CELL DIVISION

Pre-lab Preparatory Information

Know this material prior to attending your lab. You may be quizzed on this material.

Objectives, by the end of this lab, you will be able to…

…describe each stage of nuclear division (mitosis) and cytoplasmic division (cytokinesis) and identify each under the microscope.

…use a compound microscope and describe two stains used to facilitate observation.

…graph data correctly, paying particular attention to the differences between independent and dependent variables.

1. Cell Division: The emphasis of this laboratory period will be on **mitosis**, one kind of nuclear division. Mitosis is the sequence of events by which the nuclear material of one cell is distributed, by a process involving **chromosomes**, into two equal parts. (In some organisms the nuclear material divides by a process which does not involve chromosomes, or **amitosis**.) Mitosis is often accompanied simultaneously by cytoplasmic division; however, this is not always the case. For example, there are organisms in which the nuclei divide without accompanying cytoplasmic division, resulting in multinucleate cells. Therefore, we will consider mitosis and cytoplasmic division (**cytokinesis**) to be separate processes.

Although mitosis may take place in almost any living cell of an organism, it occurs primarily in relatively defined areas. Cells from the apical meristem of the onion root are most commonly used to illustrate the events of mitosis. The meristem is a region of active growth and thus a logical place to find nuclear and cytoplasmic division. The reason that the onion is often used is that the chromosomes are fairly large and distinct, and this species has a relatively small number of chromosomes.

The essentials of mitosis are the same in both plant and animal cells, but there are some differences. You will study the process in detail in the onion root and then compare the process of mitosis and cytoplasmic division of this typical plant with that of the whitefish blastula (an early stage in embryonic development) as an animal example.

In-Laboratory Exercises:

I. Mitosis with Artificial Chromosomes

Before looking at the stages of mitosis in onion root tips, it is important to understand the activities of the chromosomes during mitosis. You will be provided with the materials for making artificial chromosomes ("pop-it" beads with magnetic centromeres). Use these to follow the chromosomes through mitosis. First draw a large round "cell" and nucleus with chalk on the lab bench-top.

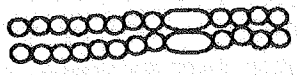
Construct a strand of red beads including a magnetic centromere. This represents an unduplicated chromosome.

Construct an identical strand of yellow beads. These two strands represent a **homologous** pair of chromosomes. In diploid organisms chromosomes of somatic cells occur in matched pairs that are similar in size, shape and location of their centromeres. One member of each pair of homologous chromosomes came from each of the parents. Therefore, in this example, the diploid number is two ($2n = 2$).

Interphase: DNA synthesis will occur during the S phase so that each chromosome will

2n = 2
diploid

red chromosome-
(pair of sister chromatids)

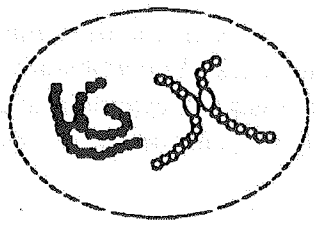


yellow chromosome-
(pair of sister chromatids)

consist of a pair of sister chromatids. Simulate this process by constructing another red strand and another yellow strand, each identical to the first. Place the centromeres of the 2 red strands together and those of the 2 yellow strands together.

Remember, the chromosomes are not actually visible during interphase.

