Chapter 6

Invertase Activity in Root Growth

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Introduction

In this lab exercise, students study the interaction between structure and function in the corn root. In Part A, students observe a prepared slide of corn root (longitudinal section). Here, they observe the regions of cell division, elongation, and maturation of corn root tip and measure the length of five cortical cells. In Part B, students will determine the activity of invertase present in specific 2-mm sections taken from different regions along the corn root. This information is used to later determine how the enzyme activity per cell changes in relation to growth in the corn root.

The principle of this enzyme assay is quite simple and straight forward. Sucrose is hydrolysed by the extracellular enzyme, invertase, into glucose and fructose, which is then measured colorimetrically by the reaction of glucose with Somogyi-Nelson's reagents. Glucose and fructose are used by the dividing and elongating cells to supply energy and carbon skeletons for growth and development. For further reading see J. A. Hellebust and D. F. Forward, The invertase of the corn radicle and its activity in successive stages of growth, *Canadian Journal of Botany,* 40:113–126, 1962.

Notes for the Instructor

This exercise has been used successfully in a first-year biology course at the University of Toronto for several years; this course contained both students who had and did not have a prior background in biology. This exercise can be completed in a 3-hour laboratory period, so long as a sufficient number of spectrophotometers are available. The experimental protocol requires many critical steps where students can potentially make mistakes. Thus, at the end of the class, if not all groups were successful in obtaining results, the instructor can combine class data. Since students are often inexperienced in pipetting procedures, their errors may be reflected in inconsistencies in optical density readings for glucose standard solutions. Thus, pooling class data for glucose standards is recommended.

It is important to grow sufficient numbers of corn seedlings with radicles of the appropriate length. It is best to grow many more corn seedlings than are required, thus students will have a large selection of seedlings to choose from. Since we purchase corn seeds that have been treated with a fungicide, seeds are not soaked in water prior to planting. Seeds are planted in perlite in

plastic trays that have drainage holes in the bottom. They are grown at room temperature away from direct light and are watered on alternate days. After 3–4 days, seedlings with hypocotyl's between 15 and 30 mm in length are collected; seedling with hypocotyl's shorter than 15 mm remain growing for use by classes on subsequent days.

Materials

Four students work as a group and each group will require the following material:

Plant Material

Corn seedlings, 3- or 4-day-old (5)

>   Keep seedlings moist between wet paper towels. *Hypocotyl must be a maximum of 30 mm and minimum of 15 mm*. Select seedlings with straight roots.

Supplies

Vials with caps (6)
Test tubes, 10 ml (20)
Cuvette for spectrophotometer, 10 ml (1)
Pipets, 10 ml (2)
Syringe with needle, 1 ml (1)
Pipet, 1 ml (1)
Beaker, 250 ml (1)
Rack for vials, plastic (1)
Rack for test tubes, metal wire (1)
Ruler, 15 cm, plastic (1)
Ruler, 30 cm (1)
Razor blades, sharp, one-sided (2)

Scissors, fine (2)
Ocular micrometer (1)
Stage micrometer (1)
Cheese cloth to wipe off tubes (1)
Prepared slide of corn root tip (longitudinal section) (2)
Dissecting needles (2)
Marking pen for glass (1)
Spatula (1)
Propipetter (1)
Masking tape
Kimwipes

Solutions

Distilled water, in squirt bottle   (1)
Distilled water, 100 ml (1 flask)
Sucrose (2%) in MES buffer (20 mM) (1 flask)
 MES = 2-(n-morpholino)ethanesulfonic acid, MW = 195.24
Somogyi's Reagent, 30 ml (1 flask)

>   Dissolve 12 g of K-Na tartarate and 16 g of $NaHCO_3$ in 250 ml of H_2O with stirring (Solution A). Dissolve 4 g of $CuSO_4.5H_2O$ in 100 ml of H_2O (Solution B). Pour Solution B into A with constant stirring. Add 24 g of Na_2CO_3. Prepare Solution C by adding 18 g of Na_2SO_4 to 50 ml of *hot* H_2O; boil to expel air, cool. Add Solution C to A+B mix. Fill to 1 litre with H_2O. (Michael Somogyi, *Biological Chemistry*, 195:19, 1952)

Nelson's Reagent, 30 ml (1 flask)

>   Dissolve 25 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ in 450 ml of H_2O. Add 21 ml of concentrated H_2SO_4. Mix and add 3 of g sodium arsenate $(Na_2HAsO_4.7H_2O)$ dissolved in 25 ml of H_2O. Mix and store at 37°C. (Norton Nelson, *Biological Chemistry*, 153:375, 1944)

