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Koch's Postulates and Yogurt

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Introduction
This laboratory project is adapted from that of Stewart, "Safely teaching Koch's postulates on the causation of infectious disease" (1990). In addition to instructions and methodology, I give suggestions for some experiments that students might perform, once the demonstration component of the laboratory is completed. I have found this project particularly useful to introduce students of microbiology to an experimental or problem-based curriculum. Students unfamiliar with laboratories in which they take control soon develop a taste for inquiry-based learning and are prepared to tackle more quantitative projects.

Some projects which students have proposed and attempted in recent years include:

1. Effects of temperatures on yogurt formation.
2. Effects of temperatures on growth of yogurt causative agents.
3. Comparison of different brands of yogurt with respect to the presence of causative agent microorganisms.
4. Attempts to prepare yogurt with pure cultures of individual isolates.

Lab Research Project
Continuing our theme of activities in microbiology which are both practical and academic, these exercises with yogurt show (1) the applications of microorganisms in food production and (2) the germ theory of disease.

Koch's Postulates
Robert Koch showed that a specific microbe (Bacillus anthracis) was responsible for a specific disease (anthrax). At this time (1877), the germ theory of disease was being tested in Koch's laboratories as well as in the laboratories of others.

In 1884, Koch set forth specific criteria known as Koch's postulates, which would establish disease etiology. These are:

(1) Individuals with the disease should harbor the microorganisms, and healthy individuals should not harbor it.

(2) The microbe should be grown in pure culture from the diseased person.

(3) When the pure culture of the microorganism is introduced into a healthy individual, that individual should acquire the symptoms which are associated with the disease.

(4) The same microorganism should be isolated from the experimentally infected individual.

Safety Statement
I recommend using a yogurt maker such as a Salton brand electric yogurt maker. The lids seal the mixture better than beakers covered with foil, and there is much less risk of contamination, particularly from water in a water bath, if that is used for the incubation. I do not require students to taste the products, but I do not discourage this, particularly when a yogurt maker is used.
Certainly there are limits to the application of these criteria. Ethical considerations disavow human experimentation. Therefore, step (3) of the postulates is usually performed only when there is no susceptible laboratory animal. Sometimes the microbe cannot be grown in pure culture. Then, other criteria or associations have to be used.

What are your ideas about the use of laboratory animals for such testing and experimentation? Do you think association data (for example correlation of smoking with the incidence of heart disease) are adequate proof of Koch's postulates?

Studying Koch's Postulates with Yogurt—Rather than employ a disease model, these experiments demonstrate a general cause and effect of microorganisms. The "disease," in this case, is yogurt. Skim milk is the healthy, uninoculated state. The texture, odor and quality of the yogurt produced are the symptoms.

Yogurt production requires two "infecting" bacteria, each with similar effects. The experiments you do will (1) isolate and identify bacteria from yogurt, (2) re-inoculate skim milk with the bacteria you isolate and (3) prepare edible yogurt of differing qualities.

About Yogurt Production—Bacteria associated with yogurt production are Lactobacillus bulgaricus and Streptococcus thermophilus. On LSD (lactobacilli-streptococci differential) medium, both colonies are colored red. Since they are both fermenting microbes, pour plates should be preformed and colony growth observed in the depth of the agar. Alternatively, skim milk agar plates can be streaked with a sample of yogurt. These plates can be grown in anaerobic chambers or in candle jars. Incubation should occur at 43-46°C in all cases.

Streptococcus thermophilus forms small (approximately 0.5 mm) round regular colonies which contain cocci or ovoid-shaped Gram positive cells. Large (1-2 mm) irregular colonies containing Gram positive rods, often in short chains, is characteristic of Lactobacillus bulgaricus.

The brands of yogurt to study will be of interest. "Natural" yogurts generally possess both types of microorganisms. Some commercial yogurts are pasteurized after fermentation, so there are few if any bacteria remaining. Yogurt used should be identified on the label as "containing active cultures." It will also be interesting to compare the numbers of bacteria in different commercial products.

Experimental Techniques

Some Experiments You Can Do

1. Compare different yogurts with regard to the numbers of microbes present.

2. The effect(s) of pure strains of bacteria alone or in combination with other isolates on yogurt production.

3. Re-isolation of bacteria from yogurt product, in order to demonstrate Koch's postulates.

4. Effects of storage conditions on the numbers of microbes present. Room temperature, refrigeration, freezing, or 37°C or higher temperatures could be tested, for example.

5. Monitor various experiments by plate count determinations on LSD plates compared to plate counts using tryptic soy or nutrient agar plates.

6. Determine pH values during the fermentation experiments you design.

Isolation of Bacteria from Yogurt

Materials:

- LSD agar plates
- Skim milk agar plates
- Sterile pipettes
- Sterile 9.9 ml (w/v) saline dilution blanks
- Spreaders
- Yogurt, preferably non-flavored
- Gram stain reagents and materials
- pH determination paper and/or pH meter

- Candle jars or anaerobic chambers
- Alcohol for sterilizing spreaders
- Glass rods
- Yogurt maker (or sterile 250 ml beakers covered with foil)
- Aluminum foil
- Saucepan or 250 ml beakers
- Heaters
- Thermometers
Procedures
1. Streak LSD and skim milk agar plates with samples of yogurt to obtain pure cultures.
2. Incubate in candle jars or anaerobic chambers at 43-46°C for 24-48 hours.
3. Describe colonies which have formed. Isolate them to pure culture by re-streaking on LSD agar plates and incubating as before.
4. Perform Gram stains on selected isolated colonies which appear different. Describe the microbes in the colonies: morphology, arrangements of cells, Gram stain properties.
5. Prepare sterile dilutions of the yogurt:
   a. Add 0.1 ml to a 9.9 ml blank (1% sterile saline) and mix. This is a 1/100 dilution. Remove 0.1 ml of the 1/100 dilution and add it to a 9.9 ml dilution blank. Mix. This is a 1/10,000 dilution.
   b. Place 0.1 ml of undiluted yogurt on each of three LSD plates.
   c. Place 0.1 ml of 1/100 dilution on each of three LSD plates.
   d. Repeat using the 1/10,000 dilution.
   e. Sterilize a glass spreader by immersing it in alcohol and igniting the alcohol. Do not hold the spreader in the heat of the flame for any length of time. While rotating the plate with its lid removed, use the spreader to distribute the samples on the plates.
   f. Invert and incubate these plates in a candle jar (or anaerobic chamber) and incubate at 43-46°C overnight.
   g. Count colonies of different types on all the plates and record your observations. Estimate the number of total bacteria per ml.

Effects of the Isolated Bacteria on Milk
1. Autoclave milk for 5 min. at 121°C in capped test tubes. Allow to cool.
2. Inoculate the sterile milk with bacteria from colonies from the pure cultures prepared previously. Inoculate tubes of sterile milk with combinations of the bacteria from sterile colonies which were also previously isolated.
3. Incubate undisturbed for 18-36 hours at 43-46°C.
4. Each day, check the inoculated tubes for coagulation, odor and acidity. Acidity can be obtained by dipping a sterile glass rod in the culture and touching it to a piece of pH paper. Match the color on the paper to the color key supplies. (Alternatively, remove a sample and use a pH meter for this measurement.) Record all observations.

Make Your Own Yogurt—This is a recipe for yogurt which you might use in designing experiments.
Take 1 liter of skim or whole milk and heat in a lidded saucepan just to boiling. Reduce the heat so that the milk remains hot but does not boil. This process will kill pathogens and spoilage organisms which may be present in commercially obtained milk. Cool, leaving the lid on (why?) by placing in a 45°C water bath. Add 100 ml (1/2 cup) of freshly purchased natural, unpasteurized yogurt to the cooled milk and stir. Decant into a clean, dry, lidded container, filled so that there is little air space above the lid. Put lids or caps on loosely (do not seal). Place in a consistently warm area (the closer to 45°C, but not above, the better). This incubation at about 30°C or better should last for 12-18 hours. Check the product for spoilage: the odors of ammonia and hydrogen sulfide (rotten egg gas) indicate this. Spoilage occurs rarely.

Additions of milk powder at the outset will yield a thicker curd product. Additions of fruit or honey should be made after the fermentation is complete. The product should be refrigerated immediately after these additions (why?). If you add more sugar at the outset of the process, more lactic acid will be formed and the product will be more tart.

