; the odds are about 1:21 that the mean ± 2 S.E. differs from the "true" value; the odds are about 1:370 that the mean ± 3 S.E. differs from the "true" value. If this is not entirely clear see below.

The standard error is given by: \( \text{S.E.} = \frac{\text{S.D.}}{\sqrt{N}} \)

That is, the S.E. is the standard deviation (S.D.) divided by the square root of N, where N= the number of replicates.

**Note** To calculate this in excel:
1) Type =
2) Click on the cell with the SD value in it OR type in the SD value.
3) Type /
4) Type SQRT(
5) Type in the sample size value
6) Type )
e.g.: “=A3/SQRT(5)”

The standard deviation describes the variability of quantities that randomly vary about the mean value in a normal distribution (see a statistics text for a detailed explanation). One useful formula for S.D. is given below:

\[
\text{S.D.} = \sqrt{\frac{N \cdot \sum (X^2) - (\sum X)^2}{N(N-1)}}
\]

where \( \sum (X^2) \) is the sum of the squares of the values for X, \( (\sum X)^2 \) is the square of the sum of the values for X, and N is the number of replicates.

**NOTE: The \((X^2)\) is NOT mathematically the same as \((X)^2\). In the case of calculations of this sort DO NOT round off any of these intermediate numbers involving \(X^2\) or \((X)^2\); carry out the numbers as far as permitted by your calculator. This is the exception to the rule about rounding off, since very large calculation errors can be made if you round off too soon. Only the final value for S.E. should be rounded off.

**Note** To calculate this in excel:
1) Type STDEV(
2) Highlight all of the cells containing values of X (the response you are measuring)
3) Type )
e.g.: “=STDEV(A3:A10)”

For the example above, only the calculation for the 10°C will be shown in detail.
### Larva # | X   | X^2   
---|------|------
1  | 0.124| 0.015376 |
2  | 0.110| 0.0121 |
3  | 0.131| 0.017161 |
4  | 0.099| 0.009801 |
5  | 0.119| 0.014161 |

\[ \sum (N = 5) \sum X = 0.583 \quad \sum (X^2) = 0.068599 \]

\[ (\sum X)^2 = (0.583)^2 = 0.339889 \quad \text{Mean} = \frac{0.583}{5} = 0.1166 \]

Calculating S.D. and S.E. for this example:

\[ \text{S.D.} = \sqrt{\frac{5(0.068599) - (0.339889)}{5(5-1)}} = \sqrt{\frac{0.342995 - 0.339889}{20}} = \sqrt{\frac{0.003106}{20}} \]

\[ \text{S.D.} = \sqrt{0.0001553} = 0.012461942 \]

\[ \text{S.E.} = \frac{\text{S.D.}}{\sqrt{N}} = \frac{0.012461942}{\sqrt{5}} = \frac{0.012461942}{2.236067978} \]

\[ \text{S.E.} = 0.005573149 = 0.006 \text{ (rounded off)} \]

**Note that standard errors of the mean values should always be shown as error bars on graphs or bar graphs.** This immediately gives the reader an impression about the precision of the data. In these lab reports, the mean value in text or tables should also always be accompanied by its standard error. The general form writing this (using the example, see below) is \( 0.230 \pm 0.009 \text{ ml/g ' hr (N = 5)} \). Note that the number of measurements for this experimental case is shown in parenthesis. This helps readers quickly evaluate how many replicates were done without searching through data tables.

**STUDENT'S T-TEST**
The t-test is used to distinguish whether two means of two sets of observations are actually different or just different due to random variation. Every time one makes a new set of measurements slightly different sets of values for the mean is found. It is not certain whether the difference is due to a real difference or just random variation that occurs in all measurements. It is particularly important to decide whether one mean differs from another when one is comparing the mean values for a control condition or at some specified initial condition (e.g. for the mealworm example, the initial condition is at 10°C) with an experimentally change condition (for example, the temperature is changed to 20°C and the rate of oxygen consumption measured again). Although the equation for the t-test is fairly complicated, it reduces to a very simple equation if one arranges the experiment to have the same number of measurements under both control and experimental conditions. In other words, if one measures each value the same number of times under each condition the t-test is much simpler to calculate. We will usually design our experiments for these laboratory exercises to meet this condition. When there are equal numbers of measurements then the "t" value (for the t-test) is given by:

\[
t = \frac{M_e - M_c}{\sqrt{\frac{(S.E._e)^2}{N_e} + \frac{(S.E._c)^2}{N_c}}}\]

where \(M_e\) is the mean value of the experimental condition, \(M_c\) is the mean of the control condition and S.E._e and S.E._c are their respective standard errors.

Also to determine statistical difference one needs to calculate one more value, the number of degrees of freedom for the experiment ("n"). Fortunately this is very simple since the degrees of freedom are simply the sum of the total number of observations for both sets of measurements less 2. That is:

\[n = N_e + N_c - 2\]

where \(n\) is the degrees of freedom, \(N_e\) is the number of measurements under the experimental condition, and \(N_c\) is the number of measurements under the control condition.

To determine whether the means are statistically significantly different, one looks up the values for "t" in the t-table (disregard the sign oft) under the appropriate row for the degrees of freedom (see attached t-table). The probability that the difference between two means is real is given by the column headings under which the t-value is found. Your T.A.s will help you become familiar with this procedure. The probability value ("p" value) given is the chance that the difference between the two means is due to random sampling and therefore not "real". If \(p = 0.05\) it means that the chances are 5 out of 100 that the difference observed is not real. If \(p = 0.01\) it means the chances are 1 out of 100 that the differences are not real. Thus small values for \(p\) indicate increased likelihood the the difference between two means is real. If the \(p\) were 0.5 it indicates that the chances are 50 out of 100 that the difference is not real and thus one should have very low confidence that there is any real difference between the experimental and control
values.

In scientific papers, the t-test and a variety of other tests are usually employed to indicate how reliable the comparisons of data under various conditions are. The values for $p$ are usually included in data tables for various conditions compared with the control or other initial state. In your reports when the exercise specifically requires a t-test you should include the $p$ value in the data table. It is generally assumed in biological research that $p < 0.05$ strongly supports the possibility that the differences between two conditions are statistically significant (or in other words, likely to be "real").

An example calculation based on our mealworm example follows:

The mean oxygen consumption at $10^\circ C = 0.117 \pm 0.00557 \text{ ml/g/hr}$ The mean oxygen consumption at $20^\circ C = 0.230 + 0.00878 \text{ ml/g/hr}$

Calculating $t$:

$$t = \frac{0.117 - 0.230}{\sqrt{(0.00557)^2 + (0.00878)^2}} = \frac{-0.113}{\sqrt{0.000031024 + 0.000077088}} = \frac{-0.113}{\sqrt{0.000108113}} = -10.87$$

Degrees of freedom $= 5 + 5 - 2 = 8$

Use the T-table following this example to see if the treatments are statistically different AND at what level (probability level). See the example on the next page as well.

1) Locate the degrees of freedom for your experiment in the left column
2) Follow the row for your DF to the column labeled 0.050 probability
3) Is your ‘$t$’ value larger or smaller than the value where your DF row and probability column meet? If larger, then the difference is statistically significant. If your calculated ‘$t$’ is smaller than the value shown, then there is no statistical difference between the treatments.
4) Now determine what probability level your ‘$t$’ value falls into. Follow the row to find where your ‘$t$’ value would fall and list the probabilities that would bound your ‘$t$’ value on the chart.
Step 3: ‘t’ value from example = 10.87. Our t value is greater than 2.306, so our treatments are statistically different from each other.

Step 4: Our ‘t’ value is greater than the largest value in the ‘8 degrees of freedom’ row. So, our value of t = 10.87 means that the probability that the difference between these two means is due to chance is very small, much less than 1 chance in 1000 (p<0.001).

We state this fact as follows: "The oxygen consumption at 10°C and 20°C is statistically different at the p < 0.001 level using a Student's t-test."
<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>0.500</th>
<th>0.400</th>
<th>0.200</th>
<th>0.100</th>
<th>0.050</th>
<th>0.025</th>
<th>0.010</th>
<th>0.005</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.000</td>
<td>1.376</td>
<td>1.307</td>
<td>2.706</td>
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<td>2.360</td>
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<td>2.648</td>
<td>2.899</td>
<td>3.435</td>
</tr>
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<td>0.823</td>
<td>1.293</td>
<td>1.581</td>
<td>1.989</td>
<td>2.284</td>
<td>2.638</td>
<td>2.887</td>
<td>3.416</td>
</tr>
<tr>
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<td>0.677</td>
<td>0.816</td>
<td>1.290</td>
<td>1.602</td>
<td>1.982</td>
<td>2.276</td>
<td>2.625</td>
<td>2.871</td>
<td>3.390</td>
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<tr>
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<td>0.815</td>
<td>1.289</td>
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<td>1.980</td>
<td>2.270</td>
<td>2.617</td>
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<tr>
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<td>1.287</td>
<td>1.595</td>
<td>1.978</td>
<td>2.264</td>
<td>2.610</td>
<td>2.857</td>
<td>3.360</td>
</tr>
</tbody>
</table>

* Parts of this table are reprinted by permission from R. A. Fisher's Statistical Methods for Research Workers, published by Oliver and Boyd, Edinburgh (1925-1950); from Maxine Merrington's "Table of Percentage Points of the t-Distribution," Biometrika, 32:300 (1942); and from Bernard Ostie's Statistics in Research, Iowa State University Press (1954).
DATA PRESENTATION

When presenting data is it important to do so in such a way that makes it quick and easy for the reader to understand. The two main forms of data presentation we will do in this class are tables and graphs.

Tables
• All tables should include:
  o A table number (sequentially ordered, table 1, table 2, etc.)
  o A detailed title/description of the contents of the table
  o Units (degrees, grams, etc.)
  o Clear headings indicating what is found in each row/column

• An example:

Table 1: Raw Data - Oxygen Consumption (µl O₂/30 min) by Mealworms at 3 Temperatures.

<table>
<thead>
<tr>
<th>Larva #</th>
<th>10°C Control</th>
<th>10°C Exp</th>
<th>20°C Control</th>
<th>20°C Exp</th>
<th>30°C Control</th>
<th>30°C Exp</th>
<th>Mealworm Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>4.29</td>
<td>3.5</td>
<td>10.04</td>
<td>6.5</td>
<td>18.50</td>
<td>0.053</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>4.53</td>
<td>5.5</td>
<td>10.84</td>
<td>9.0</td>
<td>20.54</td>
<td>0.046</td>
</tr>
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<td>1.5</td>
<td>5.36</td>
<td>7.0</td>
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<td>4.5</td>
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<td>6.0</td>
<td>15.68</td>
<td>0.042</td>
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<tr>
<td>5</td>
<td>2.5</td>
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<td>4.0</td>
<td>10.98</td>
<td>7.5</td>
<td>21.20</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Graphs
The basic structure of most graphs is that along the x-axis is plotted the independent variable, the parameter that is experimentally varied in a controlled way. The dependent variable is plotted on the y-axis. The dependent variable is the process or product that is modified by changes in the independent variable, this is what you are measuring.

There are many types of graphs including bar graphs, line graphs, xy scatter plots, and pie charts. Regardless of the type of graph, ALL GRAPHS MUST INCLUDE:
• X and Y-axis titles
• X and Y-axis labels (including units)
• Graph title (must be descriptive)
• Legend
• Error bars

Name: ___________________
Exercise 1- Data Presentation and Basic Statistics

Part A

Produce an appropriate graph for each of the following data sets. Be sure that the graph includes all necessary elements (see data presentation section of the lab manual). Attach the graphs and turn them in with this exercise. Each question is worth 1 point.

1.) You interviewed 2000 people about their favorite college football team and got the following results:
   - 630 people indicated Michigan is their favorite team.
   - 440 people indicated Illinois State is their favorite team.
   - 390 people indicated Purdue is their favorite team.
   - 220 people indicated Ohio State is their favorite team.
   - 140 people indicated Illinois is their favorite team.
   - 100 people indicated USC is their favorite team.
   - 80 people indicated Tennessee is their favorite team.

   Use a graph or chart to show your results.

2.) You have been tracking the average height of male and female students over a period of 20 years. Your results are summarized in the table below:

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Average Height of Males</th>
<th>Average Height of Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>114 cm</td>
<td>116 cm</td>
</tr>
<tr>
<td>10</td>
<td>147 cm</td>
<td>152 cm</td>
</tr>
<tr>
<td>15</td>
<td>164 cm</td>
<td>158 cm</td>
</tr>
<tr>
<td>20</td>
<td>179 cm</td>
<td>163 cm</td>
</tr>
</tbody>
</table>

   Use a graph or chart to show your results.

3.) You have completed a series of experiments that has demonstrated differences between the average mass of carrots grown in different soil mixes. Your results show that the average mass of carrots grown in collected topsoil was 197.3 g; the average mass of carrots grown in sterile, potting mixture was 236.2 g; and the average mass of carrots grown in a 1:1 mix of peat and compost was 257.6 g.

   Use a graph or chart to summarize your findings
Part B
Show your work for all calculations.