Glucose standards, each made up in 2% sucrose in 20 mM of MES buffer, pH 4.6 (20 μml^{-1}, 50 μg ml^{-1}, 100 μg ml^{-1}, 200 μg ml^{-1}) (1 set)

Student Outline

Part A:
The Root Tip: Location of Cell Division and Cell Expansion

Study a longitudinal section of the root tip of corn (*Zea mays*) in a prepared slide. Figure 6.1 illustrates the early stages in primary development in a generalized root tip. Locate the *apical meristem*, the embryonic region of the root, which is composed of relatively small cells (10–20 µm in diameter) and protected by the *root cap.* Cell division takes place a short distance beyond the apical meristem — this distance varies from species to species and within a species, depending on the age of the root. The apical meristem and nearby portion of the root in which cell division takes place are collectively referred to as the *region of cell division.* The *procambium* gives rise to the primary vascular tissues (xylem and phloem), the *ground meristem* to the cortex and pith, and the *protoderm* to the epidermis.
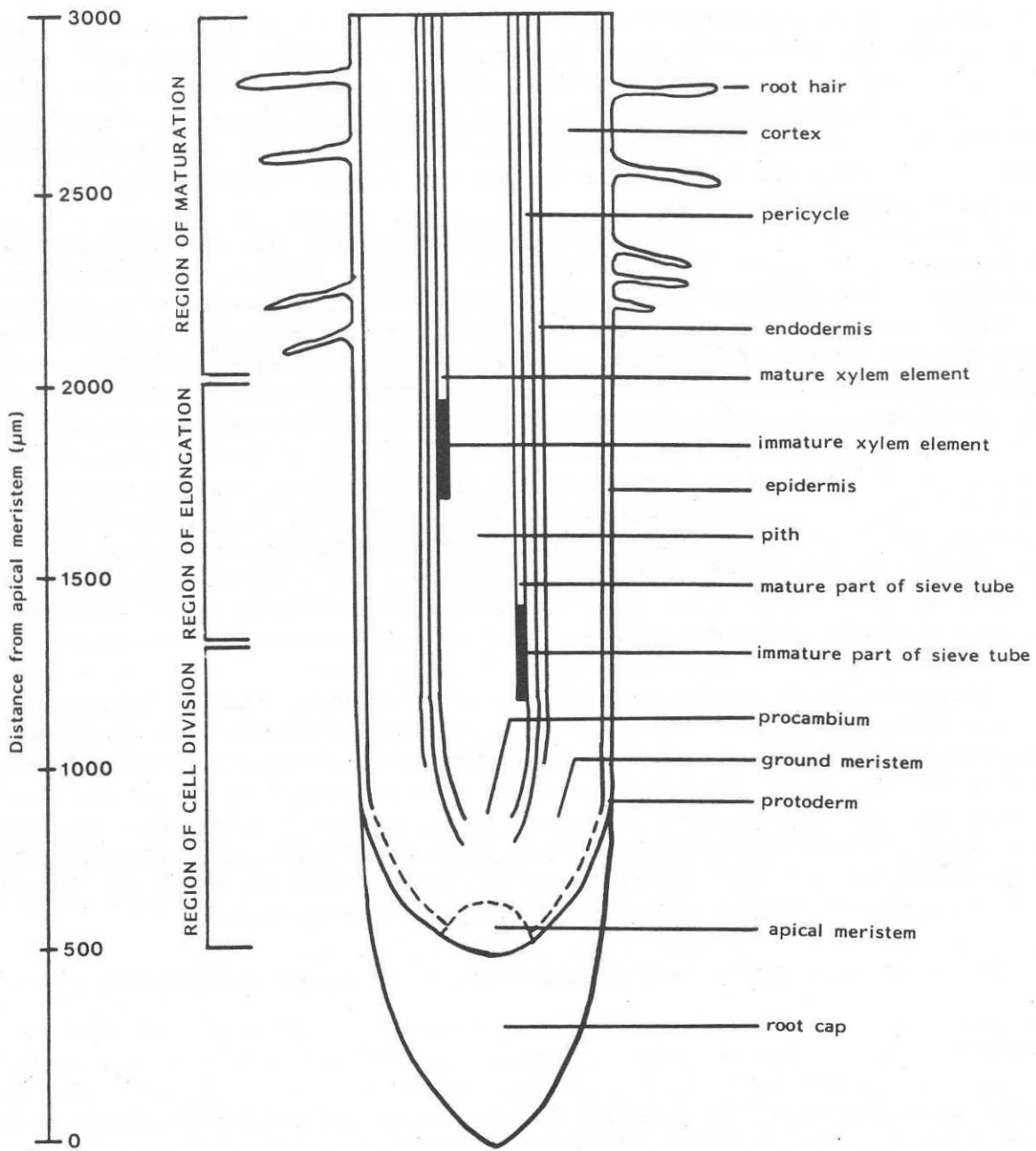
Beyond the region of cell division, but not sharply delimited from it, is the *region of cell elongation*. The elongation of cells in this region results in most of the increase in length of the root. Beyond this region the root does not increase in length. Note that as you proceed along the root away from the apical meristem the cells become larger.

