This teacher's guide includes parts three and four of the four-part third year Portland Project, a three-year integrated secondary science curriculum sequence. The underlying intention of the third year is to study energy and its importance to life. Energy-related concepts considered in year one and two, and the concepts related to atomic structure and particle phenomena considered earlier in the third year are further built upon in this volume. Chapters include: monomers and how they are built; chemistry of simple carbon compounds; polymers or stringing monomers together; polymers in 3D or the shape of things to come; where the action is - the active site; polymers to polymers; genes, proteins, and mutations; energy capture; energy consumption and metabolism; and metabolism and genes. Notes to the teacher, examples of data, and problem calculations are included. (Author/SL)
CHEMISTRY OF LIVING MATTER. ENERGY CAPTURE & GROWTH

PARTS THREE & FOUR

OF

AN INTEGRATED SCIENCE SEQUENCE

TEACHER GUIDE

1973 EDITION

prepared by
THE PORTLAND PROJECT COMMITTEE
under a grant from
THE NATIONAL SCIENCE FOUNDATION
CHEMISTRY OF LIVING MATTER, ENERGY CAPTURE & GROWTH

AN INTEGRATED SCIENCE SEQUENCE

1973 EDITION

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Dedication

This volume is dedicated to the memory of Vernon Cheldelin under whose guidance and leadership integration of the sciences for Oregon secondary school youth was begun in 1963.
Introduction

The Portland Project was initiated in the fall of 1962 when two secondary school teachers, one with background in CBA chemistry, the other having responsibility for PSSC physics, began to note and discuss the redundancy in their respective courses. Why should students be subjected to this repetitious and fragmented representation of the physical sciences? they asked. A Steering Committee met to pursue the problem further and perhaps enlist the support of a funding organization to permit its exploration in depth. Under the able and devoted leadership of Vernon Cheldelin, Dean of the School of Science at Oregon State University (deceased), two proposals prepared for support by the National Science Foundation were funded in the summers of 1963 and 1964.

Thirty-five scientists and teachers devoted various quantities of time as writers, consultants, pilot teachers, and evaluators, with the aim of ascertaining the feasibility and efficiency of the integration of chemistry and physics. Concurrently and subsequently, other groups in other parts of the country have carried on studies that are approximately parallel to this one. Though the conceptual development and points of emphasis differ, the various groups are satisfied that integration of science courses is not only feasible but highly desirable.

Dr. Michael Fiasca of the Education and Science Staffs of Portland State University conducted an evaluation which revealed that subject matter achievement in chemistry and physics and critical thinking abilities are enhanced among students who studied the integrated courses over those who study the separate disciplines of

Federation for Unified Science (FUSE) was recently organized to act as a clearinghouse of information on integrated science courses. Victor Showalter at Ohio University is the chairman of this committee.
chemistry and physics. It should be emphasized that though these differences were apparent, it could not be demonstrated that they were statistically significant.\(^2\)

A concomitant result showed that enrollments in the two-year integrated courses were dramatically greater than in the separate courses.

A survey completed April 16, 1967 showed that there were forty-four schools in twenty states using the Portland Project integrated chemistry-physics course.\(^3\)

Mounting evidence in the literature from prominent persons working in science education strongly supported this mode of organization. Dr. Jerrold R. Zacharias, the prime instigator of the PSSC physics program, exemplified the changing attitude of scientists and educators:

The division of science at the secondary school level, into biology, chemistry and physics is both unreasonable and uneconomical.

Ideally, a three-year course that covered all three disciplines would be far more suitable than a sequence of courses which pretends to treat them as distinct. Today such a three-year course would be difficult to fit into the educational system, but much of this difficulty might be overcome at once if such a course existed, and it might well be that present tendencies in education would soon overcome the rest.

In any case, a greater coordination of the three subjects is possible even within the existing framework. It is understandable that the groups which developed the existing programs, each of which faced great problems of its own as it worked toward its goals, were reluctant to embark on the larger task of giving coherence to the sum of their efforts. With the programs now complete or approaching completion, it may be that the time has arrived for this necessary step.\(^4\)

\(^2\) Detailed results of this study may be obtained by writing to Dr. Fiasca at Portland State University.

\(^3\) Detailed enrollment figures and addresses of people who are using the Portland Project courses may also be obtained from Dr. Fiasca.

\(^4\) From page 52 of Innovation and Experiment in Education, a Progress Report of the panel on Educational Research and Development to the U.S. Commissioner of Education, the Director of the National Science Foundation, and the Special Assistant to the President for Science and Technology, March, 1964.
Stimulated by the apparent success of their original work towards this kind of integrated course, persons close to the Portland Project began to discuss extension of their work to include biology with chemistry and physics in a three-year sequence. A third proposal was prepared in 1966 and granted support by the National Science Foundation. Dr. Arthur Scott, member of the Chemistry Department at Reed College who has had deep interest in the Portland Project since its inception, graciously offered his talents, energy and time to carry on the project after Dean Cheldelin's death.

A writing conference was conducted on the Portland State University campus during the summer of 1967 to develop materials such as teacher and student guides. Eight local pilot schools committed approximately five hundred students and twelve pilot teachers for testing and evaluation. Dr. Donald Stotler, Supervisor of Science for the Portland School District, has had an active part in this and other phases of this project.

Twenty-six persons whose functions were writing, consulting, analysis, and editing met on the Portland State campus beginning June 14, 1967 to begin preliminary work on the integrated course. Their first task was to formulate an outline that displayed logical content development utilizing concepts out of biology, chemistry and physics. Particular attention was paid to matching students' abilities, interest and maturity level with the sophistication of concepts as nearly as this was possible to do. Then the committee perused material developed by the national curriculum groups --PSSC, Project Physics, CBA, CHEMS, BSCS and IPS -- in search of material to implement the outline they constructed previously. In the absence of appropriate materials, major and minor writing projects were initiated.

The writing committee continued its work in the summers of 1968 and 1969 with Dr. Karl Dittmer, Dean of the Division of Science, as director. Four major projects were tackled and completed: (1) extensive revisions were effected in the three-year outline, (2) the first- and second-year courses were revised based upon
student and teacher feedback, (3) the third-year course was developed incorporating Harvard Project Physics materials as a main vehicle, and (4) an evaluation program for the three-year course was developed.
Working Committee

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Curtis Guthrie - Chemistry
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Pilot Schools

The following schools have served as pilot schools for the pilot course during one or more of the past three academic years.

