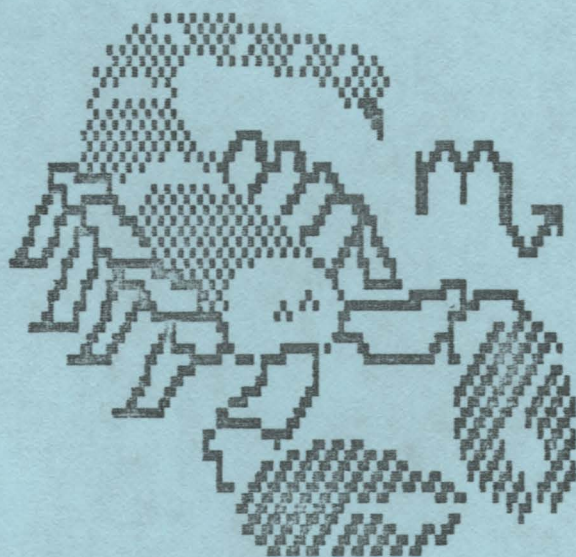


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RETIREMENT NOTICE

Paul J. Christian recently retired from teaching at Bethel College. We wish you well Paul.

Dear Members of the Association of Midwestern College Biology Teachers:

Recently I received a most gracious letter from Harold Wilkinson of Millikin University and Richard E. Wilson of Rockhurst College, thanking me for providing the after dinner talk this past fall at Augustana College. I felt honored to have been asked to give the program, and my wife and I very much enjoyed our evening with the members of the Association. I think it is commendable that this group of college biology teachers gets together to exchange information and to continue the lifelong process of learning. How quickly we could go stagnant without continuous efforts to update ourselves.

I also appreciated the fact that Dr. Wilkinson and Dr. Wilson sent a letter to my superintendent and principal. Thank you so much. I hope that someday we can meet again. Good luck in your continued efforts in the Association.

Sincerely,

Robert W. Motz, Biology Teacher
Rock Island High School

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CHINESE UNIVERSITIES: ANOTHER CHALLENGE TO AMERICAN EDUCATION

E. Russell TePaske, 411 North Francis, Cedar Falls, IA

Looking in upon a culture as an outsider and then interpreting what is occurring within it is subject always to errors of observation and of cultural bias. There is an increased risk of bias when the exposure is brief as this one was (only a month). On my side, however, was travel in many countries, both western and non-western with a resultant sense of educational proportion; a sense of educational unity amidst enormous cultural diversity. Knowing full-well that my perceptions base is limited and culturally biased, I have nevertheless decided to share some aspects of a Chinese exchange experience. In 1983, the National Commission on Excellence in Education told Americans that we were a nation at educational risk. The Chinese exchange suggested to me that America is a nation at educational risk in even more areas/ways than the 1983 report outlines.

The people of China have the most secure (least ambiguous) cultural identity of any I've ever met. The Chinese person is autonomous as an individual worker, as learner, as parent, as citizen, but most strikingly as a functional part of China's ongoing social and national history. Each person seemed to be rooted simultaneously in China's long history, in his/her local community and in an abiding faith in the People's Republic experiment. It seemed as though no person viewed him/herself either as disenfranchised or as separate from the corporate unit. In addition, there was a sense that all work had dignity and was self-actuating; that time must be savored; that recreation is intrinsically good and must be pursued aggressively but not frantically. Persons at recreation and persons at work seemed not unlike persons at leisure, at school or even at rest. The writer of Ecclesiastes may have been Chinese, as there was indeed a season

for everything under heaven...

"a time to be born, and a time to die; a time to plant and a time to pluck up what is planted;... a time to break down and a time to build up; a time to weep, and a time to laugh; ... a time to cast stones away, and a time to gather stones together; a time to embrace, and a time to refrain from embracing; ... a time to rend, and a time to sew; a time to keep silence, and a time to speak; a time to love, and a time to hate; a time for war, and a time for peace."

On several occasions I left my room at dawn, just to walk alone, separate from the University of Northern Iowa faculty exchange group with whom I traveled. Even at such an early hour senior citizens contorted in the slow-motion exercise tai gi guan and younger adults scrambled after balls on concrete, asphalt or earthen basketball and tennis courts. Bicyclists in blue (usually) people's jackets were swarming to factories, offices, and employment centers.