The *region of maturation* is beyond the region of elongation and where most of the cells of the primary tissues mature. Root hairs are also produced in this region. It is important to note that the regions described above are not sharply delimited from one another — there is a gradual transition from one to another.

☐ Once you have examined the morphology of the prepared slide of the corn root-tip, you are to measure the length of five individual cells of the cortex at different distances behind the apical meristem using an ocular micrometer. Enter your results in Table 6.1. Note that the root tip in your prepared slide must not be less than 7 mm in length.

Table 6.1. Cell enlargement in root tip of *Zea mays*.

Distance from the apical meristem (µm)	Length of five cortical cells (µm)	Mean (µm)	SD
0-1000			
1000-2000			
2000-3000			
3000-4000			
4000-5000			
5000-6000			
6000-7000			

Figure 6.1. Longitudinal section illustrating early stages in primary development in a monocotyledon root tip.

Part B:
Activity of Invertase in Relation to
the Growth and Development of the Corn Radicle

Introduction

In the dark the corn radicle grows rapidly during the first few days of germination. This growth is entirely dependent on the stored food and minerals in the corn seed. Proteins, lipids, and K, Mg, and Ca phosphate salts are found in the living peripheral *aleurone layers* of the corn grain. Most of the energy and carbon for shoot and root growth comes from the breakdown of starch to glucose which occurs in the endosperm. The *scutellum* absorbs the glucose and converts it to sucrose. The sucrose is then translocated to cells in the root and stem through the developing phloem (vascular tissue that transports food).

Before sucrose can be taken up by the dividing and elongating cells of the developing shoot and root, it must be hydrolysed to glucose and fructose by invertase. Invertase is an extracelluar enzyme secreted into the cell walls where it is optimally active at a relatively low pH of approximately 4.6. The resulting monosaccharides, glucose and fructose, are transported through the cell membrane by specific transport systems. These monosaccharides are utilized for energy and carbon skeletons for the biosynthesis of cell components. In addition, they are osmotically active substances which contribute to the turgor pressure (hydrostatic pressure) in cells — this turgor is necessary for cell expansion. In addition, a naturally occurring plant auxin, 3-indoleacetic acid (IAA) which is also necessary for cell expansion, stimulates a proton pump, located in the cell membrane, to secrete hydrogen ions into the cell wall area — this results in acidification of the water-filled cell space to about pH 4 to 5. This lowered pH is optimal for (1) invertase activity, (2) uptake of glucose and fructose, (3) weakening of hydrogen-bonds between cell wall matrix substances (a three-dimensional complex of pectins, hemicelluloses, and a special cell-wall protein) and cellulose microfibrils, and (4) increasing activity of cell wall (hydrolytic) enzymes. All four of these processes are essential for the expansion of the cell wall in response to turgor pressure.

You will determine the activity of invertase present in specific 2-mm sections taken from different regions along the corn root. Combined with the information about the numbers of cells per section (Table 6.2), you will be able to determine how the enzyme activity per cell changes in relation to growth in the corn radicle. The fact that invertase is located outside the cell membrane, makes it possible to use small sections, rather than ground up (homogenized) sections, for determinations of enzyme activity. The principle of the enzyme assay is that the substrate sucrose, a non-reducing sugar, will be hydrolysed into the reducing sugars glucose and fructose, which can be measured colorimetrically by the reaction of glucose with Somogyi-Nelson's reagents.

☐ A partial schematic outline of the experimental procedure is given in Figure 6.2.