Adams High School
Portland, Oregon
  Glen Hampshire
  Lloyd Meskimen
  Thomas Miles

Aloha High School
Aloha, Oregon
  Mary Lou Combs
  Elvis Dellinger
  Nelson Doeleman
  Ted Parker

Beaverton High School
Roger Berg
Jean Halling
Lois Helton
H. Dean Smith

Benson Polytechnic School
Howard Browning
W. B. Chase
Robert Franz
W. L. Hoffman

Central Catholic High School
Portland, Oregon
  Jacob A. Mosbrucker
  Peter Roerig

Cleveland High School
Portland, Oregon
  John Brown
  Edmund McCollough

Franklin High School
Portland, Oregon
  John Neeley
  Joseph Sklenicka

Grant High School
Portland, Oregon
  Myra N. Rose

Jefferson High School
Portland, Oregon
  Ronald Kawamoto
  Leslie Morehead
  Kenneth Starbuck

Parkrose High School
Parkrose, Oregon
  Donald Pearson

Rex Putnam High School
Milwaukie, Oregon
  Dennis Axness
  David Cox
  Jerry Fenton
  Henry Kilmer
  Jack McGoldrick
Roosevelt High School
Portland, Oregon
  Renee Bergman
  Kenneth Fuller
  Sue Storms

Sunset High School
Beaverton, Oregon
  Leonard M. Dooley

Wilson High School
Portland, Oregon
  Curtis D. Guthrie
  Norman Sipple

Woodland High School
Woodland, Washington
  Donald G. Fry
  George L. Stroud
ACKNOWLEDGEMENTS

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Cecelia Benner - Secretary
Judy Bierman - Secretary
Doni Budlong - Secretary
Alice Eyman - Secretary
Mary Grant - Artist
Ethel Hays - Secretary
Lothar Graudins - Artist
Neomia Kendrix - Technician
Peggy Munyer - Typist
Janice Page - Laboratory Assistant
Robert Poon - Laboratory Assistant
Dianne Poppino - Laboratory Assistant
Jean Sims - Secretary
Linda Tham - Laboratory Assistant
Jill Timmons - Secretary
Kathryn Ann Torvik - Artist
Shirley Tsang - Laboratory Assistant
Barbara Wiegele - Secretary

STUDENT PARTICIPANTS

Patrick Moore - Jefferson High School (sow bug experiment)
The decision to try to develop a three-year integrated science course which would replace the traditional three courses in biology, chemistry and physics is based on several considerations. Among them are:

(1) a conviction that modern developments have made the division of science under these three headings obsolete;

(2) a recognition that the traditional courses overlap in many areas, resulting in a great deal of duplication and repetition as in the gas laws, atomic and nuclear structure, calorimetry and the kinetic molecular theory;

(3) a feeling that terminal students, who take no more than one year of science, deserve to get a taste of all of science rather than just one aspect, as they do in the conventional programs; and

(4) a desire to emphasize the unity in the approach to natural phenomena and the similarity in the methods, techniques and apparatus used by scientists in all fields.

A natural question arises as to what distinguishes this course from a general science course expanded to three years. The answer is that this course does not consist of a number of unrelated topics that might be taken up in any order; rather, it treats science as a structure that proceeds from observation to the development of general principles and then to the application of those principles to more involved problems. The emphasis in a general science course is on the results of science; the emphasis here is on the methods and reasoning by which scientists have arrived at these results.

The three-year course outline shows that a number of topics such as properties of matter, energy, heat, and certain biological concepts are discussed at the first-year level and again later in the course. This re-cycling is deliberate. It is intended to introduce students in a semi-quantitative way to some of the
significant generalizations of science and to show how these generalizations arose. These topics are treated again in the second and third years when greater facility with mathematics on the part of students makes it possible for them to understand and appreciate discussions of these topics in the succinct and precise language of mathematics.

An excessively formal and quantitative approach is avoided in the first year for several reasons. Students at this level do not extract essential meaning from such a presentation of information; furthermore, first encounters with new ideas should proceed from an intuitive, non-quantitative confrontation to one that is more quantitative. Teachers have spoken out against teaching and learning methods which substitute equations, formulas and other quantitative representations for first-hand experience, word descriptions, examples and illustrations. These criticisms are just as valid for students who are very capable and very interested in science as they are for other students. Moreover, the mathematical sophistication of students at this level is such that they are unable to follow most mathematical arguments as explanations for natural phenomena.

The typical science experiences of most secondary school students consists of one or two years devoted to general science and biology. Few study physics and chemistry. A significant advantage to the course of study described here is that students are given a chance to study physics and chemistry at a level of rigor that is consistent with their ability and their mathematical maturity. Students who terminate their study of science at the end of one year get a significant exposure to the structure of biology, chemistry and physics as they are presented in the latest curricular developments. Students who might not elect science beyond the first year because of lack of interest in biology may be attracted by the chemistry or physics portions of the course and elect to take an additional year or two of science. Students who are "turned on" by biology may wish to pursue further study of biochemical topics in Years II and III.
After considering these problems and goals, the general course outline for the first year of the course was derived. It consists of four main parts:

1. Perception and Quantification
2. Heat, Energy and Order
3. Mice and Men
4. Environmental Balance?

The year begins with a study of the perceiver, moves on to the perceived, and ends with the interaction of the perceiver with the perceived. The first-year student starts out by gaining a better awareness of the nature of his perception and senses -- the faculties that make him aware of the world around him. With an increased understanding of these perceptual abilities, he can turn to the environment and then relate himself to it. He finds that his perception is limited and that he often needs to call on technological and conceptual extensions and that even these have their limitations.

The importance of organization and classification as parts of perception is emphasized. The physical properties of matter are introduced and studied as aids in organization and classification of chemicals. The identification of unknowns by study of their physical properties and use of organized data on punch cards is the culminating experiment of the Perception unit.

Apart from the great diversity exhibited in nature, which the scientist must organize in order to comprehend, certain unifying principles are essential for deeper understanding. The most powerful of these is the energy concept, which is explored in the "Heat, Energy and Order" unit in several of its ramifications -- physical, chemical and biological. The discussion begins by developing an experimentally important energy form, viz., heat. The macroscopic aspects of heat as embodied in calorimetry are related to the microscopic in terms of random molecular motion. This builds confidence in the idea of the atomic nature of matter, which
is essential to much of the unit. Various energy conversions form the vehicle for extending and generalizing the energy concept. Nuclear energy is developed in sufficient detail to underscore its environmental and social significance. Finally, the thermodynamic limitations and implications of energy conversion are explored, ending with a view of life as a supremely artful organizer in nature, a mechanism powered by energy which creates wondrous "local order", but always at the expense of influencing its environment.

The growth of a mouse colony carries the thread of the unit "Mice and Men." As the colony develops, students learn many things about the concept of population. The food and water consumed and products eliminated tie the mouse colony back to the unit "Heat, Energy and Order", and point ahead to the chapter on communities and to the unit "Environmental Balance?".

The cell concept is given prime position in this unit. It is used to enter topics on reproduction, embryology and maturation which are observed in the mice and other organisms. The mice selected for the original colony are such that an experiment in Mendelian genetics comes out of the observations students make as the colony develops. In most of the chapters man is an important organism and receives as much attention as the mouse, although the data are often secondhand.