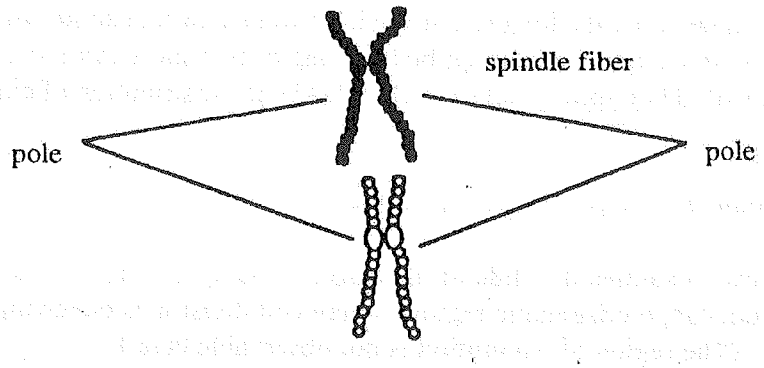
Mitosis 2n = 4



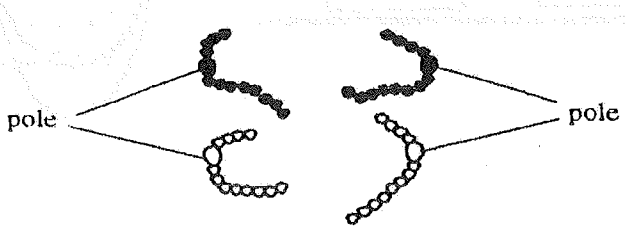
Prophase: The chromosomes are now visible and are increasingly easy to visualize as they become shorter and thicker as prophase progresses (your artificial chromosomes will remain the same, however). At the end of prophase, the nuclear envelope will disappear (so erase the chalk circle you drew representing the nuclear envelope).

Metaphase: The chromosomes line up in the central region of the cell at the equatorial plane. (It is also called the "metaphase plate", but you have to remember that it is a region, not a structure.) The centromere regions of sister chromatids are attached by spindle fibers to opposite poles of the cell. Use a piece

of string looped around the centromere and attached with tape to the pole to simulate a spindle fiber. Each simulated spindle fiber represents many actual fibers.

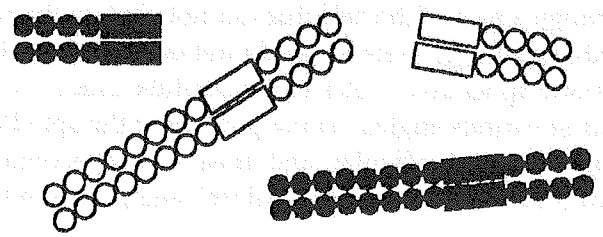


Anaphase: The sister centromeres move to opposite poles and of course the attached chromatids are carried along, trailing out behind in a v-shape. Simulate anaphase by pulling on the strings (spindle fibers) to separate the centromeres and continue pulling the daughter chromosomes to the poles. (Note - each of the daughter chromosomes was formerly a sister chromatid.)



Telophase: After the chromosomes have reached the poles the spindle disappears, the chromosomes will start to decondense and return to an interphase-like condition and two nuclear envelopes form. You should end up with two nuclei, with 2 chromosomes each (a red and a yellow). How does each of these nuclei compare with the original nucleus?

Now repeat the above process using two pairs of homologous chromosomes, the above pair and a shorter pair with the centromere at one end. One chromosome should be red; the other, yellow. In this example, the diploid number is 4 ($2n = 4$).

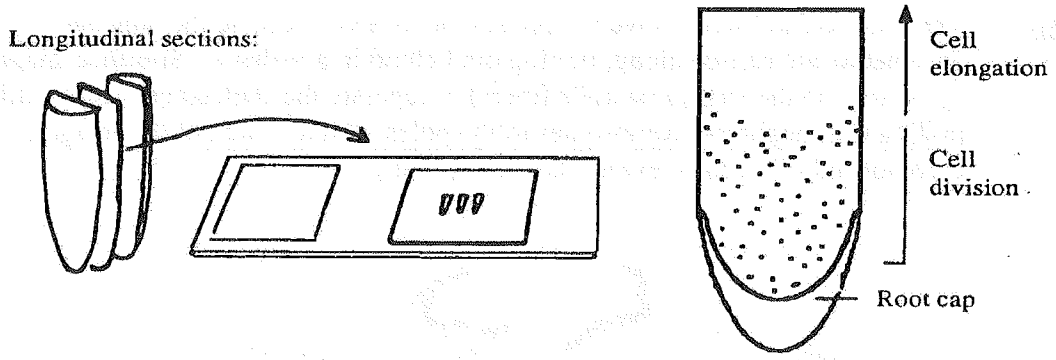


Stop at each phase to DRAW the distribution of chromosomes.

