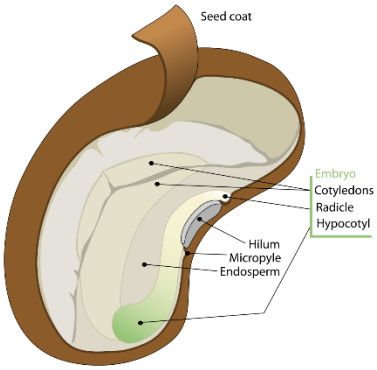
Cellular Respiration: Pea Histology and Cellular Respiration

This activity uses germinating pea seeds. Seeds are the 'offspring' of plants. While to most of us they look relatively inert or dead, seeds are actually viable organisms. Because seeds are generally NOT photosynthetic they must acquire the energy needed for growth and development by other means. Under less than favorable conditions, they at a very low rate, but they do metabolize including generating ATP via aerobic cellular respiration. Once the seed is hydrated (imbibition), germination begins. Germination includes the production and activity of many enzymes. This is a period of astounding growth for the newly developing plant.



The image to the left is a typical dicot seed. Recall the dicotyledons are a group within the flowering plants. Their seeds include 2 seed leaves or cotyledons. Peas and beans are both dicots. The seed is surrounded by a tough protective covering called the seed coat. The hilum marks the location where the seed was attached to the parent plant. The micropyle is a small pore-opening through which the pollen (plant male sex cells) passed during the fertilization of the ova, which led to the formation of the seed. The endosperm surrounds the embryo. It is produced after fertilization; starch, oils and proteins are stored in the endosperm to nourish the embryo. The embryo consists of the cotyledons, radicle and hypocotyl. The cotyledons are the seed leaves, the first leaves to appear above ground. The radicle is the fastest growing part of the embryo and will become the embryonic root. The hypocotyl becomes the stem below the seed leaves.

Just as reminder, the summary reaction for aerobic cellular respiration is shown below.

Summary Reaction for Aerobic Cellular Respiration

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + ATP$$

Glucose **Oxygen** **Carbon dioxide** **Water** **Adenosine triphosphate**

Aerobic cellular respiration is a multi-step, energy-yielding process that occurs in the cytoplasm and mitochondrion of all eukaryotic organisms, including plants. During first steps (glycolysis) of the process glucose is 'broken' in half. Enzymes in the cytoplasm perform this initial split of glucose (food/carbohydrate). Enzymes located in the mitochondrion complete the breakdown. The complete breakdown of glucose yields carbon dioxide, water, and ATP, the universal energy currency.

In this activity you will perform a functional histology (study of tissues) experiment using pea seeds that are un-hydrated and some that have been hydrated and allowed to germinate for 24 or 48 hours. Keep in mind that although pea seeds are shaped differently than bean seeds they both possess similar internal and external structures. You will be testing slices of peas for the presence of mitochondrial activity and the presence of starch at various points in development. Iodine will be used to indicate the presence of starch. 2,3,5 Triphenyl tetrazolium chloride is one member of a family of molecules called tetrazolium. Tetrazolium compounds when hydrated are colorless. If they become reduced by acquiring

hydrogen ions and electrons, they turn colors. 2,3,5 Triphenyl tetrazolium chloride when reduced turns a red color, strawberry red. So where can tetrazolium acquire hydrogen ions and electrons? Tetrazolium intercepts hydrogen ions and electrons that are stripped from glucose during aerobic cellular respiration. Hydrogen ions and electrons removed during the Kreb's cycle would normally be carried by carrier molecules such as NAD^+ or FADH, but when tetrazolium is present it intercepts these ions and electrons. A red color in a tissue when exposed to tetrazolium is indirect evidence of working mitochondria. Why do we consider this indirect evidence and not direct evidence?

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Materials

Petri dish or culture dish – 6 small (35 mm)
Pipette – Pasteur - 2
Marker
Tin foil
Beaker – 50 mL, 1
Scalpel or razor blade
2,3,5 Triphenyl Tetrazolium chloride (1.5 %)
Pea seeds* – un-germinated
Pea seeds* – 24 hour germination
Pea seeds* – 48 hour germination
Iodine
Pipette
Incubator (optional)

*other seeds can be substituted for peas. Alternatively, half the class can be asked to do the activity with pea, the other half could use another bean.

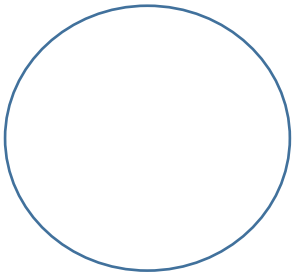
Procedure

1. At the supply table pick up 8 petri dishes. Label the Petri dishes as follows: Un-germinated IKI, Un-germinated Tetrazolium, 24 hour IKI, 24 hour Tetrazolium, 48 hour IKI, 48 hour Tetrazolium.

