Name _____ Class _____ Date_____

You Are a 19th-Century Cell Biologist

Observing Cell Division

Question What is the sequence of events that occurs during cell division?

Lab Overview In this investigation, you will prepare slides of onion root tips, observe cells in the process of dividing, and discover for yourself the important events of cell division.

Introduction In this lab, you will assume the role of a biologist in the mid-1890s. You are fascinated by the power of the microscope to reveal the inner workings of living cells. The invention of the compound microscope, along with new stains, make it possible for you to see cell structures that no one has ever seen before. You plan to collaborate with your colleagues to discover the events of cell division.

To start the lab, you will apply a fixative solution to the cells. The fixative solution will break the connections between cell walls so that the cells can be easily flattened into one layer on a microscope slide. The fixative solution will also quickly kill the cells, stopping them in various stages of cell division. Next, you will apply aceto-orcein stain to the cells so that you can observe what was happening to the nucleus and the chromosomes inside each cell.

Prelab Activity Before the modern compound light microscope, biologists had very limited capabilities to see the inner workings of a cell. Early simple microscopes (containing only one lens) could only magnify an image up to about 266×. However, with the development of the modern compound light microscope (containing an eyepiece lens and an objective lens) in the late 19th century, researchers could see images magnified up to 1000×. Researchers also took advantage of new dyes that became available in the 1800s to stain cells for observation under the microscope. These stains made it possible to see structures inside cells, such as the nucleus. The word *nucleus* means "a central point or mass." The nucleus got its name because it was the prominent stained object seen in the middle of each cell. The stains also enabled biologists to observe structures, now known to be chromosomes, that underwent changes during cell division.

Study the descriptions on the next page of the structures that your 19th-century "colleagues" saw inside dividing cells, using the new stains and microscopes. Then answer the questions that follow.

- ***Wilhelm Hofmeister*** (Germany)
  Observed lumps or nuggets that appeared inside the cell and eventually separated into two masses. In German, he called these structures *Klumpen.*
- ***Walther Flemming*** (Germany)
  Observed cell structures he called *Knauel* that looked like tufts of yarn. He also saw these structures form a star shape.
- ***Edmund Russow*** (Russia)
  Observed structures in dividing cells he described as *Stabchen,* meaning "small rods." The rods were bright and highly refractive (they distorted light) when stained.
- ***Edouard-Gérard Balbiani*** (France)
  Observed cell structures he called *batonets etroits* (narrow, little batons) of different sizes.
- ***Heinrich Waldeyer*** (Germany)
  Observed colored bodies in stained dividing cells. He coined the term *chromosome* (from the Greek words *chroma* = color, *soma* = body).

Prelab Questions

1. What were some reasons that the late 19th century was a time of many discoveries about cells?

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2. Write a sentence in your own words describing what you think was happening in the cells the 19th-century biologists were observing.

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Materials

- onion with actively growing root tips
- single-edge razor blade
- forceps
- top or bottom half of a petri dish
- test tube
- stirring rod
- transfer pipette
- warm water bath
- 2 microscope slides
- 4 plastic cover slips
- microscope
- aceto-orcein stain
- acid fixative solution

Procedure

Part A: Using Acid Fixative to Stop Cell Division

1. Use the razor blade to cut four root tips, each about 2 cm long, off an onion bulb. **CAUTION:** *Handle the razor blade carefully to avoid injury*.
2. Place the four root tips in the bottom of a test tube. You may need to use a stirring rod to gently push them to the bottom.
3. Add just enough fixative solution to the test tube to cover the root tips. **CAUTION:** *Put on safety goggles, aprons, and gloves before handling the fixative solution. This solution is very acidic. Avoid getting any on your skin or clothing. Use forceps to handle the "fixed" root tips at all times from now on.*
4. To help the fixative solution penetrate the cells, place your test tube in a test tube rack in a warm water bath at 50°C.
5. After 6 min, use a hot mitt to take your test tube out of the water bath. Carefully pour the fixative and the root tips into the petri dish.
6. Place two clean microscope slides side by side on a clean paper towel. Very gently pick up the root tips with forceps and place two on each slide.
7. Using the razor blade, cut off the upper part of each root so that only about 3–4 mm of the tip end is left. **CAUTION:** *Handle the razor blade carefully to avoid injury.* Using your forceps, pick up the upper part of the root tip that you have cut off and place it on the paper towel for disposal.

Part B: Staining the Cell Nuclei and Chromosomes

1. Add a drop of aceto-orcein stain to cover each root tip. Wait 2 min to let the stain soak into the root tip cells.
2. With the flat side of your forceps, squish each root tip flat, taking care to press straight down. Repeat this step with the other slide.
3. Let the stain soak into the flattened root tips for another 2 min.
4. Cover the flattened root tips with cover slips (2 per slide). Press gently down on the outside of the cover slip with the flat side of your forceps to squish the root tip completely flat (so that you will have one layer of cells). Be careful not to break the cover slip.

Part C: Making Observations of Dividing Cells

1. Look at one of your slides through the microscope. Locate and focus on the root tip cells under low power (40×) and medium power (100×), then switch to high power (400×) to see the cells and nuclei more closely. There is no need to clip down the slide. Scan both sections of the slide to make your observations.
2. Find a cell that is typical of the cells you have observed and make a detailed sketch of it on a separate piece of paper or in your notebook. Include as much detail as possible.
3. Now scan the slide for a cell that looks especially different from a typical cell. Search for those that have any differences in their nuclei. Look for any rodlike "colored bodies" like the ones that Heinrich Waldeyer observed. Draw sketches of these unusual cells on a separate piece of paper.
4. If you find a cell with something intriguing happening in the nucleus, share it with the classmates around you. Your teacher may ask you to redraw your sketch on the board.

Analysis and Conclusions

1. Your teacher will lead you in a discussion about the possible significance of what you and your classmates have observed about the cells and nuclei of dividing cells. Use the information from your observations and your classmates' observations to place the cell drawings your class has made in a logical sequence. Describe or sketch the sequence in the space below.

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2. Answer the questions below to communicate your discoveries about cell division.

a. Below, make your own sketch of a cell beginning to divide. Then, write a description of the main event or events that take place in your sketch.

b. Below, make your own sketch of a cell in the middle of cell division. Then, write a description of the main event or events that take place in your sketch.

c. Below, make your own sketch of a cell toward the end of cell division. Then, write a description of the main event or events that take place in your sketch.

3. Examine your sketches and descriptions. Which stage of mitosis do you think is happening in each sketch? Label your sketches appropriately.

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Extension

You can examine the effects of various substances on onion root tips. Sprout more onion root tips in plain water and some in water containing acetaminophen or aspirin. After 2–4 days, compare the onion root tips. Write a hypothesis that suggests an explanation for your observation. (**NOTE:** *Check with your teacher before carrying out any experiments.*)