Six days shalt thou labor in China and on the seventh thou shalt help children with learning tasks, go shopping and socialize in public parks -- at least so it seemed to me. In late May the streets were basically clean but a fine film of powder-like dust seemed already to be cycling into motion foreshadowing, I thought, a dustiness in the dryer months to follow. The dust moved with us as we moved from location to location under the sycamore and eucalyptus (I think) trees that lined the streets and thoroughfares.

Ten-thousand tons of night soil are collected daily by bike cart from homes without plumbing in Shanghai alone. As a result, Shanghai and other Chinese cities were well supplied with vege-

tables. The intensiveness of Chinese agriculture, the maintenance of irrigation dikes, the matter-of-fact business of harvesting a field of winter wheat one day and plowing, flooding and planting it to rice the next day demonstrated why the Chinese have become able to feed the billion plus people who live in their own borders and still have some agricultural exports. Small wonder too, that Deng Xiaopeng, China's President, has been twice named man of the year by Newsweek magazine.

Our group visited 10 universities. Our exchanges ranged from perfunctory 3-hour campus visits, inclusive of pleasantries and facilities tours to exchanges that extended over 4 days inclusive of lectures that our group presented, banquets and Chinese opera. Chinese courtesy and hospitality were always in evidence, even when our presence must have been a nuisance if not a burden. Interpreters and/or translators were experienced and remarkably effective in verbal exchanges. While translators/interpreters were employed in each exchange, administrators often showed considerable facility with the English language, their eyes and faces giving animated affirmation that comments were understood even before being translated/interpreted.

Compared to American universities, Chinese universities, colleges and technical schools seem to have low student enrollments and high faculty to student ratios. Table I lists faculty-student ratios in 9 of the schools we visited:

Institution	Enrollment	Faculty
Yunnan Institute for the Nationalities	1457	363
Yunnan University	4000	800
Kunming Teachers College	1800	450

Institution	Enrollment	Faculty
Shaanxi Normal University	4200	906
Beiging Normal University	5200	1400
East China Normal University (in Shanghai)	1032	338
Jiao Ton University (Shanghai)	5000	1600
Nanjing University	3685	1651
South China Agricultural College (Nanning)	2300	700

Table 1. Faculty-student ratios at 9 Chinese institutions of higher learning.

Political indoctrination/education is one aspect of each student's curriculum. Pamphlet-type books specified questions and their answers, apparently on matters of political and economic ideology. Students could be seen memorizing their lessons; I presumed for subsequent recitation or testing. Their study resembled ever so much that of American college students "cramming" for an upcoming test in a required liberal arts course; it was being done, but lacked conviction. Having said that, it is appropriate to add that Chinese students generally seemed broadly and knowledgeably committed to the political and economic ideology, including family planning, of their homeland. One English speaking student stated that "The most important thing is not whether we all have TVs, but how to realize the Four Modernizations," He ticked off the national goals that everyone seems to have memorized: modernization of agriculture, industry, defense, and science. "If we achieve the Four Modernizations we can have a happy life." He added, "I believe that."

The singleness of pursuit of learning by Chinese university students is difficult for westerners to grasp. Rock and disco sounds are rare; students seem not to date or dance and only a few smoke. Questions about beer busts and drugs seem to be so unreal as to be incomprehensible. Students on campus have one purpose: to study. It is assumed that attention to nutritional and fitness needs must be met to study properly. One exchange teacher from Germany stated "I'm spoiled here, I'll never have such diligent students again." Less definitive, but carrying a similar message, were the comments of a Massachusetts couple who were concluding a year's faculty exchange at Shaanxi Teachers University in Xian. "The rules are that foreign exchange faculty may not have sexual, political or religious exchanges if they're going to remain invited. Even so", said the wife, "this has been the single most significant year of my entire life, and I'm the mother of 5 grown children, so I've done quite a bit of living."

Classes meet 6 days per week and all students have some facility with a second language. Until recently some 2/3 studied Russian and 1/3 studied English, but that balance is rapidly tipping. One middle-school educated hotel worker stated "the language of commerce is English; we must understand it; read it and write it, if we are to compete in the world of commerce." University students in language courses use their day off to go to tourist sites to meet persons with whom to practice and, if possible, to elicit an offer of a dictionary from the tourist's homeland. I had delivered a lecture in English on animal behavior to some 150 Chinese students at South China Agricultural College in Nanning. It was clear from facial expressions that the concept of behavioral imprinting was being only slightly understood. I had been speaking for an hour already, so concluded my topic reasonably gracefully and asked if there were questions from the audience. I assumed it was courtesy

that had kept those eyes focused on me when there was so little light of understanding.

