Chapter 7

Simulated Laboratories and Lessons in Microbiology and Biochemistry*

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Samuel Kaplan is a professor of microbiology at the University of Illinois, Urbana, IL. He obtained his B.S. in biochemistry from Cornell University in 1959, his M.S. in microbiology from Yale University and Ph.D. under the guidance of Dr. David M. Bonner in cell biology from the University of California, San Diego in 1963. For his Ph.D. he worked on the genetics and enzymology of tryptophan synthetase from *Neurospora crassa*. Postdoctoral work on the nature of nonsense mutations and suppressors was performed in Cambridge, England with Dr. Sydney Brenner. Following a brief tenure as an assistant professor at Western Reserve University, Cleveland, OH, Dr. Kaplan joined the faculty of the Department of Microbiology at Illinois in 1967, where he has remained. Dr. Kaplan's research interests are in the genetics and biochemistry of membrane biogenesis. He has over fifty publications in refereed journals and holds research grant support from both the National Science Foundation and The National Institutes of Health. He has been awarded a Guggenheim Fellowship to study in Oxford, as well as National Cancer Society Faculty Scholar Award. Dr. Kaplan has held a National Science Foundation Faculty Science Fellowship to design PLATO instructional materials. He currently teaches in the area of general microbiology and has been awarded a National Science Foundation Scientific Equipment Grant. He is an editor for the Journal of Bacteriology.

*Supported by a National Science Foundation Faculty Science Fellowship.

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Introduction

General Comments on the PLATO System

The PLATO teaching system is an individualized computer-based (Smith 1970; Smith and Sherwood 1976) self-interactive method of providing a unique educational experience. Each student is able to work at his or her own speed. Performance is monitored continuously and individual needs, bearing on the heterogeneity of the student population, can be taken into account during lesson presentation. Because of the unique ability to simulate experimental techniques and laboratory exercises (Chabay and Smith 1977), PLATO is particularly adaptable to the natural and physical sciences.

Standard peripherals include: slide selector, touch panel, external parts, audio devices and music synthesizer.

A booklet describing the PLATO system entitled "The PLATO System" is available from Dr. Donald Bitzer, Director, Computer-based Education Research Laboratory (CERL), University of Illinois, Urbana, IL 61801. Similarly, through Bill Strutz at the CERL book office (University of Illinois, PLATO Publications, 252 Engineering Research Lab, Urbana, IL 61801) a publications list describing the PLATO system in detail is available.

Between July of 1974 and November of 1979, 7,000,000 contact hours have been logged on the University of Illinois PLATO system. Below are listed a number of sample lessons currently available on PLATO. This list is not complete.

Partial Listing of PLATO Biology-related Programs

1. pH and Acid/Base
   a. Henderson-Hasselbach Equation
   b. Amino Acids, Peptides and Proteins
   c. Buffers
   d. Applications
2. Enzyme Kinetics
   a. Michaelis-Menten
   b. Allostery
3. Scatchard Analysis
4. Proteins
   a. Amino Acids
   b. Peptide Sequencing
   c. Hemoglobin and Myoglobin
5. Nucleic Acids
   a. Structure and Biochemistry
   b. Purine Metabolism
   c. Pyrimidine Metabolism
   d. Oligoribonucleotide mapping
6. Carbohydrates
7. Lipids
8. Cofactors and Vitamins
9. Glycolysis
10. Gluconeogenesis
11. Carbohydrate Metabolism
12. Botany
    a. Spectrophotometer
    b. Experimental
    c. Plant Taxonomy

d. Tree Identification
e. Anatomy and Morphology
f. Population
g. Plant Genetics
h. Evolution
i. Induced Mutations
j. Plant Life Cycle
k. Seed Germination
l. Plant Growth
m. Tropisms
n. Photoperiod
o. Leaf Senescence
p. Hormones
q. Plant Pathology
r. Water Relations
13. Photosynthesis
14. Respiration
15. Electricity in Physiology
16. Neuron Excitability
17. Microbial Growth Curve
18. Chemical Basis of Life
19. Cell Structure and Function
20. Mitosis
21. Meiosis
22. Protein Synthesis
23. Electron Transport Chain
24. Genetic Drift
25. Natural Selection
26. Population Biology and Ecology
27. Animal Behavior
28. Human Anatomy and Physiology
29. Parasitology
30. Virology
31. Epidemiology

For further lesson descriptions two PLATO catalogues are available, namely: The University catalogue "uicat" and the Medical School catalogue "mclcat".

