

## Phagocytosis in *Tetrahymena* as an Experimental System To Study the Toxic Effects of Cigarette Smoke - Week 2

During this laboratory period you will conduct the experiment you designed last week. Organize the members of your group so you can efficiently conduct your experiment. Refer to the web tutorial on serial dilutions if that is a factor in your experiment.

### Procedural Modifications for studying the effects of smoke extracts.

Smoke extracts or extracts from filters have been dissolved in deionized water or ethanol. If using ethanol you will need to do a control to make sure it does not affect food vacuole formation. You should probably also do a control for the water.

1. Prepare microfuge tubes for killing the *Tetrahymena* by adding 100 microliters of 3% glutaraldehyde to each tube.
2. Place 2ml *Tetrahymena* culture in a glass test tube.
3. Add **20 microliters** (test substance or control substance) and mix gently.
4. **Optional:** allow a predetermined amount of time for the substance to enter the cell before adding the ink.
5. Add 2ml of 1% ink and mix gently.
6. Sample at predetermined time(s).
7. Kill organisms by pipetting 200 microliters into tubes containing 3% glutaraldehyde.
8. Count the number of food vacuoles in 25-50 cells.

**Data Analysis:** If time permits you should begin data entry and analysis before leaving the laboratory. If this is not possible you will have to do it on your own time. I suggest that you begin ASAP so that you can get help if you are having difficulty.

Organize the data so they can be easily entered into a MINITAB file. The best way to enter the data will depend on the tests you want to do. Refer to "Which Stats Should I Use" to make sure data are entered in the manner that is appropriate for your particular analysis. If you are in doubt about which stats to use check with your instructor before leaving class.

\*\*\* \*\***Note** Generally the 95% confidence interval for the mean is reported, however for this paper you have a choice of using either the mean  $\pm$  95% C.I. or the mean  $\pm$  SE. **Make sure you clarify which was used in your paper.**

Decide which tests are appropriate then use the **Stats menu** for data analysis.

1. **Two Sample T-Test** - you can either have the data for each treatment in separate columns or it can be in one column and a code column can be used to identify the treatments.

If code columns were used, use *descriptive stats* with the **BY** subcommand to determine the mean  $\pm$  SE. If you prefer to use the mean  $\pm$  95 % C.I. the data will need to be unstacked into separate columns for each treatment and then the **I-sample t** -> **confidence interval** can be used. To help you keep track of the data, unstack one data column at a time.

2. **Analysis of Variance** - you can have one code column that allows you to identify the treatments and several data columns **or** the data for each treatment can be entered into separate columns.

Calculate the means and 95% confidence intervals or SE as explained above.

3. **Two Way Analysis of Variance** - Code columns are required. Use the **GLM** command to do the analysis - The basic model is shown below.

(GLM C# {data col} = C#{code1} C# {code2} C#\*C#{code1\*code2})

Refer to “Which Stats Should I Use” for help in working with this type of data. Use two different code columns - one for each treatment. Make sure you record what the codes are. For example you may have used temperature as one variable and the concentration of smoke as the other variable. You might code these as follows:

Room temp = RT	Control	= Cont
Cold = C	High Smoke	= HS
	Low Smoke	= LS

The results of the Two Way Analysis of Variance simply tell you which factors had a significant effect. To determine the nature of that effect it will be necessary to calculate the means and 95% confidence intervals or SE. To calculate the means  $\pm$  SE, you will need to create a third code column. This column designates each combined treatment with a different code eg. use Rtcontrol for the room temperature control. Keep your headings short. Use *descriptive stats* with **By** (*combined treatment column*) to calculate the means  $\pm$  SE. Alternatively you could unstack the data using the combined code column and calculate the 95% CI. - If you decide to use the 95% confidence interval. I suggest you unstack just **one** column at a time to make it easier to keep track of what you are doing. The number of columns required to receive the data will depend on the number of different codes in the code column. TAs/instructors will be happy to help you sort this out once your data are entered.

If you have questions about entering, analyzing or graphing your data please contact your instructor or TA.

**Assignment:** Write a scientific paper to report the results of your experiment. Your first step should be to analyze the data. Then begin writing. Use "Preparing Papers" as your guide. There will be several papers for you to use when writing the introduction and discussion. **You are required to use a minimum of 2 papers.** These can be accessed from the Biology 109 Resource Page.

### Helpful Hints for Writing the *Tetrahymena* Paper

Begin with either the methods or results. Write the discussion after you finish the results and do the introduction last. This way you will already know what you have learned and it will be easier to organize your thoughts for the introduction.

#### Methods:

1. Explain the experimental design. If you are using a 3x3 or 2x4 design, you can list the concentrations/levels of the two variables and then say all possible combinations of the two variables were used. This saves having to list every treatment. Make sure you give the rationale for the concentrations chosen, the sample size, and indicate which treatment(s) serve as controls.
2. Give sufficient detail that the procedure can be repeated. Cite Bozzone (2000) for details on culturing *Tetrahymena* and preparing the growth medium.
3. Duration of the experiment and temperature at which the experiment ran.
4. Type of data collected and the statistical analysis used.

WARNING - the above list may not include everything

**Results: Enter your data into minitab.** You can work together entering the data, but the analysis should be done independently. Once the data are analyzed you should prepare your figures. If you have questions about how to manipulate the data please check with your instructor.

9. If you used a design that required doing a two way analysis of variance then you should make an interaction graph. Details for doing this are included in the section on making Excel Graphs. **Ask for help if you have questions.**
10. Use the checklist for figures/tables (page 10) when editing your graphs.

#### Analysis of the Figure

If you had a significant interaction the figure will help you visualize the nature of the interaction. Under what condition(s) is the response to one variable altered in a unique way by the presence of the other variable? Make sure you explain the nature of the interaction in the text of the results section. For example, at room temperature smoke had no affect on the rate of food vacuole formation but at 15° C it inhibited food vacuole formation.

### **Use of literature in the introduction/discussion:**

When citing the literature it is important that you make clear the reason for referring to the cited study. You need to give enough information about the study that the reader can understand its importance to your study. Some of the types of information that should be included are:

1. The species studies - different species may respond differently to the treatments.
2. Conditions of the study - point out how what the authors did differs from your design. They may have used different concentrations of related chemicals.

<p style="text-align: center;"><b>Work independently when writing your paper</b></p>
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3. If you refer to work that is cited in one of the papers you read, make sure you present it in the form of a **secondary citation** (pg.13). e.g. Sussex (1992 as cited in Ribochaud 1993) This way the reader knows that you are making an interpretation based on the interpretation of another author rather than from reading the original literature.

In the **Introduction** literature citations are used to provide background that leads to the formation of a hypothesis. For this reason the literature should be reviewed in a logical sequence beginning with the general problem and advancing to the specific questions/hypotheses that are the focus of the study. Avoid giving so much detail that you undermine your discussion.

In the **Discussion** literature citations serve three purposes.

1. They are used to point out similarities between the results of the current study and previously published studies.
2. They are used to point on differences between the results of your study and what has been reported in the literature.
3. They can be used to help explain your results.

Use of **2 primary references** is required. Check with your instructor about the location of references.

**Make sure that you adequately acknowledge the contributions of others.**

**\*\*\*Note\*\*\***

Writing this paper will probably take longer than you anticipate. Allow yourself adequate time.