When I asked for my first question, a student said in all seriousness "Please go on, you speak English like they do on the TV; most of our visitors speak Australian English." Quite obviously it was my English and not my biology or person which fascinated these students.

The cultural revolution of the 1970's was a disaster. I don't know if that was a fad word or the best descriptor of those years, but it was the one most commonly used. One staff person still thoroughly indignant with her recent memories said "For seven years I herded sheep! Now we're more than 7 years behind the West in science and technology. We need you to come and help us catch up." My memories are clearer of the fury and frustration in her voice than of the words themselves. This woman felt free both to express her opinions and to disclose the mistake her government had made without using gobble-de-gook language or requiring that I read between or beyond the lines of what she said. And she was not a solo voice in a wilderness. Faculty and staff repeatedly decried the cultural revolution calling it variously: a mistake, a disaster, a tragic error. Most stated that it had set back the country and technological advance from as little as 10 years to as much as a whole generation.

Chinese universities are nearer to being intellectual utopias than physical ones. There's the claustrophobia and congestion of too many persons using too little space; there are the odors of "schoolhouse" and of too many people serviced by too few facilities; there's too much grime and too little shine; there are too many foot paths of hard earth and too few with asphalt or concrete; there are too many thermoses; and too little water. These limitations were, of course,

creature comforts to which I had grown accustomed and each is trivial. I expect there is a dark side to Chinese academia that goes beyond creature comfort but I never experienced it. I expect its presence, because it is naive to not know of the appetite for power and of the dark side to human nature.

Both hand labor and machine labor were norms in China. Steam hammers pounded along side persons using sledge hammers and chisels; power shovels gnaw alongside persons with hand shovels; tractors plowed fields alongside workers driving water buffalo; wheat was flailed, but it was also harvested with self-propelled combines; trucks, handcarts and bicycle carts traveling side by side transported the nation's produce and refuse. "We

are technologically backward," said one young man "but we won't always be." I thought his statement too humble. The national goals of the four modernizations: agriculture, industry, defense and science, seemed to me to be progressing well. I stand in admiration and awe of a people whose industry and government have provided one-fourth of our planet's population with food, shelter, clothing, work, medical care, a sense of self-worth and a hope for a better future.

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QUANTITATIVE AND QUALITATIVE DETERMINATION OF CHLOROPHYLLS IN MUTANTS OF SOYBEAN

by

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Introduction

Chloroplasts are found in all higher green plants and are of great biological importance. This organelle contains the pigments and other molecules necessary for converting light energy to chemical energy. This conversion is made possible by the possession of a light trapping pigment, chlorophyll. The chloroplast also contains the molecules, enzymes, necessary for using this trapped energy to convert inorganic compounds (carbon dioxide and water) into simple carbohydrate molecules. These processes are collectively referred to as photosynthesis. Higher plants contain two types of chlorophyll, a and b. these pigments give the green color to plants; and although green plants contain other pigments, the carotenoids consisting of the yellow xanthophylls and the orange carotenes, these pigments are hidden by the green chlorophylls. Some cells or areas in leaves do not possess chloroplasts as evidenced by the variegated appearance of the leaves of some plants (coleus is an example). Genetic mutations are known that result in a plant producing less than normal chlorophyll concentration in each cell or no chlorophyll at all. Plants with no chlorophyll live only until they have exhausted the nutrient from the seed and then they die. The number of chloroplasts is relatively constant in the cells of each species of plant. In higher plants, there are about 20 to 40 chloroplasts per cell of the green tissue. When the number of chloroplasts is insufficient, chloroplasts divide and increase in number; if the number is excessive, degeneration of the chloroplasts reduces the number. On the basis of this information, one may hypothesize that a reduction in chlorophyll content of a plant is due to an insufficient amount of chlorophyll in each chloroplast rather than to a

reduced number of chloroplasts in each cell. A certain mutant of soybean involves a change in the chlorophyll producing mechanism. The gene for this characteristic exhibits a lack of dominance. One homozygous condition results in a plant with dark green leaves; the other homozygous condition gives a plant with yellow leaves; and the heterozygous condition gives rise to a plant with yellow-green leaves. These plants serve nicely to investigate the relationship between genetic makeup and the amount and type of chlorophylls in the plant leaf.