Procedure

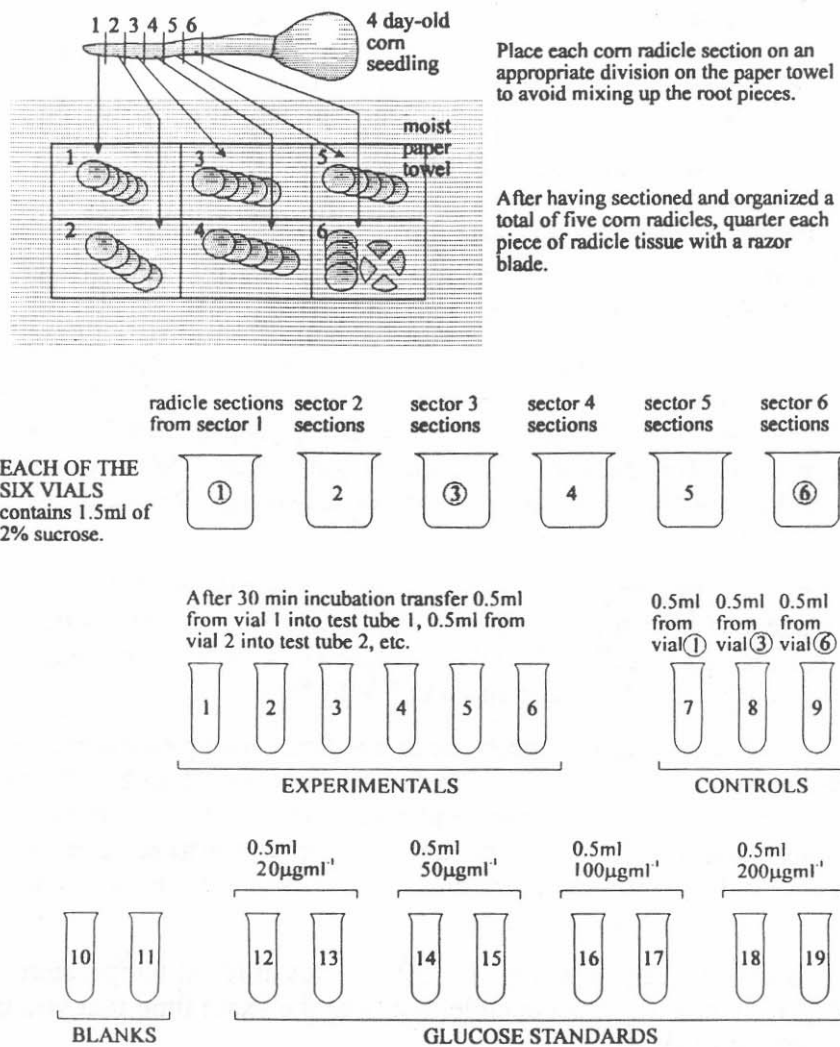1. Number six glass vials (1 to 6), and use a 10-ml pipet to transfer 1.5 ml of 2% sucrose solution (pH 4.6 and made in 20 mM of MES buffer) into each vial. Place vials in a plastic rack.

2. Place 19 20-ml test tubes (numbered 1 to 19) in a wire rack. With a 1-ml syringe transfer 0.5 ml of Somogyi's Reagent into each test tube. Rinse the syringe with water before using it again in upcoming steps.

Table 6.2. Cell number in 2-mm sections of *Zea mays* radicle.

Distance from radicle tip (mm)	Cell number
0-2	154,000
2-4	60,000
4-6	12,000
6-8	11,000
8-10	10,000
10-12	10,000

3. Obtain five uniform corn seedlings, with straight radicles at least 1.5 cm long, and put them between damp paper towels. Do not allow the seedlings to dry out.

4. Place one 4-day-old corn seedling on a plastic ruler, and cut six 2-mm sections from each radicle, starting from the tip. Place each of the sections in the appropriate numbered sectors (1 to 6) on a wet paper towel (see Figure 6.2). Repeat this sectioning with the other four seedlings. You need five sections (i.e., from five radicles) for each enzyme assay. Work quickly and handle one seedling at a time leaving the others in moist paper toweling. Do not allow the seedlings or sections to dry out.

5. Cut each set of sections into smaller pieces (approximately one-quarter of the original size) with the help of a razor blade and a spatula. This is to improve the access of substrate (sucrose) to the enzyme (invertase) located in cell walls of individual cells. (Try to cut each set the same. It is important that each set receive equal treatment. Why?)

6. *When all sections have been cut*, transfer each set of the five (cut-up) sections, using a spatula and the edge of a razor blade, to the inside of the cap of each vial (1 to 6). When all six vials are ready, quickly replace all caps, shake vials, and note time (Time = 0). Immediately transfer 0.5-ml samples of solution (use a 1-ml syringe, and be careful not to remove radicle sections) from vials 1, 3, and 6 into the 20-ml test tubes numbered 7, 8, and 9. Rinse syringe with water after use.

7. Gently replace the tops on the vials. Incubate for 30 minutes at room temperature (a somewhat shorter or longer time of incubation is acceptable, but note the exact time that you used, and use this value for your calculations).

8. Pipet 0.5 ml of a 2% sucrose solution (made in 20 mM of MES buffer) into test tubes 10 and 11 using a 1-ml pipet. These are the **reagent blanks** or "control" test tubes discussed below.

