Plant Physiology – BIOL327 name _____

Acid Growth Hypothesis

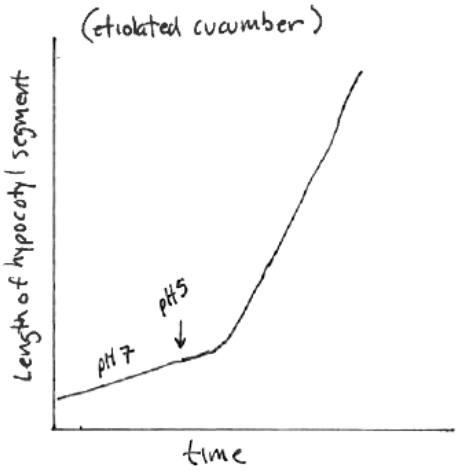
The cell wall must be strong to withstand the relatively large pressures that can develop. Yet, at the same time, the wall must be capable of growth. This presents an interesting paradox - how is the wall able to be strong, yet allow for cell growth and elongation. One answer is that the wall is "loosened" or undergoes "stress relaxation" to permit wall components to slide past one another. The following experiments are designed to test the hypothesis that wall loosening is moderated by acid (acid growth hypothesis).

Question: Does acid treatment loosen cell walls?

Prediction #1: IF cell walls can be loosened by acid, THEN treating stem or hypocotyl sections with acid buffers should stimulate elongation. The graph below plots the length of etiolated cucumber hypocotyl sections versus time. Initially the pH is 7 and at the arrow is changed to pH 5. Answer the following questions:

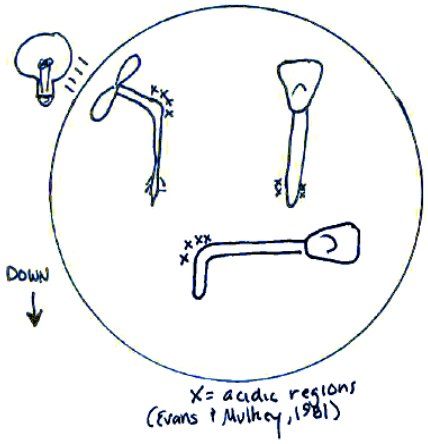
Hypothesis #1: Cell walls are loosened by acid.

1. What is a hypocotyl?
2. What does etiolated mean?
3. Suggest a reason why etiolated hypocotyls were used in this experiment.
4. True or False: Hypocotyls elongate faster at pH 7 than at pH 5
5. True or False: Cell elongation is associated with acidic pH
6. What do you conclude about the hypothesis?



Prediction #2: IF cell walls are loosened by acid, THEN regions of cell growth/elongation should be associated with acid production. The diagram below shows maize and sunflower seedlings mounted in a petri dish containing agar. A pH indicator has been incorporated into the agar. Acidic regions are indicated by an "X".

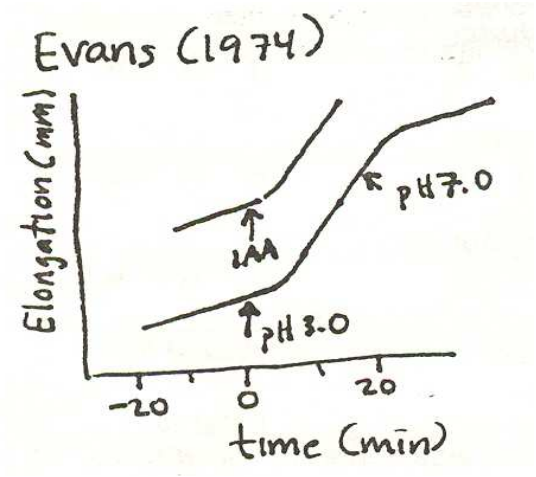
1. Why does the agar become acidified ***near*** the tip of a vertically-mounted maize seedling?
2. Why is the acidified region of the vertically-mounted seedling not at the very tip, but a few millimeters behind the tip?
3. Why is the agar acidified only on the side of the sunflower seedling away from the light?
4. Why is the agar acidified only on the upper side of the root of the horizontally-mounted maize seedling?
5. What do you conclude about the hypothesis?



Hypothesis #2: Auxin, a plant growth hormone, is associated with cell elongation and wall acidification.

Prediction: IF protons stimulate elongation and wall acidification, THEN treating seedlings with auxin should stimulate elongation in a manner similar to acids. The graph below shows the length of a stem section in various pH buffers, before and after treatment with auxin. The initial pH in both experiments is pH 7. From these data we can conclude:

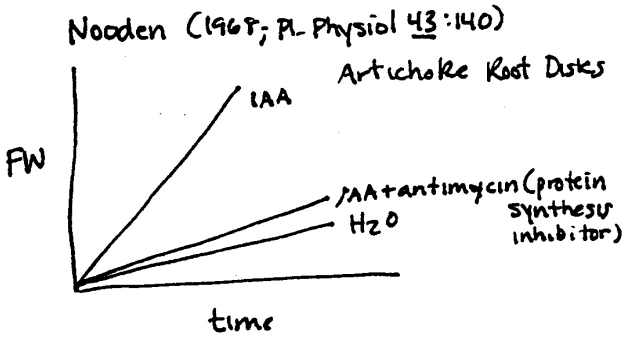
1. True or False: Acid stimulates cell elongation
2. True or False: The rate of elongation is slow but constant at pH 7.
3. True or False: The response to auxin is rapid.
4. True or False: Auxin stimulates the rate of elongation at pH 7.
5. True or False: The rate of auxin-stimulated elongation is equal to that of acid-stimulated elongation.
6. What do you conclude about your hypothesis?



