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Regeneration: Animal Growth and Development

Overview: In this laboratory, you will use planaria to study animal regeneration, as well as animal growth and development. You will learn how to collect and culture planaria from different environments to study how environmental conditions can affect regeneration and growth and development.

Objectives: Before doing this laboratory you should understand:

- what stem cells are and how they are used in both animal regeneration, and growth and development
- how different environmental conditions can enhance or reduce animal growth and development

After doing this laboratory you should be able to:

- catch and culture planaria
- how to cut planaria to trigger the regeneration processes
- how specific environmental conditions can affect cell growth and specialization

Introduction: Throughout the animal kingdom there are organisms that demonstrate the ability to regenerate lost body parts. This process involves many key steps similar to the stages of that animal's development. One of the most important of these steps is the division and differentiation of stem cells into specialized body parts.

One of the most extensive examples of animal regeneration can be found in planaria, small free living flatworms with incredible regenerative abilities. Planaria are capable of regenerating any body part that is damaged or lost due to injury (except the tip of the head), including the head and central nervous system. This regenerative ability is due to the presence of neoblasts, adult stem cells that can give rise to any type of cell. Neoblasts can include as much as 30% of all cells in the worm's body. Neoblasts are adult stem cells that share certain characteristics with embryonic stem cells and can differentiate into essentially all cells found in adult animals. A similar process occurs in adults where the neoblasts continually replace old cells. Additionally, if nutrition is limited, planarians can exhibit "de-growth" – eliminating cells by "down sizing" to redistribute its reduced resources.

When a wound is inflicted on a planaria, neoblasts at and around the wound site begin to divide rapidly, forming a blastema. The blastema is characterized as a colorless area at the tip of the growing, regenerating tissue. The blastema consists mainly of rapidly dividing neoblasts that have yet to differentiate into other cells.

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Part 1: Collection

In this section, you will collect wild planaria from the environment.

Option A: Baiting

Materials:

- a bucket, beaker, or plastic container for collecting samples
- bait: a small piece of chicken or beef liver or any other available pieces of raw meat
- cheese cloth or a worn out nylon sock
- string
- microscope

Procedure:

1. Cut the bait into relatively small pieces and wrap tightly in the cheese cloth (or other material used for a bag).
2. Tie a piece of string tightly around the bag
3. Place the bag in a stream, pond, or other local source of fresh water for 20 minutes
make sure to tie the string off so the bag won't float away
4. Scoop bait and surrounding water into the container
it is very important to collect the water around the bait
5. Examine samples under a microscope to determine the presence of planaria.

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Option B: Wading and Collecting

Materials:

- a bucket or other container
- a net
- wading boots
- microscope

Procedure:

1. Wearing boots, wade along the shore of a stream river or pond and with the net disturb the edge of the water
you can also just walk along the edge of the water
2. Collect the disturbed soil in the net and place in bucket
samples can be obtained either by transferring water in a pipette, or using the baiting technique in the bucket and taking samples from around and on the bait
3. Check for planaria with the microscope by examining samples from the bucket
planaria are small, brown flat worms with distinct arrowhead shaped heads and eyespots

Discussion:

1. What is the purpose of collecting the water around the bait instead of just the bait?

2. Aside from holding the bait, describe another purpose for the cheese cloth bag.

3. Planaria are photonegative. What does this mean and how does it affect where you can find them?

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Part 2: Culturing

In this section, you will learn how to culture planaria in a suitable environment.

Materials

- 1 small petri dish
- planaria
- water from source, or bottled water, NOT tap water
- small pieces of raw liver

Procedure

1. Prepare the small Petri dishes by pouring water either from the source or a water bottle into the dish.
2. Add planaria
MAKE SURE THE PETRI DISH IS TAPED BETWEEN HANDLINGS
3. Feed planaria about once a week with a small piece of liver (size of an eraser head)
it's a good idea to use the same food you used as bait

Discussion:

1. Why is it not a good idea to use water from a tap when culturing planaria?

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Part 3: Amputation and Regeneration

In this section you will amputate the planaria and observe their rate of regeneration under control conditions, as well as variations in temperature and pH and in the presence of certain chemicals.

Materials

- 6 small Petri dishes
- planaria
- water from planaria source, or bottle, NOT tap water
- cooler or refrigerator
- incubator
- acid solution (pH = 5)
- alkaline solution (pH = 9)
- caffeine water (crush caffeine tablet)
- razor blade/scalpel
- microscope

Procedure

1. Prepare the 6 small Petri dishes by adding 3-4ml of either bottled water or water from the planaria source and labeling them for “ideal,” “acid pH,” “alkaline pH,” “hot,” “cold,” “caffeine.”
2. Add 1-2ml of the acid solution to the “acid pH” plate and the alkaline solution to the “alkaline pH” plate.
3. Divide the planaria evenly into 6 groups and amputate them across the midsection by cutting them with the razor blade under a microscope.
4. Place the amputated planaria into each of the test plates.
5. Place the “cold” plate in the cooler and the “hot” plate in the incubator.
6. Check planaria daily for 15 days. Measure their length daily, but do not feed or remove the planaria from the test plates.

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7. At the end of the experiment, graph the average size of the planaria over time for each environmental condition on the same graph.