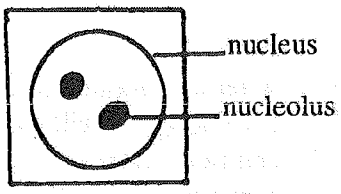
Finally, separate the above chromosomes so that you have one of each homologous chromosome. This can be either the long red or the long yellow chromosome, and the short red or the short yellow chromosome. This cannot be two long or two short chromosomes! Repeat the stages of mitosis with this **haploid** cell (n = 2). DRAW the distribution of chromosomes at each stage.

II. Mitosis: Onion Root Tip - Prepared Slides

Using low power, examine the slide of the onion (*Allium*) root tip and identify the following regions: root cap, meristematic region (where cell division is occurring), and the region of elongation. (The region of maturation is not observable here.)



The interphase cells in the region of cell division are approximately the same length and width. The nucleus takes up much of the volume of the whole cell. These cells also have very large nucleoli, sometimes several per nucleus. Do not confuse these nucleoli with the two nuclei found in telophase cells.



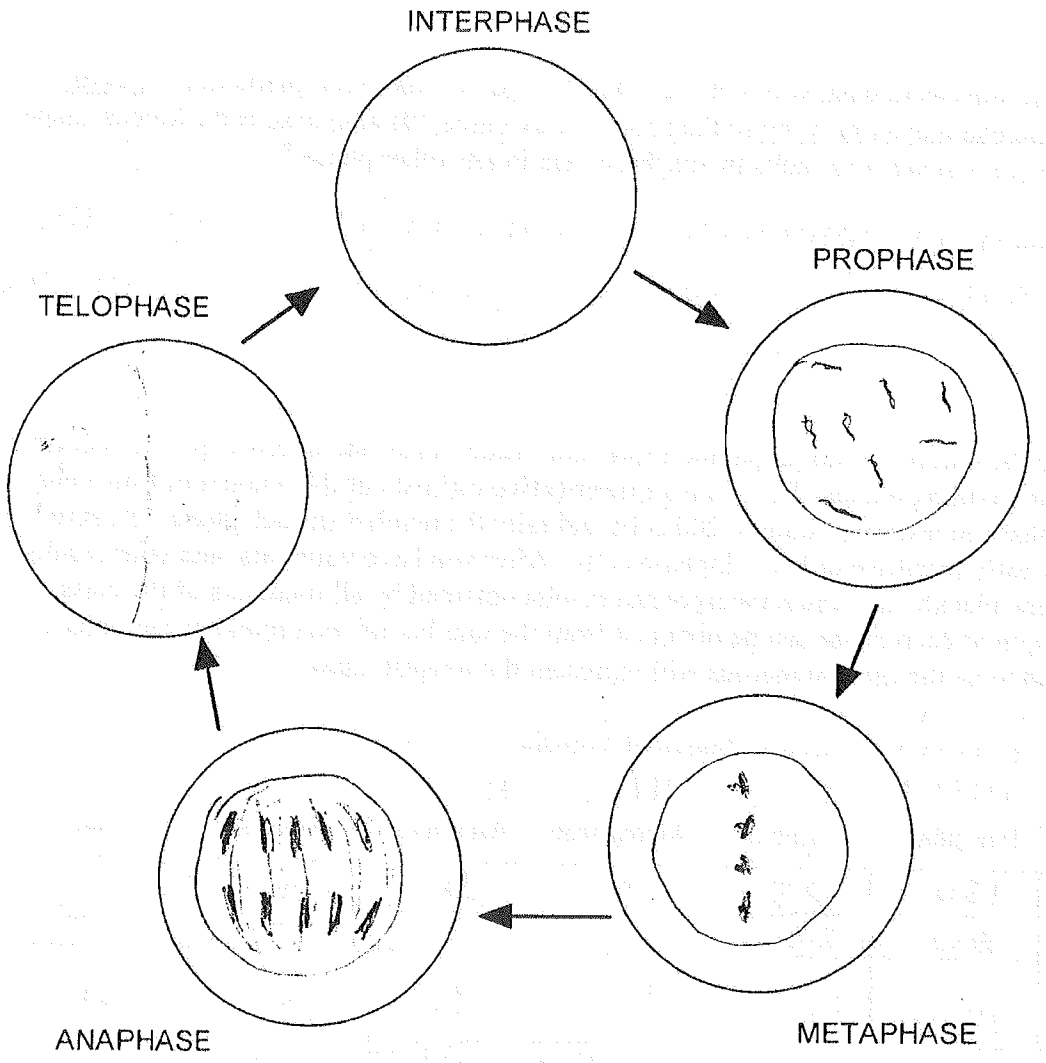
Examine individual cells in the region of division under high magnification.

