TEACHING BIOCHEMISTRY IN THE HIGH SCHOOL CLASSROOM

By

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ABSTRACT

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Recent dramatic developments in biochemistry and biotechnology have resulted in more secondary schools becoming aware of the necessity to address these two rapidly evolving areas. Citizens of tomorrow will need to stay abreast of these developments - making informed societal, political, and environmental decisions affecting the future of our planet. The need for critical thinking and data analysis skills by scientifically inclined students is crucial for furthering their science education. The decisions to be made by future scientist and potential world leaders are becoming increasingly complex. This thesis is an attempt to bring biochemistry and biotechnology to the high school classroom. Included are the background, implementation, methods of instruction, evaluation data, and conclusions of this study. The unit covers structure and function of carbohydrates, lipids, proteins and enzymes, and nucleic acids. Laboratory exercises, devoted to analysis, are emphasized.

TABLE OF CONTENTS

Introduction..... 1.
General Description of Unit 1.
Literature Survey 2.
Background 2.
Laboratory and Discussion 6.
Central Questions 8.
Goals of the Unit 8.
Key Unit Lists 10.
New or Important Terms and Concepts 10.
Real World Objects, Systems, and Techniques 10.
Unit Objectives 11.
Teaching Objectives 11.
Student Objectives 12.
Transformation..... 18.
Clinical Interviews 18.
Student Background Information 18.
Analysis of Pre and Post Test Interviews 20.
Analysis of Unit Literature 22.
Do the Teaching Materials and Text Meet Objectives 22.
Does the Literature Reflect Full Scientific Understanding 24.
Does the Literature Raise or Address Common Misconceptions 24.
Instruction..... 26.
Daily Calender and Detailed Outline of Lessons 26.
Innovative Methods 27.
Notes and Anecdotes 28.
Laboratory Exercise Outline 31.
Laboratory Techniques 32.
Laboratory Objectives 33.
Evaluation..... 34.
Do Evaluation Instruments Reflect Each Objective 34.
Analysis of Test Data 34.
Evidence that the Unit has Made a Difference 38.
Conclusion..... 46.
Pertinent Events 46.
Accomplishments of the Unit 47.
Suggested Revisions 49.
Suggested Future Units 49.
Glossary..... 51.
Appendices..... 56.
Appendix A - Outline for Chemistry II 56.
Appendix B - Daily Journal 65.

TABLE OF CONTENTS - CONT.

Appendix C - Biochemistry Pre and Post Interview 74.
Appendix D - Pre Test and Post Test 77.
Appendix E - Unit Quizzes 85.
Appendix F - Unit Test 91.
Appendix G - Special Assignments 120.
Appendix H - Sample Overheads and Visuals 123.
Appendix I - Sample Labs 133.
Bibliography..... 145.
General References..... 147.

LIST OF TABLES

Table 1 - Pre and Post Interview Results 21.

Table 2 - Pre Test Results 39.

Table 3 - Post Test Results 40.

Table 4 - Regular Classroom Test Results 42.

LIST OF FIGURES

Figure 1 - Average Pre Test and Post Test Results 41.

INTRODUCTION

General Description of Unit

This thesis is based upon two years of research in teaching an eleven week biochemistry unit to second year chemistry students. Two years ago - in the summer previous to the 1988-89 school year - the author developed a second year chemistry course for Stockbridge, Michigan schools. This course was designed to offer college bound students a much stronger and more diverse chemistry background. The biochemistry unit in the original outline for the course included: carbohydrates, lipids, proteins and enzymes, nucleic acids, and buffers. Evaluation of the original outline suggested that buffers be moved into the titration and pH unit. (see outline for Chemistry II - Appendix A) This allowed students to have a good working knowledge of buffers before beginning the biochemistry unit. Therefore, buffers will be discussed minimally.

The main approach of the biochemistry unit is to provide an introduction to the principles of biochemistry - giving students a command of the concepts, the language, and an appreciation for the process of discovery in biochemistry. The unit emphasizes a laboratory approach, with 40 percent of class time in the laboratory setting. Many modern research techniques are utilized. Pre- and post interviews were conducted for the entire unit to determine student background, the strengths and weaknesses of the unit, and student comprehension. Pre- and post tests, designed to address the

stated objectives were also used for individual sections of the unit. A Complete item analysis is included in the evaluation section.

Literature Survey

Background

The idea for this research project evolved from the initial Molecular Biology Workshop for Honors High School Teachers offered by Michigan State University (sponsored by the National Science Foundation) during the summer of 1987 and subsequent research based upon workshop outcomes during the summer of 1988. The workshop emphasized: 1) biochemistry - in which Biochemistry by Stryer (1981), was used as the textbook, and 2) laboratory technique - incorporating current technologies. Subsequent research in the summer of 1988 also involved a twofold emphasis. First, to execute, revise, edit, and publish laboratory exercises developed by the previous summer's participants, which resulted in THE WORLD OF BIOCHEMISTRY, a computer software disk containing ten lab exercises for the high school classroom. Second, to develop a specific unit using these labs and other techniques for our individual classrooms.

After the author developed the outline, objectives, and laboratory exercises, his next consideration was to find a text feasible for the secondary level. The author presently uses Modern Chemistry by Metcalf, Williams, and Castka (1986). While the text does not have a unit on biochemistry, the Teacher's Resource Book does include a complete unit with six separate worksheets covering all the

areas required. The material is fairly comprehensive and easily supplemented. Supplemental texts obtained for this unit include:

Biochemistry 2nd ed. by Lubert Stryer, 1981.

3

Schaum's Outline Series Biochemistry by Kuchel and Ralston, 1988.

Harper's Biochemistry by Murray, Granner, Mayes, and Rodwell, 1986.

Biochemistry of Lipids and Membranes by Vance and Vance, 1985.

The Chemistry of Carbon Compounds by David E. Newton, 1986.

Biotechnology In Perspective by David B. Sattelle, 1990.

Modern Biology by Otto and Towle, 1985.

Exercises in Biological Sciences by Merle K. Heidemann, 1985.

Biochemistry 200 (lecture illustrations) published by the Department of Biochemistry, Michigan State University.

While the supplemental material was not entirely limited to these publications, there is certainly more than enough quality information to be gleaned for any secondary biochemistry course.

Having compiled these sources, I surveyed current literature hoping to find pertinent information on teaching biochemistry at the secondary level. In virtually everything I read there were two recurring themes. One, that biochemistry is advancing in today's technology; and two, critical thinking and data interpretation is essential for our scientifically inclined students.

The 'explosion' of biochemistry has resulted in more secondary schools becoming aware of the need to address this area. Maler (1986) stated that, "Biochemistry is a fascinating subject formally reserved for graduate students only. ...this subject is now incorporated into

the secondary school curriculum in many places" (p. 239). Maler discussed practical illustrations of the topical approach to teaching biochemistry. She includes three separate experiments that use differing reagents to find the one that properly fits to solve 'the puzzle'. Parson (1986) writes: "In the past decade, the power and scope of recombinant DNA technology have become apparent. Media coverage of potential medical applications and financial success stories have captivated students' imaginations." Parson describes the development of a topics course that stresses laboratory techniques and methods with implications in the political, social, ethical, and economic realms. Caruthers (1985) discusses the state of DNA technology in the mid 1970's, and states:

> Even 10 years ago the chemical synthesis of DNA was a time-consuming, cumbersome task ...(yielding) short DNA segments containing 10 deoxynucleotides or less. Now simple methods are available for the rapid synthesis of relatively large DNA fragments (100-200 monomers each)... (p. 577).

Present techniques have sequenced more than 26 million bases of DNA (Smith 1989). Illustrating the need for biochemical education at the secondary level Grunwald (1986) wrote:

> ... the life sciences, especially biology and biochemistry, should be considered in connection with the development of chemical curricula, because the actual problems with which humanity is confronted are becoming increasingly complex. To teach chemistry, one must teach fundamentals... in technical chemistry, biochemistry, and biotechnology. (p. 775)

This article is a synopsis of the discussion held on chemical curricula - one of the four main topics at the 1985 International Conference on Chemical Education. Included in this article is an

application and an experiment showing how biotechnology can be integrated into a regular chemistry course.

Shapiro and James (1980) in a survey about biochemical lecture and laboratory requirements of 120 colleges and universities within the Middle Atlantic Region, found: less than one third of the institutions offered a biochemistry major, library holdings of biochemistry journals were (at best) sparse, and only a limited number of biochemistry laboratory texts had been published. The same survey given today would probably yield equally interesting results. Dramatic changes have taken place in this rapidly expanding field, as summarized in the statement by Vance and Vance (1985): "Biochemistry has matured to the point that advanced textbooks in the various subcategories are required."

Critical thinking and data analysis were key aspects of this unit and were prompted by several observations. "Scientific literacy is basic for living, working, and decision making in the 1980s and beyond" (NSF, 1982, p. 4). The obligation of today's science educators will be to develop scientific and technological literacy for all students. "In the teaching of basic biochemistry, ...it is important to draw concise, easy-to-understand diagrams and schemes, ...in order to stress fundamental aspects within a unified framework" (Macarulla and Marino, 1985, p.79). Somewhat surprisingly, the author found many students have yet to reach a well developed reasoning stage. Mensch (1989) arrived at a similar observation and also noted:

> In a world where genetic engineering and molecular biology are becoming ever more important, the development of a thorough understanding of protein synthesis is vital. Teaching this highly abstract concept to students, many whom

> have not yet reached formal reasoning... is made more difficult by the lack of good 'hands-on' instructional aids. (p. 2)

After recognizing the need for easily understood diagrams and schemes as well as manipulative models to aid in critical thinking, this author had to construct methods to help students achieve these levels.

Streitberger (1988) asserts:

> Recognizing the importance of this type of reflective and critical thinking, (science based societal issues) a number of state and national groups have encouraged the following goals for the high school curriculum. These goals, ... include: (1) "to prepare students to use science and technology in understanding and improving (students') daily lives", (2) "to apply scientific knowledge to everyday life; and to introduce social and environmemtal implications of scientific and technological development", (3) "to use current societal issues and problems to meet the needs of our society and of students", (4) "to emphasize at all levels the social and human relevance of chemistry", and (5) "to use attitudes and knowledge about science to live as an informed citizen in a scientifically developed nation". (p. 60)

Laboratory and Discussion

Literature was also surveyed to find discussion units and laboratory exercises. Many discussion units and extra readings were taken from the previous list of supplemental texts. For example:

Stryer's: Biochemistry
- enzyme substrate catalysis, pgs. 107-110.
- cholesterol as a precursor, pgs. 469-71, 473-79.
- membrane lipid bilayer, pgs. 210-13.

Vance and Vance: Biochemistry of Lipids and Membranes
- cholesterol and coronary disease, pgs. 209-10.
- membrane lipid bilayer, pgs. 25-28.

Otto and Towle: Modern Biology
- all of chapter seven on nucleic acids and protein synthesis, pgs. 99-114.

Other discussion units include: "Long Arm of the Lab," an article on DNA fingerprinting from the Detroit Free Press, Science and Medicine section, (Jan. 10, 1989) and "Construction of a Restiction Map of the Bacteriophage Lamda," an article on electrophoresis by Suelter (1989). Discussion of articles was followed by an electrophoresis demonstration. An oral report on steroid abuse by athletes was required of students, giving them the opportunity to do their own literature search.

Laboratory units were taken in most part from The World of Biochemistry and from Exercises in Biological Science. Other labs include "Qualitative Analysis Of Proteins and Alpha Amino Acids" from the Kemtec Educational Corporation, distributed by Carolina Biological Supply, and "Properties of DNA" from Modern Biology, Inc. Subsequent labs, including this one, have been ordered for next year from Modern Biology, Inc. (For a complete listing of all laboratory exercises see the laboratory exercise outline on page 31.)

Preparing for laboratory exercises that were above the typical chemistry setting, this author searched for some guidelines. The following guidelines appeared in an article by Falk (1989):

> Ideally, laboratory courses in the (undergraduate) freshman chemistry curriculum should fulfill several purposes.
> 1. The laboratory experience should reinforce chemical principles discussed in the course with "real" examples.
> 2. The laboratory experience should teach the student how to observe and describe a physical phenomenon and the logical approach to solving a scientific problem.
> 3. If given a certain amount of freedom to experiment, a student should be able to explore a chemical phenomenon to a greater depth by trying related experiments on his or her own.

There is an extensive list of adaptable high quality laboratory exercises in today's literature. The key questions in choosing these are: What will serve the objectives best? Will the exercises fit the time frame of the lab? Can the exercises be accomplished with the available materials?

Hands on laboratory experiences are highly valued by our high school Science Department. Our high school science committee has set a minimum standard of 20 percent of class time in the laboratory. Our science teachers adopted this standard in the spring of 1988 based on the following recommendation by the National Science Foundation (NSF). "Time on laboratory and field work ...Senior high school level: a minimum of one instructional period per week, 20 percent of science instruction time in the laboratory or field" (NSF, 1982, p. 4). The second year chemistry course, as stated previously, doubles this requirement. The reason for this was to better address the two re-occurring themes: the biotechnological explosion and the need for critical thinking and data interpretation.

Central Questions

Goals of the Unit

The author's main classroom goal is to produce science literate citizens. However, in Chemistry II my primary goal is to motivate students to choose a science-related area as a career. Many excellent students are turned off by "boring" high school science experiences. The hope is to rectify these previous "boring" experiences and then re-kindle an interest for those students who have been alienated by

science. In improving teaching skills, this author can use the new and innovative ideas that have been recently obtained to stimulate student curiosity and eliminate apathy in lecture, discussion, and laboratory settings. Ideally, this new background in teaching techniques and subject matter will lead to better student comprehension and make learning science fun and exciting. The author also has the conviction that high school students need to see what college classes are like. For this particular unit the author tried to teach in a manner similar to a college course, with nearly as many hours in laboratory as in lecture. Hopefully, students will be 'turned on' by the promising results of biotechnology as presented in this course and summarized in the following.

Sattelle (1990) states:

> Biotechnology's potential is enormous; its promise unrivaled. As a field of scientific research and commercial enterprise, biotechnology has grown dramatically in the past several years. Not surprisingly, the general public's understanding of what "biotechnology" means has failed to keep pace with this progress. This has resulted in a lack of full appreciation of the implications of biotechnological research and development... Improved healthcare, a more abundant food supply, safer chemicals and a cleaner environment can all be realized through the science of biotechnology. (p. iv)

The major emphases for this unit - the central themes to be addressed - are for students to acquire: a working knowledge of biochemical concepts, a command of the technical or scientific language, exposure to a wide variety of biotechnological techniques, and an appreciation for the processes of discovery in biochemistry. These themes hold

promise of achieving student feedback toward the main goal of motivating students to choose science-related careers.

KEY UNIT LISTS

New or Important Terms and Concepts for This Unit:

Upon completion of this unit students will be able to define and demonstrate an understanding of the following terms and concepts.

Alcohols
Alpha amino acids
Antibody
Anticodons
Biochemistry
Biosynthesis
Carbohydrates
Carbonyl group
Catalyst
Cellulose
Chitin
Cholesterol
Codons
Coenzyme
Cofactor
Conjugated enzyme
Conjugated proteins
Dehydration synthesis
Denaturation
Digestion
Disaccharide
DNA
Enzyme
Essential amino acids
Ester
Fats
Fatty acids
Glycerol
Glycogen
Hormone
Hydrogen bonding
Hydrolysis
Hydrophilic
Hydrophobic
Hydroxyl group
Lipids
Metabolism
Monosaccharide
mRNA
Neutral lipids
Nucleic acids
Nucleosides
Nucleotides
Oil
Oligosaccharide
Organic molecules
Pectin
Peptides
Phospholipids
Polypeptides
Polysaccharide
Prosthetic group
Protein
Regulator
Replication
RNA
rRNA
Simple enzyme
Simple proteins
Starch
Steroids
Substrate
Transcription
Translation
tRNA
Waxes

Real World Objects, Systems, and Techniques:

The student will be able to explain how the following have contributed to our understanding of biochemistry or how biochemistry has contributed to our understanding of the following:

Assays
Biotechnology
Centrifugation
Diet
Dietary Fiber
Dietary Role of:
  Carbohydrates
  Lipids
  Proteins
Digestive Processes
DNA Fingerprinting
DNA Sequencing
Electronmicroscopy
Electrophoresis
Energy Transformations
Enzymatic Catalysis
Gel Permeation Chromatography
Genetic Duplication
Heart Disease
Health
Metabolic Pathways
Nutrition
Protein Synthesis
Role of Vitamins
Spectrophotometry
Steroid Abuse
Thin Layer Chromatography
X-Ray Crystallography

UNIT OBJECTIVES

Teaching Objectives:

1. To develop a new teaching module in biochemistry covering four major areas: carbohydrates, lipids, proteins and enzymes, and nucleic acids. These topics, covering a nine to twelve week period, are to be included as part of a new second year chemistry course with a major emphasis on laboratory work.

2. To accumulate background information on students - review prior information and disseminate new, incorporating the knowledge students have from their previous biology and chemistry classes.

3. To determine student accomplishment through pre-testing and post-testing.

4. To make chemistry a real world subject - emphasizing biochemistry, thus providing a unique approach to chemistry education.

5. To increase student awareness in the diversity of chemistry and its related fields by using and/or demonstrating selected new technology available to today's researchers.

6. To provide students the opportunity to work with varied materials and multiple approaches in specific areas of biochemistry - thus planting the idea that these students are capable of 'modern' research and becoming a scientist of tomorrow.

7. To determine the overall effectiveness of the new modules - by revising and reviewing the modules to determining what worked well and what didn't - using a student survey and test analysis.

Student Objectives:

Introduction to Biochemistry

Upon completion of this section, the student will be able to:

1. Define and demonstrate an understanding of the following terms: biochemistry, biosynthesis, and organic molecules.

2. State: the essential elements of life; the importance of water and carbon dioxide to life; how minerals are absorbed; the four main classes of compounds of living matter.

3. Distinguish between organic and inorganic compounds.

Carbohydrates

Upon completion of this section, the student will be able to:

1. Define and demonstrate an understanding of: carbohydrates, hydroxyl group, carbonyl group, hydrolysis, dehydration synthesis, monosaccharide, disaccharide, oligosaccharide, polysaccharide, starch, glycogen, cellulose, pectin, and chitin.

2. List: the five common monosaccharides; the three important disaccharides; the five important polysaccharides.

3. State: the composition of carbohydrates; the three main classes of carbohydrates and their structural differences; the five important polysaccharides and their functions; the other name for glucose; the function of monosaccharides, oligosaccharides, and polysaccharides.

4. Differentiate: classes of carbohydrates; types of monosaccharides; types of disaccharides.

5. Identify: an hydroxyaldehyde and/or an hydroxyketone.

6. Explain: hydrolysis and dehydration synthesis.

7. Compare: the calorie content per gram with lipids and proteins.

Lipids

Upon completion of this section, the student will be able to:

1. Define and show an understanding of: lipids, fatty acids, alcohols, neutral lipids, phospholipids, hydrophobic, hydrophilic, waxes, steroids, fat, oil, glycerol, ester, and cholesterol.

2. List: the four classes of lipids; the five common steroids.

3. State: the composition of lipids; the four major functions of lipids; the specific functions of neutral lipids, phospholipids, waxes, and steroids.

4. Identify: the structure of glycerol; the general structure of neutral lipids, phospholipids, waxes, and steroids; the specific structure of cholesterol, testosterone, and vitamin D.

5. Explain: saturated and unsaturated fatty acids; the difference between fats and oils; hydrogenation.

6. Draw: the cell membrane and include all key components.

7. Compare: the calorie content per gram with carbohydrates and proteins.

Proteins and Enzymes

Upon completion of this section, the student will be able to:

1. Define and demonstrate an understanding of: protein, enzyme, alpha amino acids, catalyst, regulator, hormone, antibody, essential amino acids, peptides, polypeptide, simple proteins, conjugated proteins, prosthetic group, denaturation, simple enzyme, conjugated enzyme, cofactor, coenzyme, digestion, metabolism, and substrate.

2. List: the functions of proteins; the factors affecting enzyme catalyzed reactions; the characteristics of protein molecules; the causes of denaturation.

3. Give an example of: each of the functions of proteins; a cofactor and a coenzyme.

4. State: the composition of proteins; the basic building blocks of proteins; the most abundant organic molecules within living cells; the two main classes of proteins; the two main classes of enzymes; what the symbol [ ] represents; the importance of the essential amino acids; how the essential amino acids that we don't have are obtained.

5. Explain: how peptides and polypeptides are formed; protein metabolism; protein digestion; amino acid biosynthesis.

6. Describe: how proteins control cell structure and function; how the enzyme substrate complex works in biochemical reactions;

where each of the following enzymes work and which substrate they act upon: amylase, lipase, pepsin, trypsin, and peptidase.

7. Identify: the twenty common amino acids from their abbreviations.

8. Draw: the general formula for alpha amino acids and label all key components; an enzyme substrate complex showing how the enzyme causes the sustrate to break apart and how an enzyme can combine two substrate molecules.

9. Compare: the calorie content per gram with carbohydrates and lipids.

Nucleic acids

Upon completion of this section, the student will be able to:

1. Define and show an understanding of: nucleic acids, nucleotides, DNA, RNA, mRNA, rRNA, tRNA, nucleosides, replication, hydrogen bonding, codons, anticodons, transcription, and translation.

2. List: the three types of RNA.

3. State: the two main functions of nucleic acids; the contributions of Franklin, Watson and Crick; the chemical nature of nucleic acids; what each of the types of RNA represent: mRNA, tRNA and rRNA.

4. Identify: the structure of AMP, ADP and ATP.

5. Complete: the proper sequence for a given strand of DNA and a strand of RNA.

6. Describe: the composition of nucleic acids; where specific pyrimidines and purines are found; the base pairings for DNA and RNA; the formation of DNA and RNA.

7. Explain: the role of ADP and ATP; the function of DNA and RNA; the five step process of protein synthesis.

8. Compare: the structure and function of DNA to RNA.

9. Draw: a section of DNA - include at least six bonds with proper base pairing; the basic tRNA structure and label.

*** Students are expected to demonstrate an understanding of all terms on both tests and laboratory exercises.

TRANSFORMATION

Clinical Interviews

Student Background Information

The first step in conducting the student interviews was to gather background information. This information included: age, sex, grade, G.P.A., and educational background. Educational background was divided into schools attended and previous secondary science classes. Because all of my students wished to participate and the classes were small - 11 students in '89 and 12 students in '90 - all students were interviewed. The average age of the '89 class was 17.3 years with ages ranging from 16 to 18. The average age in '90 was 17.4 with the range being 17 to 19. The ratio of females to males was 3:8 in '89 and 6:5 in '90. There was one junior and ten seniors in the '89 class, while there were three juniors and nine seniors in the '90 class. The average G.P.A. in '89 was 3.47 ranging from 2.75 to 4.0 and 3.49 in '90 with a range of 3.0 to 4.0. All students, except one, had attended Stockbridge High School throughout their high school years. All students attended middle school in the district, with the same exception; one third had attended elementary school in other districts. Biology and Chemistry I were taken by the total (23 students) of both classes. Most students (14) had taken - or were taking - Physics. Approximately half had taken Earth Science. One-fifth had taken Physical Science and only two students had Anatomy and Physiology. Other nominal science classes were Human Science (Health) and General Biology.

Next, this author was curious about the students' educational and personal goals. The questions encompassed plans for furthering their education, area(s) of interest - major/minor, and career goals. Every student planned on continuing his/her education. Two were planning to attend junior college while the remainder planned on attending a four year institution. The colleges most frequently named were: Michigan State University (7), Michigan Tech (4), and the University of Michigan and Central Michigan University (3) each. Only one student was going out of state; to the University of Depaul. Upon completion of this unit, fifteen of the twenty three students chose a science as their main area of interest. They listed these majors and minors as their choices:

Chemistry - 4
Physics - 2
Environmental Engineering - 2
Biochemistry
Bio-Physics
Medicine
Sports Medicine
Dietary Nutrition
Areospace Engineering
Electical Engineering
Computer Engineering
Environmental Conservation

Other majors and/or minors were: Business Law (3), Math, Accounting, and Journalism. Three students had yet to decide but were leaning toward science. Many students hadn't reached a decision on career goals. Comments were, for the most part, that they wanted to complete a year or two of college before making such an important decision. However, there were a few students who felt they had firmly decided. One felt she would pursue a medical career, placing her

emphasis on developing new medicines. One student was certain that he was going to pursue biochemistry as a career. Another student was sure she wanted to be an Environmental Engineer. Bio-Physics was the career choice of a fourth student.