Equipment (Cost per terminal)

The current most up-to-date operating costs are the following:

Used PLATO IV Terminal	$3,000–$4,250 est.
Plasma Panel PLATO PPT Terminal	$8,500
1200 bps modem (one/terminal/phone line)	$500
4800 bps modem (one/4 terminals/phone line)(new)	$5,000–$6,000 est
(used)	$3,500 est
9600 bps modem (one/8 terminals/phone line)	$10,000
Connection fee	$600

CERL also provides a three-week training session here for new users. This is included with the installation of the terminal and is provided by the PSO group—PLATO Services Organization. All ages from grade one on up have been trained.

In addition to equipment costs there is an annual service fee which includes maintenance and consulting service as well as computer access. Further information can be obtained from Mr. F. M. Propst, Associate Director for Planning and Research, CERL, 252 Engineering Research Laboratory, University of Illinois, Urbana, IL 61801. The prices quoted are effective July 1,

1980. In addition to the University of Illinois PLATO system, several other systems are available, such as the Control Data System, the Delaware System, the Florida System, etc. Information about these can be obtained from the University of Illinois.

Below is presented the rationale for a series of simulated minilabs which we have adapted to the PLATO mode of instruction. Each laboratory has been abstracted and summarized. Figures 7.1 and 7.2 show students working at a PLATO terminal.

Rationale

In recent years various areas of biological research have advanced so rapidly and demand such specialized background and training that it has been difficult to present these findings to both our advanced undergraduate and graduate students in an integrated manner. Additionally, a firm understanding of these topics usually requires some form of graphical presentation, like a laboratory experience where data may be collected.

Aside from a specific group of graduate students either involved in or using specialized areas of research it has become difficult to effectively present up-to-date laboratory exercises and similar subject materials in either a lecture series because of format and size, or in a laboratory because of cost and time.

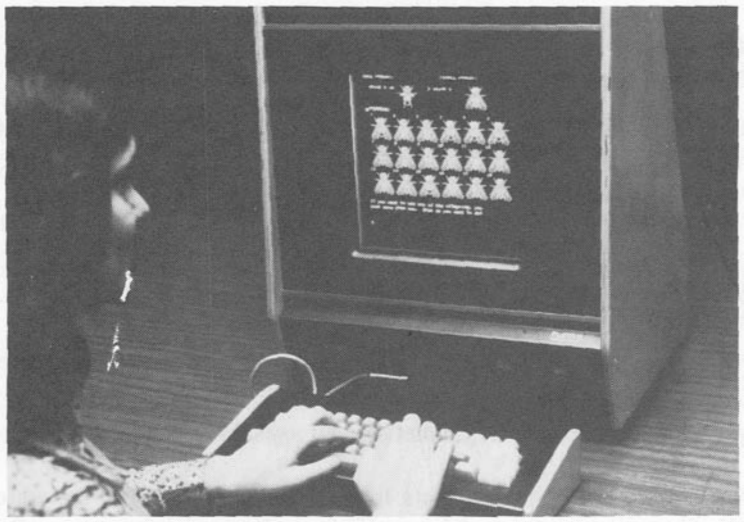


Figure 7.1. Student working on a genetics laboratory involving fruit fly crosses. Kindly provided by Ms. Janice Corum.