Various methods may be employed to determine the amount and type of pigments in plant tissue. We will utilize two of these--spectrophotometry and paper chromatography. The selective absorption of light, of a specific wavelength, is one method of identifying and quantitating chemical substances. Most biological materials absorb light in the 200 to 680 nanometer range ($1\text{nm} = 0.001\mu$). Either the wavelength (color) of light absorbed or the amount of light of a specific wavelength absorbed by a solution can be used for qualitative analysis or quantitative analysis respectively. The term photometer refers to a light meter or measuring device. If the wavelength of light can be selected over a wide range of the spectrum by the machine, the instrument becomes a spectrophotometer. In spectrophotometry, a beam of light is passed through a solution containing a light absorbing material and the amount of light transmission or the "reverse" (the amount of absorbance) is compared to the transmission or absorbance through a known solution. The known solution may be the solvent alone in which case it is called the reference solution or blank; or the known solution may be the solvent plus a known amount of the light absorbing material in which

case it serves as a standard.

Chromatography may also be used to separate and identify substances in a mixture. There are several types of chromatography including column, paper, thin layer, and gas. Depending on the type of chromatography, the two phases may be gas and liquid, gas and solid, liquid and liquid or liquid and solid. Paper chromatography is one of the most extensively used chromatographic techniques with paper acting as the solid or stationary phase and the solvent as the mobile phase. The substance to be chromatographed is placed as a small spot or line near one end of a sheet of filter paper. This end is then immersed in a solvent system which usually is composed of two or more miscible substances. In ascending chromatography, the solvent is placed at the bottom of the chamber and allowed to rise upward by capillary action. As the solvent flows past the sample, it carries individual components along with it at a characteristic rate dependent upon their solubility in the solvent. When the solvent front approaches the upper end of the paper, the paper is removed and dried. This is referred to as a single dimension chromatogram. One can produce a two dimensional chromatogram by running the material one way and then by turning the paper 90° and permitting the solvent (same one or a different solvent) to move up the paper a second time. One must use sample spots rather than lines if one wishes to use two dimensional chromatography. The substances, once separated, can be identified by their color, by examination under ultraviolet light, by spraying with a chemical which reacts with the substance to form a colored compound, or by matching the R_f value of the substance with the R_f value of a known substance. The distance traveled by each compound from the origin or base line relative to the solvent front is defined as the R_f .

$$R_f = \frac{\text{distance from base line traveled by compound}}{\text{distance from base line traveled by solvent}}$$

Spectrophotometric Determination of Chlorophyll Content of the Leaves of the Three Genotypes of the Soybean Mutant.

Members of the class will extract chlorophylls from leaves of each genotype and determine the amount or concentration present. Obtain a 0.5gm sample of leaves from one genotype; remove the petioles before weighing. Other members of the class will be performing a similar exercise with leaves from the other two genotypes. Grind the leaves with a mortar and pestle in 10ml of 80% acetone. Be careful not to spill any of the acetone solution; keep a small piece of filter paper handy to absorb any acetone solution that spills or runs down the mortar when the solution is being transferred. Use a small paint brush to keep the leaf pulp in the acetone solution. Filter the solution through Whatman #1 filter paper into a 100 ml flask. This can be done by placing a cone of filter paper into a small funnel which has the tube inserted in the mouth of the flask. Add another 10ml of acetone to the remaining leaf pulp and repeat the procedure. Repeat grinding the sample and filtering the solution until all the green color has been removed from the leaf pulp. Now take the filter paper cone and the piece of filter paper used to absorb spills and place it in the mortar and extract the pigment. The object is to get as much of the pigment as possible into the 100ml flask. Pour the solution from the flask into a graduate cylinder and add enough 80% acetone to bring the total volume to 100 ml. Place about 5ml of this solution in a Spectronic 20 tube and cover the top with parafilm to prevent evaporation of the solution. Prepare another tube with plain 80% acetone and cover. Set the Spectronic 20 to 0 absorbance with the 80% acetone blank before determining the amount of absorbance of your solution; this must be done for each wavelength. Now determine the absorbance of your chlorophyll solution at 645nm and 663nm. The values obtained from the cc (yellow)

plant are due to pigments other than chlorophylls; therefore, they should be subtracted from the Cc and CC plant values at 645 nm and 663nm. After obtaining the corrected absorbance at the two wavelengths and using the equation given below, determine the concentration of chlorophyll in your leaf sample.