2. Place 4 un-germinated peas into the Petri dish labeled un-germinated IKI. Place another 4 peas into the Petri dish labeled un-germinated Tetrazolium. Repeat this procedure for the 24-hour and 48-hour peas. Return to your bench.

3. Remove the seed coat from each pea. Squeeze the pea gently and the seed coat should tear which will allow you to remove it. The seed coat on the un-germinated peas may be dry and difficult to remove. You may be able to remove it when the peas are cut.

4. Use the razor blade to slice each pea in half between the cotyledons where the pea naturally is split. Try to make an even slice through the radicle. Make sure after you cut the pea you return it to the correct Petri dish. Repeat this procedure for the un-germinated, 24 hour germinated and 48 germinated seeds.



Unstained pea

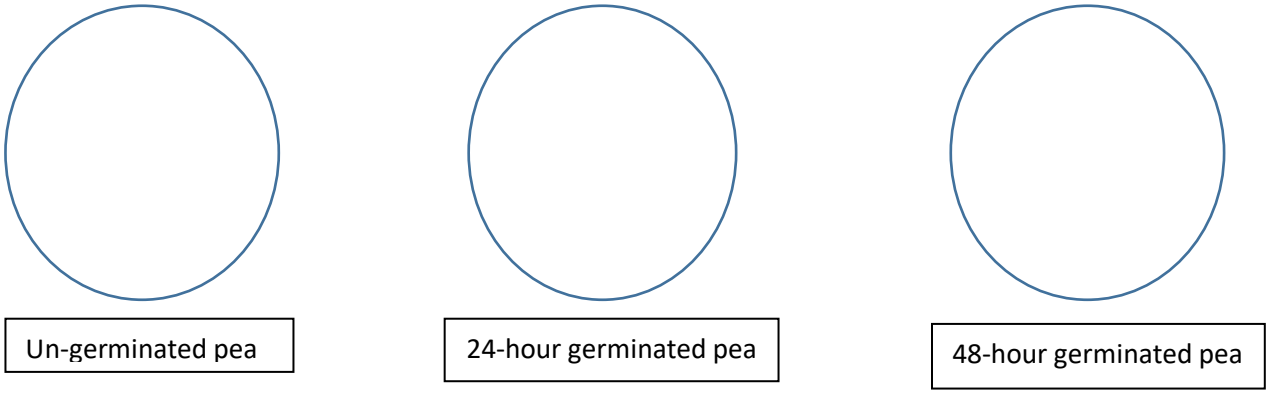
Draw a typical pea hemisphere in the circle to the right. Label the seed parts.

5. Add iodine (IKI) to the Petri dishes labeled IKI. The iodine must cover the peas. Allow the peas to incubate in IKI for 10 minutes.

6. Add tetrazolium to the Petri dishes labeled tetrazolium. Tetrazolium breaks down very quickly, therefore your instructor may not make the solution available until everyone is ready to use it. Carefully cover these plates with aluminum foil. If a 37^{o} incubator is available place your Petri dish in the incubator. Make sure you label the aluminum with your name or group identifier. Allow the tetrazolium-treated peas to incubate 45-60 minutes.

7. After 10 minutes, pour off the IKI as directed by your instructor. Rinse the peas with tap water.

Draw a representative pea (or insert photograph) from the un-germinated, 24-hour and 48-hour Petri dishes. Label the parts of each seed.



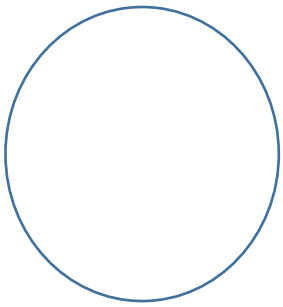
Iodine is used as indicator for what macromolecule?

What areas are stained in each specimen?

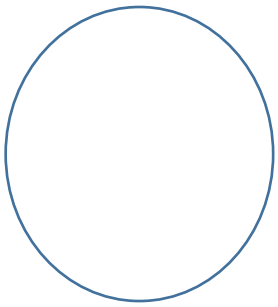
Are there differences in the regions staining with iodine at various time points?

8. After 45- 60 minutes remove the tetrazolium Petri dishes from the incubator. Remove the aluminum foil. Use a pipette to remove the tetrazolium. Your instructor will provide directions on discarding the tetrazolium solution.

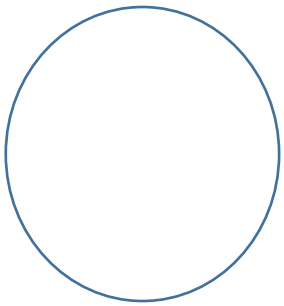
Draw a representative pea (or insert photograph) from the un-germinated, 24-hour and 48-hour Petri dishes. Label the parts of each seed.



Un-germinated pea



24-hour germinated pea



48-hour germinated pea

Tetrazolium is used as indicator for what activity?

What areas are stained in each specimen?

Are there differences in the regions staining with tetrazolium at various time points?

From your observations, in which specimen were the mitochondria most widespread or most active?