Analysis of Pre and Post Test Interviews

After gathering the background and educational goals, the author used a pre-test, post test format to determine how much prior biochemistry knowledge students had and then compared with the post test interview to see if the unit had indeed made a difference. The interview form is included in appendix C. For this section, the author wrote down student responses as close to verbatim as possible and then waited to evaluate them when all pre-interviews were completed. The same format was followed for the post interview. To protect the integrity of the interview, the author simply asked the questions and made no comments whatsoever until each response was completed. The responses were evaluated as if the students were being graded for a regular test, with the exception of no partial credit.

The interview consisted of a set of 20 questions. Average pre-test scores for '89 and '90 were 3.18 and 2.72 respectively. Average post test scores were 14.22 and 13.44 for '89 and '90. The results are given in Table 1. Recall there are 11 students in the '89 class and 12 students in '90.

Table 1 Pre and Post Interview Results

Scores are listed in the format of '89/'90 for correct responses. For example: a score of 2/5 under the heading '89/'90 would represent two students in 1989 gave correct responses and five students in 1990 answered the question correctly.

Questions (condensed)	Pre-test '89/'90	Post test '89/'90
What is Biochemistry?	3/3	6/7
What is Biosynthesis?	1/1	5/6
Four classes of organic coumpounds.	0/0	8/7
Major use of carbohydrates?	7/8	9/11
Carbohydrates contain?	9/5	11/9
Three main classes of carbohydrates.	0/0	8/6
How does glycogen differ from starch?	0/0	4/3
Largest use of lipids by humans?	4/6	8/7
Tryglycerides are commonly called?	4/2	9/6
Main function of waxes?	3/3	9/11
Two examples of common steroids.	0/0	11/10
Element found in proteins but not ...	3/3	11/11
Basic building block of proteins.	1/2	10/9
Define conjugated protein.	0/0	5/3
Enzymes are catalyst for digestion and ...	0/1	5/3
What is meant by denatured protein?	0/0	10/12
DNA and RNA are responsible for ...	0/0	6/10
Define replication, transcription ...	0/0	7/9
What are buffers?	5/6	9/10
Three key areas of physiological...buffers.	2/4	6/8
Why is Biochemistry important to you?	6/4	8/11
Why is Biochemistry important to mankind?	7/6	9/10
How has this unit aided you for college?	-	8/10
Average total scores (not including last three questions)	3.2/2.7	14.2/13.4

The results of this survey show that the students' major prior information concerned the function of carbohydrates and carbohydrate composition. Average scores on the post interview show that substantial learning had indeed taken place. The questions for the most part were consistent with the stated objectives; however, there were some exceptions. One exception was: "How does glycogen differ

from starch?" which is an ambiguous question. Students were unclear whether they were being asked for structure or function. "Define conjugated enzyme." had many responses that were close, but if students did not specifically use the term 'prosthetic group' they were not given credit. "Enzymes are catalysts for digestion and..." also had some ambiguity, with many 'respiration' responses instead of the correct response: 'metabolism'. The question "Largest use of lipids by humans?" would have been answered correctly by all students had the author accepted 'cell membranes' in addition to the expected answer: 'energy storage'. The questions covering buffers indicated the necessity for a brief review. Over 88 percent of the students had developed an appreciation for Biochemistry and considered it important to them personally and to mankind. Nearly 80 percent of the students felt this unit would be beneficial to their college careers.

One major omission of the interview was the total absence of questions concerning laboratory investigations. With 40 percent of class time being allocated to this crucial area, the author would certainly suggest laboratory questions be included. This omission was definitely a major mistake.

Analysis of Unit Literature

Do the Teaching Materials and Text Meet Objectives?

The "Teacher's Resource Book" that accompanied the course text had worksheets that covered most of the objectives. However, each

section did require a certain amount of supplemental material. The two sections that required the most additional material were lipids and nucleic acids. Supplemental materials for all sections are discussed in the following paragraphs.

Other areas the worksheets covered did not include the importance of: hydrogen bonding, buffers in living systems, the electron transport system, photosynthesis, bioenergetics, and the role of ATP, ADP, and AMP. (see Appendix H)

The lion's share of the supplemental material was presented as overheads and used primarily for discussion. The author's purpose was to introduce the complexity of biochemistry, make important connections resulting in better student comprehension, and present the complexity of biochemistry without demanding students assimilate extra information. Stressing career opportunities throughout the discussion of supplementary materials was a key point. Additional discussion elements for carbohydrates contained the digestion, absorption, and metabolism of carbohydrates, including the functional role of glycogen. The lipid supplementary material covered fatty acids, digestion and absorption of lipids, lipid transport in blood, role of cholesterol, energy from lipids, biosynthesis of lipids, and hormones. Structure of the cell membrane's lipid bilayer was covered at length as it was not included in the worksheets. Proteins and enzymes required two handouts. Protein structure was definitely deficient and needed help. A handout from Newton (1986) covered this well. Protein digestion and metabolism were also discussed as well as amino acid biosynthesis. A handout elaborating on the enzyme-substate complex

was included from Stryer (1981). A number of overheads were necessary to complement the nucleic acid section. Cellular characteristics, the flow of genetic information, nucleotides, key elements of DNA structure, the functional classes of RNA, transcription and translation, and mutations were improved upon or introduced. Students were given a Modern Biology text and required to read the chapter on nucleic acids and do the end of chapter assignment.

Does the Literature Reflect Full Scientific Understanding?

Analyzing the teaching literature for content is vital for the success of the unit. The key of the author's analysis was to see if the whole of the objectives were covered. Any omissions in the text and re-enforcing materials must be covered. This author feels the biochemistry worksheets satisfactorily meet the objectives stated in the introductory section. Supplementary materials, for the most part, were necessary to bring about a more complete understanding of biochemical processes. If students were expected to meet objectives covering the areas of metabolism and digestion for example, this would add at least three to four weeks to the unit and place demands upon them that are unrealistic. The lone exception was the omission of the lipid bi-layer of cellular membranes. This concept is well within the grasp of secondary students and the author believes it is vital to a basic understanding of biochemical processes.

Does the Literature Raise or Address Common Misconceptions?

Misconceptions are the major roadblock to student learning. Nowhere is this more apparent than in the teaching of science. Complete texts have addressed this issue. Driver, Guesne, and Tiberghien (1985) found students' conceptions have several general features. These features are: perceptually dominated thinking, limited focus, linear causal reasoning, undifferentiated concepts, context dependency, and predominant conceptions. To expect any educator to address all of these features is, of course, ludicrous. Nonetheless, any quality educator must keep these thoughts in mind when helping students tackle difficult and/or abstract concepts.

The worksheet text contained two considerable misconceptions. First, it was stated cholesterol was a steroid. This notion was misleading for students. Cholesterol is the metabolic precursor for the five major classes of steroids (Stryer, 1981). This misconception was fully addressed and hopefully corrected. The second misconception presented in the text stated that waxes were also steroids. Waxes are a class of neutral lipids composed of long chain alcohols and fatty acids. This issue was also addressed in discussion. When discussing and correcting misconceptions with students I emphasized that authors have varying opinions and texts are frequently edited, leaving out valuable information.

One benefit of teaching this unit for students was their response regarding that being a scientist or laboratory technichian is not as boring as previously perceived. The emphasis on biotechnology and the subsequent discussions certainly aided in clearing up this common misconception.

Daily Calendar and Detailed Outline of Lessons

Chemistry II Journal - Biochemistry

The journal encompasses the daily calendar of the unit and a detailed outline of the lessons and methods of presentation. The lecture outline for the entire course is in appendix A. Appendix A includes buffers which were covered earlier in the course and reviewed at the beginning of this unit. Samples of laboratory exercises, overheads, special assignments, and visuals are listed in the daily journal (Appendix B) and placed in separate appendices. (Appendices G, H, and I) The pre and post interview, pre-test and post test, regular quizzes and regular test are also included in the appendix. (Appendices C - F) Below is a sample of the daily journal.

Day - 1 Monday: 2-12-90

LESSON PLAN
- Introduction to Biochemistry
- Pre-Test: Carbohydrates (appendix D)
- Assignment: Read worksheet #1 - 'The Chemistry of Living Things'
- Review: Buffers and their physiological importance - (appendix A)
- Lecture notes and discussion: see outline (appendix A)
  - Teaching assists - overhead projector, blackboard, & visuals (appendix H)
- Homework: Questions 1-9 worksheet 1

Day - 2 Tuesday: 2-13-90

LESSON PLAN
- Correct homework and discuss
- Finish lecture
- Homework: Handout "Long Arm Of The Lab" - DNA Fingerprinting Article from The Detroit Free Press
  - Students are to read, summarize and include their

opinion of this 'state of the art technique' as permitted by the courts to use as evidence.

Day - 3 Wednesday: 2-14-90

LESSON PLAN

Collect DNA Fingerprinting summary
Discussion: article itself, grading procedure - content, grammar, spelling, punctuation, and clarity.
Demonstration: How gel electrophoresis works
Handout: "Construction of a Restriction Map of the Bacteriophage Lambda" - article on electrophoresis by Dr. Clarence Suelter, M.S.U.
Homework: Worksheet #2 'Carbohydrates' read for tomorrow.

*** Samples of visuals, overheads, special assignments, and laboratory exercises are included in the appendix. Each is listed in the daily journal for easier access. (Appendix B)

Innovative Methods

Having a small class lends itself to easier applications of innovative methods. Demonstrations can be viewed 'up close' by all students. One of the more innovative areas the author tries to maintain is the introduction of new or up-to-date laboratory techniques. In order to save money, a laboratory exercise can be performed as a demonstration rather than by the entire class. Although a demonstration is not the prefered method, for small classes it can be accomplished successfully. Three demonstrations that the author does for students, executed as demonstrations rather than 'hands on' laboratory exercises are: electrophoretic techniques, partition chromatography, and gel permeation chromatography. The author believes the requirement that students spend 40 percent of their class time in laboratory is also quite innovative. This allows

students the opportunity to pursue in depth many of the lecture topics. Students get added experience in laboratory practices benefitting them as college freshmen.

Small classes are also more manageable for small group work. Many homework and review assignments are handled in small groups when the students are 'creating' the assignment material. This small group work is implemented before all major test and for difficult homework assignments; this encompasses 15-20% of class time. Dicussion amongst peers is beneficial for increased learning because questioning students receive feedback from fellow students which for many is easier to assimilate. Learning is also aided in small groups by students having to search for answers rather than getting a quick 'in one ear and out the other' response from their teacher. Students get better acquainted with each other and are more likely to risk making an incorrect statement. Discussion is also easier to elicit from students. Discussion is a major priority with this author. When students carry out discussions however, misconceptions may occur, but they can be easily corrected. Good discussion also gives the instructor an excellent indication of what the students understand about the topic(s).

Notes and Anecdotes

DNA Fingerprinting was a true success with students. Using the two articles "Long Arm of the Lab" and "Construction of a Restriction Map of the Bacteriophage Lambda" in conjunction with the electrophoresis demonstration peaked in a high level of interest, a

lively discussion, and even a minor debate. The debate centered on the theme that even the best trained laboratory technichians can make mistakes and that lawyers can somehow always come up with their own 'experts' to refute findings. Clearly students retained a good deal of knowledge on electrophoresis and DNA sequencing.

When students were asked for someone to explain or define the term 'assay' - even though these students have some familiarity with this term - the normal response is no show of hands, or at best a roundabout guess. Therefore, I developed a way of helping them to remember. First, I simply tell them the common definition: "The qualitative or quantitative analysis of a substance." Next, I ask if everyone is comfortable with this definition; normally most students nod or respond affirmatively. Then I write the word 'ASSESS' on the board in big capital letters. I then say: "to assess something is to analyze, correct? (lots of nods) Now an 'assay' is nothing more than a way to assess (point to the word on the board) a substance. For when you do an assay, do it very carefully so you do not end up looking like ----(pause)" and I quickly erase the last 'S' in ASSESS. Now, I certainly have everyone's attention. However, the remaining word on the board is not one to be left in plain view. So, I quickly erase the word and someone invariably says: "I don't get it," which puts the rest of the class in stitches. By the time a fellow student explains to this person, order is pretty well restored. Epilogue: The term 'assay' is now well - if not permanently - etched in their minds.

The protein digestion lab led to a real eye opener for one young man. When I asked him, "Has there been much of any sign that digestion has taken place?" the student replied, "No, not much. The gelatin is still seven millimeters across." I then replied, "Determine the surface area of the piece of gelatin now as compared to the full ten millimeter square you started with." He did, and his response was non-verbal. He just looked wide-eyed and wore a very sheepish grin, to which I replied, "Do you still say there has not been much of a change?" The young man responded, "No, I'd say there's been quite a change." I simply smiled in approval and moved on to the next station to ask the same question.

While reviewing proteins, in particular conjugated proteins, my students had a good laugh at my expense. I used the term 'prostrate' group instead of prosthetic group and the classroom burst into laughter, including me. Laughter can certainly be said 'to be the best medicine'. The ensuing discussion was lively and - to say the least - full of smiles.

A major benefit these students receive is the familiarity they obtained with modern laboratory techniques and equipment. These included - but were not limited to - Qualitative Assays, Ouchterlony Diffusion, Spectrophotometric Techniques, Electrophoretic Techniques, Chromatographic Techniques, Hydrogen Ion Equilibria, Acid and Enzymatic Hydrolysis of Starch, and Determining the Physical Properties of DNA. Ouchterlony Diffusion and Hydrogen Ion Equilibria were not included in the biochemistry unit. Nonetheless, students did perform these labs earlier in the year. Student comments and

reactions were very favorable. They commented that they enjoyed the variety and the 'newness' of these techniques. Students conveyed they are more comfortable with attempting complex scientific topics and current laboratory techniques.

Laboratory Exercise Outline

Nine exercises make up the laboratory section of this unit. Two of the laboratory exercises were completed as demonstrations due to a lack of materials. Most of the exercises took multiple class periods to complete. Only the 'Fat Digestion' lab could be completed in one laboratory period. The 'Study of Saccharides' lab took five days to complete. In total, 19 days were committed to laboratory work. Dividing this number by the actual number of classroom sessions - 48, a total of 40 percent of class time was spent in the laboratory setting. The following is a list of the laboratory exercises and their location in the appendix.

1. Pre-Lab: Study of Saccharides
2. Saccharides
3. General Effect of Salivary Amylase on Starch
4. Introduction to Spectrophotometry
5. Digestion of Fat
6. Introduction to Qualitative Analysis of Proteins and Amino Acids
7. Partition Chromatography of Amino Acids
8. Protein Digestion by Papain and Bromelain
9. Gel Permeation Chromatography
10. Properties of DNA

Labs #3, #4, #8 and #9 were taken from The World of Biochemistry software disk. Lab #2 and lab #7 were obtained from Exercises in Biological Science. The pre lab #1 was developed by Sonja Michaud (my

student teacher in '89) and revised by the author. Lab #5 was developed by the author. Lab #6 was obtained through Carolina Biological Supply and lab #10 was acquired from Modern Biology, Inc.

I now have over 100 laboratory exercises covering biochemistry and biotechnology. Which I will gladly share with anyone who requests them. Lipids is the one area where laboratory exercises are scarce. A list may be obtained by contacting the author. Address: Stockbridge High School, 416 North Clinton, Stockbridge, Michigan 49285.

Laboratory Techniques

Laboratory techniques emphasized in the saccharide lab are: Iodine Test, Benedict's Test, Acid Hydrolysis, and Enzymatic Hydrolysis. For the salivary amylase lab spectrophotometry or colorimetry is the technique used. The 'Introduction to Spectrophotometry' lab stresses spectrophotometry and graphing. The fat and protein digestion labs also emphasize Enzymatic Hydrolysis. The qualitative analysis lab incorporates the use of Assays to identify specific proteins and amino acids. Paper chromatography and determination of R_f values are the key techniques used in 'Partition Chromatography'. The 'Gel Permeation Chromatography' lab is a unique separatory technique for the secondary level. Determination of the physical properties of DNA offers students techniques of spooling, denaturation, and enzymatic hydrolysis.

Laboratory Objectives.

Upon completion of this section the student will be able to:

1. Determine: whether certain saccharides are reducing sugars; if sugars are helically coiled or branched polysaccharides; the effect of enzyme hydrolysis and acid hydrolysis upon polysaccharides; the end products of fat digestion; the effect of pH on enzymatic action; the R_f value of an amino acid in a given solvent; the effects of DNAase on the structure of DNA.

2. Discover: the physical changes that occur during starch digestion; the physical properties of DNA.

3. Practice: the use of the spectrophotometer and eventually master its use upon completion of lab exercises; spooling techniques as a means of separation.

4. Graph: the visible absorption spectrum of a colored solution.

5. Plot: a graph and interpolate results; and develop a standard curve.

6. Explain: how lipase and bile work together in the breakdown of fats; the enzymatic breakdown of a protein.

7. Assay: for identification of proteins and amino acids; for determining an unknown protein or amino acid; for determining denaturation of a single strand of DNA.

8. Separate: amino acids using paper chromatography.

EVALUATION

Do Evaluation Instruments Reflect Each Objective?

The pre and post test instruments do not reflect each objective; they are shortened versions of the regular classroom test which does cover the full spectrum of objectives. Students' grades were not affected by pre and post test scores. Grades were only based upon regular classroom work. However, post test could be used as part of the overall grade. The pre and post test instruments were designed to include a broad sampling of objectives. The reasoning behind this was that pre and post test covering all objectives would take nearly a full period to complete and students would bemoan such an exercise. Full cooperation of the students to put forth their best efforts was essential for the results to be meaningful. Therefore the instruments were intentionally kept short to attain a higher level of quality student effort. Pre and post test instruments were used in previous units to acquaint students with the concept of testing for analysis. Early results indicated that some students simply wrote in an answer just to finish the test as fast as possible. Fortunately, most of the students cooperated well. While the uncooperative ones dropped the course at semester break - before the biochemistry unit. The pre and post tests are included in Appendix D.

Analysis of Tests Data

An item analysis was conducted for each of the pre and post tests. The answers were keyed on scantron sheets and an item analysis processed in the Computer Laboratory at Michigan State University was

completed. Item analysis is useful in guiding and improving instruction. According to Olson (1990), a minimum number of 50 test will yelld the best analysis. This study was by necessity, conducted on class sizes of 11 and 12 respectively. However, the item analysis statistics were beneficial to the author and did indicate several key results which are discussed later.

Item analysis breaks the students' scores into groups. The groups are divided into the upper, middle , and lower groups. This is done to determine an "index of discrimination". The index of discrimination is the difference between the proportion of the upper group with correct responses and the proportion of the lower group with correct responses. Olson (ibid) states: "It has long been accepted that optimal item discrimination is obtained when the upper and lower groups each contain twenty-seven percent of the total group." (p. 1). The higher the discrimination index, the more discriminating the question. The difficulty index is the proportion of the total group who answered the question item incorrectly. A high difficulty index indicates that the question is difficult while a low index indicates an easy question. Olson also noted: "...most test constructors desire items with indices of difficulty no lower than 20 nor higher than 80, with an average ...from 30 or 40 to a maximum of 60." (p. 2). Using these two indices, the author was able to look for ambiguity, level of difficulty, and poor option alternatives in the individual test questions.

The item analysis of the post test helped to determine student performance in meeting the objectives. Findings included: all tests

would have been more meaningful had they consisted of twenty questions (see Appendix D); and that it is very difficult to include four suitable alternative distractors (options) for certain questions. In addition, questions with low indices of difficulty (20 or less) presented a dilemma: were the questions too easy or does this indicate that objectives were met by a high percentage of students. None of the tests had items that reflected an ambiguous question. This is evident by the fact that none the lower group scores were higher than the upper group for any question. The results are very subjective due to the small size of the groups. The pre-test results give much better ranges within the indices than the post test. However, one finds it very difficult to analyze instructional methods based upon scores achieved before material was even presented. Completing the item analysis, the author arrived at the following results for each section of the unit.

The carbohydrate test consisted of 15 questions containing three items from the laboratory objectives. Five of the questions for the '89 group had low difficulty indices. In analyzing these questions, the author felt number 4 would be considered an easy question and the others simply indicate students had met the objectives. The '90 group had two questions with low difficulty indices, questions 4 and 10. This suggests that #4 is indeed an easy question. Questions 3, 4, and 12 had indices of discrimination of zero for both years. However, question #3 has a fairly high index of difficulty in '90 which means that the middle group students missed the question instead of the lower group. Question #12 is definitely a problem, with indices of

difficulty of 100 and 91 respectively and discriminations of zero. This question needs correcting. While the correct response was there (#5), the students had chosen response #2 - which was only partially correct. Rather than being an ambiguous question, which is normally indicated by such results, the responses were ambiguous and the item can easily be corrected by revising response #2. This should give a much better range in the item analysis. The remaining questions, based on the analysis, fit the desired indices fairly well.

For the remaining sections on lipids, proteins and enzymes, and nucleic acids, the analysis will be based on both years combined to give a somewhat better indication of test item quality. For the results on the tests see Table 2.

The lipid test contained ten questions with one item from the laboratory objectives. Questions 2, 6, and 9 had indices of difficulty and discrimination of zero for both years. Did these results indicate easy questions or were objectives met by all students? The author feels they are all viable questions. Pre-test scores give no indication of 'easy question' items and anyone taking the test without a background in 'lipids' would be fortunate to guess one of three. Question #5 fits the 'ideal' test item very well. Students chose three of the four other optional responses and both indices were fairly high and within the recommended range. There was no indication of a very difficult question or one that was ambiguous.

The protein and enzyme test consisted of 20 questions with only one item concerning a laboratory objective. Only question 14 had low indices for both difficulty and discrimination. This question upon

examination is easy for students based upon the very poor quality of the other options (distractors). These distractors certainly need to be changed, or at the very least, improved upon. When developing test its imperative to carefully choose all distractors. Question #4 was a difficult question. The index of difficulty in '89 was 100 and 58 in '90. Analysis of the question showed that the question was designed to be difficult and required 'critical thinking' on the part of students. The '90 group showed improvement on this question and the index of discrimination was 100, indicating only the 'thinkers' got the question correct. Item #4 was also a well balanced test item having students choose all four other options. The remaining items fit the desired indices fairly well.

The nucleic acid test was the last of the pre and post test. The test contained 15 items, none of which included a laboratory objective. Questions 4, 5, and 15 have low indices of difficulty and discrimination. The pre-test indices are all high for these three questions and upon evaluation of the items, none were considered easy. The students had met these three objectives very well. Question number 6 in the '89 group showed good option balance but in '90 all of the students but one had correct responses.

Evidence that the Unit has Made a Difference

The following tables and figure are illustrations of the results students attained after completing this unit. Pre-test and post test results for both years are included in Tables 2 and 3. Table 4 shows the results for both years of the students' regular classroom tests.

Figure 1 represents the pre-test and post test comparisons for both years. Tables 2-4 and Figure 1 reflect combined class averages. Pre test and post test are included in Appendix D.

Table 2 - Pre Test Results

Results reflect 23 combined scores for '89 and '90.

Test Topics	Carbohydrates	Lipids	Proteins & Enzymes	Nucleic acids
Pre-Test '89 & '90				
Total questions	15	10	20	15
Mean score	9.08	4.59	8.09	4.51
Percentage	61	46	40	30
St. Deviation	2.34	1.47	2.88	1.39
Range	4-13	1-7	2-14	2-7

The carbohydrate test scores on the pre-test in '89 were inflated due to students not taking the test until five days into the unit. This was the fault of the instructor as it was nearly forgotten. The pre-test scores in '90 were also somewhat inflated as the reading assignment had been completed prior to the test. Thus the average scores do not reflect a 'true' pre test score. Fortunately, these errors did not occur in the other units.

Table 3 - Post Test Results

Results reflect 23 combined scores for '89 and '90.