A rather unpleasant fact that must be faced is that as our population increases, and human activities are directed towards increasing the standard of living for this population, strains are placed upon the environment. As students discover in "Mice and Men," the size of the community has a relation to both the quantity of the food, water and energy required and the quantity of waste products produced. To develop the concept of a closed system and point out the necessity for environmental management, an analogy between the earth and a spaceship is made. Students are then introduced by a multi-media approach to the nature of some of our common pollutants (with emphasis upon air, water, heat, noise and radiation)
as well as their effects. Following this students are encouraged to undertake a rather detailed study of a particular type or aspect of pollution. Emphasis here is placed upon student activity, which may take any number of forms. The culminating activity centers around discussion of these special studies together with the complex relations involved within the environment. It is hoped that out of these studies students will become aware of threats which exist to man's future on this planet.

THE SECOND YEAR COURSE

The second year of the course is considerably more quantitative in its approach than the first. This is the case because (1) the students are one more year along in their mathematical preparation, (2) the students who elect to take a second year of science are more likely to exert the effort to master more difficult topics, and (3) many of the quantitative aspects of physics and chemistry are basic to an understanding of molecular biology, which is an important part of the following year's work.

The second year consists of two parts:
(1) Motion and Energy
(2) Chemical Reactions

Year II begins with the study of motion, going from the quantitative description of motion to a consideration of what causes motion and a discussion of Newton's laws. There follows the development of the laws of conservation of momentum and energy, including a discussion of energy in biological systems. This section, which is primarily mechanics, culminates with a discussion of kinetic molecular theory.

Due to recent advances in both molecular biology and biochemistry, the descriptive approach to biology has gradually given way to one that is primarily analytical. It is now necessary, even on the high school level, for the serious biology student to have a more thorough understanding of those concepts normally
embodied in the "modern" high school physics and chemistry courses. The major objective of "Chemical Reactions" is to build some of those basic chemical concepts that are necessary for an analytical study of "The Chemistry Of Living Matter" and "Energy Capture and Growth."

The following subtopics of this section help in the realization of the major objective: Some of the topics discussed are the mole concept, equation writing, energetics associated with chemical reactions, the dynamic nature of particles and their interactions and the application of energy and equilibrium to chemical systems.

THE THIRD YEAR COURSE

Year III consists of four parts:
(1) Waves and Particles
(2) The Orbital Atom
(3) Chemistry of Living Matter
(4) Energy Capture and Growth

The underlying rationale of the third year is a study of energy and its importance to life. The first thrust is to build the orbital model of the atom using, as background, waves, electromagnetism and historical models of the atom. Once the orbital model is established as a representation of the localization and directionalization of electronic energy, structural models are built to show how biopolymers are spatially arranged and experiments are done to give evidence of energy relationships. With shape, size and energy relationships of molecules established, the DNA molecule is introduced. The culmination of this work comes in the final section when photosynthesis is considered. With this topic, much that has gone before is brought to a logical focus.

These topics are most appropriately placed in the third year of the integrated sequence after students have developed some facility with basic ideas.
from chemistry and physics - e.g., quantitative knowledge about energy, mechanism of chemical reaction, equilibrium, rate of reaction, the photon and wave nature of light, electrical phenomena, and kinetic molecular theory. They should not now simply parrot biochemical processes such as photosynthesis and cell respiration but should understand the many chemical and physical principles which underlie these processes.

Time is allotted at the conclusion of Year III for individual investigation and studies.
Three-Year Course Outline

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* PP designation signifies materials produced by the Portland Project.
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* HP designates Harvard Project Physics material.
+ CHEMS designates material derived from the Chemical Educational Materials Study.
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CHEMISTRY OF LIVING MATTER
### Outline: Chemistry of Living Matter

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OPTIONAL

Some introduction to organic chemistry has been found to be helpful.

Chapter 18 of Chem Study, Chapters 20 and 21 of Chemistry, A Modern Course,
Smoot, Price, Barrett, or a comparable reference might be acceptable.

There are some excellent paperbacks available.

SUGGESTED READING

Really indispensable:


Fine for background:


9. McElroy and Swanson, Modern Cellular Biology, New Jersey, Prentice-Hall. (S)


(S) Suitable for use by student.
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Chapter I: MONOMERS AND HOW THEY ARE BUILT

A.1 WHAT YOU WILL BE DOING

Your studies in Chemistry of Living Matter will be a start at looking into fundamental biological processes. You will read about and discuss molecular rearrangements and energy interchanges which are bioprocesses. You will investigate these processes in two ways. Since the actual shape of a molecule is important, you will start by building molecular models. Then, you will do experiments involving the buildup, breakdown and energy transfer of these biomolecules. As much as possible, model building of similar molecules will go along with the lab exercises. In this way you will be able to study the interrelationship of structure and reaction.

A.2 THE KIT

For the model building section, you will use the "Framework Molecular Model" kit. This kit will enable you to build a model with reasonably accurate bond lengths, bond angles and bond thicknesses. At present no other type kit as accurately shows bond thickness, which is of some importance to molecular geometry. Per-

Balloons may be used as demonstration aids and for hybrid orbitals.

No other inexpensive kit is as accurate as this one.
Copper wire may be substituted for the angular fasteners in the kit. When you need extra ones, use AWG 12 Ga or any wire of \(0.080\text{ in.}^2\) diameter. Alternatively a section of tubing from the kit cut vertically into thirds yields flexible strips of the right diameter.

Hybridization as presented refers only to 2nd period elements. Third and subsequent periods involve d orbitals and are very complex (see Pauling--The Nature of the Chemical Bond).

haps your imagination will be taxed, since molecular shape must be inferred. After you have used this kit for a while you should become proficient in recognizing 3-dimensional relationships. We will begin with simple models and will ultimately try some complex models that will require cooperation of several sets of lab partners.

Open the kit. Put the enclosed instruction book aside. This booklet is for help in time of great need. Don't lose it.

There are three depressions holding two types of metal parts: clusters and fasteners. In two depressions are lengths of plastic tubing. There is a shallow groove along one side that will be used for measuring tubing before it is cut.

A.2.a METAL PARTS

There are three kinds of multiarmed metal parts called clusters. Each different metal cluster is used to represent a certain type of bonding situation for an atom and not necessarily a specific element. Silver clusters have four arms and are used to indicate bond angles near \(109^\circ\); brass clusters have five arms and are used to indicate hybridized bond angles of \(120^\circ\); the six arms of the copper
cluster represent bond angles of 90°.

Recall from Chapter 17 of CHEMS that carbon has the electron configuration

\[ 1s^2 2s^2 2p_x^1 2p_y^1 \]

When a carbon atom bonds with another atom, the carbon atom "promotes" one 2s electron so that its configuration is

\[ 1s^2 2s^1 2p_x^1 2p_y^1 2p_z^1 \]

Further, once promoted, the four valence orbitals apparently "mix" to form four new identical orbitals. This process is called hybridization, and the resulting orbitals are called \( sp^3 \) hybrids from a mix of one s and three p orbitals.