Preparation of Skim Milk LSD Agar—L-S Differential Agar (LSD Agar) is available from Oxoid, Unipath Ltd., Basingstoke, Hampshire, England or from Oxoid suppliers in the United States. Alternatively, it may be prepared as follows:

Part 1.
<table>
<thead>
<tr>
<th>nutrient broth (2X)</th>
<th>8 grams</th>
<th>500 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>agar</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>triphenyltetrazolium chloride (TTC)</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

Add HCl to pH 6.1
Add distilled water to 500 ml final volume
Autoclave, cool to touch

Part 2. 10% Skim milk
<table>
<thead>
<tr>
<th>dry skim milk powder</th>
<th>100 grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>distilled water</td>
<td>500 ml</td>
</tr>
</tbody>
</table>
Sterilize by filtration, if possible. Alternatively, sterilize water separately and add the powdered milk to it. This tends to clump as a result of sterilizing in the autoclave. Plates so prepared may be incubated in advance to check for possible contamination from the powdered milk.

Mix Parts 1 and 2. Pour into plates. Dry plates and check for contamination either by (1) incubation overnight at 37°C or (2) incubation 2 days at room temperature with lids ajar.

**Literature Cited**


This year's AMCBT Meeting will be held at Alverno College, Milwaukee. Alverno is unique among U.S. colleges and universities because of their ability-based approach to education. This approach has gained them national recognition as a leader in making college education work. Alverno is minutes from downtown Milwaukee and only minutes from Mitchell International Airport. Pat Bowne, David Ferris, and Leona Truchan invite you to participate in a conference that promises to be as unique as Alverno. For example, opportunities to discover Milwaukee's natural and cultural history will not conflict with AMCBT presentations. Additionally, a greater number of hands-on workshops - two accessing the Internet - have been added to the program.

What else might you discover? Did you know that early 20th century Milwaukee was a stronghold of Socialist thought and ground gently quake with each step? How would you like to watch blossoms, witness parallel evolution of euphorbs and cacti, and stroll among kapok, tamarind, and curare vines within a single superstructure? Where can you read The Water Street Journal while feasting upon charbroiled Usinger Bratwurst and Stuttgarter Knackwurst along with a weizglas of Callan's English Red? The gathering place by the waters" of course!
Antifungal Proteins from Grains

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These exercises adapt procedures for purification and assay of antifungal proteins from grains for the undergraduate laboratory. They introduce students to concepts of protein purification and bioassay. These experiences integrate areas of microbiology and biochemistry and afford opportunities for further independent student research projects on a topical subject.

INTRODUCTION

Plants have defenses against infection. Defenses against fungi include the production of a variety of substances which are inhibitory - phenols, melanins, tannins, salicylic acid or phytoalexins, as well as proteins which can inhibit growth of fungi. Antifungal proteins have been isolated and characterized from maize seeds (Huynh, Borgmeyer and Zobel, 1986) and from barley, wheat and rye (Roberts and Selitrennikoff, 1986 a,b). These proteins are very likely a part of a complex defense system of plants (Angier, 1992). Do all grains possess these antifungal proteins as part of a defense mechanism? Are these proteins present in all tissues of the plant as it develops? A better understanding of the roles of these proteins in plant defenses may be used in attempts to improve plants using gene engineering technologies by introducing genes determining these proteins into plants which are not resistant to fungi (Moffat, 1992). This laboratory project describes the isolation, purification, characterization and bioassay of antifungal proteins from grains.

The theories and practice of these techniques are well documented (Alexander and Griffiths, 1993; Bollag and Edelstein, 1991; Boyer, 1993; Robyt and White, 1987). However, the purification of proteins with biological activity other than enzyme activity is rarely presented. Antimicrobial assays are often part of microbiology curricula, usually as antibacterial antibiotic assays using bacteria as test microorganisms. The assay described by Roberts and Selitrennikoff (1986a) is a modification of these procedures adapted for testing antifungal inhibitors. In this paper disk assay, the inhibitor(s) diffuse towards advancing mycelia growth of the test fungus. Under the conditions described, the presence of an inhibitor stops advancing growth when compared to the growth in the absence of inhibitor.

With this background, students may design experiments for independent investigations.

MATERIALS

Isolation of Proteins

Grains (barley, wheat, rye) were purchased in bulk from Cornstalk, Ferre, and Co., Wethersfield, CT.

Electric coffee grinder

60mM acetic acid

Centrifuge

1 M Tris base

pH meter

Ammonium sulfate

Dialysis tubing

5mM sodium phosphate buffer, pH 7.0 containing 50 mM NaCl

5 mM sodium phosphate buffer, pH 7.0 containing 200 mM NaCl

10 mL column of CM Sephadex (approximately 1 cm X 3.3 cm column) spectrophotometer

1.5 x 50 cm column of Sephacryl S-200

10 mM sodium phosphate buffer, pH 7.4 containing 125 mM NaCl

Blue dextran

Ruler

0.44 micron Millipore filters and filtration apparatus
Bioassay

**Culture**: *Trichoderma reesei* ATCC #t363 was obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA.
Potato carrot agar (Medium #335, Jong and Edwards, 1991)
0.7 cm diameter sterile paper discs

METHODS

**Lab I**

**Protein purification**: isolation of proteins from grains. Extracts should be handled in the same manner one would handle enzymes (Boehringer Mannheim Biochemicals, 1985). The procedures are:

1. Weigh 120 grams of plant seeds.
2. Grind to a fine powder in an electric coffee grinder.
3. Add this powder, with stirring, to 300 ml 60 mM acetic acid cooled on ice.
4. Stir in the cold (4°C) for 1 hour.
5. Centrifuge at 7400 rpm for 30 minutes.
6. Discard pellet. Neutralize the supernatant to pH 7.6 by the dropwise addition of 1 M Tris base, using a pH meter.
7. Place at 4°C overnight. (This is an appropriate stopping point.)

**Lab II**

**Ammonium sulfate fractionation**.

8. Remove any precipitate by centrifugation at 7400 rpm for 30 minutes. Save a 3ml sample of the supernatant for further analyses. Use the remainder for the next step.

9. Ammonium sulfate fractionation:
   a. Slowly, while stirring, add ammonium sulfate to a final concentration of 30% (w/v). For the weight of salt to use, consult a table of ammonium sulfate concentrations adjusted for 40°C. Remove any precipitate by centrifugation at 7400 rpm for 30 minutes. Save the pellet, label ‘0-30% ammonium sulfate fraction’ and store at -20°C (freezer).
   b. Adjust the supernatant to 55% saturation with ammonium sulfate, keeping the solution cold (on ice). The precipitate contains antifungal protein(s) activity. Collect this by centrifugation at 7400 rpm for 30 minutes. Save a 3 ml sample of the supernatant for future analysis. Label it ‘30-55% ammonium sulfate fraction’.
   c. Dissolve the precipitate in 10 mM NaCl 5 mM sodium phosphate, pH 7.0. This may require 3 to 25 ml, depending on the amount of precipitate formed. This will vary with the extraction conditions as well as with different grain extracts tested.
   d. Dialyze against 10 mM NaCl 5 mM sodium phosphate (pH 7.0) overnight with at least two changes of buffered saline. (This is an appropriate stopping point.)

**Lab III**

**Ion exchange chromatography** using carboxymethyl Sephadex (CM-Sephadex).

10. CM-Sephadex chromatography
   a. Remove the 30-55% ammonium sulfate fraction from the dialysis tubing. Save a 1 ml sample for future analysis; label appropriately and store in the freezer.
   b. Prepare a CM-Sephadex column in 10 mM NaCl 5 mM sodium phosphate, pH 7.0 and pouring it to form a column which contains 10 ml of the resin.
   c. Add the 30-55% ammonium sulfate fraction to the column. When the protein solution descends to the top of the column bed, rinse it with 20 ml of mM NaCl 5 mM sodium phosphate (pH 7.0)
solution. Remove contaminating proteins by eluting with 50 mM NaCl 5 mM sodium phosphate (pH 7.0). Monitor the 3 to 5 ml fractions by measuring absorbance at a wavelength of 280. Continue to collect fractions of 3 to 5 ml after there is a drop in absorbance at wavelength 280. Antifungal protein(s) are then eluted from the column with 200 mM NaCl 5 mM sodium phosphate (pH 7.0)
d. Determine the absorbance of the column fractions at wavelength 280.
e. Pool fractions that have a high absorbance. Set aside a 1 ml sample and store it and the remainder of the protein solutions in the freezer. Label appropriately. (This is an appropriate stopping point.)

Lab IV
Gel exclusion chromatography.

11. Gel filtration: Sephacryl S - 200
a. Prepare a 1.5 x 50 cm column of Sephacryl S - 200 equilibrated with 125 mM NaCl 10 mM sodium phosphate (pH 7.4). Standardize the column by adding about 1 ml blue dextran solution. This will check the uniformity of the column and give the void volume. The void volume is the volume in ml required to elute the blue dextran.
b. Add 1 ml of 1 mg/ml of protein fraction from CM-Sephadex chromatography to the Sephacryl column.
c. Collect fractions (3 to 5 ml) of equal volumes. The flow rate is approximately 12 ml per hour.
d. Monitor the absorbance of the fractions eluted at wavelength of 280.
e. Pool the fractions containing the peak absorption values.
f. Filter sterilize this preparation, aliquot into sterile microcentrifuge tubes, store in the freezer and label. This preparation will be examined for antifungal activity and protein content. (This is an appropriate stopping point.) This purification scheme is presented in Figure 1.