Test Topics	Carbohydrates	Lipids	Proteins & Enzymes	Nucleic acids
Post Test '89 & '90				
Total questions	15	10	20	15
Mean score	9.73	8.39	14.03	11.71
Percentage	65	84	70	78
St. Deviation	2.58	1.33	3.73	2.82
Range	6-14	5-10	8-20	3-15

The average score on the carbohydrate post test is not reflective of student achievement. The results of the carbohydrate post test (class average of 57%) were disappointing for the class of '90. This, the author feels, is a direct result of student apathy toward post test and this 'lack of effort' was subsequently addressed. The post test results in '89 showed an average of 73 percent which is within the 'normal' percentage for the secondary classroom. Combining the two averages, a percentage of 65 was obtained. Four students did achieve a score of 14 correct indicating positive results.

The lipid test scores were better than expected on the post tests and indicate that a great deal of learning had occurred. With class percentages going from 49 to 85 in '89 and from 43 to 83 in '90 the author was very pleased with the results. However, the author's enthusiasm was somewhat tempered by the brevity of the test. A test half again as long, or better yet, even twice as long would be a much more accurate indicator of student achievement. Five of the total 23 students did receive perfect scores of 10.

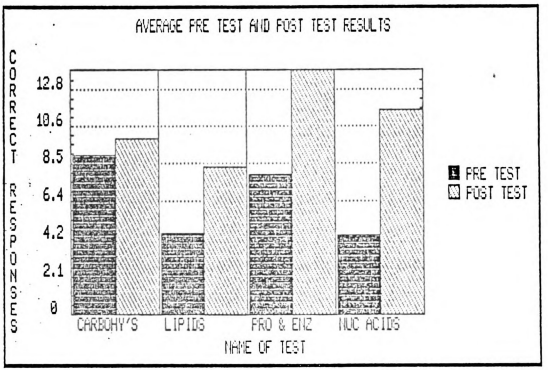


Figure 1 - Average Pre Test and Post Tests Results

The protein and enzyme post test also indicated marked improvements over the pre-test. Combined average percentages for the two pre-tests were 40 percent and for the post test the combined averages were 70 percent. One score of 19 was recorded for '89 and one score of 20 was achieved in '90. The protein and enzyme test is the better indicator of student learning as the average scores are based upon 20 questions rather than 10 or 15 as in the other tests.

The results on the nucleic acids test are also encouraging. The test scores improved from 28 to 73 percent in '89 and from 32 to 83 percent in '90. The mean score improvement on the post test of 1.59 in '90 is significant. The author feels this resulted from additional supplementary materials and an improved laboratory exercise. There were four perfect scores of 15, two in each year.

Table 4 - Regular Classroom Test Results

Results reflect 23 combined scores for '89 and '90.

Test Name	Carbohydrates	Lipids	Proteins & Enzymes	Nucleic Acids
Regular Test '89 & '90				
Total points	62	68	75	65
Mean score	46.81	54.45	55.10	51.35
Percentage	76	80	74	79
St. Deviation	6.79	9.59	11.58	9.98
Range	30-56	33-67	39-72	22-65

Table 4, summarizes the results of the regular classroom test. The author believes that regular classroom tests are accurate indicators of student performance, since these test scores greatly

affect students' grades. The regular test encompasses nearly all objectives, both lecture and laboratory. The mean for the regular test scores is 68. Multiple choice, short answer, matching, listing, explaining, defining, analyzing, and interpreting questions were used. Laboratory objectives were extensively included. The complete set of tests is included with answer keys in Appendix F.

The main purpose in including the regular test results is to add validity to the post test. By comparing the percentages on the regular tests with the post tests, one is able to see that the post test results are indeed valid. The four test result percentages of the post test and regular test match up favorably. The one exception in the results is the carbohydrate test comparison. There is a difference of 13 percent in post test and regular test percentages. The author noted this discrepancy to the class - asked them to give a complete effort on the pre and post test - and based upon the remaining test comparisons, this effort seems to have occurred.

Evidence of student interest came in 'unmeasurable' forms. Students would come into class before and after school to re-do or improve laboratory exercises. Students, interested in specific areas, would request additional readings and there were even a few requests for additional labs. The highlight of the year occurred when two seniors, who had the last week off, came back into the class for three out of the five days to do additional laboratory work; staying for two hours or more performing titrations and other analysis techniques.

A discussion during the lipid unit led to the discovery of a common misconception among high scool students. The statement was

made by one of the students that fats in any form are 'bad' for you. Many students agreed. An animated discussion ensued involving all students; after emphasizing all of the functions of lipids and the necessity of lipids for body processes, the misconception was corrected.

The final evidence came during the post interviews. Nineteen of the twenty three students gave very good to excellent answers to the questions - "Why is biochemistry important to you?" and "Why is biochemistry important to mankind?". The final question on the post interview was - "How do you think this unit will benefit you in your college science courses? Eighteen of the twenty three students felt this unit and the preceding units would be of great benefit for college. Some of their comments included: "I'm planning on going into medicine and I no longer have nearly the level of fear for the difficulty of the classwork and labs." "I was planning on majoring in Genetics but now I've switched to Bio-physics." "I was thinking about majoring in Chemistry, now I'm planning on it!" This was a good indication that students were indeed motivated to choose science as a career. The few who felt that this unit really wouldn't help in college were going into a business related curriculum. However, several of these students did comment that the study requirements were good preparation for the rigors of college classes.

Fortunately, there was not a large quantity of negative evidence. Each year there were two students who I could not seem to motivate and get them truly interested in biochemistry. Whether they did not care for the material or 'senioritis' had set in, the author remains

disappointed in their performance. Looking over the laboratory grades the author noted two students had achieved scores of only 50 percent on the "Physical Properties of DNA". This was a very low score compared with the others. As mentioned previously, the post test on carbohydrates was not very positive primarily due to student apathy, but objectives were met as evidenced by the regular test scores.

CONCLUSIONS AND REFLECTIONS

Pertinent Events

Undoubtedly, the major highlight of the unit for the students and the author is the opportunity to work with the current laboratory procedures. The DNA Fingerprinting article, the Restriction Mapping article and the subsequent Electrophoresis demonstration was an effective way to start the unit; this combination truly piqued student interest. The discussion of DNA Fingerprinting as permissible courtroom evidence gave students an opportunity to consider a science based societal issue. The saccharide lab gave students five consecutive days in the laboratory, giving them a sense of how three to four continuous hours in a college lab would 'feel'. The classwork assignment 'Looking at the Saccharides in Our Foods' gave the students the opportunity to make informed decisions about nutritional content. The oral report on steroid abuse allowed students a chance to do a brief literature survey and obtain valuable information on a major societal issue. Data analysis was the primary objective of many labs encouraging students to use 'critical thinking' procedures. The demonstrations on Gel Permeation Chromatography and Partition Chromatography were well recieved and discussed at length. The entire class period spent looking through the special visuals of Cell Ultrastructure was 'fun' for all and the time passed quickly. The extra materials in the form of overheads and visuals during review sessions led to many interesting student observations and questions, lending valuable connections for students.

Accomplishments of the Unit

The strengths inherent in this unit are many. Students were required to draw and label the basic structures of compounds in all sections, not just 'memorize to recognize'. This technique led to better understanding and comprehension. The more written biochemical exercises required of students, the higher the attainment of objectives. Group work - having students create their own review assignments, questions and problems - proved to be very beneficial. Students adapted well to new laboratory techniques. Even though a mastery level was not achieved by all, the opportunity was available. The laboratory requirement of 40 percent is believed unique in the secondary setting and is clearly one of the strengths of this unit. The assigning of work in small groups showed evidence of this technique being very helpful, especially for the 'slower' learner. Peer feedback and assistance helps increase comprehension for students. Emphasis on discussion was to involve all students; the author believes this emphasis provides a strong teaching methodology in this unit.

The unit has some weaknesses. One weakness of the unit is the complete lack of audio-visual materials. Films, videos, and filmstrips exist, but are not readily available. These audio-visual materials are unavailable in either the high school library, the science department library, or the intermediate school district library. Audiovisuals for biochemistry are available, but are costly. Another negative aspect of the unit is the lack of 'hands-on' models for students. While the high school science department does have

molecular construction models, there is a need to purchase nucleic acid and other biochemical models. Available time constraint is a third weakness of the unit. There is not enough time to cover the objectives in as much detail as the author would prefer. There is certainly enough biochemical information available to fill an entire semester. However, in establishing the Chemistry II course the author was responsible for creating a comprehensive course. For example: the biochemistry unit was shortened by two weeks in '90 to include a four week unit on Environmental Chemistry. Other units were condensed as well. Another more subtle fault is the one-hour laboratory time frame. This breaks up the continuity of lab exercises and creates difficulty in finding an appropriate place to 'stop' until tomorrow. While not a true fault of the unit - but nevertheless a problem - is the fact that students were not enthusiastic with the pre test and post test; but most complied well and results on the whole are accurate.

The evidence presented by the post test scores and the additional regular test scores is indicative of better student understanding in biochemistry. Average post test scores - based on mean scores - over the four sections in '89 of 75 percent, post test averages (3) of 79 percent in '90 (not including carbohydrates due to validity concerns) certainly lend credence that learning has been achieved. Supported by average regular test scores - again inclusive of all four sections (77 percent in both '89 and '90) - evidence is clear that the objectives were attained, and at nearly the accepted 'ideal' value of 80 percent.

Suggested Revisions

One revision is to create 20 item pre tests and post tests to be used in all four sections. This would improve the validity of the test and make subsequent analysis much easier. Secondly more laboratory objectives should be included. The pre tests and post tests should be checked to see if they are a true cross sampling. Distractors for all tests questions need to be reviewed and improvements made.

The carbohydrate unit needs improvement in two areas: first, to include more written exercises and second, to incorporate more small group work. Having students keep a daily or weekly journal including brief comments concerning content and methods - to be read by the instructor - would aid in evaluating and improving the unit. The use of appropriate hands-on models would aid all students, especially the visual learners. Field trips to biotechnology industries and/or university laboratory facilities would give students an oppurtunity to see the application of these 'real world' techniques. Finally, the author would bring in as many quality audiovisual materials as possible in fitting the time frame of the course.

Suggested Future Units

Additional units that could be included are: Photosynthesis, Physical Chemistry, Cellular Biochemistry, and a Research Project. The photosynthesis unit would emphasize: photosystems, the Dark reaction(s), and plant metabolism. Physical Chemistry's emphasis might include: reaction energies and kinetics, chemical equilibrium,

and a more in-depth study of oxidation-reduction reactions. A Cellular Biochemistry unit should consider: anatomy and physiology, molecular components, transport systems, biosynthesis, and catabolism and metabolism. The research project might become the priority unit of the four. One week of library research, six to eight weeks of written and oral preparation, and one week for oral reports would work nicely. The six to eight weeks of preparation would be done outside the classroom. How and where high school educators can sqeeze in the additional weeks necessary for just one unit is the challenge.

GLOSSARY

Alcohol A compound containing a hydrocarbon group and one or more hydroxyl (-OH) groups.

Alpha amino acids The basic building blocks of proteins; containing at least one acidic carboxyl group (-COOH) and one basic amino ($-NH_2$) group on the (alpha) carbon next to the carboxyl group.

Antibody Proteins in the blood that combine with foreign particles to neutralize them, thus producing immunity.

Anticodons An exposed triplet base found on tRNA that determines what amino acid to pick up and where to bind on mRNA.

Biochemistry The study of the structure and composition of compounds that make up the molecular basis of life.

Biosynthesis The building of organic coumpounds by living organisms.

Carbohydrates An organic compound made of C, H, and O, including sugars (energy), starch (storage), and cellulose (structure). The H to O ratio is 2:1.

Carbonyl group The structural group containing an oxygen atom double bonded to a carbon atom in a carbon chain. Example - RCOR' (keytones).

Catalyst A substance that speeds up the rate of a reaction without becoming chemically altered or consumed by the reaction.

Cellulose A polysaccharide forming the strong fibrous stucture in plant cell walls; commonly refered to as roughage or fiber.

Chitin A polysaccharide that is the major component of the exoskeleton of crustaceans and insects. It also makes up the cell wall of fungi.

Cholesterol A lipid which is the metabolic precursor for the five major classes of sterold hormones containing the 'backbone' structure - three cyclohexane rings and one cyclopentane ring.

Codons A group of three nucleotides found on mRNA that code for a specific amino acid

Coenzyme Specific cofactors that are complex nonprotein organic molecules that act with enzymes in catalyzing reactions. Example - vitamins

Cofactor The prosthetic group of a conjugated enzyme that is necessary for the enzyme to function properly.

Conjugated enzyme An enzyme that has a prosthetic group attached.

Conjugated proteins Proteins that, when hydrolyzed, yield amino acids and other organic or inorganic components.

Dehydration synthesis The formation of complex organic molecules involving the removal of a molecule(s) of water.

Denaturation The loss of the characteristic biological activity of proteins due to any of a variety of extremes.

Digestion The process by which foods are broken down into small, water-soluble molecules.

Disaccharide The sugar formed by the chemical combination of two simple sugars (monosaccharides). Example - sucrose.

DNA (deoxyribonucleic acid) A molecule consisting of alternating five carbon sugar units (deoxyribose) and phosphate molecules connected by specific nitrogenous bases. This is a double-helical structure which contains the genetic code.

Enzyme A protein catalyst produced by the cell for the purpose of speeding up specific organic reactions at body temperatures.

Essential amino acids The amino acids that must be ingested because higher animals cannot synthesize them.

Ester The compound formed by the reaction between an alcohol and an acid.

Fats Esters of glycerol and three different (mostly saturated) fatty acids; are solids at room temperature.

Fatty acids Carboxylic acids with long, even-numbered carbon chains.

Glycerol An alcohol with three hydroxyl groups on three carbon atoms; also called glycerin. Has the chemical formula $C_3H_5(OH)_3$.

Glycogen The readily available storage form of glucose that is stored in the liver and skeletal muscle.

Hormone Biological regulators that are secreted by glands or organs that control chemical reactions in other parts of the body.

Hydrogen bonding A weak chemical bond between hydrogen on one polar molecule and a highly electronegative atom on another molecule. Most commonly found in water molecules.

Hydrolysis The chemical breakdown of a substance by the addition of a water molecule.

Hydrophilic The polar head group of a phospholipid or glycolipid that has an affinity for water in the membrane lipid bilayer.

Hydrophobic The hydrocarbon tail of a phospholipid or glycolipid that avoids water molecules thus forming the nonpolar interior of the lipid bylayer

Hydroxyl group Has the symbol -OH which covalently bonds to alkanes to form alcohols. Not to be confused with the hydroxide (OH^-) ion.

Lipids Several classes of organic compounds composed of C, H, and O which are insoluble in water. Formed from fatty acids and alcohols these have a H to O ratio greater than 2:1. Examples - fats, waxes, and steroids.

Metabolism The highly integrated network of chemical reactions that enables cells (organisms) to obtain energy and synthesize molecules.

Monosaccharide The simple sugars; these carbohydrates cannot be broken down into simpler carbohydrates. Example - glucose.

mRNA (messenger RNA) A single stranded polynucleotide that carries the genetic code from the nucleus (after transcription) to the ribosomes.

Neutral lipids Commonly known as fats and oils, these compounds are esters of glycerol and three fatty acids.

Nucleic acids Polynucleotides that are responsible for making proteins (RNA) and and passing on genetic information (DNA).

Nucleosides Basic building block of nucleic acids composed of a sugar and a nitrogenous base.

Nucleotides A nucleoside that has a phosphate group attached after reacting with phosphoric acid. Thus it is composed of a nitrogenous base, a five carbon sugar, and phosphoric acid.

Oil A neutral lipid that is a liquid ester of glycerol and virtually unsaturated fatty acids.

Oligosaccharides Complex sugars containing two to ten monosaccharides most of which are disaccharides.

Organic molecules Compounds containing carbon that are essential to - or produced by - living organisms, excluding CO, CO_2, and carbonates.

Pectin Polymers of saccharides found in many fruits and plant cell walls.

Peptides Joined amino acids resulting from the process of dehydration synthesis. Two amino acids - dipeptide.

Phospholipids A class of lipids composed of glycerol, two fatty acids, and a phosphate group.

Polypeptides One step below a protein, made up of up to fifty amino acid subunits linked by peptide bonds.

Polysaccharide Carbohydrates that are very long, straight or branched chains composed of up to thousands of monosaccharide units.

Prosthetic group The nonanino acid part of a conjugated protein.

Protein Organic molecules that contain C, H, O, and N; that are complex chains of amino acids essential for nearly all aspects of cellular structure and function.

Regulator Compounds in the body that serve to control or direct a multitude of physiological functions. Example - hormones.

Replication A process that occurs only in the nucleus where a DNA molecule duplicates an exact copy of itself.

RNA (ribonucleic acid) A single stranded nucleic acid, containing the sugar ribose, found primarily in the cytoplasm that carries out protein synthesis.

rRNA (ribosomal RNA) Serves an important role in translation as the bonding site to mRNA and moves along the mRNA as tRNA puts the amino acids in proper sequence.

Simple enzymes Yield only amino acids when hydrolyzed; does not contain any cofactors.

Simple proteins Yield only amino acids when hydrolyzed; contain no prosthetic groups whatsoever.

Starch Polysaccharides that are composed of polymerized glucose units that are the most important source of dietary carbohydrates (stored energy).

Steroids Composed of three fused cyclohexane rings and one cyclopentane ring; these are important biological regulators which can cause dramatic physiological effects.

Substrate The reactant substances that the enzymes attach to and act upon in enzyme catalyzed reactions.

Transcription The process by which DNA makes mRNA to transfer information for protein synthesis.

Translation The process carried out by all three forms of RNA and ribosomes to bond amino acids in a specific sequnce to form proteins.

tRNA (transfer RNA) Shaped like a cloverleaf, this molecule has an anticodon that determines which amino acid to pick up and which codon to bind to on the mRNA; 'the workhorse of translation'.

Waxes Large esters of long chain fatty acids and monohydroxyl alcohols that form protective, waterproof coatings on the surfaces of leaves and fruits for plants and on skin, fur, and feathers for animals.

APPENDICES

APPENDIX A

APPENDIX A

OUTLINE FOR CHEMISTRY II

I. Inorganic review (time: 2-3 wks)
   A. Nomenclature
      1. Naming elements, ions, and compounds
      2. Empirical and molecular formulas
   B. Writing and balancing formulas and equations
   C. Stoichiometry
      1. Percent composition
      2. Mass to mass
      3. Gas laws
      4. Molality and molarity
   D. Solutions
      1. Solubility
   E. Ionization
      1. Electrolytes
      2. Nonelectrolytes
   F. Acids, bases, and salts
      1. Definitions
      2. Properties
      3. Indicators

II. Titration, pH and Buffers (3-4 wks)
   A. Equivalents
      1. Normality
   B. pH
      1. Ion concentration in water
      2. Calculations
      3. Neutralization reactions
   C. Titrations
      1. Molar solutions
      2. Normal solutions
      3. Indicators
      4. pH measurement
   D. Buffers
      1. Definition
         a. Function
      2. Preparation
         a. Properties and components
         b. Henderson-Hasselbach equation
         c. Titration principles
      3. Physiological importance
         a. Homeostasis
            (1). cellular level
            (2). systems
                (a) blood

(3). organisms
(4). ecosystems
(a). acid rain

III. Organic chemistry
A. Carbon coumpounds
1. Oxides
2. Bonding
3. Structures
B. Hydrocarbons
1. Structural formulas
2. Nomenclature
3. Petroleum products
a. Separation techniques
C. Hydrocarbon Substitution products
1. Alkyl Halides
a. Freon type compounds
b. Structures
2. Alcohols
a. Preparation
b. Reactions
3. Ethers
4. Aldehydes
5. Keytones
6. Carboxylic Acids and Esters
a. Preparations
b. Reactions
c. Saponification

VI. Biochemistry (9-12 wks)
A. Introduction
1. Definition
a. Biosynthesis
2. Organic molecules
a. Essential chemical elements of life
b. Compounds of living matter
(1). carbon chains
c. Four main classes
(1). carbohydrates
(2). lipids
(3). proteins and enzymes
(4). nucleic acids
B. Carbohydrates
1. Composition and function
a. C, H, and O H:O is 2:1
(1). empirical formula $C_x(H_2O)_y$
b. Energy source
(1). 4 calories per gram
c. Energy store
d. Carbon source
e. Cellular structural material

2. Definition
   a. Two or more hydroxyl groups and a carbonyl group
   b. Three main classes

3. Monosaccharides
   a. Simple sugars
      (1). the five most common
   b. Structure
      (1). open chain and ring forms
      (2). hydrolysis
   c. Nomenclature
4. Disaccharides
   a. Double sugars
   b. Natural occuring
      (1). commercial food sources
   c. Structure and composition
      (1). $C_{12}H_{22}O_{11} + H_2O$
   d. Important disaccharides
      (1). sucrose
      (2). maltose
      (3). lactose
5. Oligosaccharides
   a. Two to ten monosaccharides
   b. Disaccharides or double sugars
      (1). composition
      (2). dehydration synthesis
6. Polysaccharides
   a. Five hundred to many thousand monosaccharides
   b. Storage and structural
   c. Important polysaccharides
      (1). starch
         (a). dietary carbohydrate source
         (b). stored form in plants
         (c). composition
      (2). glycogen
         (a). stored form in animals
         (b). immediate energy source
      (3). cellulose
         (a). most abundant organic coumpound
         (b). plant structures
         (c). non-food for humans (FIBER)
      (4). pectin
         (a). fruits and plant cell walls
      (5). chitin
         (a). exoskeletons, insects and crustaceans
         (b). cell walls of fungi

C. Lipids
   1. Composition and function
      a. C, H, and O H:O is > 2:1
         (1). water-insoluble molecules
         (2). building blocks are:

(a). fatty acids
(b). alcohols
(c). other substances
b. Major energy store
(1). 9 calories per gram
c. Structural material in membranes
d. Biological regulators
e. Protective components in
(1). bacterial and plant cell walls
(2). insect exoskeletons
(3). coverings of vertebrates
f. Four main classes
2. Neutral lipids (triglycerides)
a. Commonly called fats and oils
(1). the "food" lipids
(2). fats are solid at room temp and oils are liquid
(3). chemical difference
(a). fats - mostly saturated
(b). oils - mostly unsaturated
(c). hydrogenation will solidify oils
b. Function
(1). storage of energy in both plants and animals
(2). other functions in animals
(a). insulation to prevent heat loss
(b). protection against bruises and shock
(c). support for organs in the body
c. Structure
(1). fatty acid esters of the alcohol glycerol
(a). glycerol and three different fatty acids
3. Phospholipids
a. Function
(1). structural elements of cell membranes
b. Structure
(1). glycerol, two fatty acids, and a phosphate group
c. Lecithin
4. Waxes
a. Function
(1). protection and waterproofing
(a). leaves, fruit, skin, fur and feathers
(b). beeswax and whale oil
b. Structure
(1). esters of long chain fatty acids & monohydroxyl alcohols
5. Steroids
a. Function
(1). important biological regulators
(2). have dramatic physiological effects
b. Structure
(1). three fused cyclohexane rings and a cyclopentane ring
c. Examples
(1). cholesterol, hormones, bile, poisons, and vitamin D

D. Proteins
   1. Composition and function
      a. C, H, O, and N (most also contain sulfur)
         (1). some may also contain P, Fe, Zn, and Cu
      b. Basic building blocks are alpha-amino acids
      c. Energy source
         (1). 3.8 calories per gram
      d. Most abundant organic compounds within living cells
      e. Control cell structure and function
         (1). the means by which genetic information is expressed
      f. Diverse functions
         (1). catalyst and regulators
            (a). enzymes and hormones
         (2). structural support
            (a). bones and cartilage
         (3). movement
            (a). muscles and tendons
         (4). protection
            (a). skin and hair
            (b). antibodies
         (5). transport materials
            (a). oxygen by hemoglobin
         (6). source of essential amino acids
         (7). blood clotting
            (a). fibrinogen and thrombin
         (8). storage
            (a). seed proteins and casein
         (9). can be harmful
            (a). viruses are mainly protein
            (b). snake venom
            (c). botulism (bacterial protein)
      g. High molecular weight
         (1). 6,000 to many million
   2. Amino acids
      a. Contain a carboxyl group and an amino group
         (1). general formula
         (2). alpha-amino acid
      b. Alpha-amino acid
      c. Average molecular weight of 120
      d. Twenty common amino acids
         (1). nomenclature
         (2). essential amino acids
   3. Peptides
      a. Peptide bonds
      b. Dehydration synthesis
      c. Tripeptides
   4. Polypeptides
      a. 4 to 50 amino acids
         (1). over 50, polymers are called proteins
   5. Protein molecule characteristics
      a. Each has a specific chemical composition
         (1). what amino acids compose it

b. How much of each amino acid
c. Order of amino acid arrangement
d. A definite molecular weight