You have observed the amount of time it takes 30 radish seeds to germinate. The data collected is presented below:

<table>
<thead>
<tr>
<th>Plant #</th>
<th>Time of development (in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>213</td>
</tr>
<tr>
<td>2</td>
<td>222</td>
</tr>
<tr>
<td>3</td>
<td>245</td>
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<td>259</td>
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<td>230</td>
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<td>27</td>
<td>218</td>
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<td>28</td>
<td>266</td>
</tr>
<tr>
<td>29</td>
<td>211</td>
</tr>
<tr>
<td>30</td>
<td>245</td>
</tr>
</tbody>
</table>

1. 3 points. What are the calculated mean, standard deviation, and standard error for the data in the table?

Mean: __________
Standard deviation: __________
Standard error: __________

Exercise 1
2. 2 points. Randomly select 2 subsets of data that contain only 15 and 3 radishes respectively. What are the calculated means and standard errors for each of these subsets of data?

Data set containing 15 radishes:
Mean: __________
Standard error: __________

Data set containing 3 radishes:
Mean: __________
Standard error: __________

3. 2 points. Briefly describe the effect that reducing the number of experimental samples had with respect to standard error.

_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________
Laboratory 1 - Properties of Enzymes

Objective:

The objective of this experiment is to characterize some of the basic properties of enzymes. You will study the effect of pH, temperature, enzyme concentration, substrate concentration and inhibitors on the rate of product formation by the enzyme, catalase. The factors studied here affect almost all enzymes in a similar fashion and therefore you should view these experiments as applicable to enzymes in general.

Introduction:

Enzymes are cellular proteins that function as biological catalysts. A catalyst is a substance that increases the rate of a reaction (see text for background material). There are thousands of enzymes in cells. These enzymes promote very specific chemical reactions in very specific sequences (called metabolic pathways). Some of these pathways lead to the breakdown of nutrient molecules such as glucose to CO2 and water and the subsequent formation of ATP. Other pathways lead to synthesis of common organic molecules found in cells, the synthesis of lipids and carbohydrates, the synthesis of nucleotides and ultimately DNA and RNA and even to the synthesis of enzymes themselves. In other words, even the synthesis of enzymes requires other specific enzymes. It is safe to say that virtually all chemical reactions in cells are promoted and regulated by enzymes (with a very few exceptions). The statement that enzymes regulate as well as promote chemical reactions is an important one. If an enzyme normally promotes a reaction and if the enzyme is somehow inactivated or inhibited or its tertiary structure disrupted, then you would expect the rate of the chemical reaction that the enzyme promotes would also decrease. Sometimes key points in metabolic pathways are regulated by "switching-up" or "switching-down" the activity of a particular enzyme by cellular "message" molecules that act as stimulators or inhibitors of the enzyme's activity.

Since enzymes are just an important class of proteins, all the general properties of proteins apply to enzymes as well. Therefore, the primary structure of enzymes is determined by the sequence of the 20 naturally occurring L-amino acids. Enzymes also have secondary structure (helices and pleated sheets are common but not every enzyme must necessarily have a helix or a pleated sheet), tertiary structure and quaternary structure. Recall that tertiary protein structure is its three dimensional shape as the protein spontaneously folds as the side chains of the constituent amino acids interact with other side chains of the same protein and with water in which the protein is dissolved. The forces that may contribute to tertiary structure include hydrogen bonding, hydrophobic interaction, ionic bonding and covalent bonding.

Quaternary structure refers to the fact that some (but not all) enzymes require multiple separate protein (polypeptide) chains to form an active catalytic unit. Some active enzymes may be composed of several polypeptide chains (typically 2 to 4, but larger numbers of subunits are known for certain enzymes). These polypeptide subunits are not necessarily the
same protein, some enzymes may have different polypeptide chains combined within the quaternary structure. The forces that hold the polypeptide chains together in the quaternary structure are the same that act in establishing tertiary structure - namely, hydrogen bonding, hydrophobic interaction, ionic bonding and covalent bonding.

It should also be pointed out, and you should remember that each enzyme that promotes a particular kind of chemical reaction is unique in its primary, secondary, tertiary and quaternary structure. An enzyme that promotes a particular kind of reaction apparently must have a particular three dimensional shape. It is generally the case that enzymes from diverse organisms (for example, potatoes and humans) that promote the same chemical reaction (for example, catalase promotes the breakdown of H₂O₂) are quite similar at all levels of structure. Enzymes that promote very different reactions (such as DNA synthesis or glucose breakdown) are very different structurally from catalase (or almost any other enzymes). In other words, enzymes fulfilling a certain function usually have a certain specific structure and shape regardless of the organism, tissue or cell type it is in. This structure and shape differs from almost all other enzymes. Enzymes that act on the same compounds are likely to have similarities in structure.

It should be mentioned that the same enzyme from different organisms and even from different tissues in the same organism may have very slight differences that can be measured experimentally. Sometimes these slight differences are used to construct evolutionary relationships among organisms since it can be argued that the more differences (even though slight) there are in the same enzyme from different organisms, the more genetically and taxonomically different the organisms themselves are. This fact still does not change the general statement made above that enzymes of similar function tend to have similar structure regardless of which organism was the source of the enzyme.

An enzyme is usually thought of as binding tightly (but temporarily) to the compound or compounds upon which it acts. (the general term "substrate" is used to denote those compounds upon which and enzyme acts). This binding occurs in the "active site" of the enzyme. The active site of an enzyme is defined as that specific region of the enzyme at which the substrate or substrates binds or complexes to the enzyme). The active site has a particular geometric shape and chemical properties that permit only specific molecules, to bind to it. This binding brings the substrates close together and promotes the reaction. The product or products are then released and the enzyme is free for another cycle of binding and product formation. This process is usually summarized by the reaction listed below:

\[
E + S \rightleftharpoons ES \rightarrow P + E
\]

where E = enzyme, S = substrate, ES = enzyme-substrate complex and P = product. The arrows indicate that typically the formation of the enzyme-substrate complex may be reversible but product formation is usually irreversible. This is not always true. Some enzymes work perfectly well in both the forward and backward directions.

For any enzyme to be active (promote its particular reaction efficiently), the tertiary and quaternary structures must have just the right shape in solution. Agents that inhibit enzymes
(cause them to decrease in activity) are often agents that chemically or physically disrupt tertiary or quaternary structure. Since both tertiary and quaternary structure are stabilized by hydrogen bonding; hydrophobic interaction, ionic bonding and covalent bonding, it might be predicted that any agent that alters these types of bonding or interaction should also alter tertiary structure and this alteration of tertiary structure could cause inactivation (partial or complete of the enzyme). Generally speaking, this is true.

Recall that hydrogen bonds are easily disrupted by heat. One would predict that if an enzyme is heated sufficiently, its hydrogen bonds would be disrupted. This disrupts the tertiary structure and the enzyme should become inactivated. This happens with all enzymes. The process is given a special name, heat denaturation. An enzyme that is denatured is inactive. In boiling water (100°C) the disruption of the tertiary structure is usually so severe that the enzyme precipitates (it is no longer as soluble in water as it once was). Under certain conditions some enzymes may regain activity at lower temperatures, but most remain permanently inactivated. By the way, raising the temperature of solution containing an enzyme but keeping below the denaturation temperature increases the reaction rate. This is simply due to the fact that the average kinetic energy of the reactant molecules (remember heat is just molecular movement) is increased, increasing the probability that the enzyme and its reactants will come together. Conversely, cooling and enzyme slows the reaction rate because the average kinetic energy of the reactant molecules is decreased. (One reason refrigeration preserves food is that it drastically slows down the enzymes in contaminating microorganisms and in the food itself).

Another important factor that affects tertiary structure is the pH of the solution in which the enzyme is dissolved. Recall that changing pH changes the protonation of amino acid side chains on proteins. Just as the net charge on a single amino acid may be altered by changing pH, so can the net charge on proteins be altered by changing pH. You would predict, therefore, that enzymes should be sensitive to pH. This is true. Most enzymes have an optimal pH range they function best in and if the pH is above or below this range the enzymes are inactive or very inefficient. Changing pH disrupts ionic bonds (and also ionic interaction of the enzyme with water molecules) and therefore can change tertiary structure. Small pH changes alter enzyme activity, large pH changes often lead to precipitation of the protein. Sometimes returning the enzyme to its optimal pH restores activity and sometimes (depending on the particular enzyme) it is permanently denatured. The sensitivity of enzymes to pH changes is one reason cells must have buffers (such as phosphate or bicarbonate) that prevent drastic pH change.

Organic solvents such as ethyl alcohol can disrupt tertiary structure by interfering with hydrophobic interaction (although there may be other effects as well). In analogy with the statements above, one would expect enzymes to be inhibited in the presence of organic solvents and at high solvent concentrations to be denatured (causing precipitation of the protein). This occurs with most enzymes. Interesting, many enzymes seem to be able to return to their active state after treatment with organic solvents such as alcohol or acetone. This is, in fact, one method by which enzymes are isolated from tissues for laboratory study.

Finally, agents that modify the covalent linkages at any level of structure, primary through quaternary, might be expected to alter enzyme activity. All enzymes require that specific side chain groups be present in certain positions in order that the active tertiary conformation can be
formed. Certain side chains must be present in the active site of the enzyme. For example, many enzymes require free sulfhydryl groups (-SH, the side chain of the amino acid cysteine). Heavy metals (mercury, lead, tin, etc) react covalently very rapidly with free -SH groups and can thereby alter the tertiary structure. If the -SH group is in the active site region of the protein, the heavy metal can also chemically and physically block this site. There are also many organic compounds that covalently react with a variety of amino acid side chains of proteins in addition to -SH groups (i.e. -OH, -NH2, -COOH groups). Pesticides, herbicides, nerve gas and poisons of many kinds are examples of this kind of agent. In general, these agents covalently modify a protein, thus inactivating it. Sometimes these covalent modifications are reversible if the enzyme is treated with the appropriate chemical agents, but in most cases the protein is permanently denatured.

Many enzymes (but not all) require cofactors for activity. Cofactors are organic or inorganic molecules (usually of small molecular weight) that are usually required at the active site to assist in substrate binding or other processes. Enzymes that require cofactors usually have an absolute requirement for them. Organic cofactors are also called coenzymes. For example, enzymes that act on ATP usually require magnesium ion (Mg\(^{2+}\)) as a cofactor. Apparently, Mg\(^{2+}\), a the divalent cation, assists in binding ATP which has multiple negative charges at neutral pH. Typically, cofactors for enzymes that bind oxygen or produce oxygen have organic multiple ring structured "heme" groups that have iron complexed to them. This type of iron-heme cofactor is present in hemoglobin and is also present in catalase, the enzyme you will be investigating in this experiment. Removal or chemical modification of the cofactors inhibits enzyme activity. Certain very toxic poisons (such as cyanide and azide) act by reacting with cofactors (in the case of hemoglobin and catalase, with the iron-heme group). The cofactors are absolutely essential for enzyme function and when they have been chemically modified, the enzyme ceases to function. Sometimes this type of inhibition is reversible, but often it is not.

Enzymes are very selective with regard to substrate. Sometimes there may be a single substrate upon which the enzyme normally acts or at best a few substrates. If an enzyme is exposed to two or more substrates simultaneously, they will compete for the active site. This competition results a decrease in the amount of product formation from each competing substrate. There is a limited amount of enzyme and each substrate occupies the active site for part of the time. Therefore, less product from each individual substrate is formed. This process is called competitive inhibition and is observed with all enzymes. "Artificial" substrates that block the active site but are not acted upon by the enzyme have been synthesized for many enzymes. Competitive inhibition of enzymes is used to help define the natural range of substrates upon which an enzyme acts. It is also the basis for selective drug design to target certain enzymes and leave others untouched. That is, a drug that resembles a substrate of an enzyme is more likely to react with or bind to that enzyme than some other unrelated enzyme.

**Specific Background for this Experiment**

In this experiment you will investigate the effects of various factors such as temperature, pH and inhibitory agents on the rate of oxygen formation by the enzyme catalase (extracted from potato). Catalase converts hydrogen peroxide (H\(_2\)O\(_2\)) to water and gaseous oxygen (O\(_2\)): 
Catalase occurs in most eukaryotic organisms and its function is to destroy hydrogen peroxide that is produced by certain cellular oxidase enzymes. These enzymes that convert compounds ingested by phagocytosis or that are absorbed by other means to usable cellular substrates. Hydrogen peroxide is a very strong oxidizing agent and could cause cellular damage if there was no way of getting rid of it. A good deal is known about the molecular structure of catalase. The active enzyme has a molecular weight of about 240,000 and its quaternary structure consists of four polypeptide subunits (each with a MW = 60,000; each subunit is about 500 amino acids long). Bound to each subunit is an iron-heme cofactor. Therefore, the active enzyme contains a total of 4 such iron-heme groups. Catalase is one of the fastest acting enzymes known. Each enzyme molecule can convert about $10^7$ molecules of $\text{H}_2\text{O}_2$ per minute into $\text{O}_2$ and water!