9. Using a 1-ml pipet, transfer 0.5 ml of each standard glucose solution (made in 20 mM of MES buffer) into test tubes 12 to 19 as follows: Into both test tubes 12 and 13, transfer 0.5 ml of 20 μg ml^{-1} of glucose standard solution. To test tubes 14 and 15 add 0.5 ml of 50 μg ml^{-1} of glucose standard solution. Test tubes 16 and 17 each receive 0.5 ml of 100 μg ml^{-1} of glucose. Finally, test tubes 18 and 19 each receive 0.5 ml of 200 μg ml^{-1} of glucose (see Table 6.5).

1,2,3,4,5,6 — 4 day-old corn seedling

Place each corn radicle section on an appropriate division on the paper towel to avoid mixing up the root pieces.

moist paper towel

After having sectioned and organized a total of five corn radicles, quarter each piece of radicle tissue with a razor blade.

radicle sections from sector 1 — sector 2 sections — sector 3 sections — sector 4 sections — sector 5 sections — sector 6 sections

EACH OF THE SIX VIALS contains 1.5ml of 2% sucrose.

① 2 ③ 4 5 ⑥

After 30 min incubation transfer 0.5ml from vial 1 into test tube 1, 0.5ml from vial 2 into test tube 2, etc.

0.5ml from vial① 0.5ml from vial③ 0.5ml from vial⑥

1 2 3 4 5 6 — 7 8 9

EXPERIMENTALS — CONTROLS

0.5ml 20μgml⁻¹ — 0.5ml 50μgml⁻¹ — 0.5ml 100μgml⁻¹ — 0.5ml 200μgml⁻¹

10 11 — 12 13 — 14 15 — 16 17 — 18 19

BLANKS — GLUCOSE STANDARDS

1. To each of the 19 test tubes 0.5 ml of Somogy's reagent is added.

2. All 19 test tubes are now inccubated in the boiling water bath for 5 minutes.

3. Following incubation, 0.5 ml of Nelson reagent and 4 ml of distilled water is added to each test tube before reading the samples in the spectrophotometer at 650 nm.

Figure 6.2. A partial schematic outline of the experimental procedure (steps 1–10). Refer to the text for details.

10. After the 30-minute incubation period, use a 1-ml syringe to remove 0.5-ml samples from vials 1 to 6 and put into the 20-ml test tubes with the corresponding numbers 1 to 6. Be careful not to remove any radicle sections. Rinse the syringe with water after use.

11. Place the rack with the 19 test tubes into the boiling water bath to facilitate the colour reaction with the dye. Incubate for 5 minutes.

12. After incubation in the water bath, remove the rack and place the test tubes in a sink containing cold tap water for about 5 minutes. (Do not allow water to get into the tubes.)

13. After samples have cooled, use a 1-ml syringe to add 0.5-ml of Nelson's Reagent to each tube.

14. Add 4 ml of distilled water (use a 10-ml pipet) into all 19 tubes. Mix well by gently tapping the tubes with your finger.

15. Now you should read the samples in the spectrophotometer at 650 nm.

16. Obtain a cuvette (a test tube with a small vertical line at the top). Use this tube for all of your readings and always place the tube in the spectrophotometer with the mark facing the same direction. The machine is sensitive to the imperfections in the glass tubes — different tubes will give different readings for the same sample. Readings with the same tube may also vary. Minimize this variation by placing the same tube in the machine the same way for all samples. *Caution:* Only hold the tube at the top — finger smudges also affect the readings.

17. Pour the contents of test tube 10 into your cuvette. This tube is the *reagent blank* or control for your readings. (The wavelength on the machine should have been set at 650 nm and the instrument adjusted to read 0% Transmittance without a cuvette in position.) Place your blank in the machine and adjust Absorbance to read 0. Pour the contents back into test tube 10. Repeat this reading procedure with your other blank (test tube 11); absorbance should read close to 0. Pour the contents back into test tube 11. Rinse the cuvette with distilled water, invert, and drain onto a Kimwipe.

18. Measure absorbance for the remainder of the tubes:
    (a) pour contents of large test tube into cuvette,
    (b) read absorbance and record your readings in Tables 6.3, 6.4 and 6.5,
    (c) pour the contents back into the appropriate large test tube,
    (d) rinse the cuvette with distilled water after each reading, and
    (e) drain well on a Kimwipe.

19. Using ethanol and cheese cloth, remove numbers from test tubes. Place used test tubes in plastic container on the side bench.

20. Dispose of razor blades, syringes, and needles in the labeled containers at the side bench.

Table 6.3. Absorbance of test tubes 1 to 6.