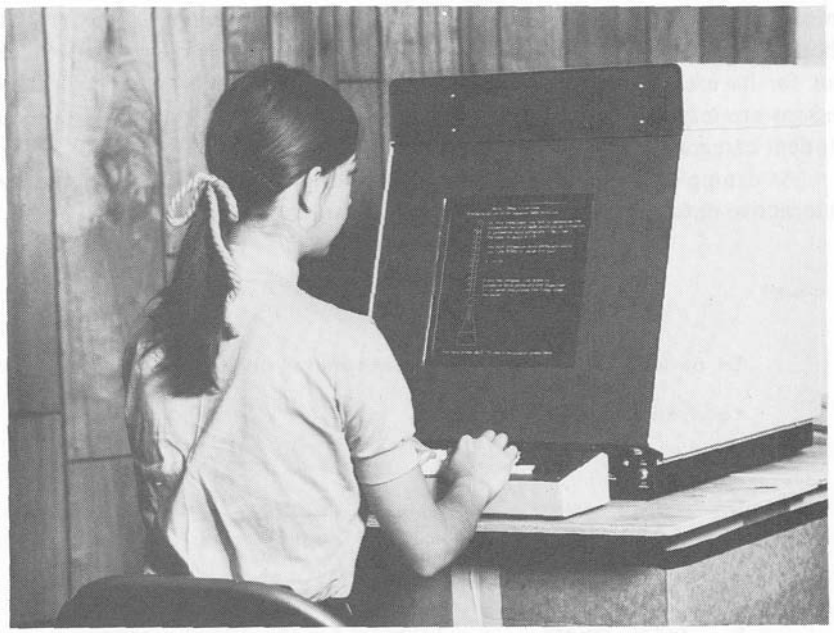


Figure 7.2. Student working on a chemistry laboratory involving acid/base titration. Kindly provided by Ms. Janice Corum.

Experience has indicated that considerable effort has been expended on behalf of improving the educational experience: for lower-division undergraduates, as well as at the research level for upper-level graduate students. However, highly sophisticated subject matter areas designed for upper division undergraduates has received little attention, either financially or in terms of new ideas.

Because these areas have advanced so rapidly and are so highly specialized, we as teachers have found it difficult to incorporate these topics into our curricula because: (i) of the time element, (ii) insertion would be at the expense of existing instructional material, (iii) of the highly specialized nature of the new material, i.e., it is simply not relevant to all students, but clearly of immense importance to many, and (iv) these topics are experimental in nature and require a laboratory format for their effective presentation.

I have used the PLATO mini-lab exercises in several ways. The Sanger oligonucleotide mapping lesson as well as the bacterial growth curve are incorporated as part of a laboratory course in biochemistry and microbiology, respectively. Lessons on the genetic code are used as an experimental supplement to a standard lecture course. Furthermore, because of the modular construction of this series of lessons, individual lessons may be incorporated

into a variety of lecture or laboratory courses. For example, colinearity although important for its contribution to the coding problem, by itself stands out for its usefulness in basic genetics, i.e. three factor crosses, etc. These lessons are intended for students in the junior, senior and first year graduate student categories.

As examples of the kinds of display material provided students and the interactive nature of the PLATO system consider Figure 7.3–7.7.

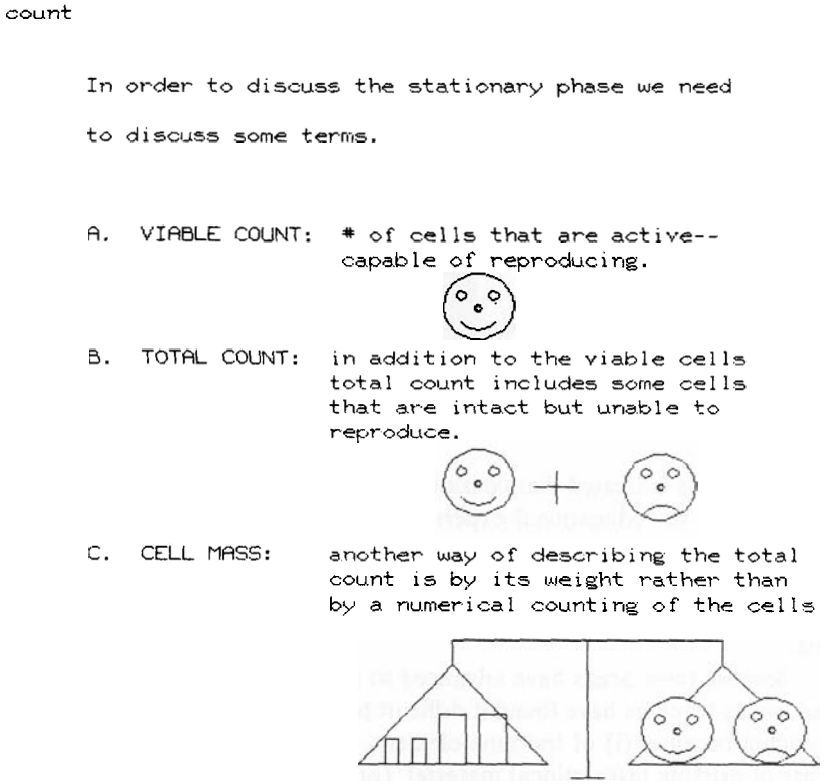


Figure 7.3. Display material taken from a PLATO lesson on the bacterial growth curve.