Chlorophyll concentration (mg/ml = $8.02 (A_{663}) + 20.2 (A_{645})^D$
 D = mg total chlorophyll solution/mg of original suspension

$$D = \frac{\text{total of 80\% acetone solution}^*}{(\text{grams suspension}) 1000}$$

*This would be 100 ml if you followed instructions, but could be whatever volume you used.

Amount of chlorophyll in CC (dark green) plant is _____. The amount in Cc (yellow-green) plant is _____. After obtaining the values from the CC and Cc plants, determine the ratio of chlorophyll in the CC plant to the Cc plant. What is your conclusion about the effect of each gene on chlorophyll production? _____

Separation and Identification of Plant Pigments Utilizing Paper Chromatography

Obtain a sheet of Whatman #1 filter paper. Take precautions not to finger the body of the paper as oil from your fingers will influence the flow of the solvent. Lightly pencil a line across the width of the paper about 1 inch from the bottom edge. Using a capillary tube place a thin line of your acetone extract of the plant pigments along the penciled line. Allow this to dry and reapply the solution. Repeat until the line appears to have a significant amount of green pigment. Obtain another piece of paper and apply the solution in a series of spots about 1 inch apart. Place both sheets in the solvent jar along with sheets containing pigments from the leaves of the other two genotypes. The jar contains solution A

(petroleum ether and benzene, 9:1).

Allow the solution to move up the paper until it is about 2 inches from the top of the paper. Take the paper out, allow it to dry and then place it in solution B (petroleum ether and benzene, 2:1). Again allow the solvent to move to about 2 inches from the top of the paper. Remove the paper and draw a line to indicate the solvent front (the highest point reached by the migrating solvent). Make drawings to show the appearance of your chromatograms. Determine the R_f value for each pigment; place a pencil mark in the center of each pigment spot to use as the distance traveled by the compound. Carotene is the most soluble in the solvent and therefore will appear close to the solvent front. The xanthophylls should be separated into two yellowish pigments below the carotene. Chlorophyll a is blue-green in color and chlorophyll b is yellow-green in color. Compare the pigments from each genotype. How do they differ as to types and amounts of pigments?

 Relate the differences to the results obtained in the previous experiment.

 Which chromatogram (the one produced from the pigment line or from the pigment spots) is better for determining the different types of pigments?

_____ The different amounts of pigment? _____

The objectives for this laboratory exercise are:

1. To determine the quantitative and qualitative differences in pigment content of soybean plants with different genotypes for chlorophyll content.
2. To become aware that plants contain pigments that are masked by the chlorophylls.
3. To learn how to use the Spectronic 20 to quantitate a substance.

4. To learn how to use paper chromatography to separate substances (pigments) from a mixture.

Materials and Methods

1. Soybean seeds may be obtained from Carolina Biological #17-8200. They provide planting instructions and estimate of time required for plants to reach a specific size.

2. The 80% acetone solution is made up on a volume to volume ratio with water.

3. The filter paper cone is constructed by folding a 9cm circular piece of Whatman #1 filter paper into four equal parts.

4. A Spectronic 20 with operating instructions should be provided. The unit should be equipped with the accessory red filter and red sensitive phototube.

5. Provide several Spectronic 20 tubes in a test tube rack with several small squares of parafilm.

6. A pan balance sensitive to 0.01 gram should be available.

7. A pair of forceps should be used for handling the filter paper to remove it from the funnel.

8. A small artist's paint brush is ideal for pushing the leaf mass into the acetone.

9. Glassware should include a 100ml; graduate cylinder, 100ml flask, small funnel, and mortar and pestle.

10. Teams of 2 to 4 members can be used to extract the pigment from each genotype of the soybean plant.

11. The equation for determination of chlorophyll concentration was taken from Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, Polyphenoloxidase in Beta vulgaris. Plant. Physiol., 24:1-15.

A RAPID METHOD FOR STUDYING ENZYME ACTION

by

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Commercially available dry yeast is a source of sucrase of β -fructofuranosidase which hydrolyzes sucrose, also of commercial origin. The products are equimolar quantities of fructose and glucose and the latter is detected with any of several test strips sold for detecting glucose in urine. The procedure requires inexpensive materials and can be applied for a variety of laboratory experiments designed to familiarize college biology students or advanced high school students with the fundamental behavior and properties of enzymes.