Lab V
Bioassay of antifungal activity.

In this laboratory, the amount of grain extract which inhibits a fungus will be determined. The fungus used is Trichoderma reesei.

Using sterile procedures, dilute the plant protein fraction to be tested as shown on Table 1.

Using a template, aseptically place a 0.7 cm paper disc in the center of a potato-carrot agar plate and four discs 1.2 cm from the central disc and equidistant from each other. Add 20 microliters of dilutions of plant protein fractions to the other discs.

Add 1 ml sterile PBS to a slant culture of Trchoderma reesei. Agitate vigorously using a vortex mixer (if available) or by hand to form a slightly turbid suspension of conidia. Add 20 microliters to the central disc. Incubate plates at room temperature in a moist, closed container. Inspect daily until mycelia growth from the central disc has enveloped the peripheral disk containing PBS only (control) and had formed crescents of inhibition around the discs containing inhibitor concentrations of antifungal proteins. Record all observations.

Lab VI
Protein Assays

Table 1. Dilution levels of plant protein fraction.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Microliters of extracts</th>
<th>Microliters of PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1:2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>1:10</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>1:30</td>
<td>10</td>
<td>290</td>
</tr>
<tr>
<td>1:100</td>
<td>10</td>
<td>990</td>
</tr>
</tbody>
</table>

Protein may be determined using several methods. However, the method recommended for use is the spectrophotometric method of Warburg and Christian (1941). Samples or dilutions of samples are
Figure 1: Antifungal proteins purification scheme

Antifungal Proteins Purification Scheme

Grind 120 g. grain seed in electric coffee grinder to fine powder

Resuspend in 300 ml 60 mM acetic acid; stir 1 hour at 40°C

Centrifuge 7400 rpm for 30 minutes

Supematant (Sample I)
(discard)

Adjust pH to 7.6;
Place at 4°C overnight

Centrifuge 7400 rpm 30 minutes

Supematant 0 - 30% (NH₄)₂SO₄

Centrifuge 7400 rpm 30 minutes ———> Pellet (discard)

Supematant 30 - 55% (NH₄)₂SO₄

Centrifuge 7400 rpm 30 minutes ———> Supematant (discard)

Pellet - dissolve in 3 - 25 ml 10 mM NaCl/5 mM sodium phosphate, pH 7.0;
dialyze overnight against this buffer (PBS) (Sample II)

CM - Sephadex chromatography

10 mM NaCl/5 mM PBS ———> discard

5 mM NaCl/5 mM PBS ———> discard

200 mM NaCl/5 mM PBS (collect fractions)

Pool fractions with O.D.₂₈₀ > 0.7 (Sample III)

Gel filtration (Sephacryl S-200)

Pool fractions with O.D.₂₈₀ > 0.2 (Sample IV)

Analyze fractions for protein and for antifungal proteins by bioassay

Figure 1. Antifungal proteins purification scheme.
examined at wavelengths 280 nm and 260 nm for UV absorption and protein concentrations are estimated by use of a nomograph (Roby and White, 1987).

Thaw the samples of fractions formed by the protein purification procedure. First, pipette 1 ml of undiluted sample into a 1 ml cuvette. If the concentration is too high, absorption may be greater than 1.00 at either (or both) of these wavelengths. In that case, dilute the sample 1:10 by pipetting 0.1 ml of the sample into 0.9 ml of water and then read absorbance at 280 nm and 260 nm. Multiply the estimation of protein concentration by the dilution factor (10, in this case) for the samples.

Record the concentration of protein in mg/ml for each fraction.

Additional Laboratories
As an additional exercise, protein purification may be analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). I have used the procedures and materials supplied by BioRad Corporation (Bio-Rad Laboratories, 220 Maple Avenue, P.O. Box 708, Rockville Center, NY 11571, USA) to perform SDS-polyacrylamide gel electrophoresis separation of the antifungal proteins in the samples. Low molecular weight standards should be separated to permit estimation of molecular sizes of isolated protein bands. The purest preparation should contain a large portion of one protein molecule (an antifungal inhibitor). The Coomassie blue staining procedure is used; samples applied to the gel contain at least 1 microgram of protein (minimum amount). Prepoured 10% gels purchased from BioRad are used.

Gels are dried using the procedure of Michael and Ford (1991). Dried gels may be copied and/or mounted directly in the laboratory notebook.

Results
The purification of antifungal protein activity from barley (*Hordeum vulgare*) seeds is summarized in Table 2. The fractions examined are indicated in the purification scheme (Figure 1). Antifungal activity is determined by bioassay. The assay of fraction IV for this preparation is shown in Figure 2. A unit of activity is defined as the smallest amount of protein from a particular fraction which causes growth inhibition of the fungus. The purification table shows that antifungal activity may be isolated by protein purification.

![Figure 2. Bioassay of antifungal activity by barley extract. Paper disks contained 25 microliters of grain extract diluted 1:30 [disk 1], 1:3 [disk 2], 1:1 [disk 3] or 25 microliters of PBS disk c. Conidia from *T. reesei* were added to the central disk. Plates were incubated at room temperature in a moist chamber for 72 hours.](image)

| Table 2: Purification of antifungal proteins from barley (*Hordeum vulgare*) |
|-----------------------------|-----------------|-----------------|----------------|----------------|
| Fractions                  | Total Protein (mg.) | Total antifungal units x 10^{-3} | % units recovered | Specific activity (U/mg protein) |
| 1. Crude grain extract     | 1157            | 897             | 100             | 775            |
| 2. 30-55% (NH₄)₂SO₄        | 250             | 150             | 17              | 600            |
| 3. CM-Sephadex pool        | 38              | 88              | 9.8             | 2316           |
| 4. Sephacryl pool          | 7               | 14              | 1.6             | 2000           |

*One unit is defined as the smallest amount of protein from a fraction causing detectable growth inhibition of the fungus.*
Using these procedures, students have examined different grains for protein content, and antifungal activity, examined germinated seedlings (roots and shoots) for antifungal protein content and analyzed fractions obtained by protein purification by SDS-PAGE. Other projects have examined different test fungi in bioassays. These student projects reproduce published data (Huynh, Borgmeyer and Zobel, 1986; Roberts and Selitrennikoff, 1986) and extend knowledge about plant-fungi interactions (unpublished results).

These laboratory exercises provide opportunities for cooperative learning, particularly if students have differing background experiences. For example, microbiology students may assist those without training in sterile procedures. Students are motivated to develop independent research projects, once the procedures are completed successfully in the structured laboratory. These laboratories require an integration of learning, and an interdisciplinary approach to scientific investigation. Additional laboratory experiences which may be performed include the analysis of protein fractions by SDS-PAGE and more precise protein analyses. However, the protein purification and bioassay procedures as outlined here provide investigative laboratory experiences for students and skills to develop independent research projects.

Literature Cited


Hormones and the Motor Response of Root Gravitropism

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Gravitropism is a growth movement which results from the response of roots and shoots to gravity. The root cap/tip (the terminal 1.0 to 1.5 mm of the root) is the site of perception of the gravitational force. Gravit curvature occurs in the elongation zone of roots, which is 2 to 6 mm behind the root cap/tip.

According to the Cholodny-Went hypothesis (Digby and Firm, 1980; Went and Thimann, 1937), the gravitropic response of roots is controlled by the lateral movement of a growth inhibitor across a root when the root is placed in a horizontal position in a gravitational field. Auxin is redistributed by lateral transport toward the lower side of the horizontally-oriented root. The accumulation of auxin in the lower portion of the root results in a supraoptimal auxin concentration. Since auxin is inhibitory to root growth, the supraoptimal concentration of auxin inhibits root growth in the elongation zone on the lower side of the root. The upper portion of the root contains optimal levels of auxin. The optimal levels of auxin stimulate growth of the upper portion of the root. This differential rate of elongation between upper and lower halves of the root results in curvature. Although this hypothesis is widely accepted, an alternative hypothesis has been proposed.

The alternative hypothesis for root gravitropism is called the Root Cap Inhibitor Model. In this model, abscisic acid (AbA) is substituted for IAA as the growth inhibitor. Pilet and Rivier (1981) found that AbA is present in root caps. They proposed that AbA accumulates in the lower hemisphere of the elongation zone of horizontally-oriented roots. Additionally, Pilet and Chanson (1981) observed that exogenously-applied AbA can inhibit root elongation in maize.