Remember that when you are observing sectioned materials, you are viewing a **"two-dimensional slice of three-dimensional reality."** The sections are thinner than the width of a cell. You may therefore see some cells with no nuclei - not because they never had them, but because the section went through a part of the cell that did not contain the nucleus. (In other words, when you slice a hard-boiled egg, some slices do not contain the yolk.) Other cells may have only portions of the mitotic apparatus. Later in the blastula slides, the sections will pass through the mitotic apparatus at various angles: some parallel to the spindle (like in the root tips - and most textbook figures), some perpendicular, and most at various oblique angles. It is your challenge to observe these various "two-dimensional slices" and put them together to imagine "three-dimensional reality".

Be sure to note at least the following: relative lengths and thickness of chromosomes; position and arrangement of chromosomes; presence or absence of nuclear membrane, nucleus and spindle fibers. You should be able to identify the phase of mitosis of any cell which you are shown.

In the following circles, draw a "typical" onion cell in interphase and in the different stages of mitosis. Draw them to the same magnification.

What is the actual width of these cells?_____μm $\frac{375}{18}$



III: Determining the Duration of Mitosis

Before you begin:

Given what you know about the events of mitosis, what is your **hypothesis** about the relative lengths of the stages of mitosis?

Hypothesis: I think that the cell will stay in prophase the longest.

State your **predictions** as to what you will see. Don't forget to make your predictions **specific** and **testable**. You can make one or more than one. For example, "**If** anaphase is the longest stage of mitosis, **then** we will see more cells in anaphase than in any other phase."

Prediction(s): If prophase is the longest stage, then we will be able to see more cells in prophase.

Observe the onion root tip preparation (prepared slide) under the microscope and identify the phases of the dividing nuclei. To obtain a **quantitative** estimate of the amount of time cells spend in each phase of mitosis, examine 200 cells and tally the number in each phase. Be sure not to overlook early prophase or late telophase cells. After you have your data, add your results to the table on the blackboard which incorporates results obtained by all members of the class. The relative length of each phase can be obtained from the number of cells found in each phase. The phase found to be the most numerous will represent the longest phase.

Table 1: Phases of Mitosis

	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total
1) Number of cells	150/200	25/200	15/200	6/200	4/200	200
2) Percent in each phase	75%	12.5%	7.5%	3%	2%	100
3) Timing*	.75 x 24	.125 x 24	.075 x 24	.03 x 24	.02 x 24	24hr.

*Assume that the cell cycle takes 24 hours from the very start of one prophase to the beginning of the next prophase. Calculate the "timing" by multiplying the percent of cells in each phase by 24 hours. This gives you the approximate amount of time a cell spends in interphase and in each section of mitosis.

IV. Mitosis: Onion Root Tips - Squash Method

You will use either acetocarmine or aceto-orcein, as directed by your Laboratory Instructor; however, make certain that you see the results for both stains.

SAFETY NOTE: Concentrated acetic acid is toxic! Do NOT breath the fumes. The staining must be performed in a hood. You must also wear your chemical splash-proof safety goggles when staining the root tips. Students without goggles may watch at a safe distance.