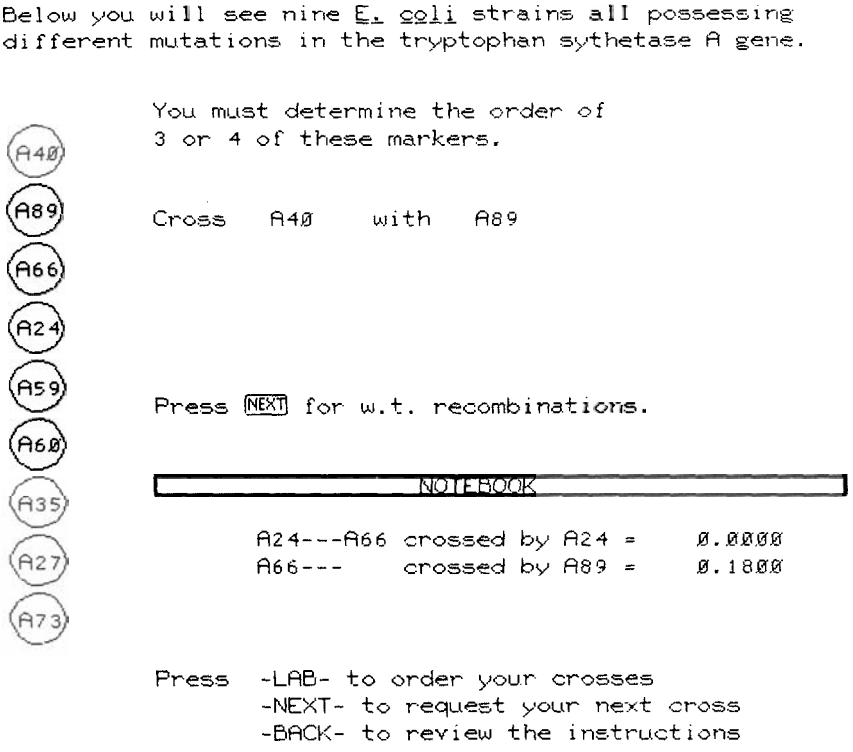
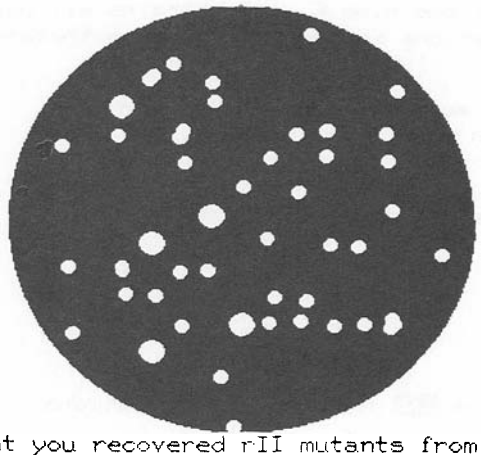


Figure 7.4. Display material taken from a PLATO lesson on genetic mapping in bacterial crosses with special reference to colinearity between gene and product polypeptide.



The fact that you recovered rII mutants from a cross of wild-types by your wild-type-like revertant implies which of the following? ⟫

a) The existence of a suppressor mutation is indicated
b) Crossing over can be hazardous to your health
c) The spontaneous mutation rate of rII mutants = 1 in 10
d) rII mutants grow faster than wild types

Figure 7.5. Display material taken from a PLATO lesson involving frame shift mutants in the rII region of bacteriophage T_4. The small plaques are the wild type virus and the large plaques are the rII mutant viruses.