In this experiment you will use the "floating disk" technique you used for the first experiment in this course. The procedures, taken from that exercise, are repeated below. Fresh catalase extract will be prepared from potato. Homogenizing the potato tissue for a minute or so, breaks up the individual cells releasing cellular organelles and cytoplasmic solutes, enzymes, etc. Twenty-five grams of potato per 100 ml distilled water will be homogenized for 1 min and then filtered through a Buchner funnel.

The following experiments will be done and the procedures are described below: 1. Effect of enzyme concentration on reaction rate 2. Effect of temperature 3. Effect of pH 4. Effect of inhibitors (mercuric chloride, sodium azide, hydroxylamine) 5. Effect of a protease on reaction rate. In the last part of the experiment, catalase will be treated with trypsin, a "digestive" enzyme that attacks peptide bonds and degrades proteins. One would expect that catalase activity should decrease as the trypsin clips off portions of the catalase molecule.

**IMPORTANT NOTE ABOUT DATA PLOTTING:** The rate of $\text{O}_2$ production by catalase using the floating disk technique is inversely proportional to the time (in seconds) that it takes the disk to rise to the surface. Obviously, if there is no catalase present the disk never rises. The more active the catalase, the less time it takes for the disk to rise indicating more $\text{O}_2$ is formed in less time. All rates should be expressed as reciprocal seconds, that is, the rate of catalase activity = $1/T$ where $T = \text{time in seconds}$. For example, assume one disk took 20 seconds to rise to the surface and another took 40 seconds. There is obviously more catalase activity in the first disk than the second. The rates would be $1/20 = 0.05 \text{ sec}^{-1}$ for the first disk and $1/40 = 0.025 \text{ sec}^{-1}$ for the second disk. Now the rate relationships are clearly expressed in these units. The first disk (0.05 sec$^{-1}$) has twice the rate of the second disk (0.025 sec$^{-1}$).

**General Procedures:**

1. The T.A. will prepare the potato catalase extract. You should watch this procedure.
   a. Homogenize 25 g of fresh potato with 100 ml distilled water for 1 minute at high speed in a blender.
   b. Pour off the homogenate and filter it through filter paper using suction filtration through a
Buchner funnel. The filtrate should be clear. This filtrate will be the catalase extract used for the following procedures (you will only need about 10 ml of this extract for the experiment).

2. Pour 100 ml of 1% H₂O₂ into a 150 ml glass beaker.

3. Making the measurements
   a. Using forceps, dip a filter paper disk (about 6 mm diameter) about half-way into the catalase extract. Let capillary action draw the extract into the rest of the disk until it is completely saturated.

   b. Carefully drop this disk (preferably edgewise) into the 100 ml of H₂O₂. It should immediately sink. Be sure not to dip the forceps in the H₂O₂ as they might be carrying extra extract and introduce more error.

   c. As soon as the disk touches the surface of the H₂O₂, start timing with a stopwatch. When the bubbles of O₂ accumulate on the disk in sufficient quantity, it will rise to the surface. As soon as the disk reaches the surface stop the stopwatch and record the time to the nearest 0.1 second.

   d. Do a trial run. The time it takes the disk to rise should be between 20 and 60 seconds. If it is faster, ask the T.A. to dilute the extract with an equal volume distilled water and test again.

   e. For every experimental condition described below, and for controls, repeat this measurement 3 times. Be sure to clean the forceps each time before picking up the next filter paper disk.

SPECIFIC PROCEDURES FOR CONTROL AND EXPERIMENTAL TREATMENTS

PRELIMINARY NOTES:

A. Start the treatment of catalase extract with trypsin immediately before beginning other parts of the experiment. Let the trypsin act on the catalase extract for at least an hour (let it incubate for as long as you can) and then test this extract after all of your other procedures are finished.

B. Do the Sodium Azide treatment LAST, as this will contaminant the H₂O₂ if done before the other treatments.

C. IF ANY TREATMENT OF THE CATALASE EXTRACT RESULTS IN TIMES LONGER THAN 5 MINUTES, TERMINATE THE OBSERVATION AND ASSUME NO ACTIVITY IS PRESENT IN THE EXTRACT. For calculation/graphing purposes, record this as 300 seconds.
1. **CONTROL**: Dip disks in a mixture of 0.75 ml distilled water and 0.25 ml phosphate buffer (pH 7) and place in H₂O₂. If no change occurs discontinue after 5 min. YOU WILL COMPARE THE CONTROL TO ALL EXPERIMENTAL TREATMENTS

2. **EFFECT OF ENZYME CONCENTRATION ON REACTION RATE:**
   a. Experimental: Test the catalase extract at 3 concentrations of extract.
      i. **Concentration 1** (label this 100%): Mix 0.75 ml catalase extract + 0.25 ml phosphate buffer (pH 7)
      ii. **Concentration 2** (label this 50%): Mix 0.38 ml catalase extract + 0.37 ml distilled water + 0.25 ml phosphate buffer (pH7)
      iii. **Concentration 3** (label this 25%): Mix 0.19 ml catalase extract + 0.56 ml distilled water + 0.25 ml phosphate buffer (pH 7)
   b. Measure the rate of O₂ production at each concentration 3 times. Plot the rate (in reciprocal seconds) of catalase activity versus concentration (percent "original" concentration).

3. **EFFECT OF TEMPERATURE:**
   a. Pipette 0.5 ml of catalase extract + 0.5 ml phosphate buffer (pH 7) into a test tube. Place it in a boiling water bath for about 10 min. Test the boiled extract 3 times.
   b. Mix 0.5 ml of catalase extract + 0.5 ml phosphate buffer (pH 7). Do 3 replicates of the extract at room temperature. Ask the T.A. for the room temperature and note it in your report.
   c. Mix 0.5 ml of catalase extract + 0.5 ml phosphate buffer (pH 7). Do 3 replicates of the extract in H₂O₂ pre-chilled on ice and kept on ice during the experiment. You can assume that the temperature of the H₂O₂, if properly chilled, is 0°C.

4. **EFFECT OF pH:**
   a. Mix 0.5 ml catalase extract + 0.5 ml of each of the following phosphate and/or citrate buffers in separate test tubes. The buffers will have the following pHs: pH3, pH 5, pH 7 (pH 7 IS THE SAME AS THE ROOM TEMPERATURE TUBE, DO NOT REPEAT THIS), pH 9.
   b. Measure the rate of catalase activity 3 times at each pH. Plot catalase activity (reciprocal seconds) versus pH (x-axis).

5. **EFFECT OF A PROTEASE ON REACTION RATE:**
   a. Mix 0.5 ml of catalase extract with 0.5 ml of trypsin solution in phosphate buffer (pH 7) in a test tube. Let the trypsin incubate with the catalase extract for as long as feasible but at least 1 hour.
b. Trypsin: Test the trypsin treated catalase extract prepared in part ‘a’ above 3 times.

6. EFFECT OF INHIBITORS:
   a. Sodium azide: Mix 0.5 ml catalase extract + 0.25 ml phosphate buffer pH 7 + 0.25 ml 1 mM sodium azide. Measure catalase activity 3 times. This compound reacts with the iron-heme cofactor required by catalase. **SODIUM AZIDE IS VERY TOXIC. BE EXTREMELY CAREFUL.**

DATA, GRAPHS and CALCULATIONS:

DATA - By the end of this experiment, you should have recorded the following data as seconds required for the disks (dipped in the solutions specified above) to rise to the surface:

1. Control
   a. Control - 3 replicates. (TO BE COMPARED TO ALL GROUPS BELOW!)

2. Effect of enzyme concentration on reaction rate:
   a. Experimental - 3 replicates at each of 3 concentrations of extract.

3. Effect of temperature:
   a. Three replicates of boiled catalase extract.
   b. Three replicates of room temperature catalase extract.
   c. Three replicates of catalase extract measured at 0°C.

4. Effect of pH:
   a. Three replicates of catalase extract measured at the following pHs:
      i. pH3, pH 5, pH 7 (USE ROOM TEMP DATA), pH 9.

5. Effect of a protease on reaction rate.
   a. Three replicates of catalase extract treated with trypsin.

6. Effect of inhibitors:
   a. Three replicates of catalase extract treated with sodium azide.

GRAPHS & CALCULATIONS - BE SURE ALL FINAL TABULATION AND GRAPHING IS DONE WITH **RECIPROCAL SECONDS** AS UNITS OF THE RATE OF CATALASE ACTIVITY. (See Specific Background Section). Use reciprocal seconds to calculate the mean ± S.E. for the 3 replicates for each experimental condition. You will have 5 graphs, one for each experiment:

1. Effect of enzyme concentration on reaction rate: Plot rate of catalase activity (mean ± S.E.) versus relative enzyme concentration (percent extract on x-axis). *Include control*
2. Effect of temperature on reaction rate: Prepare a graph comparing rates (mean + S.E.) of catalase activity at 0°C, room temperature and for boiled extract. Include control data.

3. Effect of pH on reaction rate: Plot rate of catalase activity (mean ± S.E.) versus pH (pH on x-axis). Include control data.

4. Effect of a protease on reaction rate: Prepare a table comparing rates (mean ± S.E.) of catalase activity measured under control conditions and on catalase extract treated with trypsin. Include control data.

5. Effect of inhibitors on reaction rate: Prepare a graph comparing rates (mean ± S.E.) of catalase activity measured under control conditions and in the presence sodium azide. Include control data.

**Discussion:**

Reread the introduction carefully. This exercise was designed to test the effects of enzyme concentration, temperature, pH, inhibitory reagents and trypsin on an enzyme reaction. Do your data agree with the information outlined in the introduction? For each inhibitory agent, suggest a mechanism of action. If all enzymes have somewhat similar properties, does this suggest some ways that pollutants or other toxins might affect the metabolism of cells?

**Conclusion:**

In a few sentences state your findings from this experiment.
Exercise 2- Types of Data and Standard Curves

Page 1 of 2

10 pts

Remove this page from your lab manual and turn it in with your standard curve attached

1. 4 points. For each of the following indicate whether the described procedure results in qualitative or quantitative data.

a. Determination of the amount of chlorine in a pool with a test strip and it shows there is 300ppm in the water.

b. Home pregnancy tests determine if there is Human chorionic gonadotropin (hCG) hormone in urine. If there is it will turn blue and you are pregnant.

c. Determine whether a solution is an acid or a base using litmus paper. Litmus paper turns different colors if exposed to an acid or a base.

d. Diabetics test blood glucose levels by putting a drop of blood on a special strip. The strip enters a hand-held reader that displays the blood glucose concentration expressed in mg/dl units.

2. 6 points. Generate a standard curve and determine the approximate concentration of the experimental sample in the following example. Be sure to determine and show the standard error for each set of samples.

You are a technician in a quality control lab at a major pharmaceutical company. They produce a new antibiotic called squigimycin and it is your job to determine the relative concentration of squigimycin produced in each reaction. Squigimycin reacts with Reagent Z to produce a color that absorbs light at 540nm. Therefore, you can use spectrometric analysis at 540nm to determine the concentration of squigimycin in each reaction.

You have 5 standards of known concentration that you have tested (each three times) to determine the absorbance. Your collected data are Absorbance at 540nm and are provided on the next page in a table. You will use the means that you calculate to produce your standard curve.
Absorbance of known concentrations of Squigimycin at 540nm

<table>
<thead>
<tr>
<th>Known Concentration</th>
<th>10µg/ml</th>
<th>25µg/ml</th>
<th>50µg/ml</th>
<th>75µg/ml</th>
<th>100µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading 1</td>
<td>0.11</td>
<td>0.27</td>
<td>0.49</td>
<td>0.67</td>
<td>0.89</td>
</tr>
<tr>
<td>Reading 2</td>
<td>0.14</td>
<td>0.24</td>
<td>0.52</td>
<td>0.69</td>
<td>0.87</td>
</tr>
<tr>
<td>Reading 3</td>
<td>0.10</td>
<td>0.28</td>
<td>0.51</td>
<td>0.68</td>
<td>0.93</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After creating your standard curve, use the curve and the data below to determine the approximate concentrations of the three reactions (A, B, C) that you tested below; do so by comparing the means of the three unknown reactions to the curve.

Absorbance of unknown concentrations of Squigimycin at 540nm

<table>
<thead>
<tr>
<th>Reaction with unknown concentration</th>
<th>Rxn A</th>
<th>Rxn B</th>
<th>Rxn C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading 1</td>
<td>0.74</td>
<td>0.32</td>
<td>0.59</td>
</tr>
<tr>
<td>Reading 2</td>
<td>0.68</td>
<td>0.29</td>
<td>0.60</td>
</tr>
<tr>
<td>Reading 3</td>
<td>0.71</td>
<td>0.33</td>
<td>0.59</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Laboratory 2- Isolation of DNA, Measurement of the DNA Content of Strawberries

Objective:
The objective of this experiment is to isolate deoxyribonucleic acid (DNA) from strawberries and measure the amount of DNA using a quantitative colorimetric assay.