1. Remove the bottom 2 to 3 mm from the root of an onion. Place it toward one end of a microscope slide. Do not put the unused part of the root back into the container.

2. Place 2-3 drops of dye solution over the root tip and heat GENTLY on a warm hot plate for at least one minute. Be patient for better results!

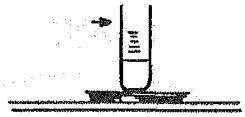
   Do NOT allow the sample to boil dry; add more stain if necessary.

3. Place the slide on a paper towel and add a cover slip. Press gently on the cover slip with your finger or the eraser end of your pencil. Do not break the cover slip.

1. Slide with root tip.

2. Add drop of stain and heat gently

3. Add cover slip, squash with pencil



The root tip should squash out into a nice thin layer of cells. If the tissue is still too tough to squash, heat the preparation a little more and try again.

4. Once you have made certain that there is NO stain on the bottom of the slide, place it on a microscope and look for the phases of mitosis. Remember, dividing cells are relatively square; elongate cells are no longer dividing, so ignore them.

!!! Compare your results to the **demonstration slide** of a squashed root tip. !!!

The "Allium test" has been used to determine whether a specific compound causes chromosomal breaks by growing onion roots in the chemical, followed by microscopic examination. Would this analysis use squashes or sectioned materials? Why? (And why is it called the "Allium test"?)

V. Cytokinesis in Plant Cells

It was mentioned that cytoplasmic division need not occur immediately following nuclear division, although it often does. In roots cytoplasmic division is first visible in telophase. By late telophase you can see a cell plate forming between the two daughter nuclei. The plate first forms in the middle of the cell and extends outward until it connects up at right angles with the original cell walls. (Try to think of the cells in three dimensions.) Each of the daughter cells will form a cell wall along the cell plate.

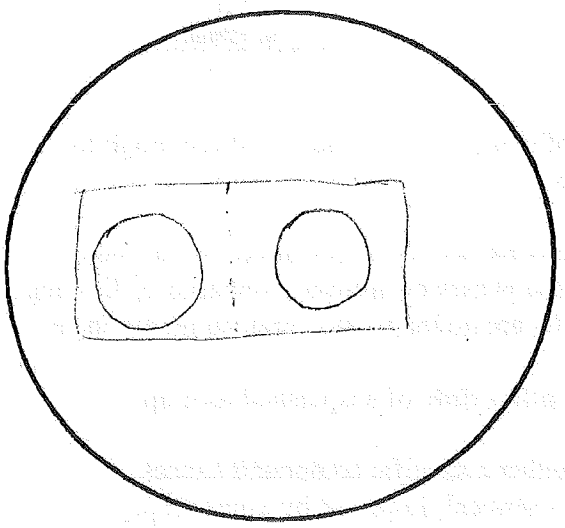
Observe some of the onion root tip cells in various stages of telophase and try to detect various stages in the formation of the cell plate and cell wall. Draw a series of pictures showing the formation of the cell plate and the new cell wall.

VI. Mitosis and Cytokinesis in Animal Cells

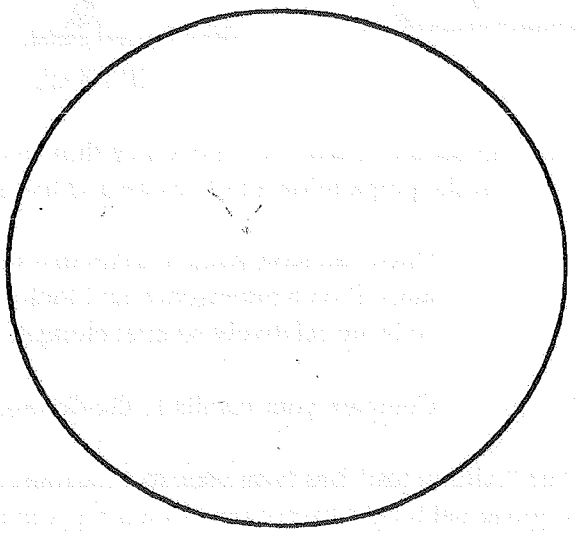
Slides of whitefish blastulae will be used to show mitosis and cytokinesis in animal cells. Although the result of these processes and many of the events are the same or very similar to that of the plant cells, there are some differences. What differences can you detect?