Introduction

Controlled catalysis is a central topic in biochemistry and the functional units responsible for every chemical reaction in cells are the several thousand enzyme proteins. Research on enzymes has moved over the past several decades from that of discovery and description to kinetics and mechanism and, more recently, to attempts at synthesis of amino acid polymers into which a catalytic site will be incorporated (1). Early introduction of students to the extraction and assay of enzymes has become more important for transmitting the concept of enzymatic catalysis in a laboratory environment, so that topics such as energy fixation and utilization, metabolism, nucleic acid synthesis and protein synthesis as well as protein structure will have greater significance when they are covered in first-year biology. The enzyme sucrase is widely distributed in bacteria, fungi, plants and animals and a ready source is the yeast Saccharomyces cerevisiae. Sucrase of β -fructofuranosidase (invertase in earlier literature) hydrolyzes the disaccharide sucrose into equal amounts of its two monosaccharides fructose and glucose. The appearance of glucose is detected with a test strip, to which is bonded a

region, onto which a stabilized enzyme, glucose oxidase, has been incorporated along with a second enzyme, peroxidase, which reacts with hydrogen peroxide, one of the two products of the action of the glucose oxidase on its substrate glucose. (The other product is gluconic acid). The peroxidase catalyzes the reaction of hydrogen peroxide with a chromogen which is oxidized to yield a product with a different color. The color change is then examined against a chart to determine how much, if any, glucose is present. Accordingly, the action of sucrase can be assayed quickly and with a high degree of specificity.

Materials and Methods

This procedure is described so that the various reagents may be obtained either from laboratory suppliers or from local commercial sources. (The author has carried out sucrase assays both in a laboratory and in the kitchen). The substrate for the enzyme, sucrose, is over 99 percent pure as purchased in a grocery store and white (colorless) household vinegar is either 4 or 5 percent acetic acid (.006 to .083 Molar). The pH optimum of yeast sucrase is between 3.5 and 5.0 so the vinegar can be brought to pH 4 or 4.5 by adding 3 ml of clear household ammonia (NH_4OH) to 96 ml of 4 percent acetic acid to produce an ammonium acetate buffer. The pH can be determined with pHdrion test paper until a pH of about 4.5 is reached. Since the optimum is broad, this value need not be precise. The sucrose (342.3 molecular weight) should be dissolved in water at a ratio of 5 grams in a total volume of 10 ml giving a substrate concentration of 1.46 Molar (1.46 Moles per liter). The yeast can be any commercial brand, also purchased from a grocer. I used "Red Star" brand instant blend dry yeast in one-quarter ounce packages. For a small-scale assay, one gram was weighed out and

stirred into 5 ml of tap water. The slurry was allowed to stand for about an hour, with occasional stirring, in order to permit some of the cells to undergo autolysis. The cells are resistant to damage and even after twelve or more hours, many are intact. During autolysis the suspension should be kept at room temperature. Subsequently the slurry can be either filtered through paper or centrifuged to remove the cells. In the event the filtrate or supernatant is slightly turbid, the remaining cells will not interfere with the assay.

For detection of enzyme activity, a typical protocol is as follows:

Tube#	1	2	3	4	5
Buffer	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Sucrose	0.1ml	0.3ml	0.5ml	—	0.5ml
Yeast extract	0.1ml	0.1ml	0.1ml	0.1ml	—
Water	0.4ml	0.2ml	—	—	0.5ml

The objective of this experiment is to determine the effect of several concentrations of the substrate sucrose on the rate of enzyme action. The experimental design need not be identical to the volumes above as long as the final volumes are the same for each tube. Also, the yeast extract should be added last just after stirring each tube to assure a homogeneous mixture of each additive. After a period of incubation at room temperature, such as thirty minutes, a test strip is briefly immersed in each tube and then the presence or absence of one of the two products of the reaction, namely glucose, is determined. For this purpose plastic test strips used to determine glucose in urine provide a rapid, sensitive and specific method. These strips are sold in pharmacies under several brand names including "Diaslix" or "Clinistix," both products of the Ames Company, a division of

Discussion

In addition to experiments of the type described above, there are a number of other assays which can be performed with ease because of the convenience and rapidity associated with the glucose test strips. The behavior of sucrose at various pH values can be studied, permitting students to verify some of the classical experiments on which the field of enzymology is based. By heating one of the tubes in boiling water for various time periods (1 through 5 minutes) the effects of temperature on the catalytic activity of the protein can be understood. Aniline is a reversible inhibitor of the enzyme as are other aromatic amines including