Hypothesis #3: Auxin stimulates cell elongation, in part, by stimulating protein synthesis.

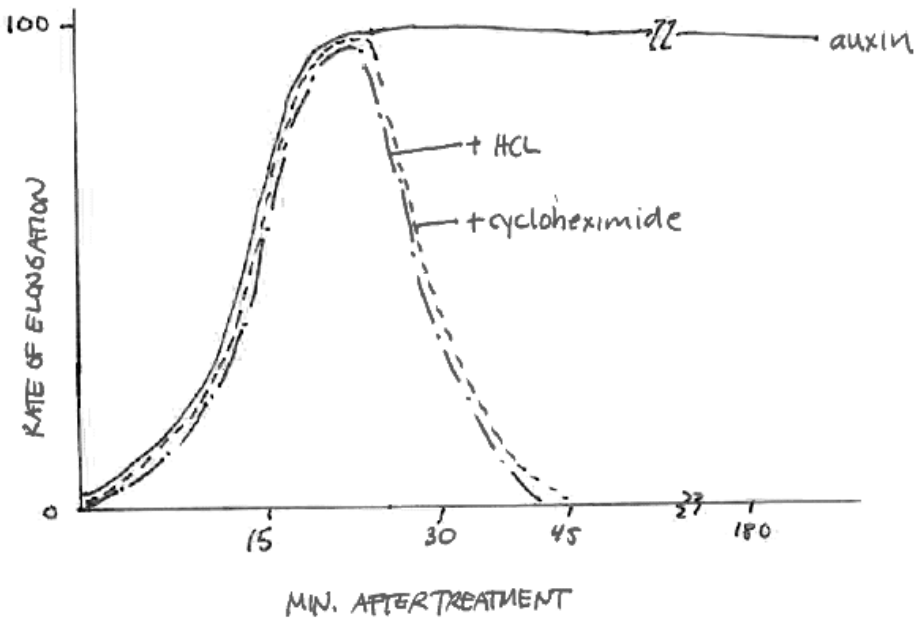
Prediction: IF auxin stimulates protein synthesis, THEN treating cells with antimycin, an inhibitor of eukaryotic protein synthesis, will block its action. The graph below shows the fresh weight increase in artichoke root disks after treatment with water, water + auxin, or water + auxin + antimycin.

1. True or False: Auxin stimulates root disk growth
2. True or False: The effect of auxin is abolished by a protein synthesis inhibitor
3. True or False: Protein synthesis is required for growth of root disks



Hypothesis #4: Auxin has two roles in cell elongation: (a) Auxin has a short-term effect that involves activating a proton pump and (b) Auxin has a longer-term effect that is involved with stimulating protein synthesis (transcription and translation).

Prediction: IF auxin stimulates protein synthesis, THEN treating coleoptiles with a protein synthesis inhibitor should prevent expansion. IF auxin stimulates a proton pump directly, THEN this should not be affected by a protein synthesis inhibitor. The graph below shows the rate of coleoptile expansion after treatment with either: (a) auxin alone; (b) the same level of auxin plus cycloheximide (a known inhibitor of eukaryotic protein synthesis); or (c) acid (HCL).



1. What is a coleoptile?
2. Why are coleoptiles used in these types of experiments?
3. Which of the following plants would produce a coleoptile? sunflower, oat, maize, wheat, bean, barley, lettuce, RCBr, turnips.
4. What does cycloheximide do? Would cycloheximide be a good antibiotic to take if we get an infection?
5. Do you think antimycin could have been used in place of cycloheximide?
6. True or False: Auxin causes coleoptile elongation by a mechanism that involves acidification of the cell wall.
7. True or false: Dilute acid mimics the initial, but not the longer-term, effect of auxin on the coleoptile cells.
8. True or False: Auxin stimulates elongation by two different mechanisms, with an initial acid-induced passive phase followed by a longer-term response that requires protein synthesis.
9. True or False: The initial phase of auxin induced elongation can occur in the absence of protein synthesis
10. True or False: Auxin-induced acidification of the cell wall does not require protein synthesis.
11. What do you conclude about your hypothesis?
12. Write a summary that explains the mechanism by which cell walls are loosened. Be sure to include the impact of auxin, acid, proton pumps, protein synthesis, short-lived and longer-term effects of auxin.

Reference: This exercise is adapted, in part (esp. hypothesis #4), from an unpublished course handout from Dr. W Becker, U of Wisconsin.