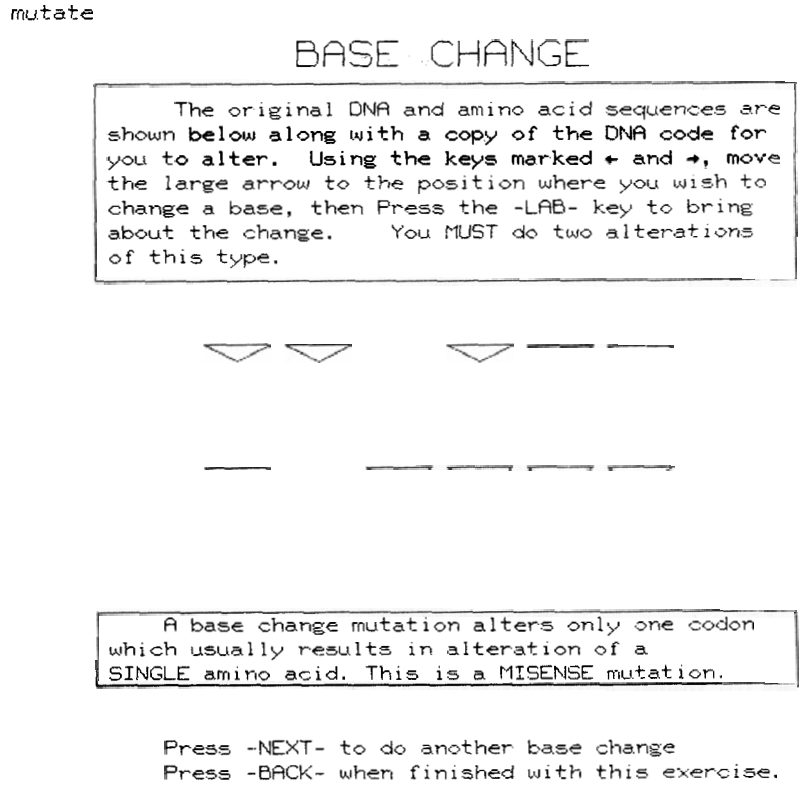


Figure 7.6. Display material taken from a PLATO lesson dealing with base pair changes in the DNA and the kinds of mutant alterations found in product polypeptides.

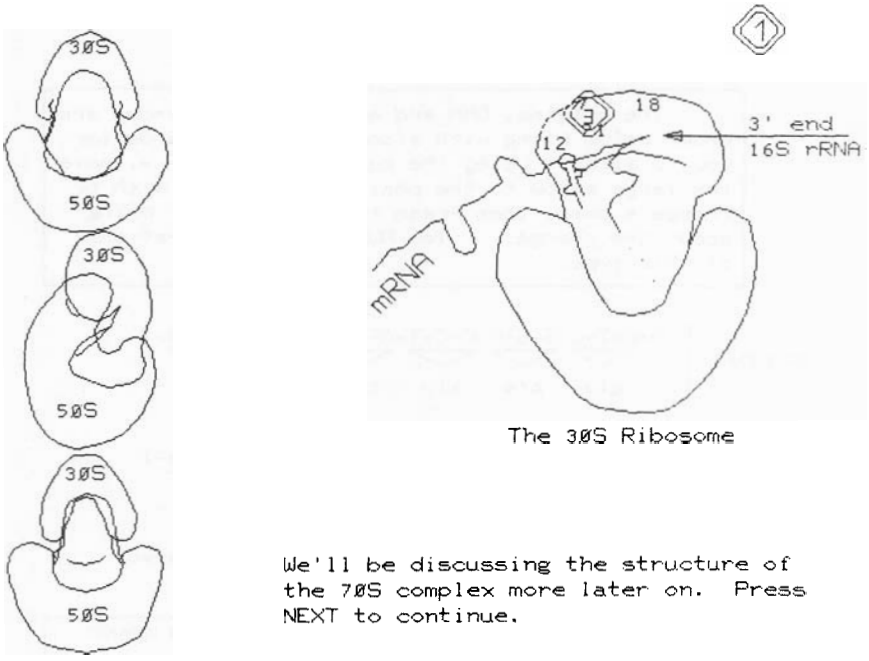


Figure 7.7. Display material taken from a PLATO lesson dealing with protein synthesis. In this figure we have the mRNA bound into the 30S ribosome initiation complex involving initiation factors and key ribosomal proteins. On the left is a rotated view of the active 70S ribosome complex.

Below are listed in succession a series of simulated mini-laboratories dealing with important biological concepts. These lessons are presented in the form of short abstracts.

Specific Example: An Experiment in Oligonucleotide Mapping:

Employing PLATO, I am able to reproduce the two-dimensional oligonucleotide patterns observed for the digestion of RNA molecules by either RNase T_1 alone or in combination with alkaline phosphatase. Sanger has shown that under precisely defined experimental conditions oligonucleotides derived from the digestion of RNA molecules will move to precisely defined coordinates (isopliths) depending upon their sequence and composition. These results are reproducible employing PLATO, by assigning known oligonucleotides to individual variables and then assigning each variable to a particular screen location based upon the results of Sanger.

Each student can proceed to PLATO where in lesson one each student is shown the structure of known RNA molecules and the mode of action of enzymes known to digest RNA.