Miles Laboratories; "Tes-Tape" manufactured by Eli Lilly and Company; and "Chemstrip" a product of the Boehringer Mannheim Company. All of these items contain a dry and stabilized mixture of components as described in the introduction above. The strips are typically packaged with either 50 or 100 separate test units per bottle and instructions along with a color scale are provided. Currently, the strips cost about 6 cents each, based on the price of a bottle of 50 or 100. The sucrose substrate, at 1.46 M concentration, contains 146 micromoles per 0.1 ml aliquot. For the 1.6 ml volume suggested for each tube in the protocol, the concentration available to the yeast extract is 91 micromoles per ml or 91 millimoles (mM) per liter. The K_m for sucrose with this enzyme has been determined to be 25-26 mM (2). Accordingly, the substrate is present at over 3.5 times the K_m assuring that the reaction will proceed at close to maximum velocity where 0.1 ml of sucrose is added. In order to more fully demonstrate the dependence of enzyme rate on substrate concentration, it would be advisable to carry a tube in the protocol to which sucrose was added after diluting a portion of the stock sucrose solution ten or twenty-fold. Stock refers to the 1.46 M solution.

3,4-xyloidine, 3,5-xyloidine and meta-toluidine. Sucrase is an enzyme which has been extensively studied because of the ease of extracting it from yeast (3,4). As noted in the introduction, the earlier literature refers to the enzyme under the name invertase based upon an early method of assay during which inversion of the direction of rotation of plane-polarized light was followed in a polarimeter. The inversion results from the fact that sucrose shows positive rotation

$$([\alpha]_D^{20} = 66.5^\circ)$$

D

while the mixture of glucose and fructose shows negative rotation since the values are

$$[\alpha]_D^{20} = +52.5^\circ \text{ and } [\alpha]_D^{20} = -92^\circ$$

D

D

respectively and the larger negative value of fructose controls the overall direction of rotation (5).

Animals contain an enzyme similar to that found in yeast. In mammals and humans the sucrase is localized in the mucosal tissue of the small intestine. Sucrose intolerance was first reported in 1964 and since then there has been a steady increase in cases. This condition is associated with symptoms of abdominal fullness, cramping pain and diarrhea. The malady is usually due to a genetic disorder, inherited in an autosomal recessive manner. The cause is a lack of sucrase in the intestinal mucosa and treatment require avoidance of table sugar (sucrose) in the diet (6).

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SAFETY PROCEDURES FOR THE MICROBIOLOGY LABORATORY

by
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Every instructor of microbiology must be concerned about laboratory safety. This is because the microbiology laboratory represents a unique environment in which accidents can and do occur. In general the hazards associated with a microbiology laboratory fall into the following categories: (1) laboratory acquired infections, (2) chemical, (3) laboratory equipment, (4) fire, and (5) handling of animals.

Laboratory Acquired Infections

This represents perhaps the greatest hazard to those working in the microbiology laboratory. Students may come into contact with potentially dangerous pathogenic agents. When working with infectious agents or biologicals (blood, urine, etc.) the following regulations should be strictly adhered to:

1. At the start and end of each laboratory session, each student must clean their assigned bench-top area with a disinfectant solution.
2. No direct mouth pipetting. Always use a rubber bulb or automatic pipettor.
3. Wear a laboratory coat, gloves, etc., when working with biological specimens to avoid skin contact.
4. If you have cuts or abrasions on the skin of your hands, cover with bandages and wear rubber gloves.
5. Do not eat, drink or smoke in the laboratory.
6. To avoid infection keep fingers, pencils and such out of your mouth.
7. Disinfect immediately all biological spills with disinfectant solution.

8. Always flame the inoculation needle or loop before setting it down.
9. Hot inoculation needles or loops should never be plunged into specimens or cultures. This may create a dangerous aerosol. Always cool inoculation loops by holding them in the air for 10 to 15 seconds or by touching portions of uninoculated media.
10. Always sterilize all discards.
11. Place test tube racks with cultures and other materials away from the edges of the work bench.
12. Do not place coats, hats or other personal articles on the work bench surface. They should be kept in lockers or on coat hangers.
13. Always wash your hands before leaving the lab and whenever you spill culture or unknown material on them.
14. Microorganisms in wet mounts or hanging-drop preparations are still alive and should be placed in a disinfectant tray.

Chemical Hazards

The most common dangers from chemicals are skin burns, fires, explosions and toxic fumes. Splattering of dangerous chemicals can easily cause extensive damage to vital organs such as the eyes or skin as well as clothing. To avoid these dangers the following precautions should be followed:

1. Store all flammable solvents in a safety cabinet. Keep open flames such as that of a bunsen burner away from the cabinet.
2. Never pipet strong acids, caustic

materials or strong oxidizing agents by mouth.