	Test tube number					
	1	2	3	4	5	6
Your absorbance						
Class absorbance (optional)						

Table 6.4. Amount of glucose at Time = 0.

Test tube number	Absorbance Time = 0
7 8 9	
Mean	
Class mean (optional)	

Blank or Control Tubes (test tubes 10 and 11)

The reagents (e.g., Somogyi's reagent and MES buffer) may affect the absorbance of the solution in all the test tubes. To control for this effect, you placed in test tubes 10 and 11 solutions containing everything except what you are testing (i.e., the amount of glucose). The blank tubes for this experiment contained 0.5 ml of the 2% sucrose solution in 20 mM of MES buffer as well as 0.5-ml of Somogyi's Reagent and 0.5-ml of Nelson's Reagent instead of 0.5 ml of a sugar (glucose and fructose) solution. The absorbance of the blank tube is subtracted from the absorbancies of the other test tubes where the reaction between glucose and the reagents occurred. You accomplished this by setting the absorbance of the spectrophotometer to zero using your blank tubes. *Note:* The sucrose solution and the standard glucose solutions were made in a buffer to maintain the necessary pH.

Standard Solutions

You determined the absorbance of known amounts of glucose (i.e., test tubes 12 to 19). You tested these standard solutions in duplicate to allow for human and technical error. Ideally you should have made several replicates of each known amount to improve your confidence in the absorbance readings. When you plot mean absorbance versus amount of glucose, the resulting graph is called a standard curve. Theoretically, since absorbance is directly proportional to the amount of glucose in the solution, the standard curve should be a straight line passing through the origin (0 μg glucose, 0 absorbance); draw your graph this way. Using this standard curve you may determine the amount of glucose in an unknown solution by determining absorbance of the solution. Once you have found the absorbances of solutions in test tubes 1 to 9, you can determine the amount of glucose present in these test tubes by interpolation from the standard curve.

You must first understand what the difference in amount and concentration:

Amount (µg) = concentration (µg ml^{-1}) × volume (ml)
Concentration = amount ÷ volume

The concentration of the standard solutions was expressed as µg (glucose) per ml (of solution). The amount of glucose added to each tube may be calculated, for example:

100 µg ml^{-1} × 0.5 ml = 50 µg (concentration × volume = amount)

Plot the graph for your standard curve: absorbance (at 650 nm) (on the ordinate) versus amount of glucose in µg (on the abscissa). Use the data in Table 6.5 to construct your standard curve for glucose.

Table 6.5. Absorbancies (*A*) of the standard glucose solutions.

Tube	Glucose concentration (µg ml^{-1})	Amount of glucose (µg)	Your data		Class data (optional)	
			A	Mean *A*	*A*	Mean *A*
12	20	10				
13	20	10				
14	50	25				
15	50	25				
16	100	50				
17	100	50				
18	200	100				
19	200	100				

Invertase Activity in the Corn Radicle

In order to calculate the rate of enzyme activity you must consider other items first:

1. The amount of "glucose" present at Time = 0. The radicle sections will convert sucrose to glucose and fructose in the presence of the necessary enzymes. The amount of glucose produced after a period of incubation must be compared to the amount of glucose present at the start of the incubation, i.e., Time = 0. In addition, the sucrose solution itself might affect absorbance readings. Correct for the effect of the sucrose solution and any glucose present at the start of the experiment by subtracting the Time = 0 absorbance from absorbance of the samples (1 to 6) after incubation.

   The amount of glucose present at Time = 0 should be approximately the same for all radicle sections. Thus, instead of sampling all six vials you took selected samples from the vials (note that you took samples from the beginning, middle, and end of the set to allow for the effect of the time lapse incurred when transferring sections into the appropriate vials).

2. The amount of glucose in each test tube represents the amount of glucose in 0.5 ml samples taken from each vial (1 to 6) after the incubation period. The amount of glucose in each test tube may be read directly off the standard curve of absorbance versus amount of glucose.

3. The amount of glucose in each vial represents the amount of glucose produced from the conversion of sucrose to glucose and fructose by five 2-mm radicle sections after a known period of incubation. The volume in each vial was initially 1.5 ml. The amount of glucose in each vial is three times the amount of glucose in the 0.5 ml sample — except for vials 1, 3, and 6; 0.5 ml was removed from vials 1, 3 and 6 at Time = 0 leaving only 1.0 ml instead of 1.5 ml solution. Thus the amount of glucose in vials 1, 3, and 6 is two times the amount of glucose in the 0.5 ml sample.

4. The amount of glucose produced by each 2-mm radicle section in each vial is the amount of glucose in each vial divided by the number of radicle sections (i.e., five).