In the next lesson, the student is introduced to the methods of oligonucleotide separation.

A third lesson then displays actual experiments and data taken from the literature involving the separation of known oligonucleotides. During this lesson the student is given a known group of oligos, and is introduced to buffer systems, materials, experimental technique and the theory of oligo separation.

The fourth lesson involves the student conducting an experiment on PLATO, using some unknown material which he/she can design based upon the number of nucleotides and base composition. The student actually generates his or her own RNA chain, digests it, analyzes the digest and then in the next lesson maps the oligos.

In the final lesson the various oligoes are mapped and the student must identify these oligoes.

Overall, the various lessons take the student approximately four hours to complete. During the course of these lessons the student is asked over 100 questions, demanding information relevant to the subject material. In appropriate sections, help sequences and review material are presented. The student can review, voluntarily, any of the lessons during the course of the exercise, which seems appropriate to his/her understanding.

As an exercise, I have illustrated below the cost-effectiveness of this approach. The capital outlay for the high voltage electrophoresis equipment, facilities and auxiliary expenses in order to perform an oligonucleotide analysis is approximately \$7,000–\$9,000. The equipment that this will buy can service no more than 20 students/week. The recurring cost for each analysis on a per student basis is approximately \$400 or, for 20 students, \$8,000/wk. No instructional or supervisory costs have been included. One PLATO terminal costs \$7,000, the recurring costs/student are less than \$20/week, but over 150 students can be effectively instructed. One terminal does not require the space or personnel involvement that is required in a laboratory.

LESSON IN OLIGONUCLEOTIDE MAPPING

TITLE: Welcome to a Simulated Exercise on Sanger Oligonucleotide Mapping.

AUTHOR: S. Kaplan

AFFILIATED WITH: University of Illinois.

PREREQUISITES: Strong background in microbiology, must read assigned reading material.

LENGTH: Approximately 1 hour.

SUGGESTION APPLICATIONS: Supplement to a college level microbiology class, nucleic acid chemistry.

Part I

BRIEF ABSTRACT: The lesson introduces the student to the techniques of Sanger mapping. Nine topics are provided for drill and practice. They are as follows:

a. enzymes used to digest RNA chains
b. methods of separating oligonucleotides
c. advantages and disadvantages of Sanger separation
d. test separation of a known oligomixture
e. explanation of Sanger grid system
f. synthesis of your own polyribonucleotide chain
g. digestion of your synthesized chain
h. analysis of oligonucleotide cleavage products
i. Sanger analysis of your oligos—demonstration of user's understanding of material

Throughout the lesson, the student must demonstrate proficiency of the material through drill and exercises. Help is not provided for questions, therefore, the student must return to text material to review certain aspects of the material.

LENGTH: Approximately 2 hours.

Part II

BRIEF ABSTRACT: The lesson is a continuation of the microbiology course in Sanger oligonucleotide pattern mapping.

The student examines how RNA is digested. First, a brief review activity and drills are presented. Then the student is presented with a discussion on the advantages and disadvantages of using the Sanger high-voltage paper electrophoresis method of mapping. The student steps through preparing, isolating and digesting polyribonucleotide chains. The oligonucleotides must be separated using Sanger's technique in the simulated electrophoresis lab.

LENGTH: Approximately 2 1/2 hours

Part III

BRIEF ABSTRACT: In the final portion of this lesson, the student generates a polyribonucleotide chain, digests the chain, and demonstrates understanding of the Sanger nucleotide mapping patterns.

The student is provided with a review of the Sanger grid, migration in the oligo, instruction of isopliths and is then presented with a question review. The index is as follows:

a. Digestion of RNA chain
b. Foresis oligos
c. Questions in digestion
d. Generating chain

LESSON ON THE GROWTH OF BACTERIA

TITLE: Cell Growth
ESTIMATED LENGTH: 2 hours
PREREQUISITES: (If any) General Chemistry, General Biology, College Algebra
INTENDED AUDIENCE: Students in General Microbiology, Nursing, Premed, Biology.

Part I

SHORT ABSTRACT: This lesson represents a general introduction to the growth of bacterial populations. The growth is analyzed on both a single-cell basis and a population basis. The concept of exponential growth is introduced as well as appropriate terminology.