3. Wear protective eye glasses when working with caustic chemicals.
4. Use the laboratory hood when working with chemicals that generate odoriferous, corrosive or toxic fumes.
5. If a chemical is spilled, clean it up immediately.
6. Know the location of the safety shower and how to use it.
7. Slowly pour acids into water. Water should never be added to acids because it will quickly generate heat.
8. Read warning labels on reagent bottles.
9. Keep bunsen burners and hot plates away from flammable liquids.
10. Never grasp reagent bottles by the neck. Instead hold it securely in both hands around the bottom.
11. When pouring reagents into a drain, always flush with a large amount of water.

Laboratory Equipment

With an increase in equipment usage seen in the microbiological laboratory, students are being exposed to a greater degree of electrical and mechanical hazards. Basically there are two types of electrical injuries. They are (1) electrical shock and (2) electrical burns.

Students must also learn to use laboratory equipment in such a manner as to avoid accidental infections. This can occur when using such instruments as a blender, centrifuge or hyperdermic syringe where infectious aerosols can be set up. The following procedure should be followed to avoid or minimize accidents due to laboratory equipment:

1. Properly ground all electrical equipment and check grounding every six months.
2. Before centrifuging carefully inspect all glass centrifuge tubes for cracks. If any are found discard them. If a tube should break while centrifuging, stop the machine, carefully remove all pieces of broken glass from the rubber cushion and disinfect the area.
3. Never pick up broken glassware with your bare hands. First disinfect and then use janitorial equipment to clean up the area.
4. Always stand to one side when opening the door of an autoclave. Some residual steam may still be in the instrument and it may cause a skin burn.
5. Always wear rubber gloves when removing broken ampoules or tubes from a deep-freeze.
6. Never touch electrical equipment with wet hands.
7. Special precautions should be taken when using the hypodermic needle and syringe. Some areas in which accidents may occur are: (1) separation of the needle from the syringe during injection, (2) accidentally injecting oneself, and (3) aerosols can form when the needle is withdrawn from a vial.
8. Keep hands, necklaces, ties, etc., away from moving parts of equipment.
9. If spillage occurs on electrical equipment, immediately turn off the instrument and dry it. Disinfect if needed.
10. Never touch a person receiving an electric shock! First turn off the circuit breaker and only then give aid.
11. If electric cords or equipment starts to sizzle, crackle, or smoke when turned on, immediately unplug the instrument.

12. Know where the circuit breakers are located in the laboratory.

13. Mix broth cultures in such a manner as to avoid wetting the plug or cap.

14. Keep all flammable liquids away from electrical contacts.

15. Before using an autoclave carefully examine pressure gauges, safety relief valve and discharge line.

Fire Hazard

Preservation of human life is the first priority in cases of fire. Procedures such as the following for the handling of fires should be established and practice drills carried out.

1. Know where fire extinguishers, fire blanket and sand bucket are located and how to use them.

2. Check all fire equipment and test it at regularly scheduled intervals.

3. Know where the alarm systems are in the building.

4. In case of fire evaluate the situation quickly. If you can put it out readily and easily, do so. Otherwise sound the alarm system.

Laboratory Animals

Proper methods should be followed when working with animals in the laboratory. Improper procedures can result in pain to the animals as well as bites and puncture wounds to the handlers. Always adhere to the following procedures:

1. Devices for holding animals are available and should be used.

2. Always wear gloves when handling animals.

3. Use animal cages designed to minimize exposure to both animals and

students.

4. Immediately treat all puncture wounds. This type of injury may be relatively painless, but microorganisms can be introduced into the body via the animal's saliva or from the surface of its skin.

Conclusion

Educators have the responsibility to warn students of the dangers that may exist in a microbiology laboratory. By adopting these procedures and presenting them in the form of a "Laboratory Safety Manual" the instructor informs students of these hazards and hopefully thereby a reduction in such accidents will occur. It is suggested that each student should be required to read the safety manual and pass an examination over its contents.

The responsibility on the part of the instructor does not end with the teaching or enforcement of safety procedures. He or she must serve as a role model, to protect both present and future students from illness, injury and even death.

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Editor's Note: I hope the publication of this article marks the beginning of a series on safety in the biological laboratory. I look forward to receiving additional articles on animal care, hazardous waste disposal, radiation safety, and handling of toxic materials.