However, several other researchers have demonstrated that AbA promotes root elongation within the time period required for expression of gravit curvature. Mulkey et al. (1983) found that the initial effect of AbA is stimulation of root growth over a wide range of concentrations of AbA (Figure 1).

The inhibition of root growth is observed only in high concentrations of AbA (0.1 to 1 μM) and with prolonged exposure to AbA (more than 12 hours). Gravit curvature is complete within 2 hr. These data discount the involvement of AbA in the motor response of gravitropism of roots.

Furthermore, the effects of auxin on root elongation are consistent with its suggested role as a growth inhibitor in gravitropism. IAA strongly inhibits root growth at concentrations higher than 0.1 μM (Mulkey, et al., 1982; Thimann, 1937). The evidence for AbA as an inhibitor of root growth is less consistent (Jackson and Barlow, 1981). AbA has been reported to inhibit root elongation (Pilet and Chanson, 1981), to have no effect on root elongation (Gaither, et al., 1975) or to promote root elongation (Abou-Mandour and Hartung, 1980; Gaither, et al., 1975; Yamaguchi and Street, 1977).

In this laboratory exercise, a simple agar block method is used to examine the effect of plant hormones on the elongation zone of a root during
gravitropism. This method is simple, but has many applications to verify the role of plant hormones in gravitropic curvature.

GOALS OF THE EXPERIMENT
1. Comparison of the effect of unilateral application of IAA and AbA on asymmetric growth of roots.
2. Examine the involvement of plant hormones (IAA and AbA) in the gravitropic response of roots.

TIME REQUIREMENT
0.25 hour (approximately) 3 days prior to experiment to soak grain
1.00 hour (approximately) 1.5-2 days prior to experiment to plant grain
1.00 hour prior to experiment to prepare agar block
2.00 hours experiment running time

MATERIALS AND EQUIPMENT
Abscisic acid (AbA)
Agar
Chamber for humidified box
Dark room
Disposable plastic petri dishes, 100x15 mm
Forceps
Grain (corn)
Hot plate
Indole 3-acetic acid (IAA)
Paper towel
Photographic paper (black and white)
Plastic trays and tub
Plexiglas
Razor blade
Screw, two machine screws (1.5" x 8/24 or 8/32)
Small block of wood
Thread
Time lapse video cassette recorder and camera
Window putty

METHOD
Seedling Preparation. Corn grains are soaked overnight in running tap water to prevent anaerobiosis. The grain germinates between wet paper towels on plastic trays in a vertical position. To obtain straight primary roots you should place the corn grains in rows on a tray covered with 2-3 layers of paper towel. Cover the grains with 3 or 4 layers of paper towels; place another tray over final layer of towels to hold the paper towels and grain in place. Position the trays vertically in a shallow tub containing 1-2 inches of water. Primary roots of approximately 1.5-2.0 cm should be used for the experiment. This should require 2-3 days of growth, depending upon the cultivar and temperature.

Incorporation of Indole-3-acetic acid and Abscisic acid. For agar plates containing IAA or AbA and plain, prepare 100 ml of 1% of non-nutrient agar solution. The solution is boiled to dissolve the agar and poured in 100 x 15 mm plastic petri dishes (10 ml of solution per plates). Plates are prepared to contain 0.01 mM IAA, 0.1 mM AbA, and no hormone. The poured plates are placed on a level surface to cool.

Preparation of Agar Block. Prepare a marking block as illustrated in Figure 2. The marking block is constructed of two machine screws which are glued to a small block of wood. The machine screws act as guides and spacers for thread, which is wrapped around the block/screws. Using this wood block, press the surface of agar plates horizontally, then vertically to make a grid of small squares on the surface of the agar plates. Using the razor blade, carefully cut the surface of agar plates along the scars to produce uniform agar blocks.

Application of Agar Block. Carefully pick up a agar block with forceps and place it on the elongation zone of root which is 4-6 mm from the root tip. The agar blocks, which may or may not contain hormones, are placed on the top or bottom surface of root depending on the experiment.

![Figure 2. Design of wood block for making the scar on the surface of agar plates to prepare the agar blocks.](image)
Preparation of Seedling Holder. A holder for the seedlings is made with pieces of Plexiglas as shown in Figure 3. The size of the bottom and top portion of holder is 50 x 70 mm, and the stand itself is 30 x 150 mm. This holder will allow placement of 10 seedlings along each side. To maintain humidity around the holders, individual humidity chambers can be prepared by removing the mouth-end from 500 ml tissue culture flasks. A square of paper towel is moistened and placed against one of the inside walls of the flask. Inexpensive humidity chambers can be made by cutting the top from 2 liter plastic beverage bottles. The lower half of the bottles can be lined with moist paper towels and inverted over the seedling holder. For large classes, a small aquarium (2-5 gallon) can be lined with moist towels; a square of window glass can be used as a lid. The key to the success of this experiment is to maintain a very high humidity level within the chamber.

EXPERIMENTAL PROCEDURE

1. Prepare the humidified chamber with paper towels and distilled water.

2. Select 50 seedlings with primary roots of 1.5-2.0 cm in length.

3. Place the seedlings in a horizontal orientation on the Plexiglas holder with window putty (Figure 3). Ten seedlings should be placed along each side of the holder. In this experiment, 3 holders should be used.

4. Place the holders into humidified chambers after applying agar block to the elongation zone of roots as follows:

a) plain agar blocks on both side (control).

b) top: 0.1 mM AbA;
   bottom: plain agar block

c) top: plain agar block;
   bottom: 0.1 mM AbA

d) top: 0.01 mM IAA;
   bottom: plain agar block

e) top: plain agar block;
   bottom: 0.01 mM IAA

5. Incubate the roots in the humidified chamber for 1 hour. Observe the curvature periodically.

6. If a time lapse video cassette recorder is available for use in observing the curvature, the roots may be allowed to respond for several hours or overnight.

7. If a time lapse video cassette recorder is not available, take the holder to the dark room after 1 hour. Place the photographic paper behind the holder, then illuminate the light for a very short time (approx. 1 sec) to get the shadow of roots. Develop the photographic paper. A shadowgraph will be produced with a black background and white root shapes. Measure the degree of curvature with a protractor.

OBSERVATION AND QUESTIONS

Measure the curvature of roots which are applied the agar blocks containing IAA or AbA. Compare the effect of IAA and AbA on the gravitropic curvature.

Is there any difference in the presentation time and rate of curvature between the treatments with the agar blocks containing hormones on top and bottom portion of roots?

What is the major difference between control and hormone treated roots?

SUGGESTIONS FOR ADDITIONAL EXPERIMENTS

1. Measure and compare the root elongation rate in the presence of 0.01 mM IAA or 0.1 mM AbA during short (up to 12 hours) and long time periods (up to 3 days).

2. Apply other plant hormones such as gibberellic acid, kinetin, or ethylene (in the form of Ethephon) to the roots.

3. Apply ethylene agonists, such as silver ions, AVG (1 μM) or cobalt ions to the gravireacting roots. The interaction of auxin and ethylene in root elongation has been well documented (see Mulkey et al, 1982).


Yamaguchi, T., and H. E. Street. 1977. Stimulation of the growth of excised cultured roots of soya
**News and Views**

**Curriculum Vitae**

**Candidates in AMCBT Elections**

**Presidential Candidates:**

**LEONA TRUCHAN**

**Office Address:**

Alverno College  
Natural Sciences, Mathematics, and Technology Division  
Milwaukee, WI 53215

**Education:**

1970  
Ph.D., Biological Sciences: Ecology, Northwestern University, Evanston, IL

1963  
M.S., Biological Sciences, Developmental Biology, DePaul University

1953  
B.A., Biology, Alverno College, Milwaukee, WI

**Recipient of Federal and Private Grants for research/study (Partial List):**

1994-1996 Philip Morris "Teaching for Tomorrow," National Program

1993-1996 Framework Development Team Member, Eisenhower National Prototype Grant, "Changing Perspectives"

1990-1992 Kellogg Grant "Reconceptualize the Integrate Science Course"

1995 Alverno Institute Fellowship (Use supertext to create prototype for Microbiology Students)

1991-1992 Alverno Institute Fellowship (create a capstone molecular biology experience)

1985 Alverno Institute Fellowship (incorporate technology into biology curriculum

1988 University of Wisconsin, Madison, "Rapid Cycling Plant Workshop"

1987 University of Chicago, Photobiology Course, National Science Foundation

1986-1988 Recombinant DNA Courses (Cold Spring Harbor), National Science Foundation and Fotodyne funded, University of Wisconsin, LaCrosse

1994, 1985 Invitational Conference on Science Education in Wisconsin, Wingspread: Johnson Foundation

**Presentation and Consultant Positions (Partial List):**

1994  
Workshop of preparing "New College Teachers for the 21st Century." at ASM and AIBS annual meeting

1992-1994 Consultant to 7 community colleges in South Carolina

1993  
Chair of Steering Committee for Coalition of Life Sciences (CELS III), Woods Hole, MA

1990 Keynote Speaker at 1st National Undergraduate Biology Workshop, Butler University;

**Honors and Awards (Partial List):**

Recipient and Director of Eisenhower Higher Ed Competitive Grant Program, 1993, '94, '95;  
Lead Consultant in year-long in-service for Science and Mathematics Specialty Teachers in Magnet Schools - Title II Grant;

Visiting Fellow, Philip Institute of Technology, Coburg Campus, Victoria Australia;

Institute of Catholic Higher Education, Mercy Campus, Ascot Vale, Australia, 1987;

Professional Merit Award: Alverno Alumnae Association, 1985;

Outstanding of Teachers of Adults Award;

Outstanding Teacher Award;

Uhrig Foundation Award for Teaching Excellence.