LONGER ABSTRACT: The growth of bacterial populations is defined by way of comparison to the growth of an individual or the growth of a city. The concept of "living" and "dead" as applied to bacteria is presented and a definition of "viable" is introduced. The growth of a single cell is contrasted to the observations of population growth as it applies to asynchronous versus synchronous growth. Terminology, such as generation time, doubling time, growth, etc., as these apply to asynchronous cell populations in exponential phase, is discussed. Numerous questions and problems are presented to the student during the course of the exercise. Further, a summary quiz is presented later (see below). The student must obtain at least 70% correct on first try in order to pass.

CONTENT AREAS: Bacterial Growth, General Terminology, Viable Cell.
GOAL(S): This lesson is intended to introduce the student to the concepts of bacterial growth, both at the cell and population levels.

Part II

SHORT ABSTRACT: A refresher exercise in the use and manipulation of exponents.

LONGER ABSTRACT: Exponents to the base (2) and base (10) are discussed. Their use and mathematical manipulation are presented. Both logs and antilogs are considered. A series of problems is presented, and a minimum correct response is required. This exercise is intended so that students who feel they are not certain of the use of exponents for later lessons might refresh their memory. This lesson *is not* required.
CONTENT AREAS: Logs, Antilogs, Drills.
GOAL(S): To refresh the student in the use of exponents.

Part III

SHORT ABSTRACT: This lesson describes the exponential growth phase of a bacterial culture, its mathematical determination and its practical application.
LONGER ABSTRACT: In this lesson the student is introduced to the concepts and use of the exponential growth phase. The fact that each bacterial cell is viable during the phase directs the student's attention to the employment of exponential cultures in common laboratory experiments. The mathematical representations of growth rate, generation time and numbers of generations are discussed. The semilog plot of bacterial growth versus time is presented. The practical uses of these growth parameters are considered and the students are able to plot their own experimental data and derive growth rate and generation time. The student is drilled on the use of the exponential growth phase in bacterial populations. The end of this lesson contains a quiz covering this lesson as well as the general characteristics of bacterial growth. Seventy percent correct on first try is passing.
CONTENT AREAS: Exponential growth, semilog plots, growth rate, generation time, numbers of generations.
GOAL(S): Following completion of this lesson the student should be thoroughly familiar with the uses of the exponential growth curve for a bacterial population.

Part IV

SHORT ABSTRACT: In this lesson the student is introduced to the practical aspects of microbial growth and how to utilize the theoretical approach discussed earlier under common everyday circumstances.
LONGER ABSTRACT: This lesson contains a simulated laboratory exercise dealing with the growth of bacterial populations. Various environmental parameters which influence the exponential phase of bacterial growth are presented, discussed and employed. Exercises and problems are presented for the student to work out and in which the student is able to use his/her knowledge of exponential growth to solve practical problems such as

food spoilage, storage, and infection. There is no pass or fail on this section, but the student should be judged on his/her ability to employ information gained from lessons 1–3 under practical conditions.

CONTENT AREAS: Bacterial growth, simulated laboratory exercise, bacterial growth in food spoilage and storage, environmental effects upon bacterial growth.

GOAL(S): The purpose of this lesson is to show the student how knowledge of bacterial growth can be employed in everyday situations.

Part V

SHORT ABSTRACT: This lesson is intended to introduce the student to the stationary phase of the bacterial growth curve.

LONGER ABSTRACT: The stationary phase of the bacterial growth curve is discussed both as to its population dynamics as well as the physiological state of the cells in culture. Various chemical and physical agents which influence the stationary phase are presented. Graphing the stationary phase and consideration of cultural conditions on graphing of the cell growth cycle are also presented. Linear growth and its implications are also considered. A summary quiz is available and the student should have at least 70% correct on the first try to pass.

CONTENT AREAS: Stationary phase, physical and chemical conditions, graphing stationary phase, population dynamics.

GOAL(S): The student should realize that the stationary phase is not a quiescent period in the life cycle, but presents its own unique population dynamics.

Part VI

SHORT ABSTRACT: This lesson introduces the student to the dynamics of bacterial death as a population phenomenon. Factors that influence bacterial death are also presented.

LONGER ABSTRACT: The death phase of the bacterial growth curve is presented as a problem in population dynamics. The environmental factors which influence the death of bacterial cultures are presented. The mathematical expression describing the death of a bacterial population is discussed and its use presented. Graphic presentations of the death phase are also considered. Further, the practical applications of microbial death to the food and drug industry are presented and employed in various drills. Problem solving is included at the end of the lesson in order that the students demonstrate a practical awareness of the bacterial death phase.