After this discussion the clusters will be referred to by color, i.e., silver, brass or copper, or by type, such as \( sp^3 \).

A.3 HYBRID ORBITALS

An hybrid orbital differs from ordinary s and p orbitals in several ways. First, ordinary p orbitals are symmetrical about the nucleus, but hybrid orbitals are asymmetric. (Figure A.1) Second, because they are asymmetric (and because these hybrid orbitals, just as any orbitals, are arranged in the lowest energy configuration, i.e., as far apart as possible), the actual arrangement of the orbitals in space is different for the hybrids than for

Silver clusters represent \( sp^6 \) hybridization.

The designation \( sp^3 \) identifies which orbitals are "mixed," one s and three p orbitals. Therefore, \( sp^6 \) hybridization means that one s and 2 p orbitals hybridize, while the remaining p orbital is not involved. \( sp^3 \) hybridization indicates one s and one p orbital hybridize, with the two other p orbitals not involved.

Figure A.1
A Hybrid Orbital
Water may be represented as an $sp^3$ hybridization of oxygen.

Electron promotion precedes all hybridization.

Remember that hybrid orbitals are regions of high bond probability—higher than ordinary $p$ orbitals.

$p$ orbitals. For example $3$ hybrid $sp^2$ orbitals will be arranged at $120^\circ$ to each other in a plane, while $3$ $p$ orbitals are at $90^\circ$ to each other. Third, these orbitals are regions of much greater bonding tendency (as can be seen by their asymmetry) than $s$ or $p$ orbitals. In fact, hybridized bonds result in much greater stability for the molecule than would be predicted from $p$ orbital bonding.

Since the four $sp^3$ hybrids of carbon are spaced as far apart from each other as are the arms of a tetrahedron, the angles between the bonds are $109^\circ 28'$. All $sp^3$ hybrid orbitals have this angle between the four bonds. This angle is also close enough to represent the bonds of oxygen in water and nitrogen in ammonia and alkyl amines. It is true, however, that most frequent use of the silver cluster will be carbon atoms that form $sp^3$ orbital bonds.

Under certain bonding conditions, a carbon atom forms $sp^2$ hybrid orbitals. The $sp^2$ hybrid orbitals are formed of one $s$ and two $p$ orbitals to make three equal hybrid orbitals. These orbitals orient themselves $120^\circ$ apart and all lie in the same plane. The non-hybridized $p$ orbital is found at $90^\circ$ to the plane of the $sp^2$ hybrids. The brass cluster illustrates this kind
of bond situation.

A.4 SINGLE BONDS

Single covalent bonds which are linear and symmetrical about the bond axis between atoms are called "\( \sigma \)" or "sigma" bonds and are the covalent bonds that exist in molecules. Figure A.2 shows two \( p \) orbitals overlapping to form a \( \sigma \) (sigma) bond. C-D is the bond axis; the dotted line above F identifies the center of the covalent bond. The distance from this center to the nucleus of atom A \( (d_1) \) is the covalent bond radius of A. The distance along the bond axis from the center to the nucleus of atom B \( (d_2) \) is its covalent radius. Note that the dotted line at F is only meant to indicate the center of the \( \sigma \) bond and not the halfway distance along the bond axis. In some cases this bond center may coincide with the halfway distance between the atoms, but it is usually displaced toward one atom of the pair. Displacement is toward the more electronegative element.

A.5 DOUBLE BONDS

When \( sp^2 \) hybridization occurs—let's say between two carbon atoms—an \( sp^2 \) hybrid from each carbon overlaps to form a \( \sigma \) bond, and the \( p \) orbitals that are at right angles to the plane...
of the \( sp^2 \) hybrids can distort and form a new bond called a \( \pi \) (pl) bond.

(Illustrations of the orbital overlap in the pl bond formation may be seen in Figures A.3 and A.6).

Note that the \( \pi \) bond is joined above and below the plane of the \( sp^2 \) hybrids. This gives rigidity to this bond so the atoms are not free to turn on the axis of the \( \sigma \) bond. This, of course, is a double bond and is found between carbon atoms (C-C), between carbon and oxygen (C-O), and between carbon and nitrogen (C-N). Since the forces holding two nuclei together in a double bond are greater than those of the single bond the nuclei are drawn closer together, and thus the C-C covalent distance is greater than the C=C covalent distance.

(Table A.1)

<table>
<thead>
<tr>
<th>Atomic Covalent Radii (Å)</th>
<th>Single Bond Radii</th>
</tr>
</thead>
<tbody>
<tr>
<td>C - single 0.77</td>
<td>H 0.30</td>
</tr>
<tr>
<td>C - double 0.67</td>
<td>F 0.64</td>
</tr>
<tr>
<td>C - triple 0.60</td>
<td>Si 1.17</td>
</tr>
<tr>
<td>P</td>
<td>Cl 1.00</td>
</tr>
<tr>
<td>N - single 0.74</td>
<td>Br 1.14</td>
</tr>
<tr>
<td>N - double 0.62</td>
<td>I 1.33</td>
</tr>
<tr>
<td>O - single 0.74</td>
<td></td>
</tr>
<tr>
<td>O - double 0.62</td>
<td></td>
</tr>
<tr>
<td>O - triple 0.55</td>
<td></td>
</tr>
</tbody>
</table>

Table A.1

Since a single bar C-C represents a single covalent bond, double bars (C=C) and triple bars (C≡C) represent double and triple bonds.
ORBITAL OVERLAP IN $\pi$-BOND FORMATION IN ETHYLENE
BY OVERLAP OF P ORBITALS, AS SHOWN IN THREE STAGES

Figure A.6
Bond strength:

- C-C 83 kcal/mole
- C=O 100 kcal/mole
- C≡O 123 kcal/mole

Morrison and Boyd, Organic Chemistry, Allyn and Bacon.

Although this is a double bond, it is not a double π bond but rather a strong σ and a weaker π bond with the result that the double bond is stronger than the single bond but not twice as strong.

A.6 TRIPLE BONDS

The copper-colored fastener illustrates the third kind of hybridization of carbon. In this case hybridization involves one σ and one p orbital, thus giving two hybrid orbitals while retaining two ordinary p orbitals. Since the hybrids are equivalent, they assume a linear configuration. The p orbitals form at right angles to the hybrids. The two non-bonded p orbitals are found at 90° to the sp orbitals and 90° to each other. The triple bond of carbon in acetylene HCECH is formed by σ bond formation between sp hybrids and π bonds between the four p orbitals left.

There are two other fasteners. One is a linear pin that is used to show bonds between unlike atoms and one an angular fastener that is used in building double and triple bonds.

A.7 PLASTIC TUBING

Interatomic distances are measured in Ångstrom units, abbreviated Å. One Å is equal to 10⁻¹⁰ meters or 10⁻⁸ cm. We shall refer...
to distances in Å. In this kit the scale
is 1 in. to 1 Å.