**Memberships:**

American Society of Microbiology; American Institute of Biological Society; American Women in Science; Association for Biology Laboratory Education (President 1989-91; Board Member 1987-
1993; Association of Midwest College Biology Teachers; Coalition of Education in Life Sciences (CELS III - co-Chaired Steering Com. 1992-1993); National Science Teacher Association (Co-chaired College Vision Task Force on Undergraduate Education 1992-1993; Member of Task Force K-16); Society for College Science Teachers (Board Member 1990-1994; chaired 4 regional meetings); Wisconsin Science Educators (President 1983-84; 1988-89)

Select Publications:

Current Research:
Effective Science Teaching and Scholarship; Science Teaching and Science Misconception Barriers; Science Teaching and Use of Technology to Enhance Learning

**************************

ETHEL STANLEY

Office Address: Millikin University
Biology Department
1184 West Main Street
Decatur, IL 62522

Education:
In Progress  Ed.D. Curriculum and Instruction, Illinois State University, Bloomington, IL
1989  Secondary Teaching Certificate, Millikin University, Decatur, IL
1976  M.S., Biology, Wayne State University, Detroit, MI
1973  B.S., Biology, Wayne State University, Detroit, MI
1968-1971  Biology Major, Thiel College, Greenville, PA

Professional Experience:
1988-1995  Visiting Instructor, Millikin University, Decatur, IL
1984-1988  Teaching Fellow, Millikin University, Decatur, IL
1980-1984  Systems Analyst/Programmer Manager, CNA, Chicago, IL
1978-1980  Programmer/Analyst, Wayne State University, Detroit, MI
1975-1978  Instructor, Oakland Community College, Detroit, MI
1972-1975  Graduate Teaching Assistant, Wayne State University, Detroit, MI
1970-1971  Undergraduate Lab Assistant, Thiel College, Greenville, PA

Select Publications:
Select Presentations:
1994  "In Search of 3 Year Old Tilia Trees: Misconceptions in Biology." NABT Conference, November.
1993  "Pass the Videocam, Please." AMCBT Conference, Decatur, IL, October.
1991  "Students in Transition." NABT Conference, Houston, TX, November.

Memberships:
Botany Society of America, Association of Midwest College Biology Teachers - Board Member, American Institute of Biological Sciences, National Association of Biology Teachers, Middle Illinois Science Educators - Board Member, Sigma Zeta, Alpha Lambda Delta - Faculty Advisor, Environmental Affairs Council - Faculty Co-Advisor

Recent Grants:

****************************

Secretary Candidates

DONALD B. HOAGLAND

Office Address: Westfield State College
               Biology Department
               Western Avenue
               Westfield, Massachusetts 01085

Education:
1988  Ph.D., Dept. Zoology, University of Vermont, Burlington, VT
      Specialization in mammalian biology
1980  M.S., Dept. Biology, Northern Arizona University, Flagstaff, AZ
      Specialization in mammalian ecology
1977  B.A., Dept. Biology, State University College at Potsdam, NY

Professional Experience:
1994  -  Assistant Professor, Westfield State College, MA
1991 - 1994  Assistant Professor, McPherson College, KS
1988 - 1991  Assistant Professor, University of Kansas, Lawrence, KS
1989 - 1991  Instructor, University of Kansas Medical Center, Kansas City, KS
1985 - 1986  Lecturer, Trinity College, Burlington, VT
1982 - 1983  Instructor, State University College at Potsdam, NY

Courses Taught:
Environmental Biology, Comparative Vertebrate Anatomy, Vertebrate Physiology, Biological Concepts, Genetics, Ecology, General Biology, Human Anatomy, Human Physiology, Animal

Memberships:
American Association for the Advancement of Science, American Society of Mammalogists, Association of Midwestern College Biology Teachers, National Science Teachers Association, Society for Molecular Biology and Evolution, Society of Systematic Biology, Southwestern Association of Naturalists

Professional Service:
1st Vice President, AMCBT, 1994-1995
Local Committee, 75th Annual Meeting of the American Society of Mammalogists, University of Vermont, 1995

Select Publications:

Select Papers Presented:

******************************

WALLACE R. WEBER

Office Address: Department of Biology
Southwest Missouri State University
Springfield, Missouri  65804

Education:
1968 Ph.D., Ohio State University
1959 M.S., Southern Illinois University
1956 B.A., Southern Illinois University

Professional Experience:
1978 - present  Professor of Biology, Southwest Missouri State University
1971 - 1978  Associate Professor of Biology, Southwest Missouri State University
1967 - 1971  Assistant Professor of Biology, Southwest Missouri State University
1962 - 1967  Instructor, Ohio State University, Columbus, OH
1962  Graduate Teaching Assistant in Botany, Ohio State University, Columbus, OH
1962  Instructor in Summer Science Training Program for high school students, Ohio University, Athens, OH
1959 - 1962  Instructor in Biology, Otterbein College, Westerville, OH
1956 - 1959  Graduate Teaching Assistant in Botany, Southern Illinois University, Carbondale, IL

Research and Scholarly Interests:
Flora of Missouri, Atlas Project on Missouri Flora Distribution, Biosystematics of Silphium asteriscus complex, Ecophysiology of Geocarpon minimum, ongoing project of manual writing for
use in local flora courses ("Missouri's Spring Flora" and "Woody Plants of Missouri"), the floristics of Camps Clark, Crowder, and Macon in Missouri

Memberships:

Professional Service:
Member of the Board of Directors of the Missouri Prairie Foundation, 1979-1981
Secretary of the Missouri Prairie Foundation, 1981-present
Member of Board of Directors of Missouri Native Plant Society, 1980-present
Co-editor of Missouri Botanical Record (a register for the update of state and county records in the Journal Missouriensis), 1981-present
Member of Steering Committee, Association of Midwest College Biology Teachers

************************************************

Steering Committee Candidates:

JOYCE V. CADWALLADER

Office Address: Saint Mary-of-the-Woods College
                Department of Science and Mathematics
                Saint Mary-of-the-Woods, Indiana 47876

Education:
1974      Ph.D., Life Sciences - Physiology, Indiana State University
1969      M.S., Psychology, Indiana State University
1967      B.A., Biology (Psychology Minor), Western College for Women

Professional Experience:
1991-      Professor of Biology, Saint Mary-of-the-Woods College, Indiana
1982-1986  Chairperson of Biology, Saint Mary-of-the-Woods College, Indiana
1984-1991  Associate Professor of Biology, Saint Mary-of-the-Woods College, Indiana
1977-1978  Adjunct Assistant Professor of Psychology, Indiana State University
1979-1981  Postdoctoral Trainee, Developmental Biology, University of Wisconsin, Madison
1976-1979  Assistant Professor of Biology, Saint Mary-of-the-Woods College, Indiana
1974-1976  Assistant Professor of Psychology, Saint Mary-of-the-Woods College, Indiana
1969-1972  National Defense Education Act Title IV Fellow, Indiana State University
1968-1969  Teaching and Graduate Assistant, Indiana State University

Courses Taught:
Biology: Basic Anatomy and Physiology, Embryology, Comparative Vertebrate Zoology, Medical Terminology, Histology, Readings in Biology, Health and Nutrition, Cell Biology, Topics in Biology, Math/Science Topics, Genetics, Principles of Biology, Medical Biology, Biology of Aging, Human Physiology and Our Bodies; Our World, Psychology: Tests and Measurements, Physiological Psychology, Human Sexuality, Psychology of Women
Memberships:
American Association for the Advancement of Science, American Psychological Association, Association of Midwestern College Biology Teachers, Association of Women in Science, Council on Undergraduate Research, Sigma Xi, Sigma Zeta

Selected Publications:

Honors and Awards:

*******************************************************************************

TERRY L. DERTING

Office Address:
Murray State University
Department of Biological Sciences
P. O. Box 9
Murray, Kentucky 42071-0009

Education:
1986 Ph.D., Ecology, Evolution, and Organismal Biology, Indiana University, Bloomington, IN
1981 M.S., Zoology, Virginia Polytechnic Institute and State University, Blacksburg, VA
1978 B.A., Biology, Mount Holyoke College, South Hadley, MA

Professional Experience:
1993 - Assistant Professor of Biology, Murray State University, KY
1991-1992 Adjunct Professor, Department of Biology, Beloit College, WI
1989 - 1991 Assistant Professor - Temporary, Hollins College, VA
1987 - 1989 Assistant Professor - Temporary, Radford University, VA

Courses Taught:
Physiological Ecology, Ethics in Biology, Comparative Anatomy, Developmental Biology, Ethology, Human Anatomy, Vertebrate Ecology, General Biology, Zoology, Genetics, Histology, Human Physiology, Vertebrate Embryology

Research Interests:
Physiological and behavioral ecology of mammals, proximate and ultimate controls of life-history variation, habitat and resource partitioning, conservation strategies for preservation of biodiversity, ethics in undergraduate education

Memberships:
Sigma Xi, American Association of University Women, Association of Midwestern College Biology Teachers, Council on Undergraduate Research, BioQUEST Curriculum Consortium, American Society of Mammalogists

Curriculum Vitae of Candidates
Honors and Awards:
1991  Sigma Xi, Election to Full Membership
1987  Gerry-Eloise Fellowship, Sigma Delta Epsilon, Graduate Women in Science
1986  A. Brazier Howell Award for Graduate Research, American Society of mammalogists
1985  Eigenmann-Eiler Summer Scholarship, Indiana University
1984  Junior Investigator Research Award, Innovative Research of America

*****************************************************************************

CLAIREE A. RINEHART

Office Address:  Western Kentucky University
                Department of Biology
                1 Big Red Way
                Bowling Green, KY  42101-3576

Education:
1977  B.S., Microbiology, Brigham Young University, Provo, UT
1979  M.S., Botany, Brigham Young University, Provo, UT
1984  Ph.D., Botany, University of Georgia, Athens, GA

Professional Experience:
1988 - present  Assistant Professor, Dept. of Biology,
                Western Kentucky University, Bowling Green, KY
1987 - 1988  Research Associate, Institute for Molecular Virology,
              University of Wisconsin, Madison, WI
1984 - 1944  Postdoctoral Fellow, Institute for
              Molecular Virology, University of Wisconsin, Madison, WI

Courses Taught:
Introduction to Molecular Biology, Recombinant Gene Technology, Molecular Genetics,
Biological Instrumentation, Virology

Research Interests:
Molecular mechanisms of the plant virus Southern Bean Mosaic Virus. Molecular/genetic
analysis of protein structure vs function

Memberships:
American Association for the Advancement of Science
Association of Mid-Western College Biology Teachers
American Society for Virology
Kentucky Academy of Science
Sigma Xi

Honors and Awards:
NIH Viral Oncology Training Grant Postdoctoral Fellow, 1984 - 1987
Brookhaven National Laboratory Symposium on Photosynthetic Carbon Metabolism, 1978

*****************************************************************************

RICHARD WILSON

Office Address:  Rockhurst College
                Department of Biology
                Kansas City, MO  64110

VOL 21(2): July 1995  BIOSCENE  23
CALL FOR NOMINATIONS

The Society for College Science Teachers and Kendall/Hunt Publishing Company are soliciting nominations (including self-nominations) for the 1996 Outstanding Undergraduate Science Teacher Award. This national award, recognizing an outstanding teacher of natural science at the undergraduate level, will be presented at the 1996 SCST/NSTA annual convention in St. Louis, Missouri in March 1996.

The Outstanding Undergraduate Science Teacher Award recognizes and rewards achievements and contributions in the enhancement of science education by teachers of undergraduate science. Nominees must have been actively engaged in teaching undergraduate science over the past five years. The award consists of a $1,500 monetary award, a plaque attesting to the awardee’s accomplishment, a complimentary joint SCST/NSTA one-year membership, an expectation to speak at the 1996 SCST/NSTA College Luncheon in St. Louis, and up to $500 for reimbursement of expenses incurred for travel to participate in the 1997 SCST/NSTA National Convention, where the recipient will present the Marjorie Gardner Lecture.

The awardee will be selected based on achievements and contributions made in the following categories:

TEACHING EXCELLENCE evidenced through teaching philosophy and effectiveness, teaching innovations, and course and curricula development;

SCHOLARSHIP evidenced through publications in science education, presentations, grants received, and other forms of scholarship, and;

SERVICE to science education, students, the profession, scientific & education organizations, the awardee’s institution, local teachers and their school systems, and the general public with the overall goal of enhancing understanding of scientific issues.

Selection of the awardee by the Executive Board of SCST is based upon recommendation by the Outstanding Undergraduate Science Teacher Award Committee. This committee consists of SCST members and a representative from the Kendall/Hunt Publishing Company, who review and evaluate the documentation provided by the nominee. Information detailing the specific materials to be submitted will be sent to all nominators and nominees.

To nominate yourself or a colleague complete the enclosed coupon and mail it no later than September 22, 1995 to:

Dr. Eileen Gregory, Chair
Outstanding Undergraduate Science Teacher Award Committee
Rollins College
1000 Holt Avenue
Winter Park, FL 32789-4499

FAX: (407) 646-2479

24 Teaching Award Nomination Form SCST
NOMINATION FORM

SOCIETY FOR COLLEGE SCIENCE TEACHERS (SCST) 
AND KENDALL/HUNT PUBLISHING COMPANY 
1996 OUTSTANDING UNDERGRADUATE SCIENCE TEACHER AWARD

I nominate the person named below for the SCST-Kendall/Hunt 1996 Outstanding Undergraduate Science Teacher Award. If nominating a colleague, I will inform him or her of my intention to nominate and will assist the candidate in assembling and forwarding the documentation to the Chair of the Award Committee in a timely manner for a confidential review by the Committee. I further agree to serve as a contact person on the candidate’s behalf.

Nominee’s Name ________________________________

Title _________________________________________

Department ____________________________________

College/University ______________________________

Street _________________________________________

City________________________ State __________ Zip __________

********************************************************************************

Nominator’s Name ________________________________

Title _________________________________________

Department ____________________________________

College/University ______________________________

Street _________________________________________

City________________________ State __________ Zip __________

Mail by September 22, 1995 to: Dr. Eileen Gregory, Chair, Outstanding Undergraduate Science Teacher Award Committee, Rollins College, 1000 Holt Avenue, Winter Park, FL 32789-4499
As of the time that *Bioscene* went to press, final room assignments, registration forms, and housing information was not received. Therefore, please expect that information to arrive separately. If you need any more immediate information, please contact the AMCBT Local Arrangements Chairperson, Dr. Pat Bowne, Department of Biology, Alverno College, 3401 S. 39th St., P. O. Box 343922, Milwaukee, WI 53224-3922; email: pbowne@omnifest.uwm.edu; FAX: (414)-382-6354; Voice: (414)-382-6077.

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**Thursday, September 28**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Location</th>
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<tr>
<td>6:00-8:00 p.m.</td>
<td>REGISTRATION RECEPTION</td>
<td>Nursing Building</td>
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<tr>
<td>8:00 p.m.</td>
<td>OPENING SESSION</td>
<td>Nursing Building, Wehr Hall</td>
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<td>Welcome for AMCBT</td>
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<td><em>Donald “Buzz” Hoagland, Program Chair</em></td>
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<td><em>Patricia Bowne, David Ferris, Leona Truchan, Local Arrangements</em></td>
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<td>WELCOME to Alverno College</td>
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<td>PRESIDENTIAL GREETING: Harold Wilkinson</td>
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<td>9:00 p.m.</td>
<td>EXECUTIVE COMMITTEE MEETING</td>
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<td><em>(to immediately follow opening session)</em></td>
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<tr>
<td>6:00-8:00 p.m. &amp; 9:00-12:00 midnight</td>
<td>OPEN COMPUTER LAB- software previews, electronic bulletin board, etc.</td>
<td>Nursing Building</td>
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**Friday, September 29**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:00 a.m.</td>
<td>REGISTRATION</td>
<td>Alumni Hall</td>
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<td>7:00-8:10 a.m.</td>
<td>BUFFET BREAKFAST</td>
<td>Alumni Hall</td>
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<td><em>(price included in registration)</em></td>
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<td></td>
<td>Interest Groups by Discipline</td>
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</table>
7:30-12:00 am  FIELD TRIPS I
1. Wehr Nature Center/Mitchell Park (Birding) [$8.00]
2. Cedarburg Bog [$9.00]
3. Boerner Botanical Gardens/Mitchell Park [$8.00]