CONTENT AREAS: Bacterial death phase, mathematical expression, influences, graphing, practical problems.

GOAL(S): The student should be familar with the mathematical expression of bacterial death and the factors which influence their expression and how they can be used.

Part VII

SHORT ABSTRACT: In this lesson the student is introduced to the lag phase of the bacterial growth curve and how the previous history of the culture determines the nature and extent of this phase.

LONGER ABSTRACT: The bacterial lag phase, its duration and dynamics are presented. The influence of present cultural conditions as well as the history of the inoculum is used to describe the nature and duration of the lag phase. The physiology of the cells during lag phase, graphics of lag phase and cell type are all presented as they deal with cells in lag phase. Special conditions of the lag phase, such as diauxie and auxotrophic mutants are further discussed in the context of the bacterial growth curve. Finally, nutrient shifts are considered, a quiz describing the lag phase is presented, and a score of 70% on first try is expected for passing.

CONTENT AREAS: Lag phase, nutrient environment, past history, culture physiology, diauxie.

GOAL(S): The student should understand that the lag phase reflects the present and past environmental history of the bacterium.

COLINEARITY: GENE AND POLYPEPTIDE

SHORT ABSTRACT: This lesson introduces the student to the concept of colinearity between gene and product polypeptide and the experimental predictions forthcoming from such a hypothesis.

ABSTRACT: The student is provided a series of mutations in either the head protein gene of bacteriophage T_4 or the tryptophan synthetase A gene of *Escherichia coli*. The student may select any three or more mutations, construct the appropriate genetic crosses and from the mapping data obtained order the three point mutations. Subsequently the student will order the product polypeptides from each mutant strain and determine whether or not a point for point relationship exists between the position of the mutation in the gene and the position of the alteration in the product polypeptide.

GOALS: The student should become familiar with standard genetic tools for ordering mutational sites within a gene and constructing and analyzing three point crosses. The relationship between protein and gene will provide the student with an insight into the mechanisms of information transfer and the genetic code.

FRAME SHIFT MUTANTS AND THE GENETIC CODE

SHORT ABSTRACT: This lesson enables the student to reproduce the legendary frame shift experiments of Crick and associates involving a genetic analysis of the proximal portion of the rII B cistron of bacteriophage T_4.

ABSTRACT: The student will mutagenize stocks of T_4 virus with acriflavin, pick rII mutants, determine if the mutational sites are the same or different and then revert each independent isolate with acriflavin. The wild type revertants will be analyzed by classical backcross techniques and those shown to contain a suppressor mutation will be analyzed further as to the identity of the suppressor mutation and the presence of the original lesion. Once the student has collected a series of independent primary and secondary mutations mapping in the proximal rII B region of the genome, these will be crossed in all possible pairwise combinations. The resulting wild type viruses will then be genetically examined in order to determine if the recombinants are true wild type or pseudo wild type. Once these data are obtained the student will be required to analyze the data to determine which mutations when together in the same gene yield pseudo wild type viruses and which do not. Such data will permit the student to formulate the general construction of the genetic code.

GOALS: The student will learn the details and concepts of genetic crosses, backcross, identical crosses, suppression, strain construction, etc. Further, the student will experience the methods leading to deductive reasoning and the formulation of broad hypotheses on the basis of very restricted data following the formulation of specific assumptions.

COMPUTER USE AND BIOLOGICAL METHODOLOGY

SHORT ABSTRACT: The analysis of RNA chains for secondary structure permits the student to experience the power of computer methodology as an adjunct to important and timely biological research.

ABSTRACT: The student can provide the computer with RNA chains of any length or to select from known RNA chains of precisely determined sequence. The computer has been programed to consider all possible base-pairing alternatives existing within the RNA chain. However, the programing is designed to weight the quality of the base-pair interactions, i.e. base-pair with a more favorable free energy of interaction are given greater weight than those with a lesser free-energy of interaction. At each step in the scan of the most favored base pairing arrangements (secondary structure) the student can choose which is to be considered, which is to

be discarded and which might be considered at a later time. The process is repeated until the entire structure of the RNA molecules is considered. At each step, the structure, local and cumulative, is graphically displayed, until the final structure is revealed.

GOALS: The student should be aware of the dynamics of RNA structure and its importance to known biological phenomena. The place of the computer in biological investigation is revealed.

References

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