Introduction:
One of the most important and intensely studied macromolecules in modern biology is DNA. The reason for this is the fact that chromosomal DNA encodes the complete set of hereditary information necessary to construct a complete organism. DNA in every species has a unique length and encodes a unique set of information for that species. As you are aware, the information is encoded as sequences of four different monomer nucleotides: deoxyadenosine-5’-monophosphate, deoxyguanosine-5’-monophosphate, thymidine-5’-monophosphate, deoxycytidine-5’-monophosphate. These nucleotides contain, respectively, the well known bases: adenine, guanine, thymine, and cytosine. Three nucleotides in a sequence ultimately specify a single amino acid in a protein. The information is transferred in small amount to messenger RNA (usually an amount equivalent to a single gene’s worth of information which specifies a single protein). Messenger RNA (mRNA) the leaves the nucleus via nuclear pores, and enters the cytoplasm where it binds to ribosomes. The code contained within the mRNA is translated on the ribosomes and the specific protein is synthesized. The proteins that result from a multitude of different mRNAs may be enzymes or structural proteins for intracellular use, proteins for export, or proteins to be incorporated into membranes or other organelles. The enzymes may be part of a critical cellular metabolic pathway involved in generating energy, DNA duplication, RNA synthesis, or other metabolic sequence. It is therefore obvious that DNA ultimately specifies cellular structure and function and thus the structure and function of the organism as a whole.

To encode the entire amount of information necessary to construct and organism as a sequence of nucleotides, DNA molecules must be very long. As an analogy, think of trying to translate the Encyclopedia Britannica into Morse code (a series of dots and dashes for every letter of the alphabet). You would need a very long sequence of dots and dashes to encode the information. Likewise, with three nucleotides specifying one amino acid, and as many as a thousand amino acids in one protein, several thousands of bases are needed to encode a single protein. Considering the possibility of thousands of proteins per cell, it is not surprising that DNA is such a long molecule. It has been calculated that if all the DNA in a single human cell were unraveled and stretched out, it would be more than a meter long!

To compact all this information into a cellular nucleus which is a few micrometers in diameter, the DNA is twisted over and over (“supercoiling”) into small compact units called chromosomes. In humans, there are 46 (23 pairs) chromosomes, each containing a fraction of the total information. Chromosomes also provide convenient units into which DNA is packaged for cell division (mitosis) and production of gametes (meiosis). By the way, the number of chromosomes an organism contains is unique for every species and does not necessarily reflect
its phylogeny. The following is a list of the chromosome number of a few common species: fruit fly, 8; pea, 14; corn, 20; toad, 22; tomato, 24; cat, 38; man, 46; potato, 48; goat, 60; horse, 66; dog, 78; duck, 80. Chromosome numbers from 2 to 380 have been observed in other species.

Ordinarily, the DNA in an active, non-dividing cell is somewhat “relaxed” or partially unraveled so that RNA synthesis enzymes can gain access to the DNA sequences. In fact, certain areas of chromosomes have been shown to open up specifically to permit transcription of specific genes in response to regulatory compounds like hormones. The compact structure of chromosomes results from the association of DNA with specific proteins called histones. Histones are proteins that have a large number of positively charged amino acid side chains. For example, lysine and arginine show this characteristic at neutral pH levels. DNA is a negatively charged molecule because of the phosphate linkages between the nucleotide subunits. This opposition of charges causes tight ionic bonds between the histone and DNA. Disrupting the histone/DNA interaction will cause the DNA to unravel and be more easily removed from a solution. An easy technique for disrupting these ionic bonds is to change the ionic conditions of the solution containing condensed DNA (adding salt, NaCl is a common practice). NaCl interferes with the histone/DNA interaction, and the DNA molecules become more linear in structure. These linear DNA molecules are so long that they can be “wound up” like yarn on a pipette tip.

**Specific Background for this Experiment:**

In this experiment, you will isolate DNA from strawberries and wind it up on a glass hook to remove it from solution. You will then re-dissolve the DNA in a NaCl solution and measure the amount of DNA you have isolated. You will measure your isolated DNA using a colorimetric assay, the diphenylamine test. To quantitatively measure your results, you will use a spectrophotometer and compare your results with known concentrations of DNA measured using the same technique. To gauge your results, you will prepare a standard curve using known concentrations of DNA isolated from herring sperm (which contains relatively pure DNA).

The first phase of the isolation procedure involves preparing a nuclear fraction from the strawberry tissue. We must first rupture the nuclear membranes to access the DNA. We will mash the strawberry tissue in a solution of detergent and EDTA (ethylenediamine tetraacetic acid). The detergent disrupts the nuclear membrane, allowing easy access to the DNA. The EDTA is added as a chelating agent, which binds to metal ions. A chelating agent, like EDTA binds to Magnesium and Potassium ions that are essential cofactors for DNases. Added EDTA to the solution disables any DNAses that may be present, preventing the breakdown of our newly recovered DNA.

The next phase of the isolation procedure removes the histones from the DNA by adding NaCl. We will then precipitate the DNA by adding isopropanol. By gently adding the alcohol to the top of the salted DNA solution, the molecules “fall out of solution.” We will then remove the strands of DNA and redissolve them in a NaCl solution. This solution will be used for the colorimetric assay using diphenylamine.

The diphenylamine reagent (also called Dische Reagent) actually detects deoxyribose rather than intact DNA. Thus, if the DNA is fragmented during the isolation process, it should
make relatively little difference in the colorimetric assay. For more sophisticated experiments, intact DNA is necessary and considerable care must be used. Human tissue (i.e. your skin) contains DNAases, enzymes capable of cutting DNA into small fragments, so it is important to use very clean glassware and other equipment for DNA isolation procedures. For this reason it is important that you DO NOT TOUCH the tips of the glass hooks, or the inside of the test tubes or beakers. The diphenylamine reagent reacts with the deoxyribose in DNA to form a blue colored product that has an absorption maximum at 600 nm. You will prepare a standard curve from a solution containing a known amount of herring sperm DNA and use this to determine the amount of DNA you have isolated from the strawberry.

**Procedures:**

1) **DNA isolation**
   a. You will prepare the strawberry extract by mashing 10g of strawberry tissue with 10 ml of 10% detergent solution (also contains EDTA) in a beaker.
   b. You will then filter the extract through gauze (to removed large, undisrupted pieces of tissue) into a clean beaker. Transfer this solution into a 50 ml conical tube.
   c. To the tube, add 2ml NaCl (2M). This will free the DNA from histones. Using the guides on the side of the graduated tube, measure the amount of solution you have. Calculate the volume of 70% of this solution. This is important for the next step.
   d. In a clean pipette, measure out the amount of isopropanol you will need to precipitate the DNA from this solution (You will need the equivalent of 70% of the total volume of the DNA/NaCl solution).
   e. Place the pipette tip along the side of the tube, and VERY SLOWLY let the isopropanol trickle down the side of the tube into the DNA solution. The isopropanol should form a layer on the top of the DNA/NaCl solution. Don’t shake, mix, or jolt the tube or you will mix the layers. Close the lid tightly.
   f. Slowly tilt the tube until it is almost horizontal. You should see a knob of whitish precipitate. This is your DNA. Slowly tilt the tube back to vertical and repeat the process.

2) **Measuring DNA Content**
   a. **Preparation of the Standard Curve**
      i. Prepare three replicates for each point on the standard curve, and use the mean as your measurement. Prepare samples of herring sperm DNA from a stock solution of 10mg/ml DNA at the following concentrations: 3mg/ml, 2mg/ml, 1mg/ml, 0.5 mg/ml. Your total volume should be 1.0 ml, they prepared for testing by combining stock DNA, NaCl and diphenylamine reagent as described in the table on the next page.

****CAUTION: The diphenylamine reagent is toxic! WEAR GLOVES and GOGGLES when preparing your solutions. ****
<table>
<thead>
<tr>
<th>Desired Final Concentration</th>
<th>ml of stock DNA (10mg/ml)</th>
<th>ml of NaCl (2M)</th>
<th>ml of diphenylamine reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>1.0</td>
<td>4.0</td>
</tr>
<tr>
<td>0.5 mg/ml</td>
<td>0.05</td>
<td>0.95</td>
<td>4.0</td>
</tr>
<tr>
<td>1.0 mg/ml</td>
<td>0.1</td>
<td>0.9</td>
<td>4.0</td>
</tr>
<tr>
<td>2.0 mg/ml</td>
<td>0.2</td>
<td>0.8</td>
<td>4.0</td>
</tr>
<tr>
<td>3.0 mg/ml</td>
<td>0.3</td>
<td>0.7</td>
<td>4.0</td>
</tr>
</tbody>
</table>

ii. Before testing each of your “known” solutions, they must be incubated in a boiling water bath for 15 minutes. Be sure to label your tubes before placing them in the waterbath.

b. Preparation of your DNA solution.
   i. While your solutions are incubating, prepare your “unknown” solution for testing. Measure 1ml each of your DNA solution into 3 tubes, and add 4.0 ml of diphenylamine reagent into each tube. Place in the boiling waterbath for 15 minutes before testing.

c. Using the Spectrophotometer
   i. To prepare your standard curve, and ultimately determine the DNA concentration of your solution, you must use the Spectronic 20 spectrophotometer.
      1. To prepare the machine, you must first use the BLANK tube. This will set the machine to zero, and ensure accurate readings for further tubes.
   ii. For each tube, record the absorbance value generated by the spectrophotometer. For your known values, you should have 12 data points, and for your DNA solution, you should have 3.

3) Preparing your report:
   a. Introduction
      i. Should have a brief background of the experiment, why we are interested in the results, and include your hypotheses.
   b. Materials and Methods
      i. Should have a brief summary of the procedures in paragraph form. The summary should include the names of reagent used, and in what amount.
         1. For example: “We precipitated the DNA from solution using 5ml of isopropanol, then dissolved the product in 6ml of 2M NaCl
   c. Results
      i. Should have in the text the results of your DNA solution. You can include a table of mean absorbance values, complete with standard error values, for both the known and DNA solutions, but you must explain them in the text as well.
         1. Simply stating the results are in table 1 is not sufficient.
      ii. Should also contain the standard curve of the mean and standard error of
the known values. Also show how you obtained the concentration of your DNA sample.

1. The graph should have the DNA concentration along the x-axis, and absorbance at 600nm on the y-axis.

d. Discussion
   i. Should include whether or not you supported your hypotheses.
   ii. Should also include the following:
      1. make some comments about the appearance and behavior of the DNA as you handled it. Does it behave as you might predict exceptionally long molecules might behave?
      2. Discuss the concentration you obtained compared to other students in the class. If you had different concentrations, why do you think this is so? Are there techniques in this experiment that might lead to error in evaluating the true concentration of DNA in strawberry tissue?

e. Conclusion:
   i. In a few sentences state your findings from this experiment.
NOTES
Exercise 3- Rf Values and T-tests
(Page 1 of 2)
10 pts

1. 5 points. You are employed by the State Crime Lab and are investigating a possible homicide. The detectives on the case believe that the victim was given coffee containing poison. You want to determine which, if any, poison(s) was/were present in the coffee. You set up a paper chromatography run. As controls you load A) regular coffee; B) commercial rat poison found at the crime scene; C) a prescription medication found at the crime scene; and D) a lawn chemical found at the crime scene. Your experimental sample is the coffee consumed by the victim. You performed paper chromatography with a hydrophobic solvent and allowed the solvent front to travel 50cm. You determined that the coffee control contains 18 distinct substances. The rat poison produced 2 bands, one band moved 10.2 cm and the other moved 22.0 cm; the prescription drug contained three substances (and therefore 3 bands) that migrated 11.1, 19.5 and 40.7 cm; and the lawn chemical produced one band that migrated 17.0 cm. The experimental coffee sample contained 21 substances 18 of which matched with the control coffee. The remaining three bands traveled 10.2, 17.0 and 22.0 cm respectively.

a) Calculate the Rf values of the bands in the three control poisons.
   Rat Poison band 1 _____
   Rat Poison band 2 _____
   Prescription drug band 1 _____
   Prescription drug band 2 _____
   Prescription drug band 3 _____
   Lawn Chemical band 1 _____

b) Calculate the Rf values of the 3 unique bands in the experimental coffee sample.
   Band 1_____  Band 2_____  Band 3_____ 

b) With the information that you have, make a recommendation to the detectives regarding any unusual contents of the victim's coffee by comparing the Rf values of your control poisons to the Rf values of the unknown compounds in the experimental coffee sample.
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

2. 1 point. What is the most appropriate method for separation of proteins by mass?
3. **4 points.** You are examining the effects of acid rain on grape size. You grew grape plants in neutral pH (control) and highly acidic soil determined the mass of several grapes. A small collection of data is presented below.