6. Two main classes
   a. simple proteins
      (1). yield only amino acids when hydrolyzed
   b. conjugated proteins
      (1). yield amino acids and organic or inorganic components
         (a). when hydrolyzed
      (2). prosthetic group
         (a). nonamino acid part
7. Common test for protein
   a. Biuret reaction
   b. Xanthoproteic reaction
8. Other protein properties
   a. Hydrolysis
      (1). strong acid or base at their boiling point
      (2). digestive enzymes at lower temperatures
         (a). both yield peptides then amino acids
   b. Denaturation
      (1). loss of biological activity
      (2). caused by
         (a). extreme pH or temp
         (b). certain chemicals: Pb, Hg, alcohol, or acetone
9. Protein metabolism
   a. 20g/day minimum
   b. Free amino acid pool
      (1). protein turnover
10. Protein digestion
   a. Hydrolysis of peptide bonds
   b. Enzyme catalyzed
      (1). proteases or proteolytic
11. Amino acid biosynthesis
   a. Plants
      (1). can make all 20 essential amino acids
   b. Bacteria
   b. Animals

E. Enzymes
1. Are proteins
2. Catalyst
   a. Review definition
   b. Usually end in -ase
4. Two main classes
   a. Simple enzymes
   b. Conjugated enzymes
      (1). prosthetic group
      (2). cofactors and coenzymes
5. Biochemical reactions
   a. Enzyme substrate complex
      (1). specific sites
      (2). lock and key

b. Digestion
(1). amylase - starch
(a). mouth
(2). lipase - fats or oils
(a). small intestine
(3). pepsin - proteins
(a). stomach
(4). trypsin - proteins
(a). small intestines
(5). peptidase - polypeptides
(a). small intestine
c. Metabolism
d. Synthesis of proteins
(1). amino acids ---> polypeptides ---> proteins
(2). two step process
6. Factors affecting reactions
a. Concentration of enzyme
(1). [ ] denotes concentration
b. Concentration of substrate
c. pH
d. Temperature
F. Nucleic acids
1. Cellular structure review
2. Two main functions
a. Making proteins
b. Passing on genetic information
3. Scientific contributions
a. Watson and Crick
b. Franklin
4. Chemical nature
a. Basic building blocks
(1). nucleotides
b. Molecular weights
(1). DNA - ten billion range
(2). RNA - twenty five thousand range
c. Composition
(1). nucleotides
(a). pyrimidines and purines
(b). base pairing of DNA and RNA
(2). five carbon sugar
(3). phosphoric acid
(4). nucleosides
d. Reactions
(1). formation of DNA
(2). formation of RNA
(3). role of ADP and ATP
5. DNA
a. Genetic information
(1). replication
(2). hydrogen bonding
(3). amino acid pairing

6. RNA
   a. Three types
      (1). mRNA. rRNA, and tRNA
   b. Protein synthesis
      (1). five step process
      (2). transcription - first step
         (a). nucleus
      (3). translation - four step process
         (a). cytoplasm
         (b). function of mRNA, rRNA, and tRNA
         (c). peptide bonds
         (d). codons and anticodons
7. Amino acids
   a. abbreviations
8. Comparison of DNA and RNA

V. Photosynthesis (2-3 wks)
   A. Photosystems
   B. Dark reactions
   C. Metabolism

VI. Projects (2 wks)
   A. One week library research
      1. Six to eight weeks to put project together
   B. One week for presentations

VII. Physical chemistry
   A. Reaction energy and kinetics
      1. Units and symbols
      2. Heat of formation
      3. Exothermic and endothermic reactions
      4. Factors influencing reactions
   B. Chemical equilibrium
      1. Reversibility
      2. Constants
      3. Solubility
   C. Oxidation-reduction
      1. Electrochemistry
      2. Electroplating

VIII. Environmental Chemistry
   A. Geology and geography of the Great Lakes
      1. Formation
      2. Water flow
         a. Great Lakes basin
         b. watershed
         c. wetlands
         d. fresh water
   B. The water cycle
      1. Man's influence
   C. Abiotic and biotic factors
      1. Physical properties

a. pH
b. dissolved oxygen (D.O.)
c. hardness
d. concentration of carbon dioxide [CO_2]
e. temperature
2. Flora and fauna

D. Energy relationships and interactions
1. Ecosystem
a. niche
b. habitat
c. pyramid of energy
(1). food chain
2. Photosynthesis and respiration
3. Cycles
a. carbon
b. nitrogen
c. phosphorus
4. Energy flow
1. lake ecosystem
2. stream ecosystem

E. Man's impact on the Great Lakes
1. Positive and negative impacts
a. use and abuse
b. effects on groundwater
2. Industrial and agricultural use
3. Regulation
4. Economic importance
a. five major areas
5. Who should control the Great Lakes
a. 'who pays to play'

IX. Additional units if time permits
A. Cellular biochemistry
B. Research projects

APPENDIX B

APPENDIX B

Daily Journal

Day - 1 Monday: 2-12-90

LESSON PLAN
Introduction to Biochemistry
Pre-Test: Carbohydrates (appendix D)
Assignment: Read worksheet #1 - 'The Chemistry of Living Things'
Review: Buffers and their physiological importance - (appendix A)
Lecture notes and discussion: see outline (appendix A)
Teaching assists - overhead projector, blackboard, & visuals (appendix H)
Homework: Questions 1-9 worksheet 1

Day - 2 Tuesday: 2-13-90

LESSON PLAN
Correct homework and discuss
Finish lecture
Homework: Handout "Long Arm Of The Lab" - DNA Fingerprinting Article from The Detroit Free Press
Students are to read, summarize and include their opinion of this 'state of the art technique' as permitted by the courts to use as evidence.

Day - 3 Wednesday: 2-14-90

LESSON PLAN

Collect DNA Fingerprinting summary
Discussion: article itself, grading procedure - content, grammar, spelling, punctuation, and clarity.
Demonstration: How gel electrophoresis works
Handout: "Construction of a Restriction Map of the Bacteriophage Lambda" - article on electrophoresis by Dr. Clarence Suelter, M.S.U.
Homework: Worksheet #2 'Carbohydrates' read for tomorrow.

Day - 4 Tuesday: 2-20-90 (2-15 snow day; 2-16 thru 2-19 mid-winter recess)

LESSON PLAN
Return summary of 'DNA' article
Discussion: restriction mapping article and

electrophoresis demonstration
Lecture and notes on carbohydrates: see outline - (appendix A)
Teaching assist - overhead projector, blackboard and visuals (appendix H)
Homework: Draw and determine the differences of monosaccharides.

Day - 5 Wednesday: 2-21-90

LESSON PLAN
Correct and discuss homework
Continue with notes on carbohydrates
Homework: Draw the structure and know the composition of sucrose, maltose, and lactose

Day - 6 Thursday: 2-22-90

LESSON PLAN
Correct and discuss disaccharide homework
Finish notes
Demonstration: cellulose is fiber - wheat, grass, cattails, rope, cereal, lettuce etc.
Homework: Questions 1-10, Worksheet 2

Day - 7 Friday: 2-23-90

LESSON PLAN
Correct homework
Classwork: complete pre-lab worksheet (appendix G)
Correct and discuss in class
Handout Saccharide Lab - four sections
Homework: Read information on Benedict's test in lab handout

Day - 8 Monday: 2-26-90

LESSON PLAN
Lab: Benedict's Test - part 1
Homework: Read Iodine test procedure

Day - 9 Tuesday: 2-27-90

LESSON PLAN
Lab: Iodine test - part 2
Homework: Read procedure for Acid Hydrolysis of Saccharides

Day - 10 Wednesday: 2-28-90

LESSON PLAN
No class: National Honor Society meeting

Day - 11 Thursday: 3-1-90

LESSON PLAN
Lab: Acid Hydrolysis - part 3
Homework: Read Enzymatic Hydrolysis of Saccharides:
Amylase Action on Starch

Day - 12 Friday: 3-2-90

LESSON PLAN
Lab: Enzymatic Hydrolysis - part 4

Day - 13 Monday: 3-5-90

LESSON PLAN
Finish Enzymatic Hydrolysis lab analysis and questions for the previous labs. Hand in completed Saccharide lab.
Homework: Bring in at least three food labels that have the nutritional (carbohydrate) content listed. Cereal packages are recommended.

Day - 14 Tuesday: 3-6-90

LESSON PLAN
Hand back and discuss Saccaharide lab
Classwork: 'Looking At The Saccharides In Our Foods' - (appendix G)
Homework: Study for carbohydrate quiz tomorrow. Test on Friday.

Day - 15 Wednesday: 3-7-90

LESSON PLAN
Hand back and discuss Saccharides in our Foods.
Take last minute questions before quiz.
Administer quiz. (appendix E)

Day - 16 Thursday: 3-8-90

LESSON PLAN
Correct Quiz and discuss.
Review: Carbohydrates - emphasis on lab work
Teaching assists - overhead and blackboard (appendix H)

Day - 17 Friday: 3-9-90

LESSON PLAN
Release day for Science Olympiad Team.
Other class members (6) aid in setting up events.
No assignment

Day - 18 Monday: 3-12-90

LESSON PLAN
Use overheads from 'Biochemistry 200' Illustrations to review stuctures emphasized in previous labs. - (appendix H)
Hand out salivary amylase lab and give background information in pre lab disscussion.
Homework: Read lab, pay close attention to procedure.

Day - 19 Tuesday: 3-13-90

LESSON PLAN
Lab: 'General Effect of Salivary Amylase On Starch'

Day - 20 Wednesday: 3-14-90

LESSON PLAN
Lab: finish 'Salivary Amylase lab'
Homework: Complete lab write-up and data analysis
Carbohydrate test on Friday 3-16

Day - 21 Thursday: 3-15-90

LESSON PLAN (Guest Teacher - 'Substitute')
Collect labs
Students are to work in groups and study for tomorrow's test. Develop questions and answers then exchange between groups. Have guest teacher collect.
Reminder: Test tomorrow

Day - 22 Friday: 3-16-90

LESSON PLAN

Administer Carbohydrate Test (appendix F)
Handout: Pick-up lipid worksheet
Homework: Read pages 10-11 of Lipid Worksheet #3 for Monday.

Day - 23 Monday: 3-19-90

LESSON PLAN
Post Test: Carbohydrates (appendix D)
Correct regular test and discuss.
Hand back labs and discuss.
Homework: Finish reading Lipid Worksheet #3 for Wednesday.

Day - 24 Tuesday: 3-20-90

LESSON PLAN
No class: Drama Club Play

Day - 25 Wednesday: 3-21-90

LESSON PLAN
Pre-Test: Lipids (appendix D)
Lecture notes and Discussion: see outline (appendix A)
Teaching assist - overheads, blackboard and visuals - (appendix H)
Homework: Questions 1-10 end of lipid worksheet, due tomorrow.
Oral report on steroid abuse due Monday 3-26.
Students are to do a literary survey finding information on an athlete who has used/abused steroids and report results. (appendix G)
Read Spec 20 lab for tomorrow.

Day - 26 Thursday: 3-22-90

LESSON PLAN
Lab: 'Introduction To Spectrophotometry'

Day - 27 Friday: 3-23-90

LESSON PLAN
Lab: finish Spec 20 lab
Homework: Complete lab write-up and data analysis.
Reminder: Oral report on steroids due Monday.

Day - 28 Monday: 3-26-90

LESSON PLAN
Hand back homework from lipid worksheet and discuss.
Oral reports on steroids
Collect Spec 20 labs
Homework: Read fat digestion lab for tomorrow.

Day - 29 Tuesday: 3-27-90

LESSON PLAN
Lab: Fat Digestion (appendix I)
Homework: Lab write-up and data analysis

Day - 30 Wednesday: 3-28-90

LESSON PLAN
Collect labs and discuss
Lecture: finish notes on lipids
Teaching assists - overheads from 'Biochemistry 200' and <u>Biochemistry of Lipids and Membranes</u> - (appendix H)
Homework: Test Friday on Lipids

Day - 31 Thursday: 3-29-90

LESSON PLAN
Review for test on lipids tomorrow. Whole class, then small groups.

Day - 32 Friday: 3-30-90

LESSON PLAN
Administer Test on Lipids (appendix F)
Post test: Lipids (appendix D)

Day - 33 Monday: 4-9-90

LESSON PLAN
Hand back fat digestion lab
Return regular lipid test and discuss
Pre-test: Proteins & Enzymes (appendix D)
Hand out Protein worksheet
Home work: Read all of Protein Worksheet #4 and answer questions 1-10 for tomorrow.

Day - 34 Tuesday: 4-10-90

LESSON PLAN
Correct homework and collect
Lecture and discussion notes on proteins: see outline - (appendix A)
Teaching assists - overheads, blackboard, and visuals - (appendix H)
Handout Enzyme worksheet
Homework: Read all of Enzyme Worksheet #6.

Day - 35 Wednesday: 4-11-90

LESSON PLAN
Oral quiz on reading assignment
Finish notes on proteins and complete enzyme lecture as well.
Teaching assists - overheads, blackboard, and visuals - (appendix H)
Homework: Do questions 1-10 on enzyme worksheet for tomorrow.

Day - 36 Thursday: 4-12-90

LESSON PLAN
Wrap up enzyme notes.
Correct enzyme homework and discuss.
Students help set up lab for tomorrow.

Homework: Read 'Qualitative Analysis of Proteins and Amino Acids' for Friday; we'll do part one of three tomorrow.

Day - 37 Friday: 4-13-90

LESSON PLAN
Demonstration: Partition Chromatography and determination of R_f values
Lab: 'Qualitative Analysis of Proteins and Amino Acids' - part I Ninhydrin test
Handout: Read Stryer's discussion of enzyme-substrate complexes.

Day - 38 Monday: 4-16-90

LESSON PLAN
Discussion of Partition Chromatography
Lab: Finish parts II and III, Biuret's and Xanthroproteic test for Qual. Anal. of Proteins
Homework: Study for quiz tomorrow over proteins and enzymes.

Day - 39 Tuesday: 4-17-90

LESSON PLAN
Classwork: Students are to share all lab data on overhead for analysis and discuss. Then determine unknown on their own and turn in lab.
Discuss Stryer's enzyme handout.
Quiz: Proteins and Enzymes (appendix E)

Day - 40 Wednesday: 4-18-90

LESSON PLAN
Correct quiz and discuss.
Review: protein structures (primary thru quaternary), enzymes, dehydration, hydrolysis, denaturation etc. Use overheads from 'Biochemistry 200', Harper's Biochemistry, and The Chemistry of Carbon Compounds as well as visuals.
- (appendix H)
Homework: Read 'Protein Digestion By Papain & Bromelain' for lab tomorrow.

Day - 41 Thursday: 4-19-90

LESSON PLAN
Lab: 'Protein Digestion By Papain & Bromelain' Do set-ups 1-3 to prepare for tomorrow.
Demonstation: Gel Permeation Chromatography
- (appendix I)
Homework: Re-read procedure for taking readings and change time to 5 minutes.

Test on Monday over Proteins and Enzymes.

Day - 42 Friday: 4-20-90

LESSON PLAN
Lab: Check and adjust pH of enzyme solutions. Run lab and take readings every 5 minutes.
Homework: Lab write-up and data analysis are due Monday.
Reminder: Test Monday

Day - 43 Monday: 4-23-90

LESSON PLAN
Collect labs
Administer Test on Proteins and Enzymes (appendix F)
Post Test: Proteins and Enzymes (appendix D)
Handout: Nucleic Acid Worksheet
Homework: Read first two pages of Nucleic Acids Worksheet #5.

Day - 44 Tuesday: 4-24-90

LESSON PLAN
Hand back regular test and discuss.
Pre-test: Nucleic Acids (appendix D)
Lecture notes and discussion on Nucleic Acids: see outline - (appendix A)
Teaching assists - overheads, blackboard, and visuals - (appendix H)
Homework: Read remainder of worksheet on nucleic acids.

Day - 45 Wednesday: 4-25-90

LESSON PLAN
Hand back protein digestion lab and discuss.
Classwork: Read Chpt 7 in Modern Biology - 'Nucleic Acids and Protein Synthesis' pgs 100 thru 111.
Continue notes on nucleic acids.
Teaching assists - overheads from Modern Biology, 'Schaum's Outline Series' and 'Biochemistry 200' - (appendix H)
Homework: Review all steps in protein synthesis.

Day - 46 Thursday: 4-26-90

LESSON PLAN
Finish lecture notes
Review: sequencing, replication, transcription, protein structures, and translation. Use overheads from Nucleic Acid Worksheet and 'Biochemistry 200' also visuals from Cell Biology (appendix H)
Discussion of above

Homework: Do questions for review and applying concepts on page 112 in Modern Biology for tomorrow.

Day - 47 Friday: 4-27-90

LESSON PLAN
Collect homework
Science Olympiad preparation for State competition tomorrow.

Day - 48 Monday: 4-30-90

LESSON PLAN
Hand back homework and discuss
Special visuals: Cell Ultrastructure - booklet of electron and light microscopy of cellular structures and features. Used to tie all units of Biochemistry together and bring in examples of biotechnology.
Homework: Questions 1-11 end of worksheet #5.

Day - 49 Tuesday: 5-1-90

LESSON PLAN
Correct homework and discuss
Classwork: Students are to develop sample questions and problems for all nucleic acid terms from list provided, include an answer key. (appendix G).
Homework: Students are to exchange developed questions and problems; answer classmate's worksheet for tomorrow. Test on Nucleic Acids on Thursday.

Day - 50 Wednesday: 5-2-90

LESSON PLAN
Collect homework
Lab: 'Physical Properties of DNA'
Collect labs and discuss
Reminder: Test tomorrow

Day - 51 Thursday: 5-3-90

LESSON PLAN
Hand back homework and discuss
Administer Test on Nucleic Acids (appendix F)

Day - 52 Friday: 5-4-90

LESSON PLAN
Post Test: Nucleic Acids (appendix D)
Correct regular test and discuss
Set up for 'Science Fair' in Media Center

APPENDIX C

APPENDIX C

BIOCHEMISTRY PRE AND POST INTERVIEW FOR CHEM II

Student's Initials_____ Age_____ Sex_____

Grade_____ Overall G.P.A. _____

Educational Background (Schools Attended)

_____

Sciences classes (H.S.)

Earth Science ____ Physical Science ____ Biology ____

Chemistry ____ Physics ____ Anatomy & Physiology ____

Other _____

Personal goals:

College _____ Other _____

Major/Minor _____ Career _____

Additional comments concerning your science education.

_____

_____

Pre-Test and Post Test Interview

What is biochemistry?

_____

_____

What is biosynthesis?

_____

_____

There are four main classes of organic molecules. Name them.

1 _____ 2 _____ 3 _____

4 _____

The major use of carbohydrates by living things is for

_____.

Carbohydrates contain which elements?

_____.

List the three main classes of carbohydrates. 1 _____

2 _____ and 3 _____

How does glycogen differ from starch? _____

_____

The largest use of lipids by humans is for _____

Triglycerides or neutral lipids are commonly called _____

and _____.

A main function of waxes in the living world is for _____.

Two common examples of steroids are _____ and

_____.

Which element is found in proteins but not carbohydrates or

lipids? _____.

The basic building blocks of proteins are _____.

Define a conjugated protein. _____

_____

What is meant by a denatured protein? _____

_____

Enzymes are the catalyst for digestion and _____ in

animals.

DNA and RNA are nucleic acids responsible for

_____ and _____

respectively.

Define: replication, transcription, and translation.

_____

_____

_____

What are 'Buffers'? _____

_____

List three key areas of physiological importance of buffers.

_____

_____

_____

Why is the study of Biochemistry important to you?

_____

_____

_____

_____

Why is the study of Biochemistry important to mankind?

_____

_____

_____

How do you think this unit will benefit you in your college science courses? _____

_____

_____

APPENDIX D

APPENDIX D

CARBOHYDRATES: PRE-TEST AND POST TEST

MULTIPLE CHOICE

1. Glucose and galactose make up a molecule of (a) sucrose (b) lactose (c) maltose (d) all of these

2. A simple sugar consists of (a) an hydroxyaldehyde (b) an acid and an alcohol (c) an acid and a keytone (d) an hydroxyketone (e) either a or d

3. The energy supplied by carbohydrates in calories per gram is (a) 9 (b) 3.8 (c) 4 (d) 6.2

4. Carbohydrates supply (a) energy (b) carbon (c) cellular structural material (d) none of these (e) all of these

5. A sugar that can be found in fruits and honey is (a) fructose (b) lactose (c) glucose (d) sucrose (e) both a and c

6. All of the following are true of mono- and disaccharides except(a) they taste sweet (b) they are white crystalline substances (c) they are soluble in water (d) they form a red precipitate in Fehling's or Benedict's solutions (e) they are known collectively as sugars

7. The process by which a dilute acid breaks down a complex carbohydrate into simpler ones is known as (a) hydrolysis (b) biosynthesis (c) dehydration (d) carbonyl grouping

8. Starch turns dark blue in the presence of iodine because the iodine reacts with what compound found in the starch molecules (a) amylase (b) amylose (c) amylopectin (d) lypase (e) none of these

9. Dehydration synthesis of glucose and fructose produces (a) maltose (b) lactose (c) pentose (d) sucrose (e) all of these

10. The structural polysaccharide found in plant cells is (a) cellulose (b) maltose (c) sucrose (d) lactose (e) deoxyribose

11. The 'souring' of milk, yogurt, and cottage cheese is produced by bacteria acting on (a) a polysaccharide (b) cellulose (c) lactose (d) sucrose (e) maltose

12. Polysaccharides are chains of how many monosaccharides (a) two or more (b) ten or more (c) fifty to on hundred (d) one hundred to five hundred (e) five hundred to many thousand

13. Which of the following is not a polysaccharide (a) glycogen (b) cellulose (c) pectin (d) chitin (e) all are polysaccharides

14. Animals store saccharides in the form of (a) cellulose (b) glycogen (c) pectin (d) chitin (e) starch

15. A carbohydrate that cannot be hydrolyzed is (a) sucrose (b) starch (c) glycogen (d) fructose (e) all of these

Carbohydrate - Key

1. b
2. e
3. c
4. e
5. e
6. d
7. a
8. b
9. d
10. a
11. c
12. e
13. e
14. b
15. d

LIPIDS: PRE-TEST AND POST TEST

MULTIPLE CHOICE

1. For lipids the ratio of H to O is (a) 2:1 (b) > 2:1 (c) < 2:1 (d) 3:1

2. Lipids are (a) a major energy store (b) structural materials in membranes (c) biological regulators (d) protective components (e) all of these

3. Triglycerides are commonly called (a) waxes (b) steroids (c) fats and oils (d) proteins (e) all of these

4. The structure of neutral lipids is (a) glycerol and two fatty acids (b) ethanol and two fatty acids (c) ethanol and three fatty acids (d) glycerol, ethanol and a fatty acid (e) three fatty acids and glycerol

5. The function of phospholipids is for (a) insulation (b) structural elements of cell membranes (c) transfer of electrical impulses (d) blood clotting (e) none of these

6. How many classes of lipids are there (a) 5 (b) 4 (c) 3 (d) 2 (e) 1

7. Lipids form waterproof coatings on all of the following except (a) eyes (b) leaves (c) feathers (d) fruits (e) skin

8. An example of a steroid is (a) hormones (b) bile (c) cholesterol (d) vitamin D (e) all of these

9. The structure of steroids is made up of (a) three cyclohexane rings (b) long chain fatty acids (c) glycerol (d) a cyclopentane ring (e) a and d

10. What is the pH of a solution of digested fats (a) > 7 (b) < 7 (c) 7 (d) could be >or< 7 (e) does not change

LIPID KEY

1. b
2. e
3. c
4. e
5. b
6. b
7. a
8. e
9. e
10. b

PROTEINS/ENZYMES: PRE-TEST AND POST TEST

MULTIPLE CHOICE

1. The element common to proteins that is not found in carbohydrates or lipids is (a) H (b) Na (c) K (d) N (e) Ca

2. The basic building blocks for proteins are (a) lipids (b) carbohydrates (c) hydrocarbons (d) triglycerides (e) amino acids