The plastic tubing is used to identify spe-
cific atoms involved in bonding. This is done
through a color key found in Table A.2.

<table>
<thead>
<tr>
<th>Hydrogen-white</th>
<th>Bromine-orange</th>
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<tr>
<td>Carbon-black</td>
<td>Iodine-brown</td>
</tr>
<tr>
<td>Nitrogen-blue</td>
<td>Silicon-light yellow</td>
</tr>
<tr>
<td>Oxygen-red</td>
<td>Phosphorus-violet</td>
</tr>
<tr>
<td>Fluorine-light green</td>
<td>Sulfur-dark yellow</td>
</tr>
<tr>
<td>Chlorine-dark green</td>
<td>All metals-grey</td>
</tr>
</tbody>
</table>

Table A.2

The length of the plastic tubing used indicates
either the covalent radius of the atom or the
van der Waals radius (the non-bond direction).
You will recall that the van der Waals distance
is usually the non-bonded distance of nearest
approach of one atom to another. Lengths of
covalent radii are given in Table A.1 and
lengths of van der Waals radii in Table A.3.

<table>
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<tr>
<td>H</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>O</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>As</td>
</tr>
</tbody>
</table>

Table A.3

The equivalence 1 in. = 1Å is unfortunate but dic-
tated by the kit.

Current usage is in nanometers:
1 nm = 10⁻⁹ m.
1 Å = 10⁻¹⁰ m.

See CHEMS Ch. 17 for van
der Waals forces.

It is very important
that the students under-
stand that the plastic
tubing represents elec-
trons involved in bonding
and the cluster represents
the nucleus.
A precise decimal inch scale is to be found in the booklet which comes with each kit.

A single-edge razor blade and a 3/4 x 4 x 4 piece of pine do nicely for cutting tubing.

A single length of black tubing 1.54 inches long is the equivalent length of two carbon covalent radii joined, i.e., one carbon-carbon covalent bond.

Any covalent bond between like atoms is made by cutting tubing of the correct color to a length exactly equal to twice the covalent radius of the atom.

All preprinted tubing should be measured to see that it is the length represented or needed.

The tubing is fastened to the metal clusters by sliding the tubing onto one of the arms of the cluster. Thus the color of the tubing on the cluster identifies the atom the cluster represents. The length of the tubing represents the covalent or van der Waals radii. The position and size of an atom are clearly visible within the framework model of the molecule.

A.8 CONSTRUCTION OF BONDS

Bonds between like atoms are cut from tubing of one color. The length of such a bond is twice the covalent radius of the atom. For example, the C-C (carbon - carbon) bond is 1.54 Å long, since the covalent radius of C is 0.77 Å. Take a length of black tubing and as accurately as possible cut a piece to 1.54 inches (remember that the scale is 1 Å = 1 inch, so 1.54 Å = 1.54 in.) by using the scale on the kit. If you are satisfied that you’ve cut this piece to represent 1.54 Å, mark it (with masking tape, etc.) to use as a pattern to cut all other 1.54 Å pieces of black tubing. Now cut out 10 or 15 pieces of 1.54 Å black tubing for later use. Take a 1.54 Å piece and join two silver clusters together. You have a bare C-C bond. Set this aside for now. All other bonds between like atoms can be made by cutting the proper tubing.
to the right length and joining the indicated clusters.

A.9 **BO**D**S BETWEEN UNLIKE ATOMS**

As an example, the C-O bond is shown. This kit provides several lengths of colored tubing already correctly printed to show C-O bonds.

![Diagram](image)

This tubing, when cut, will yield C-O bonds of the correct length. However, these are C-O single bonds, and C-O double bonds will have to be built. The building of single bonds between atoms follows a regular procedure; only the length and color of the tubing change from one bond to the next.

A.9.a **TO BUILD A C-O BOND**

![Diagram](image)

Black-blue (C-N) tubing, like the C-O tubing in Figure A.10, is also included. Other bonds will
have to be constructed as you need them.

This kit provides preprinted C-H, N-H and O-H bonds. These bonds are printed as in Figure A.12. The tubing in Figure A.12 is C-H bonding; however, N-H and O-H bonds are printed (and cut)

An N-H bond is represented by a length of tubing. Remember to check on proper length.

Figure A.12

in the same way.

A.10 ENOUGH TALK; LET'S BUILD MOLECULES

We will start with methane--CH₄. Take a silver($sp^3$) cluster and four C-H bonds (cut as previously described). Slide the black end of each C-H bond all the way onto an arm of the cluster. Do this for all four C-H bonds.

Next make water molecule. This model is made by attaching two O-H bonds and two sections of red tubing (cut to van der Waals radius of oxygen) to an $sp^3$ cluster.

One can construct the hydronium ion, $H_3O^+$ by removing one of the pieces of red tubing (which represents an unshared pair of electrons) and putting on an O-H bond.
**Figure A.13**

TETRAHEDRON

FOUR C-H BONDS

VAN DER WAALS RADIUS OF METHANE

**Figure A.14**

SP³

RED

WHITE

+ 2 O-H

+ 2 O (VAN DER WAALS)

**Figure A.15**

COVALENT RADIUS OF OXYGEN

VAN DER WAALS RADIUS OF WATER AT HYDROGEN ATOM

VAN DER WAALS RADIUS OF OXYGEN
Let's make a more complicated molecule, a model of methanol—CH$_3$OH.

To build this we will need

![Tetrahedron for Carbon + Black Tubing + Tetrahedron for Oxygen + Red Tubing + C-O Bond + Red Tubing + White Tubing](image)

Figure A.16

Complete the model by adding C-H bonds to the carbon tetrahedron and two van der Waals lengths of oxygen (red) tubing to the oxygen tetrahedron.

The unbonded electrons occupy a space represented by the van der Waals length only when the unbonded electrons belong to a singly bonded atom.

### A.11 Words About Molecules

In point of fact, you now know how to make CH$_4$ models and how to make models containing O-H and C-O-H groups. If you take the tetrahedra connected by black tubing which you constructed earlier and attach C-H bonds to the available arms you will have
which is \( \text{CH}_3\text{CH}_3 \) or \( \text{C}_2\text{H}_6 \) or ethane. As a matter
of fact, you should now build several models of
this series. Just remove a C-H bond from a tet-
rahedron, add a C-C bond and finish the molecule
by adding C-H bonds. As you do this, you'll note
that when you add a new carbon, you will also
add two new C-H bonds. An entire series of car-
bon compounds called the alkanes is built in
this way. The general formula of this group
is \( \text{C}_n\text{H}_{2n+2} \) when \( n \) is any integer.