<table>
<thead>
<tr>
<th>Neutral pH soil (Control)</th>
<th>Highly acidic soil (Experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3 g</td>
<td>2.1 g</td>
</tr>
<tr>
<td>3.1 g</td>
<td>1.9 g</td>
</tr>
<tr>
<td>4.0 g</td>
<td>1.8 g</td>
</tr>
<tr>
<td>2.1 g</td>
<td>2.5 g</td>
</tr>
<tr>
<td>3.3 g</td>
<td>2.0 g</td>
</tr>
<tr>
<td>3.9 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>4.2 g</td>
<td>2.6 g</td>
</tr>
</tbody>
</table>

a) Calculate the degrees of freedom for these data sets.

b) What is the t value for this data?

c) Using the table in your lab manual as a guide, determine the p value (may be a range) and use that information to determine if these data sets are significantly different (p<0.05).

    p value _______
    Are these groups significantly different?_______
Laboratory 3 - Chromatographic Analysis of Plant Pigments

Objective:

The objective of this portion of this exercise is to separate and identify photosynthetic (chlorophyll) and accessory plant pigments from the leaves of bean plants using paper chromatography. The primary pigments present in the leaves of most higher plants are basically the same as those in beans except the proportions differ. Therefore, these observations apply generally to most plants.

Introduction:

The basic biochemical processes that generate ATP in cells is fundamentally the same in almost all cells. The oxidation of glucose by the enzymatic pathways, glycolysis, the citric acid cycle and the electron transport cytochromes leads to the establishment of H$^+$ ion gradients that ultimately drive phosphorylation of ATP from ADP and inorganic phosphate (Pi). (See text). Plant cells as well as animal cells respire (that is, utilize oxygen for glucose breakdown and ATP generation). Plant respiration occurs in both the light and dark, but becomes easily measurable experimentally only in the dark.

As everyone knows, plants are unique in that they possess another series of important synthetic enzymes that are located primarily in the organelles (in eukaryotes) called chloroplasts. The function of chloroplasts is to harvest light energy to drive the synthesis of ATP and NADPH (nicotinamide adenine dinucleotide phosphate). This ATP and NADPH is then used to synthesize glucose from CO$_2$ and H$_2$O. The overall reaction can be written:

\[
\begin{align*}
\text{chloroplast enzymes} & \quad 6\text{CO}_2 + 6\text{H}_2\text{O} & \rightarrow & \rightarrow & \rightarrow & \rightarrow
C_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \quad \\
\text{chlorophyll, light} & \quad \text{glucose}
\end{align*}
\]

The overall process is called photosynthesis, meaning literally, light driven synthesis of organic molecules. This reaction is basically the reverse reaction of mitochondrial respiration. At the light intensities ordinarily found in the environment in which a particular species lives, there is usually an excess production of glucose. Via other metabolic pathways and with the addition of a few basic nutrients such as inorganic nitrogen and phosphate salts from the soil, glucose can be converted to fats, protein and nucleic acids. In other words, chloroplasts along with the other synthetic enzymes in plant cell cytoplasm and other organelles, can synthesize virtually all the basic organic molecules it needs from inorganic precursors using light energy to drive this process. Higher plants and algae (terrestrial and oceanic) are the ultimate primary sources of organic nutrients for almost all organisms. Animals, fungi and other non-photosynthetic organisms (with the exception of a few bacteria that derive energy from other sources) feed directly or indirectly (by eating herbivores) on plants. Thus, it is probably not an exaggeration to state that almost all living things are dependent on the processes of photosynthesis in one way or
The details of photosynthesis and the energy trapping reactions that occur will be described in lecture (Also see the text). The first portion of this exercise measured the production of oxygen by plant tissues at various light intensities. In this section, you will identify the major plant pigments that are present in most higher plants. These pigments, chlorophyll a, chlorophyll b, beta-carotene and lutein, are molecules that absorb light energy, transferring this energy to one another by way of electrons and finally to a central chlorophyll or reaction center. The reaction center is coupled to enzymes (oxidation-reduction or redox enzymes) that ultimately trap the energy in chemical form. Chlorophyll a and b are the most important pigments and they absorb light in the red and blue parts of the spectrum. Beta-carotene and lutein are called accessory pigments because they absorb light in a slightly different region of the spectrum and transfer the energy to chlorophyll, thus improving the efficiency of light energy trapping.

To understand some of the following material, it is important to recall some basic characteristics of light. Light behaves as if it is made up of both particles (photons) and electromagnetic waves. Either description is quite accurate and interchangeable, although some features of the behavior of light is best explained by one or the other character. So called "white" light is simply a mixture of colors (the spectrum) of light ranging from violet to red. Each color can be identified with a specific range of wavelengths (see text). The traditional colors of the spectrum are red, orange, yellow, green, blue, indigo, violet (easily remembered by the acronym, ROY G. BIV). The longer the wavelength, the lower the energy of the photons. The visible electromagnetic spectrum ranges from about 400 nm to 700 nm in wavelength. The distance from peak to peak of the electromagnetic waves is literally the distance 400 nm for blue light or 700 nm for red light and this can be measured directly with certain instruments. It just so happens our eyes are physiologically tuned to detect colors of light in the "visible" 400 nm to 700 nm range. You should realize that plants and animals such as insects sometimes are capable of absorbing light outside this range as well.

The structures of chlorophyll a and b, beta-carotene and lutein (or xanthophyll), which is closely related to beta-carotene, are shown on the following page. These four compounds are the most common in plants and are the ones you will attempt to identify in extracts from bean leaves. Notice that chlorophyll a and b are almost identical in structure. Both contain the complex magnesium containing porphyrin ring with a long hydrocarbon "tail". The only difference lies in the fact that chlorophyll a contains a methyl (-CH$_3$) group whereas at the same location on the molecule chlorophyll b contains an aldehyde (-CHO) group. Slight difference significantly changes the light absorbing characteristics of the molecule. Beta-carotene and lutein closely resemble one another although their structures are very different from that of the chlorophylls. Beta-carotene is entirely composed of carbon and hydrogen atoms. Lutein has a nearly identical structure but has two polar -OH groups substituted on the terminal carbon rings.

If you look closely at these molecules you will realize that both contain long stretches of alternating single and double bonds, either in ring form (the porphyrin ring) or in linear form (beta-carotene). It turns out that this type of structure is quite suitable for absorption of light. The electrons of these systems can be "activated" or raised to higher energy levels by impinging
photons of light. The longer or larger a molecule is that contains this type of bond arrangement, the longer the wavelength of light that is absorbed. In a sense these molecules act as "antennas" that absorb light. Radio and TV antennas absorb radio waves (which are, in fact, just electromagnetic waves similar to light) but with much longer wavelengths. Just as radio antennas can be "tuned" to certain wavelengths by making their physical size larger or smaller, these molecular antennas for light can also be "tuned" by increasing or decreasing the size, polarity or nature of substituents on the molecules.

Chlorophyll a and b absorb light in the red and blue regions of the spectrum and permit yellow and green light to pass or be reflected. Thus most plants look green, not because they absorb green light but because they absorb red and blue light and do not absorb green light. Beta-carotene (common in carrots) and lutein are yellow-orange in color because they absorb somewhat less of these wavelengths of light than other wavelengths. The precise light absorption properties of these pigments at any wavelength can be measured using a spectrophotometer (such as the Spectronic 20 used in previous exercises). By changing the wavelength dial in small steps and measuring the absorbance of a solution containing pure pigments, one obtains what is called
an absorption spectrum. Shown on the next page are the absorption spectra for chlorophyll a and b, beta-carotene and lutein. The peaks indicate where maximum light absorption occurs and the valleys indicate where relatively little light is absorbed. These spectra are unique to each compound and in some cases can be used experimentally to specifically identify these compounds in extracts.

In intact plant cells, chlorophylls and carotenes are associated with specific proteins (by the various types of weak bond interactions) and may be bound to membranes. Having a mainly hydrocarbon structure, it might be expected that they are not particularly water soluble. To extract these pigments from plants, one needs to use solvents such as ethanol or petroleum ether. The extraction process disrupts the associations with proteins and frees the pigment from the protein. The extracts we will prepare from plant tissues uses this principle.

The technique we will use to separate these pigments in crude extracts of plant tissue will be paper chromatography. Chromatography is used to separate structurally similar (and also structurally diverse) compounds in complex mixtures. There are a wide variety of chromatographic techniques that separate compounds using one aspect or another of their physical or chemical differences. For example, differences in size, charge or hydrophobicity are commonly used to separate structurally similar compounds such as amino acids, sugars, proteins, nucleotides, lipids or plant pigments. Chromatography, in one form or another, is one of the most commonly used and most powerful techniques in modern cellular biology. Some of the varieties of chromatography you might come across are: paper, thin layer, size-exclusion, gel filtration, ion exchange, affinity, reverse phase, high pressure or high performance chromatography. Typically, a cellular extract is applied to a "bed" material such as cellulose and a solvent (called an eluent) is then permitted to flow through this bed material, carrying with it at different speeds the various molecules in the extract. The speed depends on whether a particular molecule in the extract sticks to the bed material (by ionic or weak interactions), associates preferentially with the solvent or is of the right size range to penetrate the bed material at a reasonable rate. Like all good experiments, positive controls (also called standards) of known pure compounds are necessary to accurately establish the chromatographic behavior of the compounds to be analyzed and to assist in identifying these compounds in complex biological extracts. The bed materials actually used depend on the type of molecule to be separated. The bed material may be in sheets (such as paper) or in powder or bead form and packed into tubes (columns) through which solvent is passed (by gravity or sometimes using mechanical pumps). In this experiment, you will use filter paper as the bed material through which the solvent (90% petroleum ether: 10% acetone) will flow via capillary action.

Specific Background for this Experiment:

In this experiment you will use paper chromatography to separate chlorophyll a and b, beta-carotene and lutein from ethanol extracts of bean leaves and carrots. In addition, you will also include in your assay positive controls for chlorophyll a, chlorophyll b and beta-carotene. These molecules are quite insoluble in water and thus extraction of tissue must be done in organic solvents. A piece of paper is cut to fit into the chromatographic tank (containing the solvent), a line (the "origin") is drawn with lead pencil 3 cm from the bottom and small volumes of extract
or standard (a few µl at a time) are "spotted" on the paper along this line 2 cm apart. Great care must be used at this stage to be sure the spots of extract are as small as possible as they spread considerably after being exposed to solvent. The paper should always be handled by the edges so that contamination from your hand is minimized. The paper should also be raised off the table during the spotting procedure by placing it on a book or a piece of folded paper so the origin line is exposed to the air on both sides (ask the T.A. for advice if this is not clear).

Once the spotting procedure is completed for all samples, gently bend the paper in a circle and staple the two ends together (ask T.A. to show you this step). Each tank should contain 100 ml of 90% petroleum ether: 10% acetone mixture. Gently lower the paper into the solvent in the bottom of the tank, the edge with the origin line and the spotted samples oriented horizontally on the bottom. Cover immediately with a glass plate. Development time (time for the solvent to climb near the top of the paper) should be about 30 min.

Paper is made of cellulose which is a polymer of glucose. Cellulose has many polar -OH groups in it. The pigments will only associate with these polar groups (by weak interactions) if they have polar groups themselves. Most of these pigments being very lipid soluble and water insoluble, tend to associate with the solvent instead as it flows through the paper upward by capillary action. You would predict that those pigments with some polar character (polar groups) might lag behind compounds that are entirely hydrocarbon and therefore associate with the solvent almost entirely. Look at the molecular structures given above. You would predict that beta-carotene should be carried further by the solvent than lutein (which has two -OH groups) and both carotenes should probably migrate faster than the chlorophylls that contain more polar groups and ionic magnesium as well. This prediction is correct. The sequence of separation as
you proceed from the origin to the top of the chromatogram will be chlorophyll b, chlorophyll a, lutein and beta-carotene. Because there may be other contaminating compounds in the tissue extracts, positive controls will be necessary to confirm the location and identity of some of these compounds.

To quantify in a consistent way the location and rate of migration of molecules in chromatography, the relative rate of migration is measured in relation to the migration rate of the solvent (or solvent front, the upper edge of the solvent wetting the paper as moves through it by capillary action). The relative migration rate of a pure compound is called the Rf of that compound and is given by the simple formula:

\[
RF = \frac{\text{distance the spot of compound migrated from origin}}{\text{distance solvent front migrated from origin}}
\]

The Rf for an particular solvent mixture for a particular compound may be unique. Thus the Rf for a chromatogram of chlorophyll a run in 90% petroleum ether: 10% acetone is likely to be different than the Rf for chlorophyll a run in petroleum ether alone (or any other solvent). The Rf also varies with different bed materials. Therefore, one almost must specify the exact conditions used for a particular experiment. With a particular solvent/bed material combination the Rfs are quite reproducible. For this reason, chromatography is often used as strong evidence in research to purify compounds of unknown properties and is sometimes taken as strong evidence of the chemical uniqueness of a compound.

In this laboratory, you will also measure the absorption spectrum of ethanol extract of bean leaves. You will use a Spectronic 20 spectrophotometer and simply read the absorbance of ethanol bean leaf extract at various wavelengths. However, one must blank the spectrophotometer at each wavelength using a cuvette containing ethanol before reading the sample containing bean leaf extract. This is quite important since at each wavelength setting the instrument blank value may change.