5. The enzyme activity is the amount of glucose produced per 2-mm section per minute. Divide the amount of glucose produced per radicle section by the incubation time.

6. The rate of enzyme activity is the amount of glucose produced per cell per minute. Use Table 6.2 to determine the number of cells per section. Plot these data as enzyme activity per cell versus position of cells along radicle. Which variable is independent? Dependent? Remember that the independent variable is plotted on the abscissa, the dependent variable on the ordinate. Which type of graph is most appropriate? Are the data discrete or continuous? Which is more appropriate, a histogram or line graph? Are the data partitioned into classes?

Note: The source of invertase in this experiment is the corn radicle sections. Because the same amount of sucrose is added to each of the six vials, the differences in measured absorbances for each of these vials (as observed in test tubes 1 to 6) is due to different activities (amounts) of invertase in each radicle. Thus, the amount of glucose produced (i.e., the intensity of colour or absorbance) is correlated with invertase activity. That is, the more glucose produced, the greater the activity of the invertase present in the corn radicle section.

Laboratory Report

Purpose: Each student is expected to independently analyze the results of this experiment and present his/her findings in the form a laboratory report.

Format: It is important to present the results of scientific investigation in a clear and concise manner. The objective is to communicate a problem, subject it to the scientific method of inquiry, present the relevant data collected, and offer an interpretation of these data. Also consider the parameters of experimental design. Your assignment should include the following sections: Title, Introduction, Materials and Methods, Results, Discussion, Conclusions, and Literature Cited (where appropriate).

Length: This assignment should not exceed 1,000 words in length, excluding tables, graphs, and literature cited (where appropriate). Your assignment should be double-spaced and, if possible, typed.

Content: Your report should include the following (marks per section in parentheses, total = 150):

1. Introduction (10 marks)

2. Materials and Methods (0)

3. Results
   (a) Graph of standard curve of glucose (10)

(b) Tables of the following (consolidate where possible and show how values were derived): (35)

     i.   amount of glucose and absorbance for glucose standards
    ii.   glucose at Time = 0 (calculate mean amount)
   iii. amount of glucose in tubes 1 to 6
   iv. amount of glucose in vials 1 to 6
    v.   amount of glucose per radicle section for each vial 1 to 6
   vi. amount of glucose per section per minute for each vial 1 to 6
  vii. amount of glucose per cell per minute for each vial 1 to 6
 viii. cell size in corn root tip

(c) Present in graph form: (15)

    i.   enzyme activity per cell
    ii.   cell enlargement in root tip (data from Table 6.1)
   iii. number of cells per 2-mm section (data from Table 6.2)

4. A discussion of your data in reference to the relationship between invertase activity per cell and cell growth in the corn root.   In other words, what do the results calculated above suggest about corn root growth? (70)

5. Remember to include a brief concluding paragraph. (10)

APPENDIX A
Sample Results

Table 6.6. Glucose standards.

	Test tube							
	12	13	14	15	16	17	18	19
Absorbance (*A*) at 650 nm	0.156	0.149	0.709	0.742	1.263	1.273	1.889	1.882
Glucose conc. (µg ml^{-1})	20		50		100		200	
Amount glucose (µg)	10		25		50		100	

Table 6.7. Glucose at Time = 0.

	Test tube			
	7	8	9	Mean*
Absorbance	0.078	0.282	0.552	0.304 ± 0.29

* This value is subtracted from each T = 30 value for tubes 1 to 6. The amount of glucose at Time = 0 is basically 0 µg; such minute absorbances cannot be read from the standard curve.

**Table 6.8.** Calculations of amount of glucose/cell/minute (*A* = absorbance)*.

Tube #	*A*	*A* Time = 0	Amount of glucose (µg)				
			in tube (from graph) (iii)	in vials 1 to 6 (iv)	per radicle section (v)	per section / minute (vi)	per cell/ minute (vii)
1	0.566	0.262	9.42	18.84	3.77	0.126	80.0×10^{-8}
2	0.439	0.135	2.74	8.22	1.64	0.039	60.0×10^{-8}
3	1.482	1.178	57.63	115.26	23.05	0.768	64.0×10^{-6}
4	1.095	0.791	37.26	111.78	22.36	0.745	67.7×10^{-6}
5	1.441	1.137	55.47	166.41	33.28	1.109	110.9×10^{-6}
6	1.464	1.160	56.68	113.36	22.67	0.756	75.6×10^{-6}