After building several of the molecules in
the series \( \text{CH}_4, \text{C}_2\text{H}_6, \text{C}_3\text{H}_8, \text{C}_4\text{H}_{10}, \text{C}_5\text{H}_{12} \) there
are several things you should note. One im-
portant factor is that because of the 109° bond
of carbon these molecules twist around and are
far from linear. Another point is that there is
more than one way to put \( \text{C}_4\text{H}_{10} \) together. In
fact the greater the number of carbons, the more
possible shapes there are. These different pos-
sibilities are called isomers; they have the same
emperical formula but are actually different com-
pounds. A good example might be \( \text{C}_2\text{H}_6\text{O} \), where
one isomer involves C-O-C bonds (an ether) and

See pages 35a,b for
FUNCTIONAL GROUPS.
In the Newman Projection for ethane, one carbon is represented by

\[ \text{\textcircled{C}} \]

the other by

\[ \text{\textcircled{Y}} \]

Looking along the C-C bond we have

\[ \text{\textcircled{Y}} \]

Also, consult any recent book on conformational chemistry.

another C-O-H bonds (an alcohol).

You cannot twist the molecules too tightly together. Since the hydrogen atoms repel each other, they will stay as far away from one another as possible. For large molecules this can be a very complex situation. You also should note that some rotation about bonds is possible. While there is rotation about the bonds, it may be restricted by intramolecular repulsive forces.

Make a model of \( \text{C}_2\text{H}_6 \). Note that in one position the hydrogens are as close as possible while in another, where one end has rotated 60°, they are as far apart as possible. In ethane molecules 3 kcal/mole are required to produce this rotation. Indicating or representing these carbon structures on paper is difficult.

In the structures shown so far, covalent bonds are represented by a line between atoms. Another very important type of bond is the hydrogen bond. This bond is not as strong as the covalent bond and results from hydrogen's ability to simultaneously bond to two atoms of O, N or F. This simultaneous bond has two parts. One part is covalent; the other part is electrostatic and is represented by a dashed line, O-H---O. This bond is linear, as can be seen by building some water molecules and hydrogen-bonding sev-
eral together.

What is ice? Why does ice float on water?

**A.12 BUILDING MULTIPLE BONDS**

A carbon-carbon double bond occurs in the compound ethylene, \( \text{CH}_2=\text{CH}_2 \) or \( \text{C}_2\text{H}_4 \). To build double bonds correctly, we must be sure we work with double-bond distances, so check Table A.1 on page 13. Next we have to use the right bonds; \( sp^2 \) clusters (brass) are used. Begin this construction by assembling the following:

- 2 \( sp^2 \) clusters (brass)
- 4 C-H bonds
- 3 lengths tubing 1.34 in. long
- 4 lengths black tubing 1.54 in. long
- 4 angle fasteners

The \( sp^2 \) clusters have three prongs that lie in the same plane and are 120° apart. These prongs represent \( sp^2 \) hybrid orbitals. Take one of the pieces of black tubing 1.34 inches long and attach the two \( sp^2 \) clusters to the tubing by \( sp^2 \) hybrid prongs. Now attach the four C-H bonds to the other \( sp^2 \) prongs with the black end of the tubing on the prong. (See Figure A.17 - exploded and Figure A.18 - assembled.)

The hydrogen bond is electrostatic. Bond strength is dependent on angle. A linear bond, of course, is the strongest.

Water hydrogen bonds to form crystals. If the students build a small water crystal, they can see the expansion that takes place.

To build a hydrogen bond, any odd colored tubing will do. The length of a hydrogen bond is from \( 2.6 \text{ Å} \) to \( 3.0 \text{ Å} \) for water.

A \( C=C \) bond is cut from black tubing. Cut it twice the double bond covalent radius (from Table A.1), i.e., 1.34 in. long.
Figure A.17

Figure A.18
Now attach the four 1.54-inch lengths of tubing to the "unbonded" prongs that represent p orbitals. Your model should now look like this:

![Diagram of a molecular model with p orbitals and bonding]

We will complete the model (and the π bond) by taking two angle fasteners and sticking them into the ends of a piece of 1.34 inch-long black tubing, then joining this assembly to the open ends of two of the pieces of black tubing representing the p orbitals. Take the other two angle fasteners and the last piece of tubing (1.34 in. long) and attach it to the other p orbitals. Thus you've completed the π bond, and your model should look like this:
The reason you used 1.54 inch-long tubing pieces on the axial $p$ orbital is because the actual $\pi$ bond between carbon atoms is 3.08 Å thick, and therefore this makes an accurate representation of the molecule.

When the $\pi$ orbital is complete, you will not be able to rotate the ends of your ethylene molecule as you could the ends of the ethane molecule. In fact, the double bond severely restricts any rotation in the actual molecule of ethylene. We usually assume such rotation does not happen.
Now let's make a double bond between two unlike atoms, carbon and oxygen. In constructing a π bond between a carbon atom and an atom of oxygen, the π bond thickness at the oxygen atom is represented by using tubing of twice the covalent radius of oxygen as the axial p orbital. For a C=O bond double the covalent radius of nitrogen for the axial p orbital. In both cases the axial p orbital of carbon will be 1.54 Å long.

The simplest organic molecule that contains such a double bond is formaldehyde, structural formula $\text{H}_2\text{C}=\text{O}$. You need the following:

- 2 $sp^2$ clusters
- 2 lengths black tubing (carbon) 1.54 in.
- 2 lengths red tubing (oxygen) 1.48 in.
- 3 C=O bonds from kit or black 0.67 in.
  + red 0.62 in.
- 2 C-H bonds
- 2 lengths red tubing (oxygen) 0.74 in.
- 4 angle fasteners

If your kit contains C=O bonds, then proceed as follows; if not, first construct three bonds of this type.

Take one C=O and attach an $sp^2$ cluster (hybrid prongs) to each end. To the other 2 hybrid prongs on the carbon (black) side, add...
the C-H bonds. Then add the two black 1.54-inch lengths to the $p$ orbital prongs on the carbon cluster. Join two angle fasteners to a C=O bond and attach the whole thing to a pair of $p$ arms, one black and one red. Remember to attach the black end of the C=O bond to the black $p$ bond. Now add the other C=O bond and put the two 0.74-inch lengths of red tubing on the remaining hybrid prongs of the oxygen atom. Thus you've completed the formaldehyde molecule, and it should look like Figure A.21.

![Figure A.21](image-url)
You used the van der Waals radius to model the unbonded electrons of the oxygen atoms in water. For the formaldehyde molecule you used the covalent radius because the carbon-oxygen double bond in effect "pulls" or distorts the oxygen electron cloud. Also, using 1.48-inch red tubing for the oxygen end of the \( \pi \) bond and 1.54-inch black for the carbon end should have indicated a certain strain on this double bond.

The above points on distortion of the electron clouds and strain should be kept in mind when building other double bonds.

Remember that half of a covalent bond belongs to one atom, the other half to the other atom, and use tubing and color correctly. The C=N bond is built just as the C=C and C=O bonds, differing only in tubing colors.