3. The amount of calories available per gram of protein is (a) 3.8 (b) 9 (c) 4.2 (d) 6 (e) 5.2

4. Proteins are most important for (a) structural support (b) expressing genetic information (c) antibody formation (d) storage (e) protection

5. Long chain polymers of 50 amino acids or more are commonly referred to as (a) peptides (b) polypeptides (c) proteins (d) prosthetic group (e) protease

6. The name given to the amino acids that higher animals need to ingest, since animals cannot synthesize them, is (a) necessary (b) photosynthetic (ç) essential (d) peptides (e) ingested

7. Every amino acid contains at least one (a) carboxyl group (b) alcohol group (c) ethyl group (d) amino group (e) both a and d

8. Amino acids are joined together by (a) dehydration synthesis (b) hydrogenation (c) hydrogen bonding (d) hydrolysis (e) both a and d

9. The name given to the nonamino acid part of a conjugated protein is a(n) (a) peptide group (b) amino group (c) carboxyl group (d) prosthetic group (e) conjugate group

10. Proteins have many diverse functions except (a) being found in snake venom (b) being the main component in viruses (c) being found in the bacteria that causes botulism (d) being responsible for blood clotting (e) being the most abundant organic compound found in nature

11. Proteins have molecular weights ranging from (a) 6,000 to many million (b) 100 t0 6,000 (c) 600 to 10,000 (d) 1 to 6 (e) 6 million to 100 million

12. The name given to the C-N bond between two amino acids is (a) alpha bond (b) amide bond (c) peptide bond (d) hydrogen bond (e) acidic bond

13. The test given for proteins that results in a violet color when an alkaline suspension of a protein is treated with a solution of copper II sulfate is called (a) BCA assay (b) Hydrolysis (c) Dehydration (d) Biuret (e) Xanthroproteic

14. Proteins exposed to extremes of pH, temperature, and/or chemicals lose their biological activity. This condition is called (a) death (b) prosthesis (c) functional inhibition (d) denaturation (e) constipation

15. Which of the following chemicals would cause a protein to lose its characteristic biological activity. (a) alcohol (b) mercury salts (c) lead salts (d) acetone (e) all of these

16. The substance that an enzyme causes to react is called the (a) coenzyme (b) cofactor (c) substrate (d) catalyst (e) complex

17. The primary function of enzymes is to (a) increase the rate of reactions (b) aid digestion (c) allow metabolism to take place (d) create a 'buffered' environment (e) break down vitamins

18. The name given to the prosthetic group of a conjugated enzyme, necessary for the enzyme to function properly, is (a) coenzyme (b) metabolite (c) cofactor (d) active site (e) substrate

19. All of the following are responsible for the hydrolysis of proteins except (a) trypsin (b) pepsin (c) peptidase (d) amylase (e) none of these

20. The complex reactions that take place in our bodies occur at relatively low temperatures. The loss of enzyme activity due to a temperature increase begins at celsius temperatures of _____ degrees (a) 37 (b) 45 (c) 30 (d) 55 (e) 50

PROTEIN/ENZYME - ANSWER KEY

1. d
2. e
3. a
4. b
5. c
6. c
7. e
8. a
9. d
10. e
11. a
12. c
13. d
14. d
15. e
16. c
17. a
18. c
19. d
20. b

NUCLEIC ACIDS: PRE-TEST AND POST TEST

MULTIPLE CHOICE

1. Which of the following is not a basic component of a nucleotide? (a) nitrogenous bases (b) a five carbon sugar (c) acetic acid (d) phosphoric acid (e) both c and d

2. Which of the following statements is incorrect? (a) DNA is a double strand helix, RNA is a single strand (b) the sugar in DNA is deoxyribose whereas RNA has ribose (c) DNA adenine bonds to thymine, RNA adenine bonds to uracil (d) DNA is a much bigger molecule than RNA (e) all of the above are correct

3. The major portion of which of the following is found outside the nucleus of the cell? (a) DNA (b) chromosomes (c) RNA (d) nucleolus (e) none of these

4. DNA is produced by which part of the cell? (a) cytoplasm (b) nuclear membrane (c) plasma membrane (d) ribosomes (e) nucleus

5. mRNA is produced from DNA by the process known as: (a) transcription (b) replication (c) ribofraction (d) synthesis (e) none of these

6. What is the difference between a nucleic acid and a polynucleotide? (a) There is no difference (b) a nucleic acid has a minimum of three polynucleotides (c) polynucleotides do not have acids attached (d) nucleic acids have the base removed (e) both c and d

7. ADP and ATP are very important for cellular function. Both are classified as (a) polynucleotides (b) nucleic acids (c) vitamins (d) mononucleotides (e) proteins

8. In DNA which bases bond to each other? (a) adenine bonds to thymine (b) glycine bonds to tryptophan (c) cytosine bonds to guanin (d) cytidine bonds to isoleucine (e) both a and c

9. Pyrimidines and purines are commonly referred to as (a) nucleic acids (b) nitogenous bases (c) sugars (d) amino acids (e) nucleoside

10. Which type of RNA does not exist? (a) messenger RNA (b) transfer RNA (c) ribosomal RNA (d) chromosomal RNA

11. DNA is responsible for (a) cellular control (b) passing genetic information (c) energy storage and release (d) synthesizing amino acids (e) all of these

Nucleic Acids - cont.

12. When DNA 'unzips' itself this process is known as (a) replication (b) transcription (c) hydrolysis (d) translation (e) self-revelation

13. RNA is responsible for (a) making ribosomes (b) passing genetic information (c) protein synthesis (d) monitoring the cytoplasm (e) all of these

14. Codons and anticodons are found respectively on (a) cRNA and tRNA (b) mRNA and rRNA (c) rRNA and tRNA (d) cRNA and rRNA (e) mRNA and tRNA

15. Anticodon GCA would match with codon (a) CAU (b) AAU (c) CGU (d) AGC (e) CUG

NUCLEIC ACID - ANSWER KEY

1. c
2. e
3. c
4. e
5. a
6. a
7. d
8. e
9. b
10. d
11. b
12. a
13. c
14. e
15. c

APPENDIX E

APPENDIX E

SACCHARIDE QUIZ

CHEMISTRY II NAME_____

1. List the four functions of saccharides. (4 points)

2. Explain what occurs during the Benedict's test, when testing a reducing sugar. Describe what happens chemically and to the color of the solution. (2 points)

3. Explain what occurs during the iodine test, when testing a branched polysaccharide. Describe what happens chemically and visually. (2 points)

4. Write the general chemical formula for saccharides. (1 point)

5. Compare and contrast monosaccharides, oligosaccharides, and polysaccharides. Keep in mind structure and function. (6 pts.)

SACCHARIDE QUIZ

6. You are to test an unknown sugar; sugar 'Z'. First you test with Benedict's and iodine. According to the following data what type of sugar is sugar Z? Explain how you reached your conclusion. (5 pts.)

	Benedict's	Result	Iodine	Result
Sugar Z	blue	neg. (-)	dark blue	pos. (+)

7. Taking your original unknown add HCl. After waiting for an hour you again run the Benedict's and iodine test. Using the new data shown below, determine what has happened to sugar Z. Has hydrolysis occured? Explain. (5 points)

	Benedict's	Result	Iodine	Result
Sugar Z	red	pos. (+)	yellow	neg. (-)

Carbohydrate Quiz - Key

1. Energy source, Carbon source, Energy storage, and Cellular material.

2. The linear sugar reduces the free Cu II to Cu I. The solution changes from blue to red indicating a positive test.

3. Iodine only complexes with helically coiled polysaccharides. Therefore, it does not complex with brached polysaccharides and the test yields negative results. The yellow color does not change.

4. $C_x(H_2O)_y$

5.

Mono-	1 sugar	quick energy
Oligo-	2-10 sugars	energy storage
Poly-	> 10 sugars	structure and storage

6. Sugar Z is a helical polysaccharide. This is indicated by the positive iodine test. The Benedict's test is negative which tells us sugar Z is not a monosaccharide.

7. The positive Benedict's test indicates that the sugar has indeed been hydrolyzed. Sugar Z is no longer a polysaccharide due to the negative iodine test so we know it has been broken down.

PROTEIN AND ENZYME QUIZ

CHEMISTRY II NAME _____

1. List three functions of proteins.

2. In an amino acid where is the alpha carbon located ?

3. A peptide bond forms between what two atoms (elements) on adjacent amino acids ?

4. The chemical process by which amino acids are joined together is called ?

5. The adition of which element distinguishes proteins from carbohydrates and lipids ?

6. How many polymers of peptides are necessary to distinguish a protein from a polypeptide ?

7. The order in which peptides are put together, (the sequence of amino acids), has no bearing upon the function of the proteins they make up. True or False

8. Protein chains such as hemoglobin are long because if there is an amino acid substituted or out of place this will have no effect, due to the lengthy chain. True or False

9. Simple proteins yield _____ when hydrolized.

10. Denaturation is caused by ? Give two examples.

11. Define enzyme.

12. List the four factors that affect enzyme reactions.

13. Describe fully how an enzyme would break down a substrate.

PROTEIN QUIZ - KEY

1. Any three of the following: catalyst & regulators, structural support, movement, protection, transport oxygen, source of amino acids, blood clotting, antibodies, compose - viruse, poisons, and food toxins.

2. The carbon atom next to the carboxyl group.

3. Between the nitrogen and carbon atoms.

4. Dehydration synthesis.

5. The addition of nitrogen.

6. Greater than fifty (50).

7. False

8. False

9. amino acids only

10. Any two of the following: extreme pH, temperature extremes, lead or mercury salts, acetone, and alcohol.

11. An enzyme is a protein that acts as a catalyst.

12. [enzyme], [substrate], pH, and temperature

13. The enzyme substrate complex forms like a lock and key. This takes place at the active site on the enxzyme. The enzyme acts on the substrate, releases it and the substrate is broken down into two or more pieces. Ezymes can also act on substrates in a similar manner to put two substrates together forming one compound.

APPENDIX F

APPENDIX F

CARBOHYDRATE TEST

CHEMISTRY II NAME _____

CHOOSE THE BEST ANSWER (1 POINT EACH)

____ 1. The sugar ribose is found in a) starch b) chitin c) RNA d) DNA e) pectin

____ 2. A carbohydrate that cannot be hydrolyzed is a) maltose b) starch c) lactose d) galactose e) sucrose

____ 3. The sugar that can be found in the exoskeleton of crustaceans is a) cellulose b) chitin c) pectin d) glucose e) lactose

____ 4. A molecule of maltose is made up of a) glucose and fructose b) chitin c) two glucose molecules d) two fructose molecules e) glucose and galactose

____ 5. Only one of the following is true of polysaccharides:
a) they form a red precipitate with Benedict's solution
b) they are less than ten monosaccharides
c) they taste sweet
d) they are only used for quick energy
e) they are insoluble in water

____ 6. Pectin is a sugar found as:
a) fruit cell walls
b) plant storage material
c) animal storage material
d) the exoskeleton of insects
e) a water source in fruits

____ 7. The structure shown on the overhead is a simple sugar that consists of: a) an acid and a ketone b) a base and an aldehyde c) an hydroxyketone d) an acid and alcohol e) hydroxyaldehyde

____ 8. The ring form that has four carbons and one oxygen making up the ring is: a) maltose b) furanose c) glucose d) pyranose e) pentanose

____ 9. The enzyme that breaks down starch is called: a) amylose b) amylopectin c) starchase d) amylase e) galactose

FOR THE FOLLWING SECTION, BE AS COMPLETE AND SPECIFIC AS POSSIBLE

10. In the salivary experiment, explain what happened to the starch - iodine mixture when saliva was added to the tube. Was there a color change? If so, what color and why or why not? (2 pts.)

11. What two forms of starch are there and how do they differ from one another ? (2 pts.)

12. Compare how glucose and sucrose react in the Benedict's solution. Explain what type of reaction occurs, the color change involved and the type of sugar each one is. (2 pts.)

13. What did we use as a control for all the saccharide experiments. What is the control's Benedict's reaction ? The control's iodine reaction ? Why did you get those results ? (3 pts.)

14. For the following sugars, identify whether the sugar is a monosaccharide, oligosaccharide, or a polysaccharide; also where you might find the sugar and its function. (5 pts.)

Sugar	Type of sugar	Where found	Function
Lactose			
Galactose			
Sucrose			
Starch			
Fructose			

15. What is the general formula for all polysaccharides ? (1 pt.)

16. What is the chemical formula for maltose and for fructose ? (2 pts.)

Maltose Fructose

17. What is the source of fiber for humans ? What type of sugar is fiber ? (2 pts.)

FILL IN THE CHART BELOW FOR THE RESULTS OF EACH TEST AND THE TYPE OF SUGAR. THEN USE THE CHART TO ANSWER THE FOLLOWING QUESTIONS.(6 pts.)

Sugar	Benedict's	Results	Iodine	Results	Type
A	red		yellow		
B	blue		yellow		
C	blue		dk blue		
D	red		dk blue		
E	blue		dk blue		
F	red		yellow		

18. Which sugar(s) are reducing sugars ? Using the above results, explain your answer. (3 pts.)

19. Which sugar(s) are helical polysaccharides ? Explain you answer according to the results. (2 pts.)

20. Which sugar(s) are oligosaccharides without free aldehyde or keytone groups ? (1 pt.)

21. Compare the type of sugars present in sugars B and D ? (2 pts.)

FILL IN THE CHART BELOW FOR THE RESULTS OF EACH TEST AND THE TYPE OF SUGAR. THEN USE THE CHART TO ANSWER THE FOLLOWING QUESTIONS. (9 pts)

Sugar	Benedict's	Results	Iodine	Results	Type
X	blue		dk blue		
X + saliva	red		yellow		
Y	blue		yellow		
Y + HCl	red		yellow		
Z	blue		dk blue		
Z + HCl	blue		dk blue		
W	red		yellow		
V	red		yellow		
W + V	blue		dk blue		

22. What happened when sugars W and V were mixed ? What is the mechanism of this reaction ? What type of sugars are W and V ? (3 pts.)

23. What happened to sugar X when saliva was added ? Refer to the type of sugar before and after the reaction. (2 pts.)

24. What happened to sugar Y when HCl was added ? Explain in terms of the types of sugar before and after. (2 pts.)

25. What happened to sugar Z when HCl was added ? Again, refer to the structure of the sugar before and after. (2 pts.)

Carbohydrate Test - Key

1. C

2. D

3. B

4. C

5. E

6. A

7. C

8. B

9. D

10. Yes, there was a blue color at first that became lighter as the helical structure of the starch reacted with the iodine over time.

11. Amylose - which is helical in structure.
    Amylopectin - which has a branched structure.

12. Glucose has a positive test and turns red because it is a monosaccharide.
    Sucrose yields a negative test and remains blue because it is a disaccharide (oligosaccharide) which does not react with the Benedict's solution.

13. The control was distilled water.
    The reaction with Benedict's is negative - remains blue.
    The iodine reaction is also negative - remains yellow.
    The control is a check to make sure the reagents have not changed the solutions. This is called a negative control if a positve result is obtained something is wrong with the solutions.

14.

Lactose	di-/oli-	milk	energy/storage
Galactose	mono-	pectins	energy
Sucrose	di-/oli-	sugar	energy/ storage
Starch	poly-	potatoes	storage
Fructose	mono-	fruits/honey	energy

15. $C_x(H_2O)_y$

16. $C_{12}H_{22}O_{11}$; $C_6H_{12}O_6$

17. Cellulose; Polysaccharide

Carbohydrate Key - cont.

CHART

Sugar	Results	Results	Type
A	+	-	mono-
B	-	-	oli-
C	-	+	poly-
D	+	+	oli-/poly-
E	-	+	poly-
F	+	-	mono-

18. A & D & F; These three sugars have a positive Benedict's test and a negative Iodine test.

19. C & E; These two sugars are positive for the Iodine test and negative for the Benedict's test.

20. Sugar B

21. Sugar B is only an oligosaccharide without a free aldehyde or keytone group.
Sugar D is an oligosaccharide or polysaccharide with a free aldehyde or keytone group.

CHART

Sugar	Results	Results	Type
X	-	+	poly-
X + saliva	+	-	mono-
Y	-	-	oli-/poly-
Y + HCl	+	-	mono-
Z	-	+	poly-
Z + HCl	-	+	poly-
W	+	-	mono
V	+	-	mono-
W + V	-	+	poly-

22. They have formed a polysaccharide.
Dehydration synthesis.
Both sugars are monosaccharides (reducing sugars).

23. Sugar X has undergone hydrolysis.
X was a polysaccharide before the saliva was added; now X has been converted to monosaccharides.

24. Sugar Y has been hydrolyzed.
Y has changed from an oli-/polysaccharide to monosaccharides.

25. Hydrolysis may or may not have occurred, probably not.
The sugar is still a polysaccharide; therefore if hydrolysis did occur, it was not complete.

LIPIDS TEST

CHEMISTRY II NAME _____

SHORT ANSWER and COMPLETION

For questions #1 thru #4, identify which lipid group (class) is drawn. 1 pt. each

1.

_____

2.

_____

3.

_____

4.

_____

5. Which elements are lipids composed of? Why are lipids called organic molecules? 4 pts.

6. Compare and contrast lipids and carbohydrates. Refer to how many calories per gram, the differing ratio of elements, and list at least three functions of each. 4 pts.

7. For the two structural formulas drawn below, identify each diagram with the specific lipid name. In which lipid class are these two lipids found? 4 pts.

8. Explain what happens chemically during the hydrogenation of an oil. 1 pt.

9. Compare and contrast fats and oils. State lipid class, physical state at room temperature, and type of bonds between carbon atoms. 3 pts.

10. List the five general functions of lipids. 5 pts.

11. At your age, what percentage of your daily caloric intake should be in the form of lipids? As you get older should your lipid intake increase or decrease? Why? 3 pts.

12. Compare and contrast neutral lipids and phospholipids. Discuss the chemical make-up of each, two functions of each, and give an example of each. 4 pts.

13. What type of lipid makes up the plasma membrane and causes the plasma membrane to remain flexible? 2 pts.

14. Draw the plasma membrane and explain how and why the membrane is arranged in this way. 3 pts.

15. State the major function of the plasma membrane. 1 pt.

16. Name the other lipid found in the membrane that gives the membrane some rigidness. 1 pt.

17. Compare and contrast waxes and steroids. Include structure, functions, and give three specific examples of each. 5 pts.

18. What causes arteriosclerosis? Explain this disease. 2 pts.

19. What are steroids, vitamin D, estrogen, and bile salts derived from? What is the primary metabolic precursor? 1 pt.

20. What is the purpose of vitamin D in your body? What kind of lipid is vitamin D? 2 pts.

21. Compare and contrast testosterone and estrogen. Identify the type of lipid, describe the general structure, and state each one's function in the body. 3 pts.

22. What do bile salts do in the small intestine? 1 pt.

23. State the name of the enzyme used in the lab to break down fats. 1 pt.

24. What did the enzyme break the fats into? Name the pieces. 2 pts.

25. Discuss how we tested the laboratory solution to determine if the fats had been broken down. 1 pt.

26. Compare the results in the following situation. Test tube X has bile salts and fat; tube Y has bile salts, fat, and the enzyme to break down fats. What would you expect the results to be? Why? 4 pts.

YOU RUN A FAT DIGESTION EXPERIMENT AND HERE ARE YOUR RESULTS:

Tube	Bile acid	Lipid enzyme	Fat	pH before	pH after
A	yes	no	yes	7	7
B	yes	yes	yes	7	5
C	yes	yes	yes	7	7
D	no	yes	yes	7	6
E	no	yes	yes	7	7

Answer the following according to the above results.

27. Which tube had the most fat digestion? 1 pt.

28. Which tube should have had fat digestion but did not? 1 pt.

29. Why does tube D show a change in the pH? Expain what has happened. 1 pt.

Lipid Test - Answer Key

1. Phospholipid

2. Wax

3. Neutral lipid

4. Steroid

5. C, H, and O. Lipids are called organic molecules because they contain carbon and compose living tissue or are produced by living organisms.

6.

Lipids	Carbohydrates
9 cal/gram	4 cal/gram
H:O is > 2:1	H:O is = 2:1
Energy store	Energy source
Protection	Energy store
Insulation	Structural material
Cell membranes	Carbon source
Biological regulators	Plant cell walls

7. Testosterone; Cholesterol. Both are steroids.

8. The addition of hydrogen breaks the double bonds between the carbon atoms making the oil saturated. This process turns the oil into a fat.

9.

Fats	Oils
neutral lipid	neutral lipid
solid	liquid
saturated	unsaturated

10. Energy storage, insulation, protection, structural, regulators

11. 30% ; decrease; Lipids have a high calorie and cholesterol content which leads to circulatory problems in later years.

12.

Neutral lipids	Phospholipids
glycerol + 3 fatty acids	same + a phosphate group
energy store, insulation, support	cell membranes, non-stick sprays
body fat, vegetable or seed oil	lecithin (PAM)

13. Phospholipid

Lipid Key - cont.

14. The plasma membrane is a lipid bilayer which contains protein molecules. The hydrophobic tail ends are non-polar so they 'repel' water and align toward the center of the membrane. The hydrophilic head ends are polar so they align on the outside where the fluids (water) are. This arrangement allows for the regulation of water into and out of the cell.

15. Forms a protective layer of cellular regulation.

16. Lipopolysaccharide

17.

Waxes	Steroids
long chain fatty acids & alcohol	3 cyclohexane rings & one cyclopentane ring
protection, waterproofing	biological regulators
beeswax, whale oil	testosterone, vit D, bile acids

18. Arteriolsclerosis is caused by an abundance of cholesterol in the arteries. This clogs the arteries and forces the heart to work harder.

19. Cholesterol or 3 cyclohexane rings and 1 cyclopentane ring

20. Vit. D is essential for strong bone development. A sterold.

21.

Testosterone	Estrogen
steroid	steroid
3 cyclohex. rings and 1 cyclopent. ring	(same)
develop male sex. charact.	female sex. characteristics

22. Break down fats into smaller droplets called globules.

23. Lipase was the enzyme contained in pancreatin.

24. Fats were broken into fatty acids and glycerol.

25. Determine the pH; if it's acidic then the fat was broken down.

26. Tube X - no digestion, just smaller globules.
Tube Y - would expect complete digestion as the enzyme would be able to affect all globules.

27. Tube B

LIPID KEY -cont.

28. Tube C

29. Enzyme was able to slightly digest the fat. The digestion is not as complete as in tube B due to the absence of bile causing less surface area.

PROTEINS AND ENZYMES TEST

CHEMISTRY II Name _____

CHOOSE THE BEST ANSWER FOR THE FOLLOWING MULTIPLE CHOICE QUESTIONS. MARK YOUR ANSWERS ON THE SCANTRON SHEET.