* Columns labeled (iii), (iv), (v), (vi), and (vii) refer to the parts of the results section in each student's laboratory report. See the Laboratory Report section in the Student Outline for details of the calculations. For example, the "amount of glucose/section/minute" for tube 1 in column (viii) is divided by 154,000 cells (from Table 6.2) to obtain an "amount of glucose/cell/minute" of 80.0×10^{-8} µg.
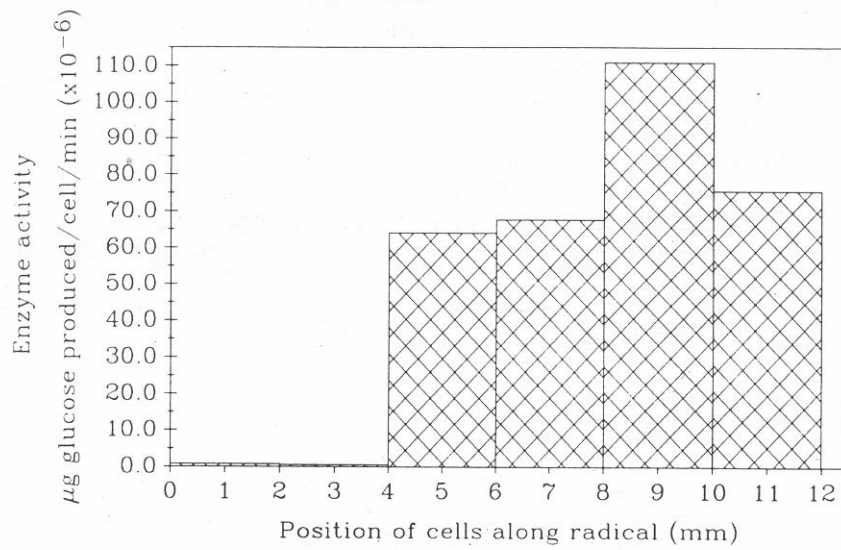
Figure 6.3. Enzyme activity per cell (amount of glucose/cell/minute/vial).
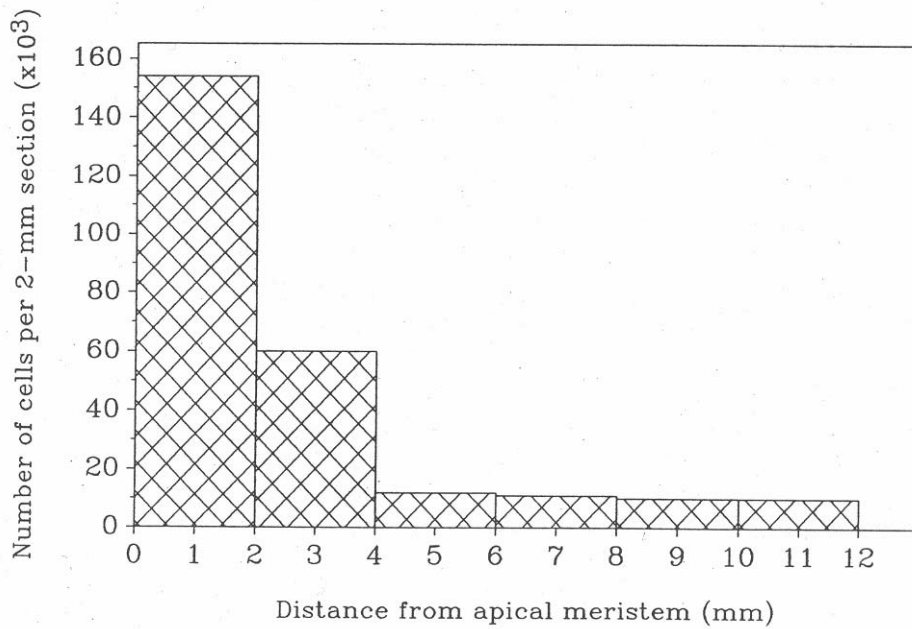
Figure 6.4. Number of cells per 2-mm section in corn root tip (data from Table 6.2)

Discussion

In the student's report, invertase activity should be discussed in relation to the three regions of growth in the monocot as shown in Figure 6.1. The student's discussion should identify the rapid increase in invertase expressed on a per cell basis in root sections at increasing distances from the root tip, that is, its correlation with rapid increase in cell expansion during root growth. It should be noted that enzyme activity per cell levels off, and even becomes constant, implying a switch from growth to differentiation.

Students should point out the important role this hydrolytic enzyme lays in converting sucrose to fructose and glucose, which are subsequently taken up by the rapidly growing cells. This results in the cells being supplied with (a) osmotically active substances that will allow water uptake (turgor pressure), (b) carbon skeletons for biosynthesis of cell material, and (c) energy through respiration for growing processes.