A more difficult model is that of allene (1, 2 - propadiene). Allene has the general formula \( \text{C}_3\text{H}_4 \) and structurally is \( \overset{\text{H}}{\text{H}} \overset{\text{C}=\text{C}}{\text{C}=\text{C}} \overset{\text{H}}{\text{H}} \). Here we have one carbon atom, the middle one, double bonded to the other two carbon atoms. This means that the middle carbon atom is \( \pi \) bonded to each of the other carbons. Forming two \( \pi \) bonds is accomplished through \( sp \) hybridization. In the kit this is represented by a

---

*Emphasize this point.*

![Allene - exploded view](image)

Figure A.22
Cyclopentane = 5 carbons in a ring.

copper colored cluster. To build allene you will need:

1 copper cluster (sp) 
2 brass clusters (sp²) 
6 C=C bonds 
4 C-H bonds 
8 C-C bonds 
8 angle fasteners

Begin by connecting the sp cluster to an sp² cluster by a C=C bond (σ bond). Now build the π bond between these two. Next connect the other sp² cluster to the sp with a C=C bond and build the other π bond. Complete the model by attaching the C-H bonds to the sp² clusters.

Another kind of multiple bond is the triple bond. In this case the carbon is sp hybridized, a σ bond is formed between sp hybrids, and π bonds are formed between p orbitals. Build C₂H₂ (acetylene) by using the sp clusters and the correct bond lengths from the table.

A.13 RING STRUCTURE

One of the very interesting aspects of using this kit comes when we build ring structures. The first ring structure to be built is cyclohexane. Hexane means six carbons and cyclo means in a circle. (What would cyclopentane be?) Take six sp³ clusters and join them
Figure A.23: Acetylene--exploded view

together in a ring using 1.54-inch sections of black tubing. You will note that there are two possible positions for this structure:

"CHAIR"

"BOAT"

Figure A.24

Try making cyclopentane. Note that this structure also is not flat. This bent kind of conformation is called "puckering."
B. FUNCTIONAL GROUPS

As you know by now, in the language of structural diagrams the bar (or line---) represents a bond; for example

\[
\begin{array}{c}
\text{H} \\
\text{H} \text{C} \text{H} \\
\text{H} \\
\end{array}
\]

is methane, a carbon atom bonded to four hydrogen atoms. Some other useful conventions follow.

B.1 ALCOHOLS

Consider this series of compounds: methanol (CH₃OH), ethanol (CH₃CH₂OH) and propanol (CH₃CH₂CH₂OH). These compounds have in common the reactions of the -OH group. To discuss the reactivity of the -OH group we can usually ignore the specific carbon chain attached to it. We ignore everything other than the -OH by using the catchall letter R to stand for C₂H₅-, C₃H₇-, C₄H₉- and so on.

B.2 OTHER REACTIVE GROUPS

A comparable situation is found with

\[
\begin{array}{c}
\text{H} \text{C} \text{O} \\
\text{H} \\
\text{H} \text{C} \text{H} \\
\text{H} \\
\end{array}
\]

\[
\begin{array}{c}
\text{H} \\
\text{H} \text{C} \text{H} \\
\text{H} \\
\end{array}
\]

All of these enter into basically similar reactions, due apparently to -C\text{=O}.

Similarly

\[
\begin{array}{c}
\text{H} \text{C} \text{O} \\
\text{H} \\
\text{CH₃} \text{C} \text{O} \\
\text{H} \\
\end{array}
\]

\[
\begin{array}{c}
\text{CH₃} \\
\text{CH₂} \text{C} \text{O} \\
\text{H} \\
\end{array}
\]
all seem to rely on \(-C=O\)
as their source of reactivity. In other words, the molecule is reactive at the site of these different groups. The groups mentioned together with others are called functional groups. See Table B.1.

B.3 ALKYL RADICALS

In section B.1 reference was made to use of the symbol \(R\) to stand for a group of atoms attached to the reactive group. Examples of these are the alkyl radicals:

- \(\text{CH}_3^-\) is methyl as in \(\text{CH}_3\text{Cl}\) methyl chloride
- \(\text{C}_2\text{H}_5^-\) is ethyl as in \(\text{C}_2\text{H}_5\text{OH}\) ethyl alcohol
- \(\text{C}_3\text{H}_7^-\) is propyl and
- \(\text{C}_4\text{H}_9^-\) is butyl etc.

<table>
<thead>
<tr>
<th>Functional Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-OH</td>
</tr>
<tr>
<td>R-CHO</td>
</tr>
<tr>
<td>R-COOH</td>
</tr>
<tr>
<td>R-O-R</td>
</tr>
<tr>
<td>R-CO-R</td>
</tr>
<tr>
<td>R-COOO-R</td>
</tr>
<tr>
<td>R-O-C-O-R</td>
</tr>
</tbody>
</table>

Table B.1
Using the kit you should build models of all of the above structures for practice.

B.4 AMINES

Later you will learn the importance of certain nitrogen compounds. Some of these include the amine functional group.

\[
\begin{align*}
\text{H} & \quad \text{N} & \quad \text{H} \\
\text{H} & \quad \text{N} & \quad \text{H} \\
\text{H} & \quad \text{N} & \quad \text{CH}_2\text{CH}_3
\end{align*}
\]

an amine  ammonia  ethylamine

B.5 MODELS OF REACTIONS

Using the kit you can demonstrate not only molecules but also reactions. After building methyl alcohol and acetic acid, manipulate the models to show this reaction:

\[
\begin{align*}
\text{CH}_3\text{OH} + \text{CH}_3\text{C} & \rightarrow \text{CH}_3\text{C} + \text{H}_2\text{O} \\
\text{methyl alcohol} + \text{acetic acid} & \rightarrow \text{methyl acetate} + \text{water} \\
& \text{(an ester)}
\end{align*}
\]

Take the methyl acetate you have just made and "react" it with ammonia.

\[
\begin{align*}
\text{CH}_3\text{C} + \text{NH}_3 & \rightarrow \text{CH}_3\text{C} + \text{CH}_3\text{OH} \\
\text{methyl acetate} + \text{ammonia} & \rightarrow \text{acetamide} + \text{methanol}
\end{align*}
\]