Finally, a demonstration will be conducted to show the phenomenon of chlorophyll fluorescence. Fluorescence is the process by which light of a particular wavelength is absorbed by a molecule (such as chlorophyll a or b) and re-emitted at a lower wavelength. This is the phenomenon familiar to most people in "black light" posters which "glow in the dark" when being illuminated with ultraviolet (UV) light. The short wavelength (high energy) UV light is absorbed by the pigments and the energy is re-emitted as light in the visible range. A good deal of energy is also lost as heat. Chlorophyll a and b absorb light in the blue range (and UV) and re-emit it in the red wavelengths. Under UV light the normally intense green solution appears blood red. In intact plants, some of this re-emitted energy is reabsorbed by chlorophyll and used for photosynthesis.

**Procedures**

1. **Chromatography of Plant Pigments:**
   a. The T.A. will prepare an extract of bean leaf for analysis. About 20 grams of plant
material will be homogenized in 50 ml absolute ethanol for 2 min.

b. Prepare a similar extract with carrot.

c. Use only the clear supernatants for spotting on the chromatogram.

d. Obtain a piece of chromatographic paper about 18 cm x 28 cm. Draw a line with lead pencil 3 cm from one edge along the longest dimension (the 28 cm edge). (Do not use pen to draw this line).

e. Beginning 2 cm from the edge, place a mark every 2 cm along the origin line. Number these lightly with lead pencil from 1 to 12.

f. Spot on a very small volume of standards or extracts at each numbered mark. Be very careful at this stage. Be sure to raise the paper up off the table using a book or folded paper underneath the chromatogram. Use a Pasteur pipette ("eye dropper") without a bulb. Dip it in the extract. A small drop will be held in the tip by capillary action. Touch this to the paper on the origin line very briefly. The fluid will flow quickly out of the pipette. Try to keep the spot formed as small as possible. Allow the spot to dry. Repeat this spotting procedure at least 7 times for each sample location on the origin. Be sure that the previously spotted material is completely dry before adding another droplet. Otherwise the spot will flow outward and become rather large in diameter.

g. There are a total of 12 locations on the origin for samples. Do 3 replicates of each of the following extracts. Bean leaf extract, carrot extract, chlorophyll a standard and beta-carotene standard. (3 replicates each x 4 samples = 12 locations on origin). Half the class will use chlorophyll b and lutein standards instead.

h. After all the spots are completely dry, gently bend the paper so the the 18 cm edges are almost touching and staple them together. Be sure that there is a space between these edges and that they are not overlapping or touching. This could disrupt the solvent flow seriously. (See the T.A. for advice).

i. Gently lower the stapled paper, origin side down into the solvent. Rest the paper standing up on the bottom of the tank. Cover and let develop for 30 min or until the solvent is 1 to 2 cm from the top edge of the paper.

j. Remove the paper from the tank and immediately mark the entire solvent front with a lead pencil.

k. The colors of the pigments will fade fairly rapidly so it is necessary to mark all spots with lead pencil. Draw around the spots and also mark your best estimate of the center of the spot.

l. With a millimeter ruler measure the distance from the origin line to the solvent front and to the spots at each sample location. Record these data for calculation of Rf.
2. **Demonstration of Chlorophyll Fluorescence:**

   a. The T.A. will darken the room and show you the red emission fluorescence of chlorophyll extract under ultraviolet light.

   b. **Do not** directly look at the UV light. Wear safety glasses if you plan to look at this demonstration for a prolonged period of time.

**Data, Plots and Calculations:**

DATA - You should have the following data recorded.

1. **Chromatography of Plant Pigments**

   a. Measurements of distance from the origin line to the solvent front at each of the 12 sample locations to the nearest 0.1 cm.

   b. Measurements of the distance from the origin line to the center of the spot or spots at each of the 12 sample locations.

**GRAPHS & CALCULATIONS –**

1. Calculate the Rfs for each spot and all locations on the chromatogram. Each extract or standard run in triplicate. Calculate mean ± SE for the Rfs for each type of sample (four different types were spotted on the chromatogram). Enclose the chromatogram or a drawing (or xerox) of the chromatogram with your report. Compare the Rfs for your standards (i.e. chlorophyll a and beta-carotene) with those of other groups in the class that used chlorophyll b and lutein as standards.

2. Identify which spots are the following: chlorophyll a, chlorophyll b, lutein and beta carotene for all samples. In a table compare the Rfs ± SE for the standards and the plant extracts.

**Discussion**

(A) Discuss the reasons you think plant pigments are effectively separated by this combination of bed material and solvent. Is there a correlation between Rf and the structure of these molecules? Which pigments were detected in bean leaf and carrot extracts based on your data? Discuss any experimental problems that might have complicated your results.

**Conclusion:**

In a few sentences summarize the main results of your experiments.
Laboratory 4 - Measuring the Rate of Photosynthesis in Plants

NOTE: Please read the Introduction section for Laboratory 3 in your laboratory manual and appropriate sections in your textbook for some elementary background material on photosynthesis.

Objective:

The objective of this experiment is to measure the rate of oxygen production by bean leaves in dim light and bright light.

Specific Background for this Experiment

Plants and other photoautotrophic organisms such as cyanobacteria use light energy to convert carbon dioxide to organic compounds that can ultimately be used to produce glucose. This process is called carbon fixation. Energy is trapped by plant pigments (esp. chlorophylls, see Experiment 7) and used to synthesize ATP. This energy can then be used to "fix" CO$_2$, converting it to organic compounds. As a by-product of the energy trapping process, water is split, yielding gaseous oxygen. A large number of enzymes are involved in this process, but the overall reaction can be written as follows:

$$\text{chloroplast enzymes} \quad 6 \text{ CO}_2 + 6 \text{ H}_2\text{O} \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow $$

$$\text{C}_6\text{H}_12\text{O} + 6 \text{ O}_2$$

chlorophyll, light

One comparatively easy way to measure the overall photosynthetic rate is to measure the amount of oxygen released under standard lighting, temperature and pressure conditions (STP). Ideally we would also measure the specific light intensity, but for the purposes of this experiment we will use two conditions, very low light intensity and high intensity light. Since light is the primary energy supply for photosynthesis, one would predict that at the high light intensity the rate of photosynthesis (and therefore the rate of oxygen production) would be much higher than at low light intensity. In the previous experiment we corrected the final data to STP and we will obviously need to do the same type of correction in this experiment.

The text that follows was adapted from Experiment 5. It is similar in many places since the methodology is quite similar. But be aware there are important differences since we are measuring oxygen production rather than oxygen utilization. Please read these procedures carefully.

In this experiment we will be measuring oxygen production by pieces of bean leaves at two light intensities. The device we will use to measure oxygen consumption is simply a gas tight 1.0 ml syringe fitted with a calibrated 100 µl glass capillary tube (One µl is equal to $10^{-6}$ liters or $10^{-3}$ ml). A droplet of low viscosity oil is carefully placed in the capillary tube, sealing the syringe gas (air, in this case) from the outside air. As oxygen is produced the gas pressure within the syringe increases slightly. In response to this increase in pressure, the oil droplet moves up the capillary 'pushed' by the slightly higher pressure of the internal gas. In fact, the pressure in the syringe nearly stays equal to that of the external atmospheric pressure as the oil drop moves up. Since the capillary tube has a known volume per unit length, by measuring the distance the oil drop moves we can calculate the volume of oxygen released.
There are some factors that must be must be taken into account using this method. First, the volume of gas sealed within the syringe is very sensitive to temperature changes. Therefore, we must control temperature carefully by placing the syringe in a water bath that has as constant a temperature as practical. The whole apparatus, whether in the control condition without a bean leaf or the experimental condition with a bean leaf, must first be equilibrated to the water bath temperature for at least 10 minutes without the oil drop and prior to sealing of the system with the oil droplet. For the same reasons, once the apparatus is sealed it must not be handled during the experiment and all measurements of oil drop movement done very gently.

Second, the actual amount of oxygen per unit volume of dry air varies with temperature and barometric pressure. It is therefore standard practice to correct oxygen uptake measurements to standard conditions (STP = standard temperature and pressure). Standard conditions (STP) are: temperature, 0°C and a pressure of 760 mm Hg (pressure at sea level). These are comparatively small corrections but it is good practice to do them. Pure (unpolluted) air has the following composition: N₂, 78.03%; O₂, 20.99%; A, 0.94%; CO₂, 0.03% and traces of other gases, 0.01%. All the other gases in air with the exception of O₂ and CO₂ are usually inert biologically, so our measurement of gas uptake needs only account for these two gases.

Third, the calculations assume that dry air is present in the syringe chamber. The presence of water vapor or other liquids contributes to the total vapor pressure of gas in the chamber. It is certain that the gas within the syringe is close to saturation with water vapor after the experiment commences. The oily substance we will be using (dibutylphthalate) has a very low vapor pressure (<< 1 mM Hg) and therefore we will make no correction for its presence.

Fourth, the amount of oxygen released depends on the weight of the bean leaf tissue. Larger pieces of leaf will release more oxygen than smaller pieces of leaf under identical conditions of temperature, pressure and light intensity. To compare data acquired from different sized leaf segments, one must correct for this weight factor. It is best to cut leaf pieces about the same size to begin with and then accurately weigh them to allow correction to a standard weight, usually 1 gm.

Fifth, the amount of time over which the measurement of oxygen production is made is obviously important. A leaf segment evolving oxygen for 60 minutes would obviously release twice as much oxygen as a leaf segment of identical weight and structure photosynthesizing for 30 minutes under identical environmental conditions. It is therefore necessary to express the rate of oxygen production per unit time (usually per hour).

Sixth, for photosynthesis to proceed efficiently an adequate supply of CO₂ is needed. The concentration of CO₂ is usually quite low in air (see above) and therefore we need a way of providing extra CO₂ to the leaf segment. This is done by bathing the leaf tissue in 0.05 M sodium bicarbonate (NaHCO₃). Bicarbonate is in equilibrium with CO₂ and this provides a convenient source of CO₂ for photosynthetic measurements.

Finally, when all the above corrections are applied oxygen production is expressed as volume (or sometimes moles, which can be directly calculated from oxygen volume at STP) per gram per unit time. For this experiment the units will be: µl/g/hr at STP.

Procedures

General Experimental Design: You will use six syringes (labeled 1-6), three for controls (which has everything EXCEPT bean leaf-segments) and three containing one leaf segment each. The leaf segments are placed in the syringes with their attached capillary tubes. All six syringes should also contain about 0.5 - 0.6 ml of 0.05 M NaHCO₃. The syringes are then equilibrated in a water bath with a carefully controlled temperature (room temperature in this case, but you MUST measure what this temperature actually is with a thermometer). This water bath will also be covered in aluminum foil to exclude most room light with only the tips of the syringes and capillaries protruding. After the equilibration period, a drop of oil is injected into the end of the capillary-tube using another syringe with a fine needle. This is drawn down the capillary tube to a point about 2 cm from the syringe needle fitting by slightly withdrawing the
syringe plunger. After a minute or two of equilibration, the distance from the end of the capillary to the bottom meniscus of the oil drop (the edge facing the syringe) should be recorded and a stopwatch started. Record the position of the drop every 10 min for 60 min for EACH syringe. Next, remove the aluminum foil light shielding and shine a high intensity microscope light directly on the leaf segments in the syringes. Repeat the same steps as above, recording oxygen production every 10 min for 60 min. Do all manipulations GENTLY and keep the apparatus clean and intact.

A. The Water Baths: The water baths will be beakers with 1 liter or more of water in them. Two light intensities will be used, very low light with the water bath shielded from room light by aluminum foil and high light intensity in the water bath without foil but with an added light source. Be sure to direct the microscope lights directly at the syringes containing the leaf segments. Temperatures of the water bath that will be used is ROOM temperature. However, this may vary somewhat throughout the day and you should monitor the temperature of the water bath with a thermometer.

B. Syringe Preparation: You will need six 1.0 ml syringes for each group of students (2 to 4). The syringes for this experiment SHOULD NOT have the capillary tubes already inserted. Three syringes will be used for the control (without leaf segments) and three will be used with leaf segments. This control is VERY important in that it allows you to monitor the movement of the oil droplet that may be due to temperature and pressure changes, etc. BEFORE inserting the capillary tube into the needle fitting of the syringe, measure the glass 100 µl capillary tube. Near one end of the glass capillary tube there will be a colored ring (usually blue). The capillary will also be marked with a fine black ring. The distance from the black ring to the end of the capillary has a volume of 100 µl (the end of the capillary referred to here is the one opposite the colored blue ring). The capillary is a cylinder and thus the volume it contains is proportional to its length. You need to determine the µl/cm of length empirically (we will call this factor, K). Measure the distance from the black ring to the end with a cm ruler and calculate the following:

\[ K_v = \frac{\mu l}{cm} = 100 \mu l/(length \ to \ end \ in \ cm) \]

In addition, the plunger of the syringe should have a piece of heavy wire or a metal washer attached to it as a weight

C. Bean Leaf Segments: Prepare three bean leaf segments for the syringes as follows: Select a bean leaf from the flat containing plants and cut a segment about 4 cm long and 1 cm wide with a razor blade or scissors. It is easier to handle if this is cut so that the midrib of the leaf is in the center of the leaf strip. That is the midrib is about 0.5 cm from each edge of the 1 cm wide dimension and it runs the entire 4 cm length of the segment This stiffens the segment a bit and makes it easier to manage when inserting the segment into the syringe. Weigh each leaf segment and record each weight. This weight will be used in later calculations.