Discussion:

1. How did the results of each test plate differ from the ideal conditions?

2. What are the effects of temperature on planaria regeneration?

3. What are the effects of pH on planaria regeneration?

4. Caffeine usually acts as a stimulant. What is its effect of regeneration in planaria?

5. How do different environmental conditions affect regeneration in planaria and how can we benefit from this knowledge?

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Regeneration: Animal Growth and Development – Teacher Guidelines

Preparation:

1. If students are not collecting planaria in the wild, order them prior to beginning the lab.
2. If students are collecting planaria in the wild, provide them with a uniform bait to use so they are less likely to catch different species of flatworms.
3. During the regeneration section, the cooler, or refrigerator should be kept at approximately 4⁰C and the incubator at 30⁰C. Make sure they are at, or close to these temperatures before starting this section (ice should not be in direct contact with the Petri plates).
4. Prepare the acid and alkaline solutions before starting Part 3 by mixing 50% vinegar and 50% bottled water. For the alkaline solution, use ammonia (1-1 ratio)
5. Prepare the caffeine water by dissolving a caffeine tablet in approximately 150ml of bottled water.

Time Table:

Before starting Part 3, make sure the cooler and incubator are at the right temperatures. All other test plates should be kept at room temperature.

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Materials: For 10 Groups of 2

Part 1: Collection

- 10 small buckets or other plastic containers
- a medium sized piece of raw liver (bait)
- a roll of cheese cloth
- a spool of string
- 10 transfer pipettes
- 10 microscopes (1 per group)
- 10 pairs of wading boots (option B only if students are wading through the water)
- 10 small nets (option B)

Part 2: Culturing

- 10 large Petri dishes
- planaria
- 10 transfer pipettes
- 1 bottle of bottled water should be sufficient if that is being used
- use any left over liver from Part 1 to feed the planaria (they only need to be fed about once a week, leave liver in the Petri dish for a 20-30 minute feeding. Remove any uneaten liver, and clean excess using cotton swabs)

Part 3: Amputation and Regeneration

- 60 test plates
- planaria from Part 2
- 240 transfer pipettes (use different pipettes for acid, alkaline, and caffeine solutions)
- 240ml water: either bottled or from planaria source
- 120ml acid solution
- 120ml alkaline solution
- 120ml caffeine water
- 20 razor blades
- 10 microscopes (1 per group)
- 1 cooler(with ice and container for test plates) or 1 refrigerator
- 1 incubator

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Expected Results

Ideal Conditions: 6-10 days, full regeneration

Heat: 15 days, partial regeneration

Cold: 15 days, partial regeneration

Acid: 15 days, partial regeneration

Alkaline: 15 days, partial regeneration

Caffeine: 15 days, partial regeneration

Answers to Discussion Questions:

Part 1: Collection

1. What is the purpose of collecting the water around the bait instead of just the bait?
Not all the planaria will be trapped or eating the bait. Collecting the water around the bait will increase the chance of catching planaria that were attracted to it.
2. Aside from holding the bait, describe another purpose for the cheese cloth bag.
To trap planaria that enter it to eat the bait and make it harder for them to escape.
3. Planaria are photonegative. What does this mean and how does it affect where you can find them?
Animals that display photonegativity tend to avoid light, preferring to live in darker areas. This can affect looking for planaria during the day because they will usually be buried in mud and unwilling to come out, even if bait is placed near them. Therefore it is important to disturb the soil at the bottom in order to release the planaria hiding there.

Part 2: Culturing

1. Why is it not a good idea to use water from a tap when culturing planaria?
Tap water can contain minerals or chemicals that are harmful to planaria and can either alter the results or kill the planaria altogether.

Part 3: Amputation and Regeneration

1. How did the results of each test plate differ from the ideal conditions?
In each test plate, the planaria did not fully regenerate over a period of 15 days, while under ideal conditions it only took 6-10 days.

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2. What are the effects of temperature on planaria regeneration?
If an environment is too hot or cold, it slows down the rate of regeneration in planaria.
3. What are the effects of pH on planaria regeneration?
If an environment is too acidic or alkaline, it slows down the rate of regeneration in planaria, and can affect the regeneration of certain structures.
4. Caffeine usually acts as a stimulant. What is its effect of regeneration in planaria?
In planaria, caffeine slows down the rate of regeneration and can cause damage to the planaria because it stimulates other body functions that take energy and nutrients away from regeneration.
5. How do different environmental conditions affect regeneration in planaria and how can we benefit from this knowledge?
Many environmental conditions can slow down or alter regeneration in planaria. Some can cause damage during the regeneration process as some parts fail to form properly. This can show the effects of many environmental conditions and drugs on both regeneration and healing, as well as growth and development. It can also help us further understand the delicate nature of these processes and develop new techniques to help them in animals and humans.

Tips and Suggestions:

1. These techniques for catching planaria do not always work. Use other techniques if they seem more effective.
2. Demonstrate how to amputate the planaria prior to having the students do it.
3. When culturing planaria, add small, gradual amounts of bottled water over time in order to ease the transfer and prevent shocking them. Allow a few days before running the experiment to allow the planaria to adapt to their new environment.

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