Lab 4

Pre-AP

# How does the environment affect mitosis?

Mitosis is the division of the nucleus of eukaryotic cells followed by the division of the cytoplasm (cytokinesis). If the division proceeds correctly, it produces two cells that are genetically identical to the original cell. Mitosis is responsible for the growth of an organism from a fertilized egg to its final size and is necessary for the repair and replacement of tissue. Anything that influences mitosis can impact the genetic continuity of cells and the health of organisms.

How do environmental factors affect the rate and quality of mitotic division? Scientists are perhaps most keenly interested in this question from the perspective of disease, specifically, the uncontrolled division of cells known as cancer. This investigation will allow you to make a simplified study of the relationship between the environment and mitosis.

## Objectives

In this investigation, you will

- prepare squashes of onion root tips to observe mitosis.
- make a hypothesis to describe the effect of caffeine on mitosis.
- compare growth of onion roots in water and in caffeine.

## Materials

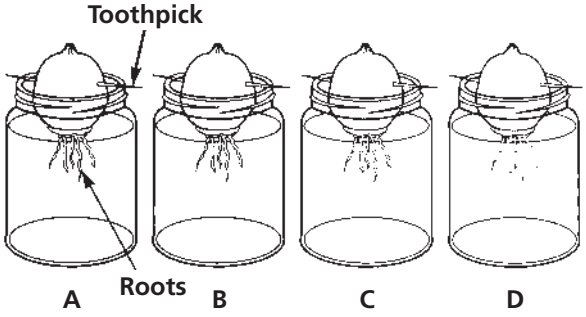
onion bulbs (4)
toothpicks (16)
150-mL glass jars (4)
concentrations of caffeine (coffee): 0.1%, 0.3%, 0.5%
metric ruler
wax pencil
scalpel
paper towels
distilled water
microscope slides (4)
coverslips (4)
Feulgen stain
methanol-acetic acid fixative
3% hydrochloric acid
45% acetic acid in a dropper bottle
forceps
microscope
25-mL graduated cylinders (2)
test tubes (8)
test-tube holder
test-tube rack
thermometer
hot plate
water bath
clock or watch

## Procedure

### Part A: Comparing Rates of Growth

1. Put on a laboratory apron and goggles. Label the small glass jars A, B, C, and D.
2. Insert a toothpick into opposite sides of each onion bulb so that each bulb can be balanced over the mouth of a jar, as shown in Figure 1. Then pour water into each jar until just the root area of the bulb is immersed. Wash your hands thoroughly.
3. Examine the bulbs each day. In Table 1, record the number of roots that emerge from each bulb and the average of their lengths.

**Figure 1**



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**PROCEDURE** continued

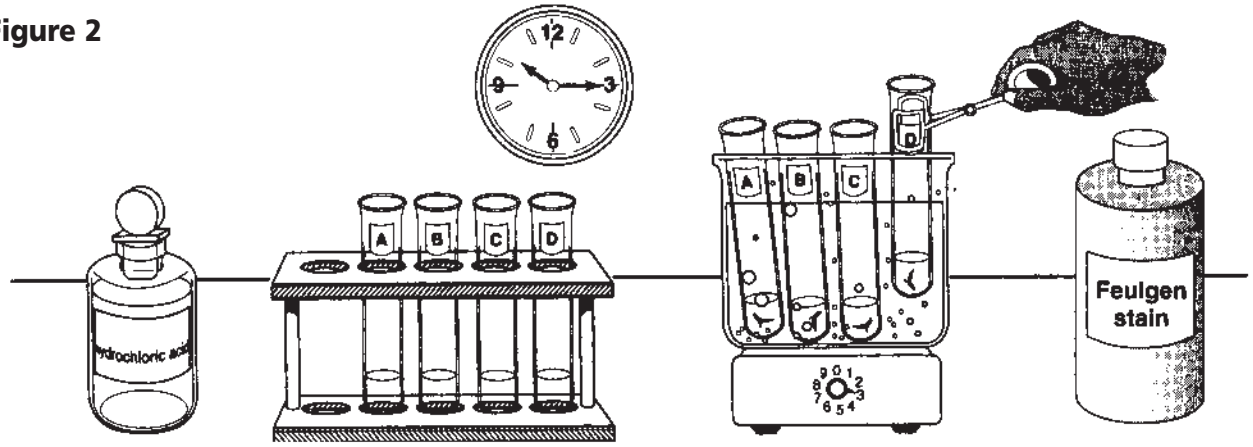
4. When the roots have grown to 1 cm in length, pour the water out of jars B, C, and D. Your teacher will provide you with caffeine solutions of three different concentrations. Fill jar B with the 0.1% solution, jar C with the 0.3% solution, and jar D with the 0.5% solution. Once again, balance the bulbs over the mouth of jars B, C, and D so that the roots are immersed.
5. Measure the roots for 3 more days, each time recording the average length of the roots for each of the treatments (that is, water and the three concentrations of caffeine) in Table 2.