D. Starting the Experiment at the Low Light Intensity: For each control (3 controls), draw up about 0.5 ml of 0.05 M NaHCO₃ by withdrawing the plunger while the needle fitting end is immersed in the solution. Then remove the syringe, hold it needle fitting end upward and withdraw the fluid a bit more so there is a clear air gap at the top. While holding the syringe in this upright position, GENTLY insert the calibrated capillary tube with the BLUE RING down (closest to the needle fitting). The 0.05 M NaHCO₃ solution MUST NOT (I repeat MUST NOT) touch the end of the capillary in the needle fitting. Place the syringe, plunger side down, in the water bath and equilibrate for 10 min.

For the experimental condition-with leaf segments, use the following procedure: GENTLY roll the 1 cm x 4 cm leaf with the darker green upper surface OUTWARD and insert this into a syringe. Gently shove this down to the end with a pipette or capillary tube. Then cover the needle fitting end of the tube with parafilm and turn the needle fitting end of the syringe DOWNWARD. Pipette into the syringe about 0.5 ml of 0.05 M NaHCO₃. The leaf segment
should be COVERED with the solution. With your finger over the needle fitting end, carefully insert the plunger just a short way into the barrel of the syringe and briefly apply pressure to the syringe. Keep your finger over the needle fitting end so no fluid escapes. Repeat the "pressurization" step 3 or 4 times. This step forces 0.05 M NaHCO₃ into air spaces in the leaf tissue and insures that CO₂ can reach the photosynthesizing cells. Do not crush the tissue, avoid nudging it with the plunger. Invert the syringe (needle fitting end UPWARD) and release your finger allowing bubbles to escape. Remove the parafilm and withdraw the plunger SLIGHTLY to create an air space in the needle fitting and slightly below it. Now insert the capillary tube into the syringe with the BLUE RING toward the syringe fitting. Do this gently and cautiously, since the tube can break at this stage. Ask your instructor for assistance if necessary. As stated above: the 0.05 M NaHCO₃ solution. MUST NOT (I repeat MUST NOT) touch the end of the capillary tube. Transfer the syringes to the water bath and place them PLUNGER END DOWN into the water. The needle fitting with the attached capillary tube should NOT be below the water level, but the body of the syringe should be fully immersed. Adjust the water level if necessary. Equilibrate for 10 min.

To begin oxygen production measurements, place a drop of oil in the end of the capillary tubes with another syringe with a fine needle. The droplet should be about 1.5 cm long in the capillary tube (if it is too small it might coat the surface of the tube as the droplet moves and break the seal to the outer air). Now, GENTLY and SLOWLY withdraw the plunger and draw the droplet down the capillary to within about 2 cm of the needle fitting but NO closer. We are trying to measure oxygen evolution and therefore the gas volume will increase and the droplet will move up the capillary. DO NOT draw the drop down too far or too fast. You will notice the droplet appears to get smaller as it coats the walls of the capillary tube. It is important to have a large enough droplet when starting so the seal is not broken during this process and therefore it should be about 1.5 cm long when starting.

Place the syringes (plunger end down) in the water bath SHIELDED FROM LIGHT with aluminum foil. Allow the tip of the syringe and capillary to poke above the foil but cover everything else well (including the open top).

Allow things to settle down for a minute or two and then start a stopwatch and begin readings (Call this first point "0" time. The "0 time" reading will be subtracted from all the others to measure change in volume). Using a ruler GENTLY measure the distance from the BOTTOM meniscus to the OPEN END of the capillary tube. Record this measurement for each of the six syringes every 10 minutes for 60 minutes. Also record the temperature in the water bath at 10 min intervals.

E Starting the Experiment at the High Light Intensity: After the measurements in low light intensity have been completed, GENTLY remove the aluminum foil shielding. Try not to jostle or bump the syringes. KEEP THEM UPRIGHT, with plunger end down. Obtain a microscope light and aim the beam at the highest intensity through the side of the water bath beaker directly at the leaf segment containing syringes. Allow things to settle down for a minute or two and then start a stopwatch and begin readings (Call this first point '0' time. The '0 time' reading will be subtracted from all the others to measure change in volume). Using the attached scale or a ruler GENTLY measure the distance from the BOTTOM meniscus to the OPEN END of the capillary tube. Record this measurement for each of the six syringes every 10 minutes for 60 minutes. Also record the temperature in the water bath at 10 min intervals.

F. Clean-up: Remove the oil drops and NaHCO₃ solution. With a long narrow forceps remove the leaf segment and discard it. DETACH the capillary tube from the syringe and place it in a container containing cleaning solution as directed by your instructors. This apparatus has to be shared with other classes. Treat it gently.
Data

You should have collected the following data (in neatly organized tables, of course):
1. Twelve temperature measurements for the water bath at 90-minute intervals.
2. Six measurements of the position of the oil droplet from the end of the capillary made at 10-minute intervals for each syringe (Total: 6 x 6 = 36 measurements per light intensity. Total for two light intensities, 36 x 2 = 72 measurements). The controls are in triplicate and experimental condition in triplicate in this design).
3. You should have the barometric pressure recorded as measured by the barometer in the lab. Your instructor will show you the instrument or provide the barometer reading.

Calculations and Plots

1. Calculate the mean temperature of the water bath over the period that measurements were taken. Use this mean temperature ($T_m$) for the calculations below.

2. Calculate the correction factor (CF) which corrects your data measured at local atmospheric pressure and temperature during your experiment to standard conditions (STP).

\[
CF = \left[\frac{273}{(273 + T_m)}\right] \times \left[\frac{(P_b - P_w)}{760}\right]
\]

Where:

$CF =$ correction factor (to STP)
$T_m =$ mean temperature in the water bath
$P_b =$ atmospheric pressure measured with a barometer in mm Hg. Note, however, that our barometer reads atmospheric pressure in inches of Hg NOT mm of Hg. Therefore, you need to convert inches of Hg to mm of Hg BEFORE using the formula (mm Hg = inches Hg x 25.4 mm/inch).
$P_w =$ vapor pressure of water which contributes to the pressure within the syringe chamber.

The following table gives the vapor pressure of water (in mm Hg) for 2°C intervals in the range of our experiment (interpolate the values for intermediate temperatures):

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Pw (mm Hg)</th>
<th>Temp (°C)</th>
<th>Pw (mm)</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>9.2</td>
<td>20</td>
<td>17.5</td>
</tr>
<tr>
<td>12</td>
<td>10.5</td>
<td>22</td>
<td>19.8</td>
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<tr>
<td>14</td>
<td>12.0</td>
<td>24</td>
<td>22.4</td>
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<tr>
<td>16</td>
<td>13.6</td>
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<tr>
<td>18</td>
<td>15.4</td>
<td>28</td>
<td>28.3</td>
</tr>
</tbody>
</table>

The number 273 in the formula is 0°C expressed on the Kelvin scale (i.e. Kelvin = 273 + °C) The number 760 in the formula refers to atmospheric pressure at sea level, 760 mm Hg.

For example:
If \( P_b = 29.85 \text{ inches Hg} \) (29.85 inches x 25.4 mm/inch = 758.2 mm)  
\[ T_m = 24^\circ C \]  
\[ P_w = 22.4 \text{ mm Hg} \) (for saturated air at 24° C) 

Then:

\[
CF = \left(\frac{273}{273+24}\right) \times \left(\frac{758.2 - 22.4}{760}\right) = 0.919 \times 0.968 = 0.889 \quad CF = 0.889
\]

3. **Calculate the amount of oxygen production in each syringe.** In the controls this value might be rather small or even negative if the barometric pressure or temperature equilibration of the water bath has changed. The syringes with the bean leaf segments should be very clearly different than these controls.

(Data from a single syringe is used for this calculation)

Select one time point after 60 minutes (or the longest time point available) and calculate the change in volume of gas in the syringe using the calibration constant \((K_v, \text{ see above})\).

Let:

\( L_0 = \) length (in cm) from the end of the capillary to the bottom meniscus of the oil droplet at '0 time'

\( L_T = \) length (in cm) from the end of the capillary to the bottom meniscus of the oil droplet after a time period \((T)\).

\( K_v = \mu l/cm \) of capillary tube (as determined above)

\( T = \) time period (in min) during which oxygen utilization was measured

\( W = \) weight of the bean leaf segment in grams

\( CF = \) correction factor calculated above

\( O_p = \) uncorrected volume of oxygen produced in \( \mu l/\text{gram bean leaf segment/hour} \)

\( P = \) corrected volume of oxygen produced in \( \mu l/\text{gram bean leaf segment/hour at STP}. This is also called the photosynthetic rate. \)

Then:

\[
O_p = \frac{\left( L_0 - L_T\right) \times K_v \times 60}{W \times T}. \\
P = O_p \times CF
\]

For example: (using our CF calculated above)
Assume that over a period of 30 minutes the oil droplet moved from a position at '0 time' at 3.3 cm (L<sub>a</sub>) to a position 1.6 cm (L<sub>T</sub>) up the capillary tube. The temperature (T<sub>r</sub>) was 24.0°C. The calibration constant K<sub>r</sub>, previously calculated (100 gl/6.1 cm = 16.4 gl/cm = K<sub>r</sub>). The weight (W) of the leaf segment was 0.150 grams. The CF calculated previously was 0.889. (NOTE: In the case of oxygen production using this apparatus, the length the oil droplet moves is (L<sub>0</sub> - LT). This differs from the equation in Exp. 5).

Then:

\[ O_2 = \frac{(3.3 \text{ cm} - 1.6 \text{ cm}) \times 16.4 \text{ gl/cm} \times 60 \text{ min/hr}}{(0.150 \text{ g} \times 30 \text{ min})} = 371.7 \mu\text{l/g/hr} \]

\[ P = 371.7 \times 0.889 = 330.4 \mu\text{l oxygen produced/g bean leaf/hr at STP} \]

4. **Statistical Calculations:** You will have three values for the control syringes and three values for the syringes with bean leaf segments. Calculate the mean ± S.E. for the control set and experimental set at each of the two light intensities. Therefore you will have 2 sets of controls and 2 sets of experimental measurements. Do t-tests to test the statistically significant differences between the low light intensity measurements and the high light intensity measurements. Also do a t-test for each of the control measurements compared with the bean leaf containing syringe measurements. You will therefore have to do 3 sets of t-tests of the following pairs: 1.) Control vs. Bean leaf (low light) 2.) Control vs. bean leaf (high light) 3.) Low light bean leaf vs. high light bean leaf. Are these statistically different?

5. **Plot the mean + S.E from above on a bar graph.** Be sure to label the graph with the light intensities at which the measurements were made.

6. **Plot the uncorrected oxygen production (L<sub>0</sub> - L<sub>T</sub>) x K<sub>v</sub> versus incubation time for each syringe in the high light intensity condition.** In other words, make a line graph with time as the x-axis and with uncorrected oxygen production on the y-axis. You should have 6 curves on this graph, one for each syringe.

**Discussion**

Discuss the effect of light intensity on the rate of photosynthesis by bean leaves. Is the hypothesis supported by your data? What sources of error in your experiments might have affected your values? Speculate on why individual bean leaf containing syringes might have different rates of oxygen production? What purpose did the controls with empty syringes serve in this experiment? Rather than choosing a single time point (such as 60 min) to calculate the rate of oxygen production, could you think of another way to calculate this rate using the data from the line graph for uncorrected oxygen production in part 6 above?

**Conclusion**

Briefly describe the major results of your experiments.
Exercise 4- Patterns of Inheritance
(Page 1 of 2)
10 pts

For all questions the following traits of cacti will be used:

<table>
<thead>
<tr>
<th>Trait</th>
<th>Alleles</th>
<th>Dominant Phenotype</th>
<th>Recessive Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>G and g</td>
<td>Dark green</td>
<td>Light green</td>
</tr>
<tr>
<td>Height</td>
<td>T and t</td>
<td>Tall</td>
<td>Short</td>
</tr>
<tr>
<td>Needles</td>
<td>N and n</td>
<td>Pointy</td>
<td>Blunt</td>
</tr>
<tr>
<td>Shape</td>
<td>B and b</td>
<td>Bent</td>
<td>Straight</td>
</tr>
<tr>
<td>Flower</td>
<td>R and r</td>
<td>Red</td>
<td>Pink</td>
</tr>
</tbody>
</table>

All of the genes encoded by these traits reside on different chromosomes, so not traits are linked.

1. For the following questions you are considering the results of a cross between the following parent plants: GgTt x ggTt

   a. What percentage of offspring should have the genotype Ggtt?

   b. What percentage of offspring should have the same genotype as either parent?

   c. What percentage of offspring should be light green?

   d. What percentage of offspring should be tall?

2. For the following questions you are considering the results of a cross between the following parent plants: GGTt x ggTt

   a. What is the ratio of dark green, tall plants to dark green, short plants?

   b. What is the most common genotype of the offspring?
Exercise 4
(Page 2 of 2)

3. For the following questions you are considering the results of a cross between the following parent plants: GgTtBb x GgTtBB

a. For which trait will all of the offspring share the same phenotype?

b. What is the ratio of offspring with the genotypes GGTTBB to ggTtBb?

c. What percentage of the offspring will be bent?