____ 1. What are the basic building blocks for all proteins?
a. polypeptides
b. alpha amino acids
c. beta amino acids
d. simple sugars

____ 2. What is the approximate molecular weight for protein molecules?
a. 100 - 100
b. 1000 - 10,000
c. 6,000 - many million
d. many million - billions

____ 3. What kind of bonds link amino acids together?
a. carboxyl
b. glycosidic
c. amino
d. peptide
e. hydrogen

____ 4. Which solution, that we used in lab, combines with the free amino group of amino acids and turns a purple color?
a. Ninhydrin
b. Xanthroproteic
c. Pancreatin
d. Bradford's

____ 5. Which of the following is made up of two amino acids and has one peptide bond?
a. tripeptide
b. protein
c. dipeptide
d. polypeptide

____ 6. Which of the following is composed of fifty or more amino acids?
a. tripeptide
b. protein
c. dipeptide
d. polypeptide

____ 7. The class of proteins that yields only amino acids when hydrolyzed is:
a. complex
b. conjugated
c. simple
d. polypeptide

____ 8. Which of the following protein substances listed below is a very harmful protein to our bodies?
a. viruses
b. fibrinogen
c. antibodies
d. arginine
e. phenylalinine

____ 9. Which proteins listed below are very important structures within our immune system?
a. viruses
b. fibrinogen
c. antibodies
d. arginine
e. phenylalinine

____ 10. What is the type of reaction that occurs between amino acids leading to peptides and eventually proteins?
a. hydrolysis
b. denaturation
c. renaturation
d. dehydration

____ 11. The prosthetic group on the amino acid is commonly the:
a. amino part
b. carboxyl part
c. R group
d. first carbon

____ 12. This test is used for determining aromatic amino acids. The test uses nitric acid and yields a characteristic yellow color.
a. Biuret's
b. Xanthroproteic
c. Ninhydrin
d. Bradford's

____ 13. What is the minimum amount of protein that should be ingested (eaten) every day?
a. 3 g
b. 12 g
c. 50 g
d. 20 g

____ 14. This protein structure is formed when several globular proteins are joined together.
a. primary
b. secondary
c. tertiary
d. quaternary

____ 15. This structure of a protein is just the amino acid sequence chain.
a. primary
b. secondary
c. tertiary
d. quaternary

____ 16. This particular protein structure is the globular form of a single protein.
a. primary
b. secondary
c. tertiary
d. quaternary

____ 17. This is the term given to a protein that has lost its biological activity.
a. renaturation
b. denaturation
c. hydrolysis
d. dehydration

____ 18. We can best obtain all twenty essential amino acids from eating a variety of:
a. bacteria
b. fish
c. meat
d. plants
e. each other (cannibalism)

____ 19. Which of the following would not be an example of a prosthetic group?
a. nucleic acid
b. mercury II chloride
c. iron III hydroxide
d. sugars
e. phospholipids

____ 20. When we cannot produce an amino acid within our own bodies, this type of amino acid is called:
a. useless
b. nonessential
c. essential
d. antigen

____ 21. This type of protein yields amino acids and organic or inorganic components when hydrolyzed.
a. simple
b. conjugated
c. cofactor
d. coenzyme

____ 22. This is the chemical process used to break down proteins into amino acids:
a. catalyst
b. dehydration
c. hydrolysis
d. denaturation

____ 23. Amino acid biosynthesis can occur in:
a. animals
b. bacteria
c. plants
d. all of these
e. only a and c

____ 24. This test can be used to detect the presence of proteins. An alkaline solution of copper II sulfate reacts with proteins and yields a purple color.
a. Xanthoprotic
b. Biuret's
c. Ninhydrin
d. Bradford's
e. all of the above

____ 25. This type of substance speeds up reactions without being used up in - or consumed by - the reaction.
a. catalyst
b. carbohydrate
c. amino group
d. prosthetic group

____ 26. If enzymes were not present in our bodies, then:
a. reactions would occur at faster rates
b. reactions in the body would never occur
c. carbohydrates would be the only substances broken down
d. reactions would occur but at very slow rates

____ 27. This protein yields amino acids and a prosthetic group when broken down.
a. conjugated
b. simple
c. complex
d. quaternary

____ 28. If an enzyme has a prosthetic group that is a complex organic molecule, this group is called:
a. a cofactor
b. the active site
c. an amino group
d. a coenzyme

____ 29. What part of an enzyme does a substrate bind to?
a. the cofactor
b. the active site
c. the coenzyme
d. the amino group

____ 30. Most enzymes are capable of binding to how many substrates?
a. one
b. two
c. three
d. many

____ 31. This enzyme acts in the small intestine to digest polypeptides into amino acids.
a. trypsin
b. pepsin
c. peptidase
d. lipase

____ 32. This enzyme digests fats and oils into fatty acids and glycerol.
a. trypsin
b. pepsin
c. peptidase
d. lipase

____ 33. The enzyme that digests proteins in the stomach into polypeptides.
a. trypsin
b. pepsin
c. peptidase
d. lipase

____ 34. This is the enzyme found in pancreatin that we used in the protein digestion lab. The enzyme acts on proteins in the small intestine that have made it through the stomach.
a. trypsin
b. pepsin
c. peptidase
d. lipase

____ 35. Enzymes are responsible for:
a. digesting food
b. synthesizing proteins
c. speeding up reactions
d. all of the above
e. a and c only

____ 36. The enzyme and substrate reaction works like:
a. a chain of beads
b. two cubes coming together
c. a lock and key
d. 'congress' - functionally decrepit

FOR THE FOLLOWING SECTION MATCH THE CORRECT AMINO ACID TO ITS ABBREVIATION.

____ 37. glycine
____ 38. methionine
____ 39. tryptophan
____ 40. phenylalinine
____ 41. isoleucine
____ 42. asparagine

a. Asn
b. Phe
c. Try
d. Asg
e. Ile
ab. Gln
ac. Iso
ad. Gly
ae. Met
bc. Phl
bd. Trp

THE FOLLOWING SECTION IS A SHORT ANSWER SECTION. YOU MAY WRITE IN THIS TEST.

43. List four ways to denature a protein. 4 pts.

44. Explain what is happening to a protein when it is denatured. Include a definition and discuss what happens to the structure and bonds. 2 pts.

45. Draw and label the basic building block of amino acids. Label all four parts. 5 pts.

46. Compare and contrast dehydration synthesis, hydrolysis, and denaturation as they relate to proteins. Keep in mind the mechanism (what's occurring). 6 pts.

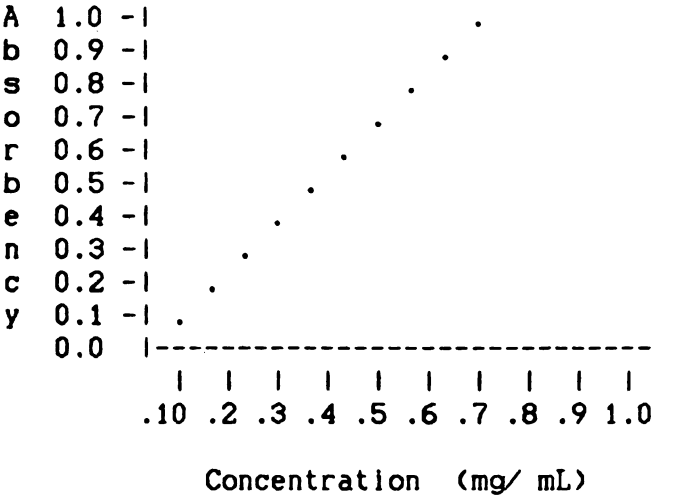
47. List four factors that affect an enzyme's ability to function. 4 pts.

48. At what temperature will an enzyme begin to denature? _____
At what temperature will an enzyme be totally denatured? _____
2 pts

49. What is the two step process used by an enzyme during <u>protein synthesis</u>? If you wish, you may draw and label this process. 2 pts.

50. According to the information in the lab on protein digestion, proteases such as pepsin function best in low pH (acidic) environments. Considering the stomach secretes a strong acid and the duodenum receives a strong base from the pancreas, how does this acid-base situation affect the pepsin's activity in these two parts of the body? 2 pts.

FOR THE FOLLOWING SECTION, ANSWER THE QUESTIONS BY REFERRING TO THE GRAPH AND THE CHROMATOGRAPHY PAPER. (Draw a line to connect the plotted points.)

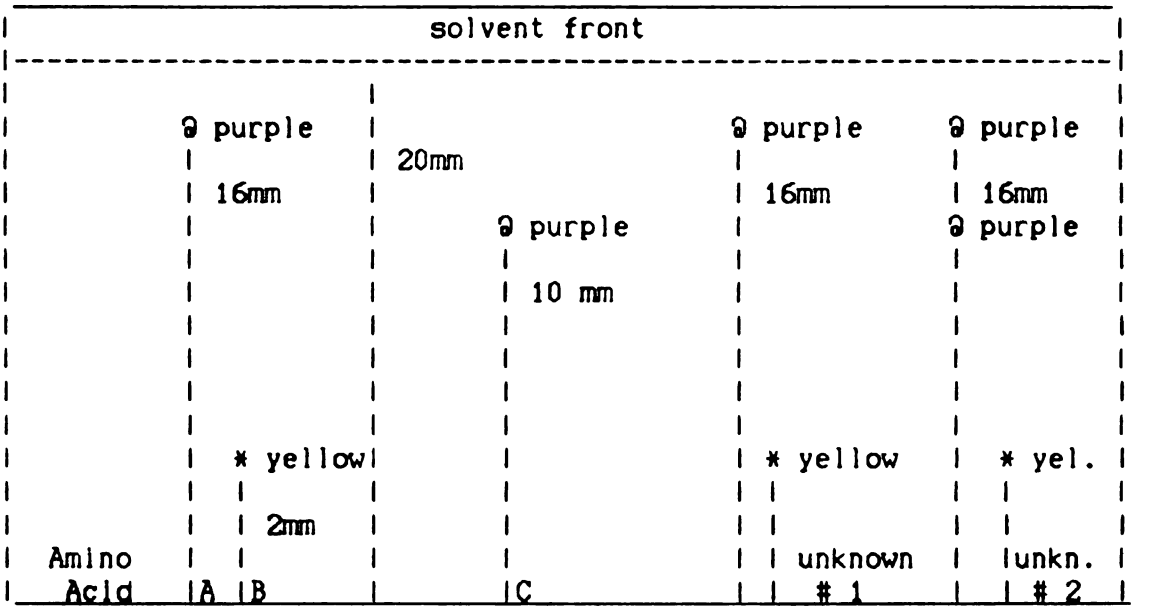


51. Using the graph above, what is the concentration of protein A [A] if the absorbency is 0.7 ? 1 pt.

52. What would be the absorbency of protein B if the concentration is 0.25 mg/mL ? 1 pt.

53. What is the name given to this type of graph drawn above? 1 pt.

SAMPLE CHROMATOGRAPHY PAPER



54. Using the sample chromatography paper above, determine the Rf factor for each known amino acid. 3 pts.

_____ A _____ B _____ C

55. Which amino acids (A,B,C) have free amino groups? How do you know? 2 pts.

56. Which amino acids are found in unknown # 1 and in unknown #2 ? 2pts.

Protein and Enzymes Test - Key

1. B
2. C
3. D
4. A
5. C
6. B
7. C
8. A
9. C
10. D
11. C
12. B
13. D
14. D
15. A
16. C
17. B
18. D
19. B
20. C
21. B
22. C
23. D
24. B
25. A
26. D
27. A
28. D
29. B
30. A
31. C
32. D
33. B
34. A
35. D
36. C

37. AD
38. AE
39. BD
40. B
41. E
42. A

Protein and Enzyme Key - cont.

43. Any four of the following: temperature, pH, lead salts, mercury salts, alcohol, and acetone.

44. Denaturation is the loss of a protein's biological activity. The proteins lose their structure (from 4° to 1°) which causes this loss. However, the peptide bonds are not broken.

45. Alpha carbon

Prosthetic group Carboxyl group

Amino group

46. Dehydration synthesis - amino acids bond to form peptide bonds which yield proteins plus water.
Hydrolysis - proteins plus water yield amino acids by breaking the peptide bonds.
Denaturation destroys the structure of proteins, thus losing activity, yet the proteins are not broken down.

47. [enzyme], [substrate], pH, and temperature.

48. 45°C; 55°C

49. 1) The enzyme plus two amino acids (substrates) join at the active site on the enzyme.
2) The enzyme catalyzes the reaction forming a peptide bond and releases the bound substrates as one new protein.

50. The pepsin is very active in the stomach breaking down proteins. However, when the pepsin-protein mixture enters the alkaline conditions of the duodenum, the pepsin loses its ability to catalyze.

51. .5

52. .3

53. A standard curve

54. Amino acid A 16/20 = 0.8
Amino acid B 2/20 = 0.1
Amino acid C 10/20 = 0.5

55. A and C; This is indicated by the purple color.

56. Unknown #1 has amino acids A & B
Unknown #2 has amino acids A, B, & C

NUCLEIC ACIDS TEST

CHEMISTRY II NAME _____

CHOOSE THE BEST ANSWER FOR THE FOLLOWING MULTIPLE CHOICE QUESTIONS. PLACE YOUR ANSWERS ON THE SCANTRON SHEET.

____ 1. A section of a nucleic acid is composed of a nitrogenous base and a five carbon sugar. This section is called a:
a. nucleotide
b. nucleoside
c. polynucleotide
d. polynucleoside

____ 2. This type of nucleic acid is found as a double stranded helix.
a. tRNA
b. rRNA
c. DNA
d. mRNA
e. mDNA

____ 3. This type of nucleic acid looks like a cloverleaf, has an anticodon end, and an attachment site for an amino acid.
a. tRNA
b. rRNA
c. DNA
d. mRNA

____ 4. This type of nucleic acid combines with proteins and forms the ribosomes that carry out translation.
a. tRNA
b. rRNA
c. DNA
d. mRNA

____ 5. This type of nucleic acid has deoxyribose and thymine.
a. tRNA
b. rRNA
c. DNA
d. mRNA

____ 6. This nitrogenous base is a pyrimidine found only in RNA.
a. adenine
b. cytosine
c. guanine
d. thymine
e. uracil

____ 7. This nitrogenous base is a purine found in both DNA and RNA. This purine is also a major component of the mononucleotides responsible for storage and energy release within the cell.

a. adenine
b. cytosine
c. guanine
d. thymine
e. uracil

____ 8. This basic building block of a nucleic acid is composed of a five carbon sugar, phosphoric acid, and a nitrogenous base.
a. nucleoside
b. polynucleotide
c. nucleotide
d. polynucleoside

____ 9. Protein synthesis occurs in this part of the cell.
a. nucleolus
b. nucleus
c. mitochondria
d. cytoplasm
e. membrane

____ 10. This nitrogenous base is a pyrimidine found only in DNA.
a. cytosine
b. guanine
c. thymine
d. uracil

____ 11. This nitrogenous base is found only in RNA.
a. cytosine
b. uracil
c. guanine
d. thymine

____ 12. This type of nucleic acid has codons.
a. tRNA
b. dRNA
c. rRNA
d. mRNA

____ 13. DNA has the ability to copy itself. This process is known as:
a. translation
b. duplication
c. transcription
d. replication
e. dehydration synthesis

____ 14. When small pieces of the DNA code are copied into an RNA code this process is called:

a. translation
b. transcription
c. dehydration synthesis
d. duplication
e. replication

____ 15. When mRNA is 'read' and used to make proteins this process is referred to as :
a. translation
b. transcription
c. duplication
d. replication
e. redundancy

____ 16. RNA molecules have molecular weights that vary greatly in size. The highest molecular weights are around:
a. 250
b. 2,500
c. 25,000
d. 250,000

____ 17. DNA molecules are some of the largest known having molecular weights in the _____ range.
a. one million
b. ten million
c. one billion
d. ten billion

____ 18. Which process requires the presence and use of mRNA, tRNA, and rRNA?
a. replication
b. hydrolysis
c. transcription
d. duplication
e. translation

____ 19. What would be the complementary strand of DNA if one partial strand has the sequence AACGTTCG?
a. UUGCAACG
b. TTCGUUCG
c. TTGCAAGC
d. CCTUGGTU

____ 20. What is the anticodon sequence if the codon sequence is AUC?
a. UAG
b. TAG
c. UTA
d. TGA

SHORT ANSWER SECTION

21. Explain what semiconservative replication is. You may use a drawing to help explain if you wish. 2 pts.

22. Explain why the genetic code (codons) is said to be redundant. 1 pt.

23. State the major functon of DNA and the major function of RNA. 2 pts.

24. The sequence shown below is a SINGLE strand of DNA. Below it write the second (daughter) strand. 2 pts.

A T G T A C C A A A C A T T A A T C A T A C T A A A A

How many codons are present is the above strand ? _____ 1 pt.

25. For the sequence that I have drawn in #4, write below the mRNA sequence. On your mRNA sequence, circle where the start and stop codons are. 3 pts.

26. Using your sequence in #5 and the given table, show what each anticodon would be for each mRNA codon. Now draw the amino acid sequence for the protein. (Hint: remember about the stop and start codons.) 5 pts.

Nucleic Acids Test - Key

1.	B	11.	B
2.	C	12.	D
3.	A	13.	D
4.	B	14.	B
5.	C	15.	A
6.	E	16.	D
7.	A	17.	C
8.	C	18.	E
9.	D	19.	C
10.	C	20.	A

21. Parent DNA 'unzips' itself and picks up amino acids to form two new daughter strands. The parent DNA makes up half of the new strands thus it is conserved.

22. The codons are in triplets and have 64 possible combinations of three. But there are only 20 amino acids - so virtually all amino acids have more than one anticodon sequence.

23. DNA has the genetic code and passes on this information.
RNA is responsible for making proteins .

24. TAC ATG GTT TGT AAT TAG TAT GAT TTT ; Nine

25. UAC AUG GUU UGU AAU UAG UAU GAU UUU
Start is AUG; Stop is UAG

26. AUG UAC CAA ACA UUA AUC AUC AUA CUA AAA
met-val-cys-asn
(protein)

APPENDIX G

APPENDIX G

LOOKING AT THE SACCHARIDES IN OUR FOODS

1. Describe this food product.

2. What is the size of a typical serving?

3. How many grams of carbohydrates are in a serving?

4. Look carefully at the ingredients. List each type of carbohydrate in this food product. For each carbohydrate state its particular function -- energy, carbon, storage, or structural. Then identify the type of saccharide -- monosaccharide, oligosaccharide, or polysaccharide.

CARBOHYDRATE	FUNCTION	TYPE

5. Which sugars in the above list would you think are reducing sugars? Explain your reasoning.

6. Which sugars in the above list (#4) would give positive results in the iodine test? Explain your reasoning.

7. Knowing that the best carbohydrates to ingest are the structural and energy ones, which of the above carbohydrates are good for you?

8. When would a storage type saccharide be good for you?

Oral Report on Steroids

Complete a literary survey to find an athlete who has used or abused steroids and report the following.

Identity of user

The type of steroid used

The purpose for taking the steroid

The potential side effects

Other pertinent information

Author and/or references

Possible sources include:

- Newspapers
- Sports magazines
- Health/Nutrition magazines
- Exercise magazines

Nucleic Acids - Key Terms

Develop questions and/or problems for each of the following:

DNA & RNA

- Comparison
- Components
- Structure

Nucleoside

Nucleotide

Pyrimidines

Purines

AMP,ADP, and ATP

mRNA, tRNA, and rRNA

Replication

Transcription

Translation

Protein synthesis

Codon

- Initiator
- Terminator

Cellular structure

Protein structure

DNA sequencing

Daughter strands (cells)

Hydrogen bonds

Molecular weights of DNA and RNA

Base pairings of DNA and RNA

APPENDIX H

APPENDIX H

The only difference between these two reactions is in the kind of linkage by which glucose units are joined in amylose and in cellulose. In amylose, the linkage is by an α-1,4- bond; in cellulose, the linkage is by a β-1,4- bond.

This may not seem like a very big difference. After all, most of the atoms in both molecules are in exactly the same relationship to each other. In fact, there is a very great difference. If you think about the role of starch and cellulose in your everyday life, you'll begin to realize that. Humans can eat starch and digest it easily. Bread, cereal grains, flour, and many other parts of our diet contain starch. We require these foods as sources of carbohydrates in our diets. But cellulose-containing materials—leaves, grass, and cotton, for example—are *not* parts of our diet. If we should happen to eat any of these materials, they pass through and out of the body unchanged. What this means is that the human body has no way of digesting cellulose, although it can easily break apart its cousin, starch. How can we explain this difference?

Our bodies contain just the right enzymes. The enzymes in this case are compounds that will attack the α-1,4- linkage and break it apart. The reaction is shown here. It is the first step in digestion that occurs whenever we eat a food containing starch.

You'll notice that the enzyme chops off two glucose units at a time. In other words, maltose is one of the first products of the digestion of starch.

But the enzymes that *can* break apart an α-1,4- linkage *cannot* do anything with a β-1,4-linkage. Can you think of any reason that this might be so? (A partial answer will be found on page 203.) The enzymes that take starch apart for us, then, are no help in trying to digest cellulose.

Those animals other than humans that *can* digest cellulose do so for one of two reasons. First, they may have the right enzymes themselves. This means having enzymes that work

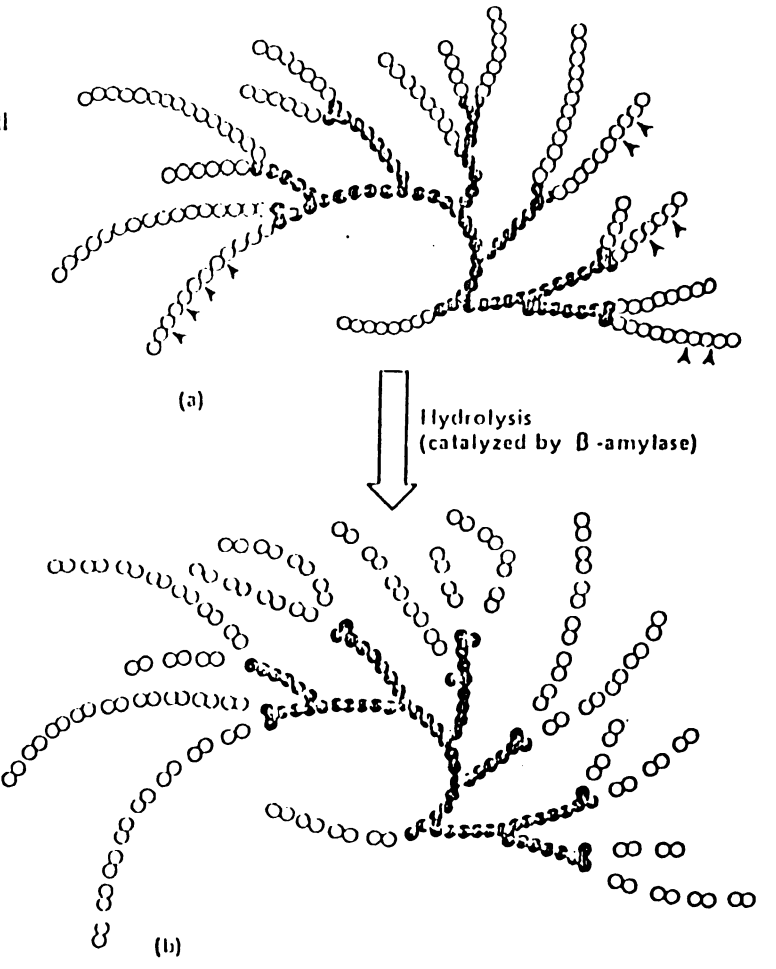
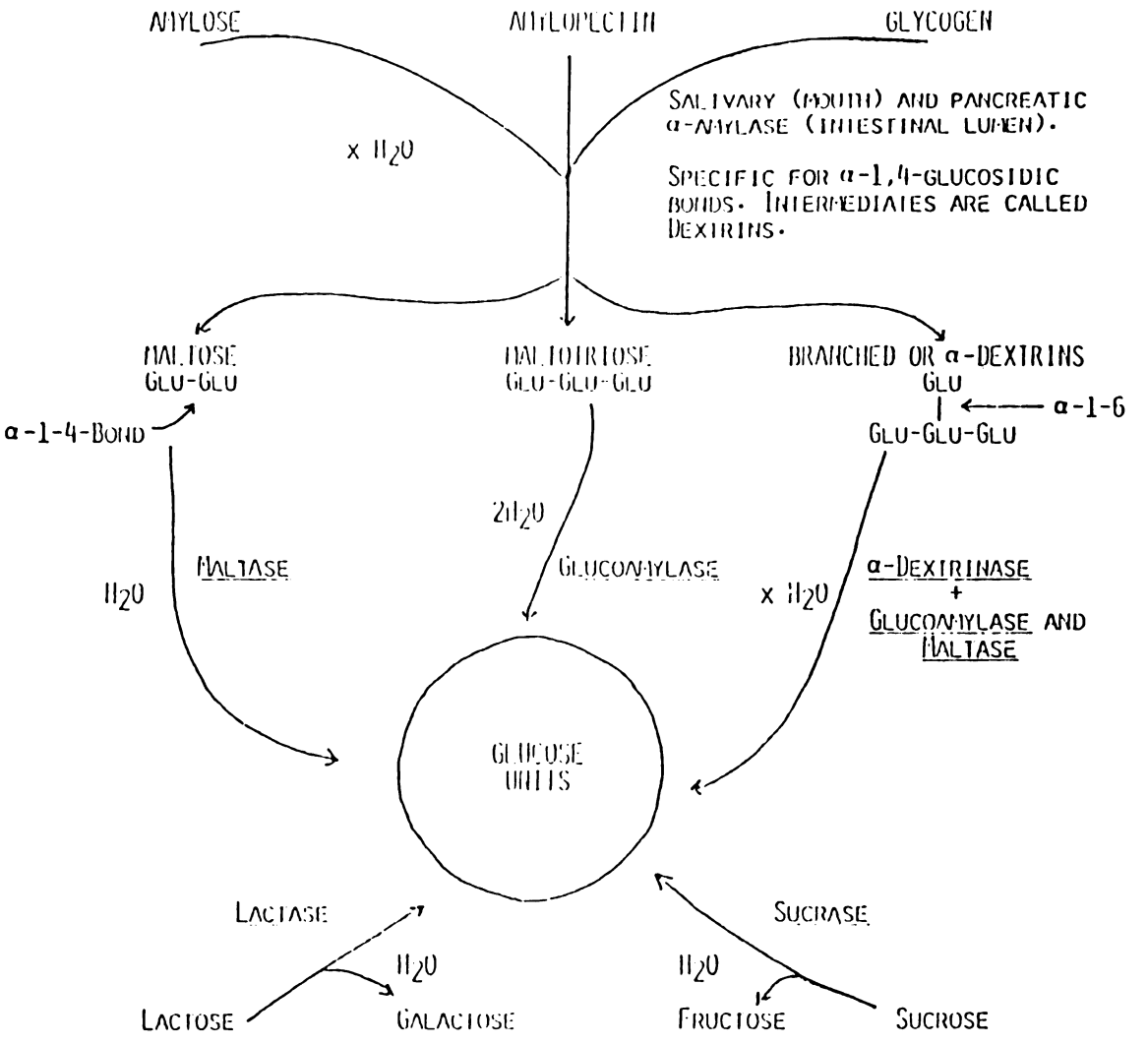


Figure 10.6 Digestion of Starch

DIGESTION OF CARBOHYDRATES



NOTE: ALL ENZYMES UNDERLINED ARE IN THE BRUSH-BORDER CELLS OF THE INTESTINAL WALL

LIPIDS

Definition:

Other terms to understand:

Hydrophobic

Hydrophilic

Polar

Non-polar

FATTY ACID: A fatty acid is an aliphatic acid of the general structure $R-C(=O)-OH$ where R is generally a hydrocarbon containing anywhere from 1 to 21 carbon atoms. The short-chain fatty acids are sometimes called the volatile fatty acids (VFA). Nearly all fatty acids have an even number of C atoms

membrane proteins are often inserted into and through the lipid matrix have further contributed to our present understanding of membranes, resulting in the Singer and Nicolson (1972) *fluid mosaic model*, a refined version of which is shown in Figure 2.1.