## Part B: Comparing Phases of Mitosis

Note: READ ALL STEPS BEFORE YOU START.

1. Label 4 test tubes A, B, C, and D to correspond to the treatments to which the onion bulbs are being subjected. Then pour 5 mL of methanol-acetic acid fixative into each of the tubes.
2. Set up and begin heating the water bath to 60°C.
3. Use the scalpel to remove all of the roots from each of the onion bulbs. **CAUTION:** ***Use the scalpel with care. Cut away from your fingers.*** Then use the scalpel to cut a 3 mm piece from the *tips* of each root. Immediately place these tips from onion bulbs treated in A, B, C, and D jars into the corresponding test tubes containing the methanol-acetic acid fixative.
4. Use the test-tube holder to place test tubes A–D into the water bath at 60°C for 15 minutes.
5. Carefully pour the fixative from each tube into a labeled container to be disposed of by the teacher. Transfer the root tips from each tube to four new test tubes labeled A–D.
6. Pour 5 mL of 3% hydrochloric acid into each of the new test tubes in order to prepare the DNA for staining. **CAUTION:** ***Hydrochloric acid is a strong acid and causes burns. Avoid contact with skin or eyes. Flush with water immediately if contact occurs and call the teacher.*** Place the test tubes into the water bath at 60°C for 10 minutes.
7. Carefully pour the acid into a labeled empty beaker that the teacher has set aside for the acid. Add enough drops of Feulgen stain into each test tube to cover the roots. **CAUTION:** ***The stain can discolor your clothes and skin. Use it with care.*** Let the tissues sit in the stain for 15 minutes.
8. From tube A, remove one root tip with a pair of forceps. Place the root tip in the center of a labeled slide. Add one or two drops of acetic acid. **CAUTION:** ***If acetic acid is spilled, flush with water immediately and call the teacher.*** Then place a coverslip over the specimen.
9. Place the slide on a paper towel cushion and cover the slide and coverslip with a piece of paper towel. Push down onto the coverslip with the eraser of a pencil. This is called a squash. *Do not press too hard or you will break the coverslip.*
10. Repeat steps 8 and 9 for treatments B, C, and D.
11. Make a hypothesis to describe the effect of caffeine on the stages of mitotic division. Write your hypothesis in the space provided under Data and Analysis.

**Figure 2**



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# How does the environment affect mitosis?

## PROCEDURE continued

12. Look at your slides under the microscope at low and high powers for cells undergoing mitosis. The cells will not be as neatly arranged as they would be on prepared slides. Examine the size, shape, and position of chromosomes in each treatment in order to help you identify phases of mitosis. In comparing treatments, do you notice differences in the number of cells in each phase? In the stronger caffeine solutions, do the chromosomes in any particular phase seem especially distinct? Count and record in Table 3 the number of cells in each phase of mitosis.
13. On a sheet of paper, sketch the stages of mitosis observed from roots in each treatment.

## DATA AND ANALYSIS

**Table 1**

| Number of Roots and Average Length in Water | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bulb A | | Bulb B | | Bulb C | | Bulb D | |
| Day | Number | Avg. Length | Number | Avg. Length | Number | Avg. Length | Number | Avg. Length |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |

**Table 2**

| Number of Roots and Average Length | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Bulb A (water) | | Bulb B (0.1%) | | Bulb C (0.3%) | | Bulb D (0.5%) | |
| Day | Number | Avg. Length | Number | Avg. Length | Number | Avg. Length | Number | Avg. Length |
| 1 | | | | | | | | |
| 2 | | | | | | | | |
| 3 | | | | | | | | |

**Table 3**

| Number of Mitotic Phases in Each Treatment | | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Interphase | Prophase | Metaphase | Anaphase | Telophase | Cytokinesis |
| Bulb A | | | | | | |
| Bulb B | | | | | | |
| Bulb C | | | | | | |
| Bulb D | | | | | | |

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# How does the environment affect mitosis?

## DATA AND ANALYSIS continued

**1.** Write your hypothesis.

**2.** Identify the control and variable for the experiment.

**3.** Study Tables 1 and 2. Compare the rate of growth of the roots immersed in water with the rate of root growth in the various concentrations of caffeine.

**4.** Describe any differences in the number of cells in each mitotic phase among the four squashes.

**5.** How do your observations about mitotic phases in Part B relate to your observations about rate of root growth in Part A?

**6.** What are some conditions or factors in the environment that might have an effect upon the rate or quality of mitotic division?

**7.** Was your hypothesis supported by your data? Why or why not?