4. For the following questions you are considering the results of a cross between the following parent plants: GgTtBbNn x GgTtBbNn

a. What percentage of offspring will have the genotype ggTTbbNN?

b. What is the most common genotype of the offspring?

c. What is the least common phenotype of the offspring?

5. For the following question you are considering the results of a cross between the following parent plants: GgTtBbNnRr x ggTtBBNnRr

a. List ten possible genotypes that could result from this gross
Laboratory 5 - The Cell Cycle and Mitosis

Objectives:

The purpose of this laboratory is (1) to observe and identify stages of the cell cycle, particularly the various mitotic stages and (2) to observe gametogenesis in plant and animal tissues.

Introduction:

The sequence of events occurring between successive cell divisions is known as the cell cycle. As the cycle proceeds, the cell roughly doubles its volume of cytoplasm, duplicates genetic information (DNA is replicated), and divides that genetic information equally into two daughter nuclei. As depicted in Figure 1 (See illustration on page 2), the cell cycle is divided into 2 phases: (1) interphase and (2) the M or mitotic phase. The relative time spent in these phases depends upon the type of cell and its particular developmental or physiological state. Cells comprising rapidly growing tissues, like those in the root tip of a plant, spend a comparatively long period in the mitotic phase, whereas those cells comprising slow-growing tissues would spend most of their lives in interphase. Non-dividing cells remain in interphase and never enter the M phase.

Interphase is characterized by a particular sequence of events. During the G1 phase (or "first gap" phase) biological molecules (carbohydrates, fats and proteins) are synthesized for incorporation into the cytoplasm of the daughter cells following cell division. It is in the G1 stage that most of the cell's metabolic and growth activity occurs. In animal cells, structures called the centrioles replicate during this part of interphase. Non-dividing cells remain in the GI phase. The next phase of interphase, the S-phase (or "synthesis" phase) is characterized by the synthesis of DNA. The G2 phase (or "second gap" phase) is defined as the phase existing between the S and mitotic phase. The synthesis of new biological molecules and the duplication of cell organelles like mitochondria occur in all three phases of interphase, but predominantly during the GI phase. The M phase of mitosis is divided into 5 stages: 4 of which are mitotic stages (prophase, metaphase, anaphase and telophase) and the 5th, known as cytokinesis, in which cytoplasmic division occurs. It should be emphasized that the process of mitosis is associated with the division of previously replicated genetic information into two new daughter nuclei. Mitosis is usually followed by cytokinesis, but need not be. For example, skeletal muscle cells in vertebrate animals are in a multinucleate condition; the result of repeated mitoses without subsequent cytoplasmic divisions.

**NOTE:** In lecture, we will devote time for discussions of interphase, DNA replication, and the mechanism of mitotic division. For this week's laboratory, we will emphasize microscopic recognition of the 4 mitotic stages as well as interphase.
Specific Background for this Experiment:

Interphase: (i) Synthesis of biological molecules including DNA; (ii) Newly duplicated DNA and associated proteins comprising the chromatin begin to condense toward the end of interphase, but are not yet visible - presence of nucleoli indicative of this stage; (iii) The nucleoplasm has a fairly uniform granular appearance.

Prophase: (i) Chromatin condenses into more distinct elongate strands early in prophase; (ii) Nucleoli begin to disappear as does the nuclear membrane; (iii) Mitotic spindle begins to form; (iv) Chromosomes appear as shortened rods near end of stage; (v) The appearance of chromatin in visible threads is probably the best indication of prophase.

Metaphase: The most distinctive of the stages of mitosis; characterized by the regular arrangement of chromosomes at a central region called the metaphase plate.

Anaphase: Anaphase begins as duplicated chromosomes separate, beginning their movement to opposite poles of the cell.

Telophase: (i) A nuclear membrane begins to form around each of the two new daughter nuclei; (ii) Chromosomes uncoil and become less distinct; (iii) The process of cytokinesis begins at this stage, as shown by the development of a cell plate in plant cells.

**NOTE: Distinguishing Telophase and Prophase will probably be the most difficult for you.
The presence of two comparatively small adjacent nuclei would indicate telophase. A nucleus with distinct chromatin strands which is larger than most of its neighbors would be a good indication that the nucleus is in Prophase.

**Procedures:**

During this laboratory we will examine prepared microscope slides of the rapidly growing meristematic tissue of the root tip of the onion *Allium*. This tissue is particularly suitable for us since the tissue showing the greatest mitotic activity is quite localized. Using the illustrations below and/or others available to you, examine the cells of the root tip and be able to find examples of each of the mitotic stages and interphase.

1. Using the illustration at the end of the laboratory exercise (See Figure 4), examine a variety of nuclei within the root tip cells until you feel confident that you can identify the various stages of mitosis.

2. With the root tip pointing downward in the field and the microscope on **low power** (100X - yellow objective) place the pointer at the root tip in region just behind the root cap where the cells first begin to arrange themselves into columns.

3. First Data Set:

   (a) From this region, proceed upward in the center of the root (toward more mature root tissue) 50 cells along a single column of cells. This procedure is designed to place each student in approximately the same location on the root tip before counting and recording the various mitotic stages.

   (b) Without moving the slide, change the objective to **high power** (400X - blue objective).

   (c) With the pointer of the ocular placed in the **12 o'clock position**, pointing downward, proceed down the column of cells indicated by the tip of the pointer and record the mitotic stage of the first 25 cells you encounter in that column. If you encounter cells that do not contain nuclei or nuclei too out of focus to allow a determination, continue your examination along another column of cells either to the right or to the left of the column you are examining. To determine which of these alternate columns to choose, flip a coin. If the coin lands heads, use the column to the right of the one you are on; if tails, use the one on the left. Proceed along that column, bypassing "empty cells", until you encounter cells with nuclei.

   (d) Record your data in a table with headings for each of the 4 mitotic stages (prophase, metaphase, anaphase and telophase) and interphase. After completing your count of these first 25 nuclei, return to low power and the beginning position just above the root cap.

4. Second Data Set:

   As described previously, you should proceed 50 cells upward, and switch to 400X. (b) For the second count of 25 nuclei, place the pointer in the **3 o'clock position** (pointer pointing toward the left) and begin classifying nuclei as you proceed **upward**.

5. Third Data Set:
With the pointer in a **6 o'clock** position, examine nuclei by proceeding **downward**.

6. Fourth Data Set:
The last 25 nuclei will be examined with the pointer at a **9 o'clock** position and in an **upward** direction.

**Data, Graphs, and Calculations:**

**DATA -**
1. **Your Group Data:** Construct table with the column headings: interphase, prophase, metaphase, anaphase and telophase. Record the number of nuclei observed in each stage. (N=100 nuclei)

2. **Class Data:** After each observer has entered his or her data on the board for the class, record the entire classes data for later analysis in the table provided on page 8.

**GRAPH -** Using data for the entire class, construct a pie chart depicting the proportion (or percentage) of time cells remain in each stage of the cell cycle. Your pie chart should differ from, Fig. 1 in that it should be in a circle rather than a disk and will not include G1, S and G2 of interphase or cytokinesis. From a practical standpoint it will be impossible to distinguish between telophase and cytokinesis. As a result, the portion of the cell cycle spent in cytokinesis will appear as telophase.

**CALCULATIONS -**
1. A typical mitotic cycle in onion root tip cells requires about 16 hours to complete. Using the percentage values in each "slice" of your pie chart, calculate the time in hours and minutes that a typical cell would spends in each stage.

2. **Statistical Analysis:** For each of the M cycle stages and interphase, calculate a mean number of observations of 100 for the class and express each ± the standard error. To get a crude measure of the variability among the means, divide the standard error by the mean. For example, if the mean number (of 100) cells found in prophase was 27.7± 1.9 for the class, a measure of its variability would be 1.9/27.7 = 0.0686. A larger value for prophase, compared - with, say metaphase, would indicate a comparatively high variability among the class observers in making determinations that a given cell is in prophase as compared to their ability in making a determination of metaphase.

**Discussion and Drawings:**
Which of the 4 M stages did you initially expect to have the longest and shortest durations? What biological events in each stage would tend to give that stage a longer or shorter duration? What particular cytological features do you think might contribute to a relatively high or low variability among observers in determining each of the mitotic stages? Briefly describe the major results from the exercise identifying the various mitotic stages.
<table>
<thead>
<tr>
<th>Observer #</th>
<th>Interphase</th>
<th>Prophase</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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Figure 4. Mitotic stages in onion root tip. Vodopich, D. & Moore, R. Laboratory Manual to accompany Raven & Johnson, Biology, Mosby, 1986.
BSC 197 - Molecular and Cellular Basis of Life

Name:_________________  Section #:_________________

Exercise 5- PCR Exercise
(Page 1 of 2)
10 pts

1. **5 points.** Attached you will find the DNA nucleotide sequence of a portion of an adhesion gene from *Haemophilus influenzae*. The sequence presented shows only one strand (5' terminus is top left, 3' terminus is bottom right). Use the guidelines in your handout to design PCR primers that would amplify a fragment that is 200-300bp in size. Remember that you will need to find the reverse compliment sequence to design the downstream primer. **Underline** the sequences from the gene that correspond to the locations where your primers (there should be two sections, one for each primer) will anneal.

   Primer 1 sequence (forward primer; be sure to write it 5’-3’)

   Primer 2 sequence (reverse primer; be sure to write it 5’-3’)

2. **1 point.** What would happen if you forgot to add the buffer to a PCR reaction?

3. **1 point.** Explain what would happen if you performed PCR using a dNTP mixture that contained only dGTP, dTTP, and dCTP.

4. **1 point.** Explain what would happen if you accidentally programmed your thermocycler to carry out the Elongation step at 95°C.

5. **2 points.** You need to perform an important PCR reaction, however, you are out of Taq polymerase. You realize that your lab does have purified DNA polymerase isolated from *E. coli* (lives at 37 degrees Celsius). You decide to attempt PCR with the *E. coli* DNA polymerase-what will happen?
**Exercise 5**
(Page 2 of 2)

Portion of hia sequence from *H. influenzae*:

*Note that the number on the left side of each row indicates the base number that begins the row. For example, the first row contains the first 85 bases in the sequence, whereas the second row begins with base number 86 and contains bases 86-172. This means that this is one sequence (1487 bases long) written 5’ to 3’.

5’
1 cacaaacgtggactgtctgttctgaacttggcagactgaaggttaaaccaagtcataaaagatcgttaaatgtgacgcact
86 gcgggtctgtgtattggtctttgttcactctactgaataatctgtcactgcaagaacttggtatctgggtatcgactgtacgttaagaattggtgagtctagggtgagttgctgtttgctttggtggtcgacgactgtgaaggtggtgatcagttgctgttataagttctgttaattcttggtggtgtgatctagtttattgtttgtctgtattttcttggtgtgtgtgtgttggtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgt
1.) Construct a pedigree for the following family with respect to jaw clicking. Individuals that express the jaw clicking trait should be represented with shaded symbols and those without should be represented with clear symbols. Be sure to number the pedigree correctly.

- No. 1 - A Male expressing jaw clicking
- No. 2 - Female not expressing jaw clicking
- Nos. 3 and 4 - Offspring of Nos. 1 and 2
- No. 3 - Female not expressing jaw clicking
- No. 4 - Female expressing jaw clicking
- No. 5 - Unrelated male not expressing jaw clicking
- No. 6 - and 7 - Offspring of 4 and 5
- No. 6 - Male not expressing jaw clicking
- No. 7 - Female expressing jaw clicking

Draw your pedigree here:
Exercise 6
(Page 2 of 4)

2. (2 points) You have been examining a family with respect to ear lobes. Individuals with lobed ears are represented with clear symbols and those with lobeless ears are represented by shaded symbols. You believe lobeless ears is an autosomal dominant trait.

a) Is the information contained in this pedigree consistent with your hypothesis that the trait is autosomal dominant? Why or why not?

b.) What are the predicted genotypes of each member of the pedigree above?

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<th>Predicted genotype(s)</th>
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3.) Human ABO blood types can be tracked with pedigrees. As a reminder, ABO blood types are determined by three different alleles. The A and B alleles encode distinct surface structures on red blood cells. The O allele does not encode a surface structure and therefore, the presence of an A or B allele will mask that of the O allele. As a result individuals with genotypes of AA or AO will have type A blood (only A structures); individuals with BB or BO will have type B blood (only B structures); individuals with AB genotypes will have AB blood type (both A and B structures); and individuals with OO will have type O blood (neither A nor B structures).

![Pedigree Diagram]

a.) Use the pedigree to predict the **genotype** of each individual.

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Exercise 6
(Page 4 of 4)

b.) If Nos. 10 and 3 had another child would it be possible for the child to be type B? Why or why not?

c.) If Nos. 6 and 7 had another child would it be possible for the child to be type 0? Why or why not?

d.) If Nos. 6 and 7 had another child would it be possible for the child to be type B? Why or why not?