8:30-12:00 am  FIELD TRIPS II
1. Milwaukee Public Museum [$10.00]
2. Miller Brewery & Jones Island Milorganite Plant - no cost

12:00-1:00 p.m.  OPEN LUNCH

1:00-2:00 p.m.  KEYNOTE ADDRESS  Alumni Hall
Eating the Apple, Knowledge and Responsibility
in the Age of Science
John Devereux, GCG Technologies, Madison, WI

2:10-5:00 p.m.  CONCURRENT WORKSHOP SESSION I
1. MACROMEDIA DIRECTOR AS AN AUTHORING TOOL
   Mark Bergland and Karen Klucz, University of Wisconsin, River Falls
2. PROTEIN AND NUCLEIC ACID ANALYSIS
   BY COMPUTER AS A TEACHING TOOL IN
   UNDERGRADUATE MOLECULAR BIOLOGY
   Andrew S. Hopkins, Alverno College, Milwaukee, WI
3. USING FYRITE BRAND GAS ANALYSERS TO MEASURE
   HUMAN METABOLIC RATE
   Mindy Mymudes, Alverno College, Milwaukee, WI

3:25-4:15 p.m.  COFFEE, POSTERS, EXHIBITS  Alumni Hall

4:15-5:00 p.m.  CONCURRENT PAPER SESSION I
1. FRACTAL GEOMETRY IN BIOLOGY
   John R. Jungck, Beloit College, Beloit, WI
2. CONSTRUCTING ONE-PAGE TAXONOMIC KEYS
   Norman Waldow, Maryville University, St. Louis, MO
3. PC MULTIMEDIA TOOLS FOR FIELD BIOLOGY
   Ethel Stanley, Millikin University, Decatur, IL
4. "INSPIRATION": CONCEPT-MAPPING SOFTWARE TO
   ASSIST STUDENTS TO COPE WITH INFORMATION
   OVERLOAD
   Leona Truchan, Alverno College, Milwaukee, WI

5:00-6:00 p.m.  BIOSCENE EDITORIAL BOARD MEETING

6:00-7:00 p.m.  SOCIAL HOUR  Alumni Hall

7:00 p.m.  BANQUET (price included in registration)
8:00 p.m.  BANQUET SPEAKER
"Methanol Toxicity"
Janis Eells, Medical College of Wisconsin, Milwaukee, WI

9:00-12:00 midnight  INTERNET WORKSHOP  (World Wide Web; CDC, NMFS, THOMAS & govenern Home Pages; AMCBT Home Page; etc.)
Tim Mulkey, Indiana State University, Terre Haute, IN
Buzz Hoagland, Westfield State College, Westfield, MA

Saturday, September 30

8:00-9:15 a.m.  CONTINENTAL BREAKFEAST  Alumni Hall
Interest Groups by Discipline

8:30-10:30 a.m.  BALLOTING  Alumni Hall

9:20-11:00 a.m.  CONCURRENT WORKSHOP SESSION II
1.  TEACHING HUMAN BIOLOGY: A WORKSHOP FORMAT
Marc M. Roy and Marion Field Fass, Beloit College, Beloit, WI
2.  JUMP-STARTING STUDENT CONCEPT MAPS
Suzanne L. Martin, Moberly Area Community College, Moberly, MO
3.  POWERPOINT: USER-FRIENDLY PRESENTATION GRAPHICS SOFTWARE
Dianne Y. Bell, Avila College, Kansas City, MO

9:25-10:10 a.m.  CONCURRENT PAPER SESSION II
1.  DOES WRITING ABOUT BIOLOGY ENHANCE LEARNING ABOUT BIOLOGY?
Randy Moore, The University of Akron, Akron, OH
2.  MOLECULAR BIOLOGY AND ON-LINE CURRICULA
Claire Rinehart, Western Kentucky University, Bowling Green, KY
3.  VIRTUAL RESEARCH IN A VIRTUAL LIBRARY
Arthur Messier, Westfield State College, Westfield, MA

10:10-10:30 a.m.  BREAK

10:30-11:00 a.m.  CONCURRENT PAPER SESSION III
1.  DATA ACQUISITION IN THE PHYSIOLOGY LABORATORY
Steven H. Mills, Central Missouri State University, Warrensburg, MO
2.  DARWIN'S FINCHES AND BEYOND: EVOLUTION AND CONSERVATION BIOLOGY IN THE GALAPAGOS ISLANDS
David J. Hicks, Manchester College, N. Manchester, IN
3.  RESOURCE FOR SCIENCE EDUCATION PROGRAM AT NCSA
Umesh Thakkar, NCSA Education and Outreach, Champaign, IL
4.  SEQUENTIAL USE OF CASE STUDIES TO TEACH INVESTIGATIVE SKILLS AND INTERDISCIPLINARY VIEWS OF SCIENTIFIC QUESTIONS
Terry L. Derting, Murray State University, Murray, KY
11:00-12:30 p.m. **LUNCHEON** (price included in registration fee)
**BUSINESS MEETING**

12:35-1:15 p.m. **EXECUTIVE COMMITTEE MEETING**
[N.B. Remember that newly elected officers must attend this very important planning meeting.]

## Abstracts of Paper/Workshop Sessions

**OPENING ADDRESS: MAKING SENSE OF NEW MEDICAL TECHNOLOGIES.**
Suzanne Amador, Haverford College, Haverford, PA
Physicians can now diagnose and treat disease using medical physics devices such as ultrasound imaging, computer tomography (CAT) scans, and magnetic resonance imaging (MRI). These exciting new medical technologies provide an excellent tool both for motivating students to study science, and for conveying much basic introductory physics. This talk will explain how one physics department uses medical physics and biophysics in different levels of the curriculum, as a way to interest premedical students and biology majors, and to broaden the training of its own majors.

**Workshop Session I**

**WI.1. MACROMEDIA DIRECTOR AS AN AUTHORIZING TOOL FOR THE DEVELOPMENT OF EDUCATIONAL SIMULATIONS.**
Mark Bergland and Karen Klyczek, University of Wisconsin - River Falls, River Falls, WI
This workshop will enable participants to get first-hand experience with one of the most powerful authoring systems for either the Macintosh or Windows operating systems. After a demonstration of basic techniques, participants will create their own interactive simulations and will discuss ways in which computer simulations can be used to enhance courses which they teach.

**WI.2. PROTEIN AND NUCLEIC ACID ANALYSIS BY COMPUTER AS A TEACHING TOOL IN UNDERGRADUATE MOLECULAR BIOLOGY.**
Andrew S. Hopkins, Alverno College, Milwaukee, WI
This workshop will explore the utilization of nucleic acid and protein sequence databases to create novel and challenging opportunities for students. Participants will be introduced to methods for accessing, searching and retrieving information from these databases, and to computer analysis of the data. Implementation of this information in laboratory experiments will be covered. Examples will be presented of searches conducted by e-mail through the "Blaster" server at the National Center for Biotechnology Information (NCBI) and of a variety of analyses available with the Genetics Computer Group (GCG) collection of programs. These include: searching for sequence by acquisition number, keywords or specific elements of sequence information; analysis of sequence for specific motifs; generation of multiple sequence alignments; analysis of phylogenetic relationships and generation of phylogenetic trees; downloading of files to a local terminal; generation of maps of restriction endonuclease cleavage. The sequence files contain references to original literature where the methodology of the derivation of the sequence is presented. Students can review this information and learn specific applications of modern techniques to the analysis of human genetic disease. Computer design of Polymerase Chain Reaction (PCR) primers will demonstrate the creation of laboratory experiments where students can; i) acquire and analyze sequence data, ii) design PCR primers to amplify a gene incorporating restriction targets flanking the amplified fragment, iii) clone the amplified DNA into an expression vector, iv) screen and recover recombinants, and v) isolate and characterize the expressed gene product. If participants come with information on particular genes, searches can be conducted to acquire and analyze those sequences.
Concurrent Paper Session I

PI.1. FRACTAL GEOMETRY IN BIOLOGICAL SYSTEMS.
John R. Jungck, Beloit College, Beloit, WI
Fractal dimensions of 2.73 seem of little use to the average science student or teacher used to Euclidean dimensions; however, such fractal "fantasies" have enormous utility in measuring distinguishing features of a wide variety of biological systems. Fractal mathematics and computer software tools will be illustrated to share the beauty and utility of fractal analyses form the molecular to the morphological level. The aesthetics of "irregular," missshapened," "fractured," "asymmetric," etc. objects will be elaborated for new appreciations.