In the circles below, draw "typical" plant and animal cells dividing by cytokinesis.

PLANT



ANIMAL



If you want to review the stages of mitosis, check out a slide set at the MSLC and try again. Or – even better - use the TV camera set up with one of the microscopes in the MSLC and go over the slides as a group!

VI. Tables, Graphs, and Charts: Presenting and Making Sense of Your Data

In last week's lab, we discussed the scientific method: developing a hypothesis and a series of testable predictions, and using scientific tools to collect different forms of data. This week we will look in more depth at the tools of data tabulation, data analysis, and data presentation.

1. Tables: One way to start working with your data is with a simple table, such as Table 1 above or the plasmolysis table from Lab 1. If you were to conduct a larger experiment, or have additional trials of the same experiment, you would enter these data into a spreadsheet program to keep it organized. Don't forget that, in addition to simply recording the data, you also need to get some kind of meaning from it. For example, if you record that you see 15 cells in metaphase, this does not tell us anything unless we know how many cells you counted in total as well. You can then calculate the fraction or percentage of cells in a given stage of mitosis. This is a preliminary form of analysis, which is how you make sense of your data; this is the start of finding patterns and correlations.

2. Graphing: In this week's experiment of the duration of mitosis, there are many options for visualizing the data. You could draw a "pie graph" to show the relative amount of the time in each phase in a 24-hour cycle. You could also draw a bar graph, where the height of each bar represents the number of cells you counted in each part of the cycle. Other types of data are best represented by x-y plots, three-dimensional surfaces, or color-coded maps. What these diverse methods have in common is that they are all tools for visualizing and evaluating large quantities of data.

depen-Yaxis Ind-X axis

Regardless of the form of your graph, in formal presentations, all figures (graphs, tables, pictures, maps) require:

1. **A title**: All figures must have a title that describes the content of the figure. A good title summaries the point and content of the figure.

2. **Proper Graph Format**: When constructing a graph, the **independent variable** is plotted on the X-axis while the **dependent variable** is plotted on the Y-axis. You determine which variable is which by considering which one depends of the value of the other.

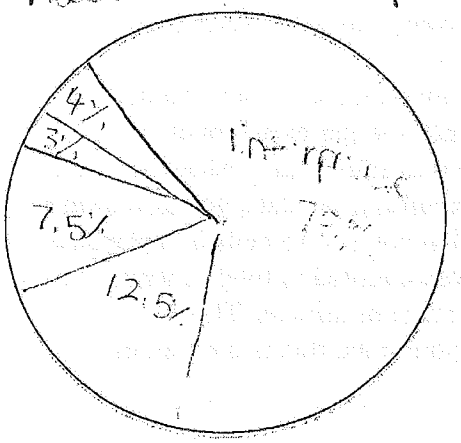
In last week's plasmolysis analysis, which would be the dependent and independent variables? Did the sucrose concentration depend on the number of plasmolyzed cells or did the number of plasmolyzed cells depend on the sucrose concentration?

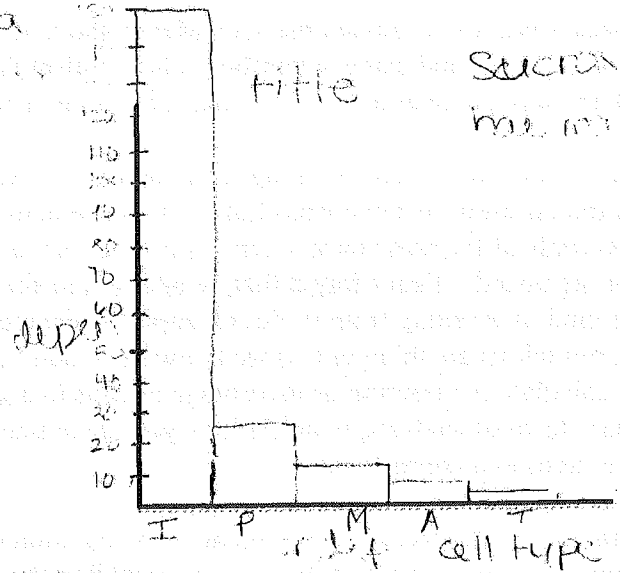
Dependent variable: # of cells that were plasmolyzed
Independent variable: Sucrose concen