The ability of lipids to assume the basic bilayer organization is dictated by a unifying characteristic of membrane lipids—namely, their *amphipathic* character, which is indicated by the presence of a

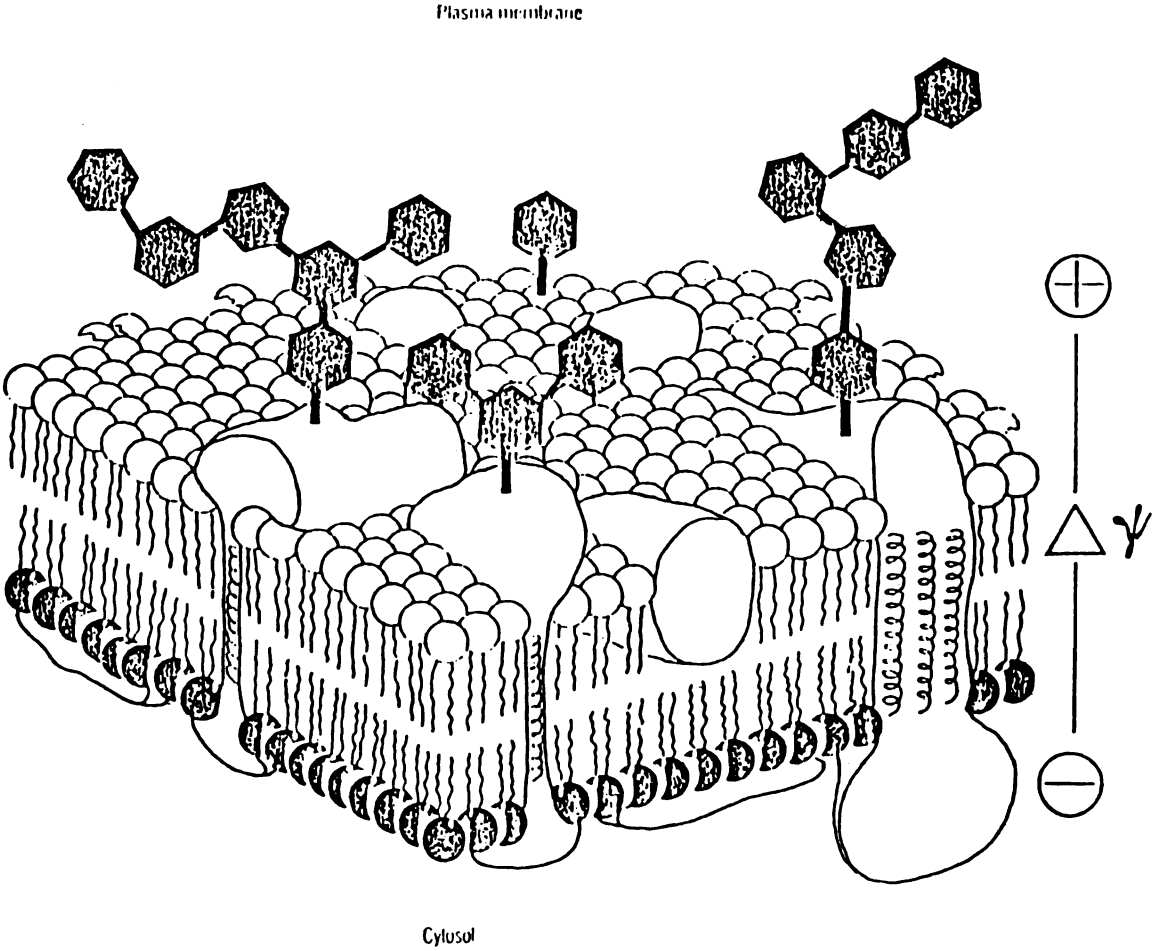


Figure 2.1. The topography of membrane protein, lipid, and carbohydrate in the fluid mosaic model of a typical eucaryotic plasma membrane. Phospholipid asymmetry results in the preferential location of phosphatidylethanolamine and phosphatidylserine in the cytosolic monolayer. Carbohydrate moieties on lipids and proteins face the extracellular space. $\Delta\psi$ represents the transmembrane potential, negative inside the cell.

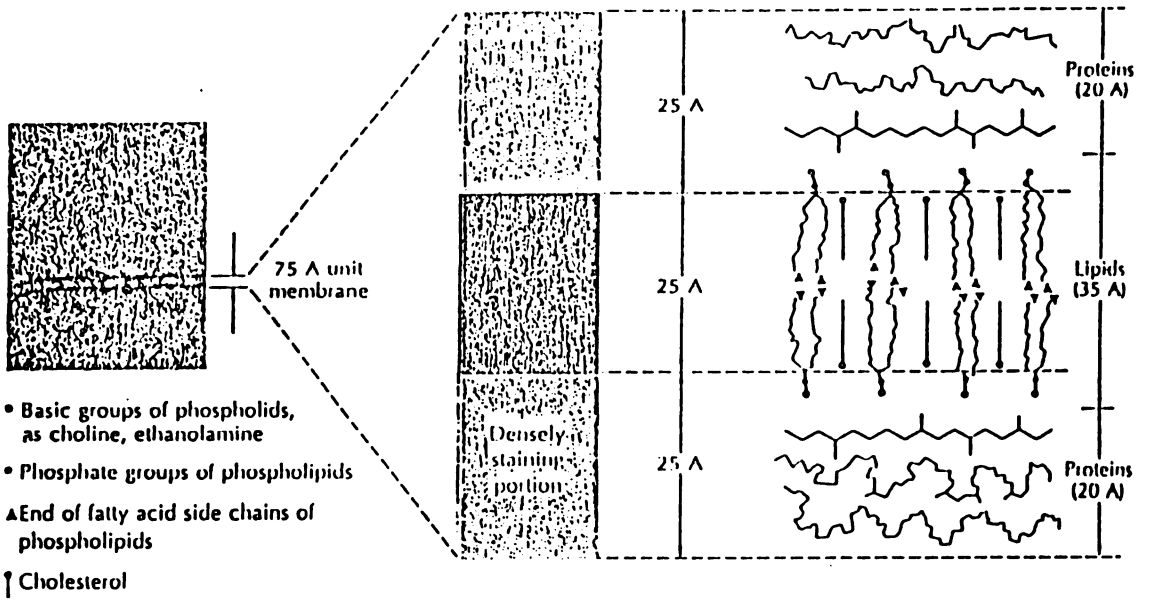


Fig. 17-26. Chemical diagram of unit membrane and membrane as seen in electron microscope. (After Davson, Danielli, Robertson; micrograph courtesy of W. Stoeckenius, Rockefeller University.)

of the cell (Fig. 17-27). This is what one would expect if the middle layer of the sandwich is made of a bimolecular leaflet of lipid because such a layer would be held together only by the relatively weak van der Waals forces between the nonpolar lipid side chains.

In recent years, however, workers in the field of membrane structure began to have some second thoughts regarding the biological relevance of the Davson and Danielli model and the general applicability of the unit membrane theory. This has led to a variety of different, though as yet imprecise, models of membrane structure (Fig. 17-28). Let us summarize the evidence in favor of a more complex formulation of membrane structure.

1. *The chemistry of membranes varies considerably.* It must be obvious by now that membranes can be, and have been, isolated. We can strip off the plasma membrane of mammalian cells and we can get the outer membrane of the bacteria in the form of spheroplasts. We can isolate endoplasmic reticulum membranes in the form of microsomes, as detailed in Chapter 15; nuclei can be isolated and their membranes obtained. Mitochondria and chloroplasts have been isolated: in the case of the former the outer, limiting membrane of the mitochondria has been separated from the inner, cristae membranes with their knobs (see Chapter 13); whereas in the case of the latter the chlorophyll-containing lamellae have been obtained. Only a few membrane systems have been studied so far from the point of view of their protein-lipid content, but the

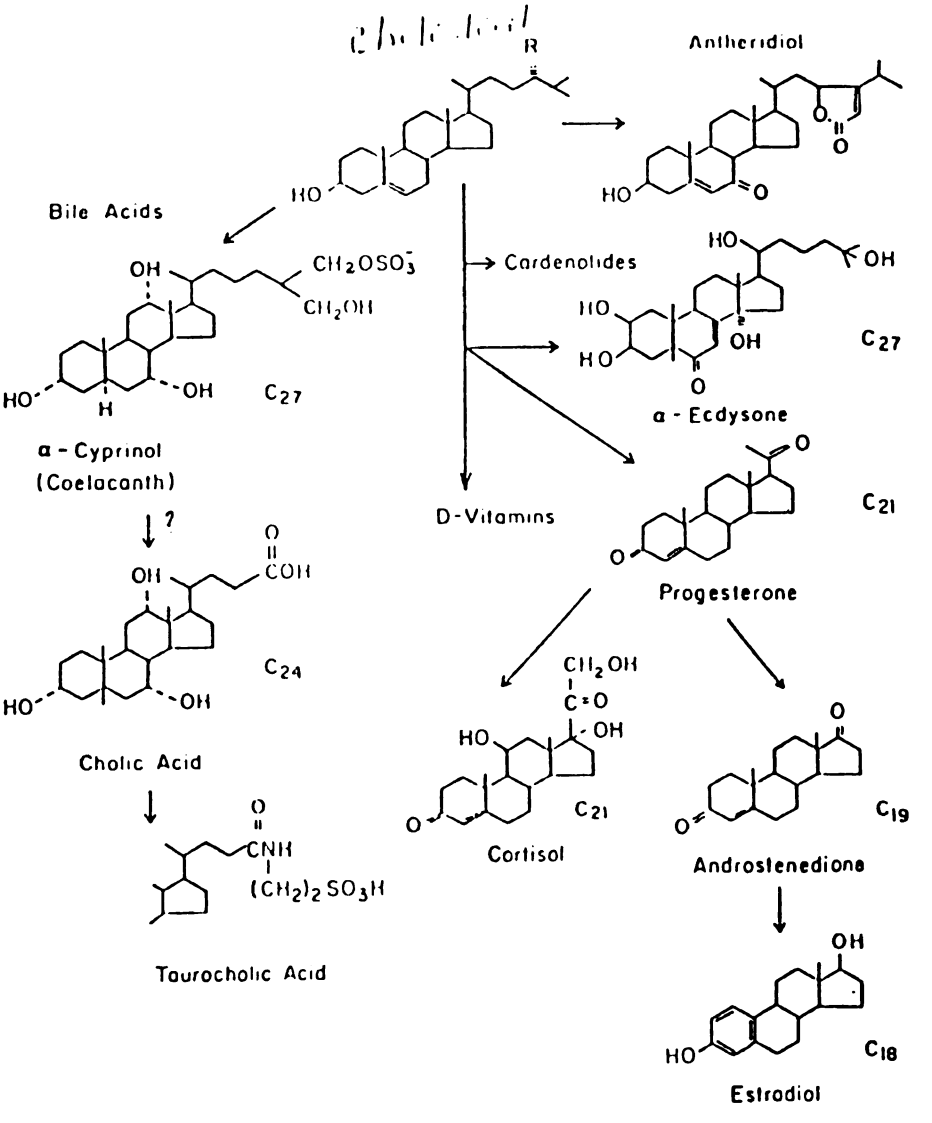


Figure 1.1. **Functional evolution of the sterol molecule.**

side chain. Thus the omnivorous cockroach dealkylates ergosterol or C_{29} plant sterols to 22-dehydrocholesterol, while *Dermestes vulpinus*, an obligate carnivore, lacks—because it does not need—the requisite dealkylating enzymes (Clark and Bloch 1959).

An extensive examination of marine invertebrates (sponges, gorgonians) has uncovered a bewildering variety of side-chain-modified sterols. Structures bearing additional alkyl or cyclopropane groups at six of the eight isooctyl sterol side-chain positions have been identified (Djerassi et al. 1979). It has been suggested, and to some extent documented, that the phospholipids of such marine organisms

Table 5–5. Modified non-α functional groups of proteins.*

Group Modified			
–OH	Non-α-N		
PO_3H_2	N-Methyl	N-Dimethyl	N-Trimethyl
Ser Thr Tyr	Arg His Lys	Arg Lys	Lys

*Modified and reproduced, with permission, from Uy R, Wold F: Posttranslational covalent modification of proteins. *Science* 1977;198:890. Copyright © 1977 by the American Association for the Advancement of Science.

yond the scope of this chapter, their presence can complicate the determination of primary structure.

Primary Structures of Insulin & Ribonuclease

Insulin consists of 2 polypeptide chains linked covalently by disulfide bonds (Fig 5–9). The A chain has an N-terminal Gly and a C-terminal Asn; the B chain has Phe and Ala as the N- and C-terminal residues, respectively. When insulin is oxidized with performic acid, the disulfide bonds linking the A and B chains are ruptured. Both chains are biosynthesized as a single polypeptide chain, **proinsulin**, which after formation undergoes proteolytic processing, forming insulin. (see Chapter 51).

Ribonuclease consists of a single chain of 124 residues with Lys as the N terminus and Val as the C terminus. Eight cysteine residues are joined by disulfide bonds, forming 4 cross-linkages in the protein (Fig 5–10).

DETERMINATION OF SECONDARY & TERTIARY STRUCTURE BY X-RAY CRYSTALLOGRAPHY

While various techniques (eg, optical rotatory dispersion, tritium exchange of labile protons) formerly were much used to infer the presence of helical structures in proteins, these have been largely supplanted by the powerful technique of x-ray crystallography.

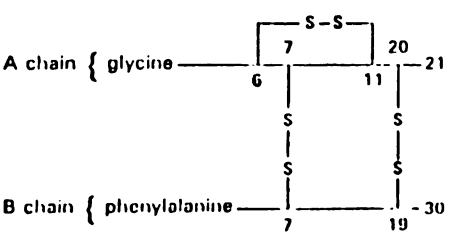


Figure 5–9. Relationship of the A and B chains of human insulin.

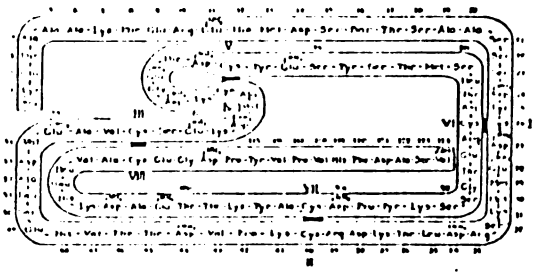


Figure 5–10. Structure of bovine ribonuclease. Two-dimensional schematic diagram showing the arrangement of the disulfide bonds and the sequence of the amino acid residues. Arrows indicate the direction of the peptide chain starting from the amino end. (Reproduced, with permission, from Smyth DG, Stein WH, Moore S: The sequence of amino acid residues in bovine pancreatic ribonuclease: Revisions and confirmations. *J Biol Chem* 1963;238:227.)

X-rays are directed at a crystal of protein and generally also at a derivative of that protein which contains an added heavy metal ion. The rays are scattered in a pattern that depends upon the electron densities in different parts of the protein. Images, collected on a photographic plate, are translated into electron density maps which, when superimposed one on another, permit the crystallographer to construct a faithful model of the protein in question. Although time-consuming, expensive, and requiring highly specialized training, x-ray crystallography reveals detailed, precise views of the orientations of all the amino acids in many proteins. Its contributions to our present-day concepts of protein structure can hardly be overstated.

DETERMINATION OF QUATERNARY STRUCTURE

Determining the quaternary structure of oligomeric proteins encompasses determining the number and kind of protomers present, their mutual orientation, and the interactions that unite them.

As long as oligomers do not undergo denaturation during the procedure used to determine their molecular weight, many methods can yield molecular weight data for oligomers. These same techniques may be used to determine protomer molecular weight if the oligomer is first denatured.

Ultracentrifugation

Developed by Svedberg, this method measures sedimentation rate in an ultracentrifugal field of around $10^5 \times g$. It has tended in recent years to be replaced by less complex techniques.

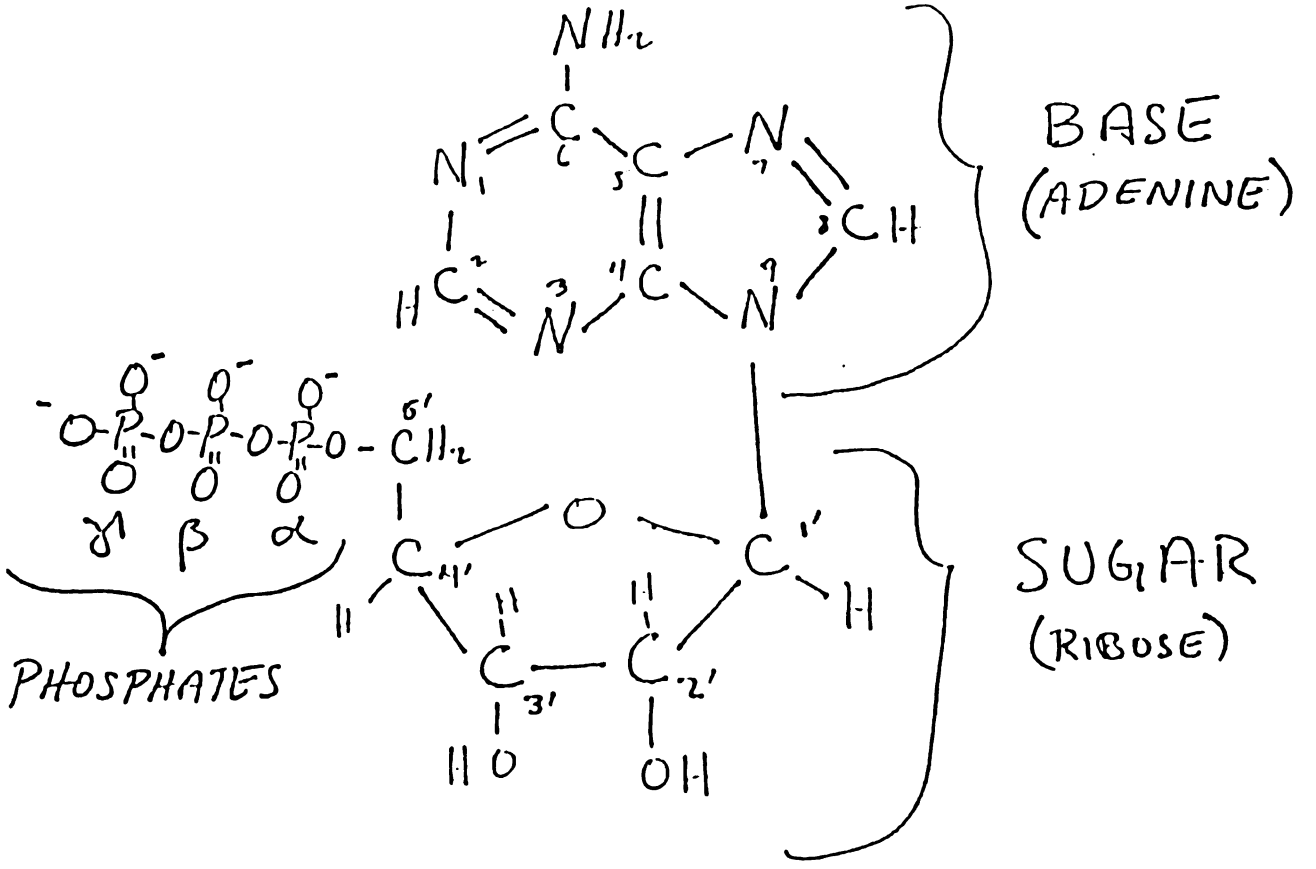
Sucrose Density Gradient Centrifugation

Protein standards and unknowns are layered over a 5–20% sucrose gradient in a plastic tube and cen-

THE BUILDING BLOCKS OF NUCLEIC ACIDS ARE NUCLEOTIDES

A TYPICAL NUCLEOTIDE:

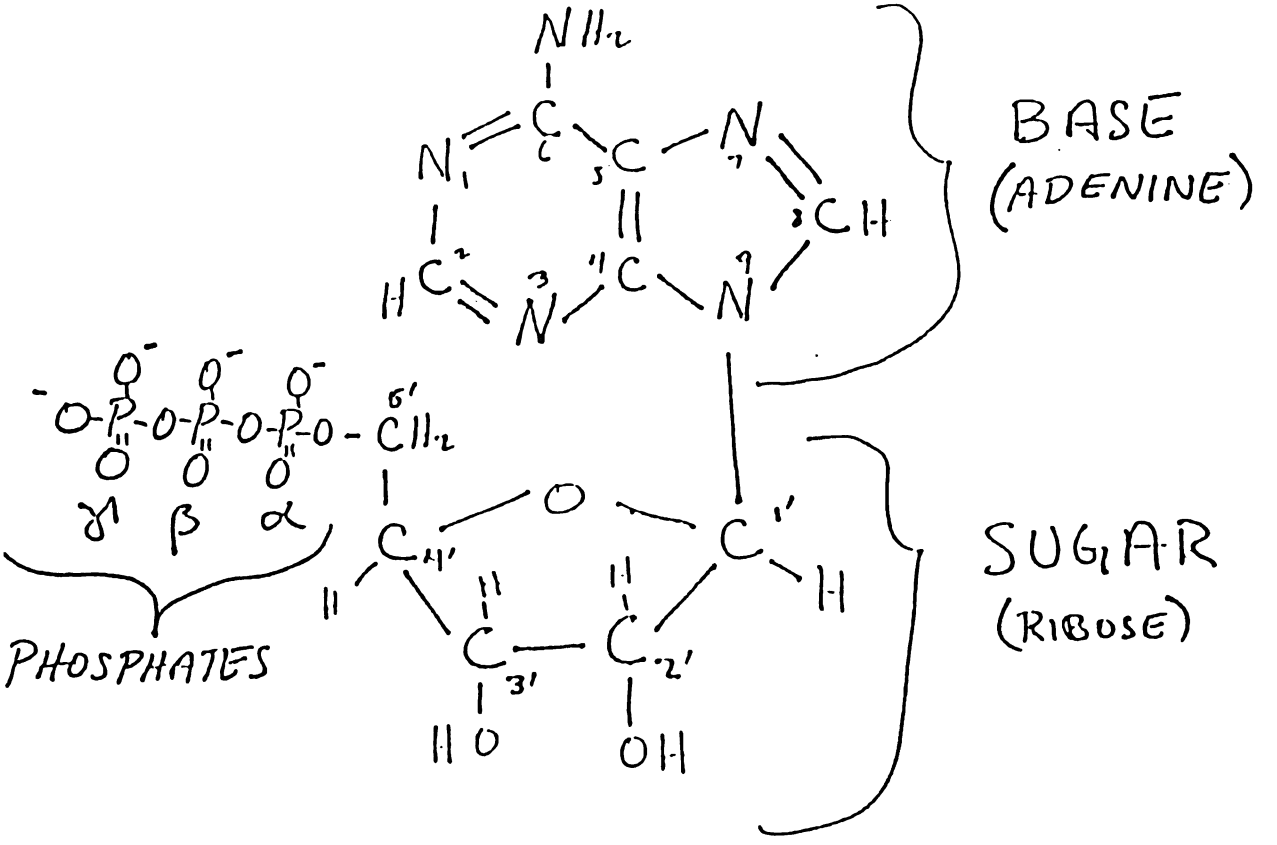
ATP



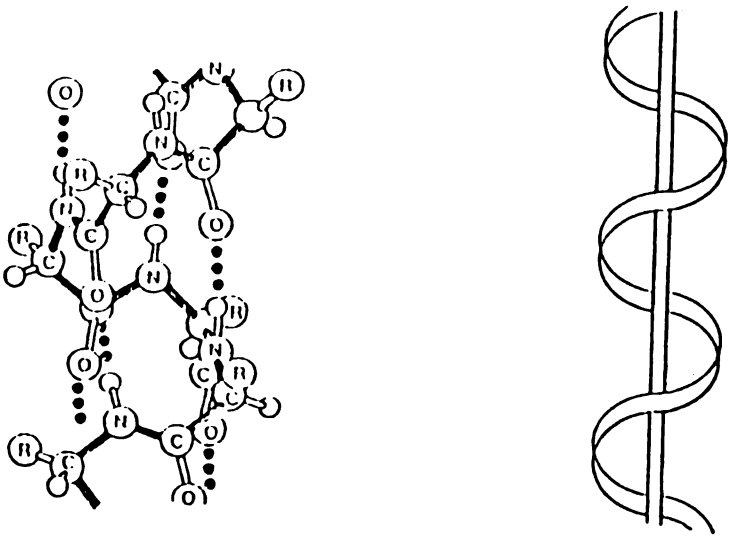
THE BUILDING BLOCKS OF NUCLEIC ACIDS ARE NUCLEOTIDES

A TYPICAL NUCLEOTIDE:

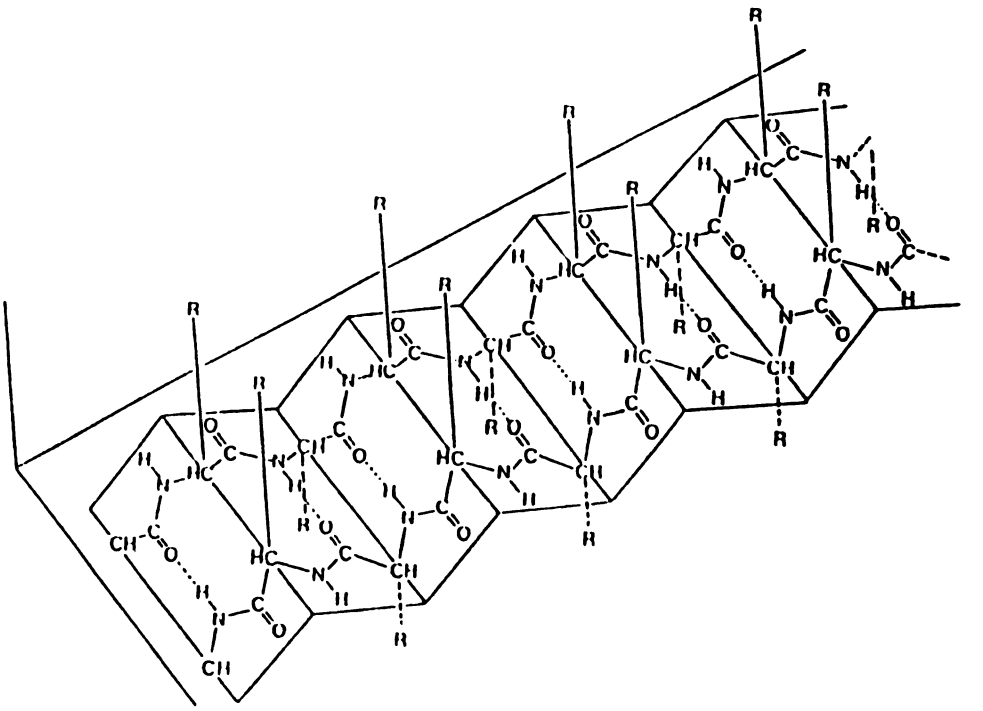
ATP



Part II
Biochemistry
Figure Page



(a) Helix (right-handed or α-arrangement)



(b) Pleated sheet (β -arrangement)

Figure 12.3
Geometric Configurations of a Protein Molecule

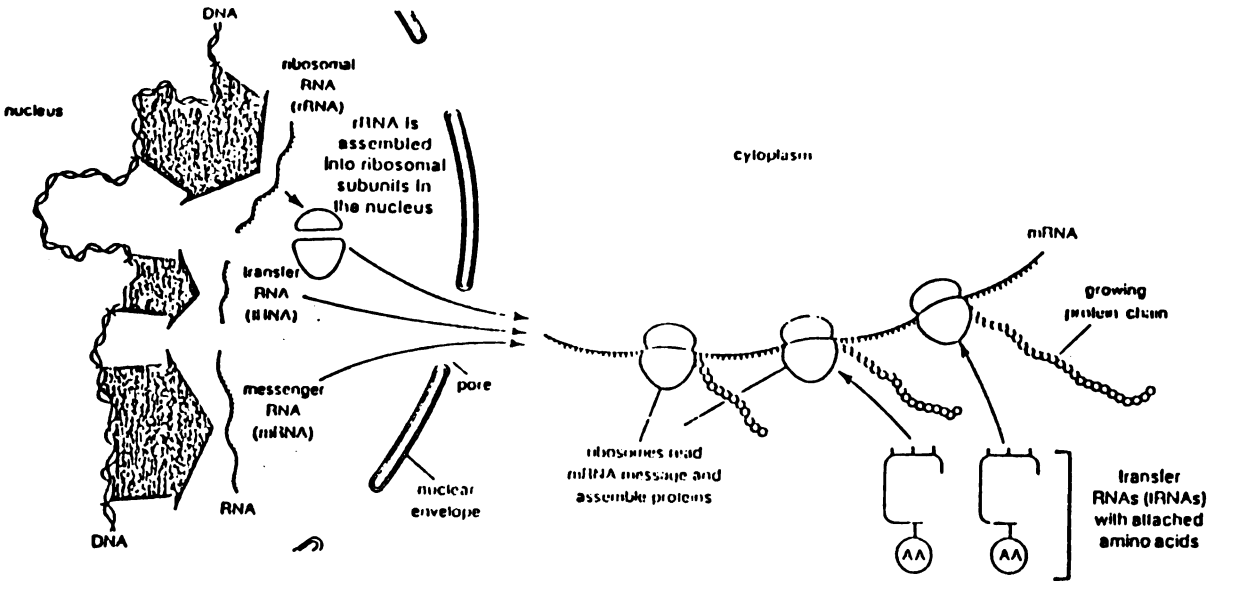


Figure 4-14. *The major events in cellular synthesis (see text).*

DNA) and replication (duplicating the DNA) and proteins that regulate or control these activities. Although many of the enzymatic nonhistone proteins active in transcription and replication have been successfully isolated and biochemically characterized, little is known as yet about the nonhistone proteins regulating nuclear functions.

Suspended within the chromatin of the nucleus are one or more irregularly shaped bodies, the *nucleoli* (singular, *nucleolus*; Figs. 4-1 and 4-13). The nucleolar material is so densely packed that its boundaries are easily traced even though no membranes separate the nucleolus from the surrounding chromatin. Two major components, called simply the *nucleolar fibers* and *nucleolar granules*, are visible inside the nucleolus. The nucleolar fibers are indistinct and difficult to trace in electron micrographs; generally they appear to be somewhat thinner than chromatin fibers. The nucleolar granules, in contrast, are distinctly visible inside the nucleolus as small spherical particles about half the size of cytoplasmic ribosomes. Frequently, spaces containing chromatin fibers extend into the nucleolus, giving parts of the structure a more open, coarsely coiled appearance. Investigations into nucleolar structure and function have established that the fibrils and granules are the morphological forms taken by successive steps in synthesis of the RNA of ribosomes and the assembly of ribosomal subunits, both of which take place in the nucleolus. The overall size and shape of the nucleolus and the distribution of fibrils and granules inside it change as cells go through their cycles of growth and division.

The nucleus functions as the ultimate control center for cell activities (Fig. 4-14). Within the chromatin, the information required to synthesize cellular proteins is coded into the DNA. Two primary kinds of information are coded in the DNA sequences. The directions for making proteins are spelled out by a code that uses the four DNA nucleotides, three at a time, to form the code words. Each three-nucleotide code word stands for an amino acid. Reading the code words in sequence along the DNA spells out the sequence of amino acids in a protein. These protein-encoding regions are duplicated into RNA copies called *messenger RNAs (mRNAs)*, which carry the directions for making proteins to the cytoplasm.

Other DNA regions store the directions for making two types of accessory RNAs that act in parts of the protein synthesis mechanism. One, *ribosomal RNA (rRNA)*, forms a part of the ribosomes, the RNA-protein structures that assemble amino acids into proteins in the cytoplasm. The second, *transfer RNA (tRNA)*, binds directly to amino acids during protein synthesis and provides the necessary link between the nucleic acid code and the amino acid sequence of proteins.

Synthesis of the mRNA, rRNA, and tRNA copies of DNA, called transcription, occurs within the cell nucleus. Following transcription, the RNA copies pass through the nuclear envelope and enter the cytoplasm. Messenger RNA, once in the cytoplasm, attaches to one or more ribosomes. The ribosomes then assemble amino acids into proteins, using the information carried in the attached mRNA as a guide. In this synthesis, called *translation*, a ribosome starts at one end of an mRNA molecule and moves along

APPENDIX I

APPENDIX I

CHEMISTRY II PRE LAB: STUDY OF SACCHARIDES

PURPOSE:

To determine the chemical and physical properties of saccharides.

OBJECTIVES:

1. Using the Benedict's test, determine whether certain saccharides are reducing sugars or not.
2. Using the iodine test, determine if sugars are helically coiled or branched polysaccharides.
3. Test known sugars in conjunction within unknown plant samples to determine if the sample is a reducing sugar or a helical polysaccharide.
4. Assay to determine the effect of the enzyme amylase and acid hydrolysis on saccharides.

BASIS FOR THE FIRST TWO CHEMICAL TESTS

Benedict's Test

The Benedict's reagent contains Cu^{++}, a strong oxidizing agent.

$Cu^{++} + e^- \longrightarrow Cu^{+}$ reduction rxn

Basically the copper II is in an alkaline solution which promotes a reaction with the linear forms of sugars which cause the copper II to be reduced. When this occurs the Benedict's solution turns from blue to red and forms a precipitate. (see lab for complete discussion)

Iodine Test

Iodine is able to complex with helically coiled polysaccharide chains (as opposed to linear chains) which converts the reddish-yellow color of iodine to a bluish-black color. (see lab for details)

Assignment

After you have completed reading the lab, use your notes and the lab to complete the following outline. Wherever you see a hyphen (-) complete the statement or a colon (:) write in the definition. You may use examples or write in any additional information you choose.

I. Saccharides are -

A. The general formula is -

B. The three main functions of saccharides are -
1.
2.
3.

II. There are three major groups of saccharides

A. Monosaccharides are -
1. Main function is for -
2. Two forms are -
3. Most are white crystalline monomers that are soluble in H_2O
4. Examples
a. glucose:
b. fructose:
c. galactose:

B. Oligosaccharides are-
1. Function is mainly for -
2. Most are disaccharides
3. Have glycosidic bonds which are -
4. Examples
a. sucrose:
b. maltose:
c. lactose:

C. Polysaccharides are -
1. Main function is -
2. Have glycosidic bonds
3. Can have three forms -
a.
b.
c.
4. Hydrolysis of polysaccharides may yield -
5. Examples
a. starch:
b. dextrin:
c. glycogen:
d. cellulose:
e. chitin:
f. inulin:
g. pectin:

DIGESTION OF FAT

PURPOSE

To demonstrate the relationship of the pancreatic enzyme lipase and the steroid bile.

OBJECTIVES

To explain how lipase and bile work together in the breakdown of fats.

To determine the end products of fat digestion.

PROCEDURE

1. Identify three test tubes as 1, 2, and 3. Put 10 mL of distilled water in each of the test tubes, then add a few (3-4) drops of Olive oil.

2. To test tubes 1 and 2 add 2 mL of the 5% bile salt solution (sodium taurocholate).

3. Add 5mL of the 2% pancreatin solution to test tubes 1 and 3.

4. Check each of the solutions with Hydrion paper. Solutions should be neutral. If not, adjust with a minimal (trace) amount of sodium bicarbonate if acidic. (use more enzyme if basic)

5. Place the three tubes in a water bath (40°C) for 30 minutes.

6. Check the solutions for a change in pH with the Hydrion paper.

7. Once tubes are in water bath, repeat the same procedure using coconut oil and place in water bath immediately and check the time. Compare your findings.

RESULTS

1. What changes occurred in tube 1? _____

_____.

2. In tube 2? _____

_____.

3. In tube 3? _____

_____.

4. Account for the differences; use your worksheets as a reference.

_____

_____

_____.

TEACHER'S GUIDE TO GEL PERMEATION CHROMATOGRAPHY

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BEHAVIORAL OBJECTIVES:

The student will be able to define and discuss gel permeation chromatography.

Upon completion of this lab the student will be able to separate small molecules from large molecules using gel media.

Students will be able to collect equal samples of solutions and detect concentration variance of contents using a spectrophotometer.

TIME REQUIRED:

Teacher preparation: Sephadex takes a minimum of three hours to swell. Sephadex may be made in advance because it stores well providing it does not dry out. If the teacher prepares columns, the lab can be completed in 50 minutes.

Student lab time: If students prepare columns two lab periods will be needed. With columns prepared beforehand, the separation and spectrophotometric work can be easily completed in one lab period (50 minutes). Stopping between parts I and II is ideal for a two day lab.

MATERIAL PREPARATION:

1. To prepare each 100 mL Tris buffer of pH 8.0, place 0.117 g of Tris base in 50 mL distilled water and 0.236 g of Tris HCL in 50 mL distilled water and combine to make 100 mL of buffer (Check pH). You will need to make

100 mL buffer for rinsing each column. You will also need 100 mL of buffer for each 50 mL of gel bed volume.

2. Use 1 g Sephadex G-25 per every 4-6 mL of bed volume. 50 g of Sephadex will make 200-300 ml bed volume. Use twice the volume of buffer as bed volume, place Sephadex in buffer and set aside to swell. (Cover! Do not allow to dry.)

3. To prepare 200 mM $(NH_4)_2SO_4$ use 0.246 g in 10 mL distilled water.

4. Mix Hemoglobin in a ratio of 5 mg/mL of 200 mM $(NH_4)_2SO_4$. 5 mL of this solution will be enough but 10 mL may be more practical for dispensing.

5. To prepare the 200 mM $BaCl_2$ use 0.416 g of $BaCl_2$ per every 10 mL distilled water.

BACKGROUND INFORMATION:

Gel permeation chromatography separates materials based strictly on size. Charge and solubility of polar or nonpolar solvents has no effect on these separations. In biochemical laboratories gel permeation chromatography is commonly used to exchange the buffer or salts in a protein solution, or used in the final stages of protein purification to separate small protein molecules from large protein molecules.

Using a gel permeation column is relatively easy but does require attention to several details. These details are:

1. Particle (bead) size. The important point of particle size is that the smaller the particle the larger the resistance to solvent flow. On the other hand , the smaller particles give better resolution (separations) than the larger molecules. The smaller beads also have smaller pores to "detour" the smaller molecules.
2. Fractionation range. The fractionation range indicates the size of molecules that can pass through the matrix of the media. Molecules that are too large cannot diffuse through the matrix and therefore, are excluded.
3. Swelling time. Gel permeation media in the dry state requires a finite time to take up water. The media with large porosities take longer to swell. Heating, to 90° C, can greatly decrease swelling time.
4. Maximum operating pressure. Because gel permeation media are not rigid structures, exceeding the maximum operating pressure will cause the media to collapse.
5. Bed volume. The bed volume allows one to calculate the amount of dry media to weigh out to fill the column. By using the formula "Pi"r^2h the volume of the column can be determined by measuring the inside diameter and height.

Dividing the column volume by the bed volume determines the weight of the dried gel needed.

Columns may be reused after re-equilibrating with buffer. To prevent microbial growth during storage, add an antimicrobial agent, such as 0.02% sodium azide, to the equilibration buffer. The antimicrobial agent may be eluted from the column before another run. If you are planning on doing further investigations using molecules that are close in size you will need a much longer column to get a good separation.

ANSWERS TO QUESTIONS:

1. Tubes 4 & 5 and possibly 6. Tube 5 has the higher concentration.

2. Tubes 7 - 10, with 6 a possibility.

3. Tube 6. (These answers are not necessarily restricted to these tube #'s but are relative in the order of molecular separation.

4. Hemoglobin is the larger molecule. The larger molecules pass through the column faster as the smaller molecules enter pores between the beads and pass through more slowly.

5. The smaller the bead (particle) size the greater is the resistance to the solvent flow and the better the resolution (separation) of the collected molecules.

SOURCES OF MATERIALS:

1. Sephadex G-25-300 can be obtained from Sigma Chemical Company, phone 1-800-325-3010. P.O. Box 14508, St. Louis Mo. 63178. (Bio-Gel P-6, coarse, 50-100 mesh is an equivalent that is available from Bio-Rad Lab, 32nd and Griffin, Richmond, Ca.)

2. The Tris HCL and Tris base for the buffer solutions are available in many supply catalogs. (Fisher, Carolina, and Sigma)

3. Hemoglobin (powder) is available from any biological supply catalog.

GEL PERMEATION CHROMOTOGRAPHY

INTRODUCTION:

Gel permeation chromatography separates molecules based strictly on size. The charge and solubility of polar or nonpolar solvents has no effect on the separation. In biochemical laboratories gel permeation chromatography is commonly used to exchange the buffer or salts in a protein solution, or in the final stages of a protein purification to separate small proteins from large proteins.

Using a gel permeation column is relatively easy but does require attention to several details. These details are: particle (bead) size, fractionation range, swelling time, operating pressure, and bed volume. (Your teacher may wish to discuss these with you.) In this lab particle size is important to you. The smaller the particle size the larger the resistance to solvent flow. The smaller spaces created by smaller particles, as opposed to large spaces between large particles, yield better resolution (separation). Therefore, the size of the beads (particles) in the column determines the efficiency in separating various sized molecules.

This laboratory exercise demonstrates the separation of hemoglobin and ammonium sulfate by gel permeation chromatography. The separation occurs as the molecules travel through layered beads. The beads used for these columns are porous. As the mixture passes through the column, the smaller molecules get "detoured" in these pores and proceed down the column more slowly. The larger molecules do not enter the pores and are carried along with the solvent, passing out of the tube before the small molecules.

OBJECTIVES:

To separate small molecules from large molecules using gel permeation chromatography.

To determine the order of molecular collection by using gel permeation chromatography and spectrophotometry.

To explain how bead size affects the separation of molecules in gel permeation chromatography.

CAUTIONS AND PITFALLS:

Be sure to maintain a liquid level above the column bed at all times. DO NOT ALLOW COLUMN TO GO DRY !!! The bed column would collapse.

Wipe test tubes clean of fingerprints and smudges before placing into Spec 20.

MATERIALS:

Ammonium sulfate $(NH_4)_2SO_4$
Barium chloride ($BaCl_2$)
Hemoglobin (5 mg hemoglobin/mL of 200 mM $(NH_4)_2SO_4$)
Sephadex G-25 in buffered solution
Tris buffer (25 mM, pH 8.0)
Column (25 cm x 10 mm) or equivalent
Disposable syringe or funnel (50 cc or larger)
Rubber tubing (2) 10 cm and 30 cm lengths
Pinch clamps (2)
Test tubes (10) 13 mm x 100 mm
Test tube rack
Spectrophotometer (Spec 20)
Ringstands (2)
Buret clamps (2)
Micropipette or equivalent
Marking pencil
Pipette (1 mL)
Dropper
Distilled water

PROCEDURE:

Part I Setting Up The Column

1. Place disposable syringe above column as the source for buffer flow. Attach disposable syringe to highest point on ring stand with buret clamp. Attach the longer rubber tubing to the syringe bottom; place pinch clamp on far end of tubing.

2. Attach column below syringe using buret clamp. Leave enough clearance at base of column to draw off samples into test tubes. Connect the shorter tubing to the bottom of column and place pinch clamp on far end.

3. Be sure filter disc is in bottom of column. If gel permeation columns are not available, a small cotton plug in the bottom of a buret works well as an alternate column.

4. Stir the Sephadex and slowly fill column. Allow the liquid to run through column by opening pinch clamp. Continue stirring mixture in beaker as you fill column to within 1-2 cm from top adapter. Keep Sephadex bed covered with liquid at all times. (Do NOT allow column to go dry).

5. Place two times the column's volume of buffer into the syringe and attach upper tubing to top of column. Open top pinch clamp and allow two column volumes of buffer to flow through column (open bottom clamp) to insure uniformity and stabilize bed. Close top clamp and allow buffer level to drain to within 1 cm of column bed and stop flow with bottom pinch clamp.

Part II Separating The Molecules

(Turn on the Spec 20, allowing it to warm up before beginning part II).

1. Carefully add 100 uL of the hemoglobin solution to the surface (top) of the column bed. Solution must be added slowly so that the surface of the bed is not disturbed. Open bottom pinch clamp slowly and allow hemoglobin to just enter bed and stop flow.

2. Gently add buffer to fill column and attach upper tubing. Put more buffer into disposable syringe if needed. You will be drawing off ten 1 mL samples.

3. Using a 1 mL pipette place 1 mL of distilled water into one of the tubes and mark the surface level on the tube. Discard the water and mark all remaining tubes at the same height.

4. Allow column to flow slowly and collect ten 1 mL column fractions (samples).

Part III Analyzing The Fractions

1. Add 1 drop of 200 mM $BaCl_2$ to each tube. Record color change or formation of precipitate.

2. Add 2 mL distilled water to each tube. Remove the level mark from all test tubes.

3. Set Spec 20 to 450 nm and blank with distilled water. Insert each of the ten tubes into the Spec 20 and record the percent transmittance. Be sure to follow proper Spec 20 procedures and wipe off tubes with a tissue to remove smudges or fingerprints before inserting.

DATA:

QUESTIONS:

1. Which tubes contain hemoglobin? Which tube has the higher concentration?

2. Which tubes contain the precipitate $BaSO_4$?

3. Are there any tubes that contain both hemoglobin and the precipitate? If so, which ones?

4. Which is the larger molecule, the hemoglobin or the ammonium sulfate? Upon what information is your answer based?

5. Explain how bead size affects the separation of molecules in gel permeation chromatography.

CONCLUSIONS:

FURTHER INVESTIGATIONS:

Separate other small molecules from other large molecule, such as hemoglobin and an indicator.

Separate proteins using much finer meshed beads.

Separate proteins in meats.

Separate hemoglobin and myoglobin.

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