PI.3. PC MULTIMEDIA TOOLS FOR FIELD BIOLOGY
Ethel Stanley, Millikin University, Decatur, IL
A visual twig key, introduction to spiders, identification of insect orders, interactive tree map of campus, pre-field trip to Lake Michigan, and introduction to plant families are presented as examples of both instructor and student authored programs designed for field investigations. A short demonstration of ToolBook to create a runtime program for the PC using a photoCD will be highlighted.

INTERNET WORKSHOP
Tim Mulkey, Indiana State University, Terre Haute, IN and Buzz Hoagland, Westfield State College, Westfield, MA
This workshop will be divided into two simultaneous sessions where participants will access the Internet and the World Wide Web via PCs or Macintosh Computers. Participants will learn how to establish a PPP and/or SLIP connection to the Internet. Freeware WWW browsers, including Mosaic, Netscape, and ElNet’s Web search will be used by participants to search the WWW during this late night foray into cyberspace. Cyberjunkies will search ElNet Galaxy, WWW Virtual Library, GNN - Whole Internet Catalog, and Lycos for . . . . Tim and Buzz’s favorite WWW sites will be demonstrated and lists of URLs (addresses) for these sites will be made available. We will also attempt to demonstrate the relevance of the WWW to classroom instruction.

Workshop Session II

WII.1. A HANDS-ON, EXPERIMENTAL APPROACH TO TEACHING HUMAN BIOLOGY
Marc M. Roy and Marion Field Fass, Beloit College, Beloit, WI
In order to involve beginning students in the process of science, we redesigned our introductory Human Biology course to focus on question posing, problem solving and communicating about Biology. Participants in this session will experience an abbreviated version of a day in our new Human Biology course, which is now taught in a workshop format. We will first engage the participants in several activities that typify our approach. We will then discuss why we implemented a workshop format for the teaching of human biology, our goals and strategies for the course, and the results of the first year. Participants are encouraged to share ideas for the improvement of the course.

WII.2. JUMP-STARTING STUDENT CONCEPT MAPS
Suzanne L. Martin, Moberly Area Community College, Moberly, MO
Concept maps are diagrams consisting of concepts (terms) connected by explicit relationships (links). Participants in this workshop will construct and analyze concept maps in cooperative activities which they can adapt for their own students. Building concept maps helps students acquire strategies for synthesizing, retaining, and applying information. Students working alone often resist learning to build maps because they have trouble getting started. Interaction with peers and the instructor overcomes resistance by helping the students determine the relative significance of concepts and construct meaningful relationships. The workshop includes guidelines for using maps and student-generated examples.

WII.3. POWERPOINT: USER-FRIENDLY PRESENTATION GRAPHICS SOFTWARE
Dianne Y. Bell, Avila College, Kansas City, MO
Presentation software may be used to produce professional-looking slides, overheads, and handouts for the classroom. One popular and user-friendly package is PowerPoint, a Microsoft product which may be purchased separately or as a part of the MSOffice package. PowerPoint contains over 100 professionally-designed templates which can be used to quickly produce
slides, overheads, outlines, speaker’s notes and handouts, all in full color or black and white. Clipart from its extensive catalog or from other commercially available packages, text, spreadsheets, and graphs can be imported from external sources. A lecture prepared in PowerPoint converts readily into student handouts. Workshop participants will learn the basics of creating a presentation in PowerPoint, including how to customize, import artwork, and prepare overheads and handouts. Each person or small group will prepare a short PowerPoint sampler and present it to the workshop. All participants will receive a diskette with their sampler, the workshop slides, and a Runtime version of PowerPoint.

Concurrent Paper Session II

PIII.1. DOES WRITING ABOUT BIOLOGY ENHANCE LEARNING ABOUT BIOLOGY?
Randy Moore, The University of Akron, Akron, OH
Many biologists use writing to help enhance students’ ability to write and learn about biology. This teaching strategy comes at a “cost” as namely, the time required to grade the term papers, essays, etc. associated with a writing-intensive course. But does all this writing really enhance learning? In this talk, I will summarize the results of ongoing studies of the use of writing as a tool for teaching biology. Specifically, I will discuss the importance of teaching students to write effectively about biology, the “costs” and benefits of using writing to teach biology, how to teach students to use writing as a tool for learning biology, and how to handle the paperwork associated with writing to learn biology. I hope to convince you that 1) much of what students write about biology does not enhance learning about biology, and 2) writing about biology enhances learning only when students first understand how to use writing as a tool for learning. The approach that I will advocate differs significantly from that of a typical “writing intensive” course.

PIII.2. MOLECULAR BIOLOGY AND ON-LINE CURRICULA
Claire A. Rinehart, Western Kentucky University, Bowling Green, KY
Example of molecular biology course materials distributed over the World Wide Web for use in lectures and in out-of-class student reviews. Demonstrates how various media and program types can be integrated into a coherent hypermedia package. Shows advantages of providing links to current on-line resources.

PIII.3. VIRTUAL RESEARCH IN A VIRTUAL LIBRARY
Arthur Messier, Westfield State College, Westfield, MA
So, your campus finally established a connection to the Internet, or you took out a second mortgage on your home and purchased a computer with a modem because new astro-turf was needed for the Administration’s outdoor putting green. You turn on the ignition switch, watch the lights in the neighborhood grow dim, and feel the awesome power as your machine inches it’s way onto the Information Superhighway. Watchout, packets of 8 bits at parity are zooming by at 56,000 bps! It is dangerous out here in cyberspace. The roadsigns, when present, are written in cyberspeak.

However, after much frustration and more patience than was required for meeting the margin requirements of the Graduate College, you finally arrive at your destination, Big Midwestern University Library to begin your search. Thirty minutes later, after traveling the beltway with no recognizable exit signs, you give up and go home. What happened?

Searching the World Wide Web can be tedious at best and frustrating or a pain in the . . . at worst. Often when you find a site that sounds like it could be the Holy Grail, you type in the URL only to find that it is not available at this time. On those occasions when you do connect, you find that the system was designed for insiders and outside cyberpunks, which you are not. The frustrations and successes of a Head Reference Librarian, at a small undergraduate liberal arts college, assisting undergraduates conduct research via the Internet will be discussed.
rating both as useful or very useful compared to the “physiograph” chart recorders. Both CDAS were found to be more convenient and easier to use in preparing lab reports. Requested instructions for using the CANDS were made available on HyperCard which is immediately available as the MacLab CADS is used. “On-line” processing of data (i.e., data stream averaging) makes recording of evoked potentials possible in “noisy” laboratories without electronic filters or “noise” isolation devices. Network connection to all recording stations to a file server not only simplifies retrieval, evaluation, and compiling of data, but also permits analysis of the compiled data via the campus network on a 24-hour basis. Support was provided by the National Science Foundation’s Division of Undergraduate Education through grant DUE-#9452535.

**PIII.2. DARWIN’S FINCHES AND BEYOND: EVOLUTION AND CONSERVATION BIOLOGY IN THE GALÁPAGOS ISLANDS**

*David J. Hicks, Manchester College, N. Manchester, IN*

Despite more than a century and a half of research since Darwin’s visit, the Galápagos continue to provide inspiration for research. Some recent studies of speciation and evolutionary radiation of plant and animal groups will be reviewed. Despite the protected status of the archipelago, the native biota faces strong impacts from human activities, and these will also be discussed.

**PIII.3. RESOURCE FOR SCIENCE EDUCATION PROGRAM AT NCSA**

*Umesh Thakkar, National Center for Supercomputer Applications Education and Outreach, Champaign, IL*

The Resource for Science Education (RSE) program is intended to foster the development, by visiting educators working with NCSA staff and researchers, of curricula, programs of study, course modules, software, publications, and/or strategies that enhance and increase the use of visualization and networking methodologies in education. The goals of the RSE Program are: 1) discussion, dissemination, implementation, and evaluation of the uses of high performance computing in science and technology education; 2) exchange of ideas on software and network access needs for computational science education between researchers, educators and students; 3) collection and distribution of high performance computing educational strategies and programs; 4) raising awareness of and providing training in high performance computing and communications (HPCC) tools and techniques for interested educators and their organizations; and 5) development of collaborative proposals to develop resources that facilitate discovery-based learning and teaching.

**PIII.4. SEQUENTIAL USE OF CASE STUDIES TO TEACH INVESTIGATIVE SKILLS AND INTERDISCIPLINARY VIEWS OF SCIENTIFIC QUESTIONS**

*Terry L. Derling, Murray State University, Murray, KY*

Case studies have become an increasingly popular teaching tool. Most frequently, the cases studies published for use in biology courses are a series of independent scenarios with or without a set of discussion questions. I will present a teaching method in which related case studies are used sequentially; thereby stimulating more in-depth thinking and critical evaluation by students than the use of isolated cases. Using sets of related case studies students can gain insight into key similarities and differences among related scientific problems and the complex relationship between science, economy, cultural history, etc.
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