3. **Labels on all axes**: Be sure to indicate what variable that each axis represents. In addition, you must include units for all variables. For example, the variable may be "time," but you must also tell what unit of time you used, days, minutes, milliseconds, *etc.*

4. **A legend**: Every figure must have an explanatory paragraph of text at its base that summarizes the content of the figure.

3. Graph Development: In the space below-left, draw and label a pie chart of the time spent in each stage of mitosis based on your group's data. To the right, draw and label a bar graph of the same data.



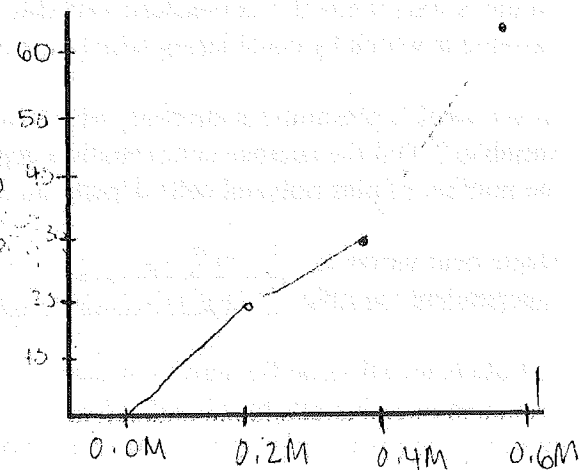


Which of these graphs do you think is clearer? Why?
What are some other ways you might represent this data?
Are your graphs properly presented?
Do these results support or reject your hypotheses about the duration of mitosis?

In most scientific studies, you would perform additional statistical tests on your data. We will not do any of these tests today, but keep in mind that data tabulation, reduction, and statistical analysis are essential tools in the process of science. They can be part of the analysis of experimental results or a way to summarize discovery science data such that new patterns are discovered. In a formal report, the statistical procedures and findings would be reported in the Methods and Results section. Presentation of any conclusions you can make about your hypothesis and its relationship to other knowledge based on your analysis is done in the Discussion section.

2. Graphs as a tool: along with its use in the presentation of data and the trends and relationships they contain, graphs can also help with predicting future events or providing information.

Use the axes to the right and your plasmolysis data from last week and develop a line graph of that data.



Can you use your graph to predict the percentage of cells that would be plasmolyzed in a 0.3 M sucrose solution?

If you found a leaf that had 40% of its cells plasmolyzed, could you estimate the solute concentration of that plant's environment?

Remember that you can preview and/or review much of the information in this laboratory by using the VIRTUAL LABORATORY FOR BIOLOGY 101. For example, you can practice identifying the phases of mitosis with real photographs of an onion root tip.

This week's assignment:

Most professional journals require that an abstract be included with any submission. An abstract is a brief summary of the goals, methods and the results of the investigation. A paragraph or two is used to tell the reader why the study was undertaken, how it was conducted, and what conclusions were made. Abstracts are generally expected to be between 150 – 250 words. 4 to 6 sentences.

Write an abstract of the experiment you did this week on the time spent in each stage of mitosis. Include a graph of your results. what we did include graph label axis which ever kind (pie) legend key

COMING ATTRACTIONS:

In this lab you used a microscope to study mitosis and cytokinesis, using both thin sections (prepared slides) and whole cells (squash method). In the next lab you will use another common instrument, the spectrophotometer, to quantify the amount of materials in a sample.