With the experience you've gained, you should be able to build any structure, given its structural formula.
<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Type of Compound</th>
<th>IUPAC* Ending</th>
<th>Example</th>
<th>IUPAC Name</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkane (saturated hydrocarbon)</td>
<td>-ane</td>
<td>H-C=C-H</td>
<td>ethane</td>
<td>ethane</td>
<td></td>
</tr>
<tr>
<td>alkene (olefin)</td>
<td>-ene</td>
<td>C=C</td>
<td>ethene</td>
<td>ethylene</td>
<td></td>
</tr>
<tr>
<td>alkyne (acetylenic)</td>
<td>-yne</td>
<td>H-C≡C-H</td>
<td>ethyne</td>
<td>acetylene</td>
<td></td>
</tr>
<tr>
<td>R-OH</td>
<td>alcohol (hydroxyl)</td>
<td>-ol</td>
<td>H-C-OH</td>
<td>ethanol</td>
<td>ethyl alcohol</td>
</tr>
<tr>
<td>R-0-R</td>
<td>ether</td>
<td>---</td>
<td>H-C-O-C-H</td>
<td>methoxyethane</td>
<td>methyl ethyl ether</td>
</tr>
<tr>
<td>O</td>
<td>aldehyde</td>
<td>-al</td>
<td>H-C</td>
<td>ethanal</td>
<td>acetaldehyde</td>
</tr>
<tr>
<td>O</td>
<td>ketone</td>
<td>-cne</td>
<td>H-C-C-H</td>
<td>propanone</td>
<td>acetone</td>
</tr>
<tr>
<td>Functional Group</td>
<td>Type of Compound</td>
<td>IUPAC$^*$ Ending</td>
<td>Example</td>
<td>IUPAC Name</td>
<td>Common Name</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>---------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>R-C</td>
<td>carboxylic acid</td>
<td>-oic</td>
<td>ethanoic acid</td>
<td>acetic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[Ethanoic acid structure]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-N</td>
<td>amine</td>
<td>-</td>
<td>2-aminoethane</td>
<td>ethyl amine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[2-Aminoethane structure]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8.2

*International Union of Pure and Applied Chemistry
THE IUPAC SYSTEM OF NOMENCLATURE

On the following page is a summary of basic IUPAC rules for naming compounds. It would pay to read through them and then go through the following examples, referring back to the rules as needed.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Application</th>
<th>Rule #</th>
</tr>
</thead>
<tbody>
<tr>
<td>a: pent-</td>
<td>5 carbon atoms</td>
</tr>
<tr>
<td>b: pentan-</td>
<td>saturated chain (all single C-C bonds)</td>
</tr>
<tr>
<td>c: pentanol</td>
<td>ol-alcohol ending</td>
</tr>
<tr>
<td>d: 2-pentanol</td>
<td></td>
</tr>
</tbody>
</table>

(a)

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Application</th>
<th>Rule #</th>
</tr>
</thead>
<tbody>
<tr>
<td>a: hexa-</td>
<td>6 carbon atoms</td>
</tr>
<tr>
<td>b: hexadiene</td>
<td>2 unsaturated bonds</td>
</tr>
<tr>
<td>c: 1,4-hexadiene</td>
<td>as opposed to 2,5</td>
</tr>
<tr>
<td>d: 3-methyl-1,4-hexadiene</td>
<td>methyl group on 3 carbon</td>
</tr>
</tbody>
</table>

On the following page is a summary of basic IUPAC rules for naming compounds. It would pay to read through them and then go through the following examples, referring back to the rules as needed.
IUPAC RULES

1) Using the longest continuous chain of carbon atoms (containing the functional group or groups) as the basis, name the compound as a derivative of this parent hydrocarbon. (Table A.4, p. 2)

2) Use the appropriate ending to indicate the principal functional group present. (Table B.1, p. 34, Table B.2, p. 35) This includes the unsaturated hydrocarbon endings (-ene, -yne).

3) Number the basic carbon chain, starting at the end which will give the principal functional group the smallest possible number.

4) Name and locate by number all other substituents (other functional groups, atoms, and carbon groups not part of the basic chain) attached to the longest continuous chain.

5) Substituents are listed alphabetically.

The above rules represent the most basic and should suffice in naming the majority of compounds you will run across. The following refer to more specific and less commonly occurring situations (some do occur fairly frequently though and you probably will encounter them occasionally).

6) Multiple, identical substituents are indicated by the appropriate prefix (di-, tri-, etc.) in front of its name and each is numbered.

7) Cyclic compounds are indicated by the prefix cyclo- in front of the basic name. Numbering starts with a carbon bearing a substituent and proceeds in the direction which will make the sum of the numbers of other substituents the lowest possible.

8) Complex substituents are themselves named by IUPAC rules, with this stipulation: that the carbon which is attached to the main chain is the number one carbon. To avoid ambiguity the substituent name may be placed in parentheses.
Exercises for Home, Desk and Lab (HDL's)

The first seven questions are based on the molecular models kit:

1. What tubing color represents oxygen?  
   What tubing color represents carbon?  
   What tubing color represents nitrogen?  
   What tubing color represents hydrogen?

2. Why cut tubing to 2x covalent radius? That is, why not cut covalent radii and join them?

3. In a C-O bond, why is part of the tubing black and the rest red in color?

4. Which metal part is used in the representation of a C-C bond?

5. What part of a metal cluster corresponds to the nucleus of an atom?

6. Why do sp³ clusters have 4 legs?

7. Why does a p orbital have two arms to represent it, while the hybrid orbitals only have one?

8. Why isn't a double bond twice as strong as a single bond?

9. Why is the C=C bond distance less than that of the C-C bond?

10. Are the bonds as rigid as the clusters?

1. red  
   black  
   blue  
   white

2. Fewer cuts, less time and models are stronger.

3. Part of the tubing represents the carbon atom (black) and the rest represents the oxygen atom (red).

4. Silver cluster

5. Center of the cluster

6. Each leg represents one orbital of the four sp³ hybrids.

7. Ordinary orbitals are symmetrical thus two armed; hybrids are not and are represented by only one part of the orbital.

8. A double bond is not really a 'double' anything; actually a double bond is the sum of a σ and a π bond.

9. The force of multiple bonds on a nucleus is greater than that of single bonds.

10. Not quite; almost, but not quite.
(11) For carbon, all hybrids are equivalent. If oxygen hybridizes to form $sp^3$ hybrids, then six electrons form four hybrids. Two of the hybrids must be "filled;" thus the four hybrids are not equivalent, and we might expect the bond angles to be different.

(12) $CH_3OH$

(13) 

\[
\begin{align*}
&H-H-H-H-H \\
&H-H-H-H-H \\
&H-C-C-C-C-C-C-C-H \\
&H-C-C-C-C-C-C-C-H \\
&H-C-C-C-C-C-C-C-H
\end{align*}
\]

Above are the simplest; any isomers are satisfactory.

(14) 

\[
\begin{align*}
&H-H-C-C-OH \\
&H-H-C-C-H \\
&H-C-C-C-C-C-C-C-H \\
&H-C-C-C-C-C-C-C-H \\
&H-C-C-C-C-C-C-C-H
\end{align*}
\]

(15) a. 

\[
\begin{align*}
&H-C-N \\
&H-H-H
\end{align*}
\]

b. 

\[
\begin{align*}
&H-C-N \\
&H-H-H
\end{align*}
\]

(11) In water the H-O-H bond angle is about $104^\circ$. Can you think of a reason why this angle is not $109^\circ 28'$ if the oxygen is really $sp^3$ hybridized?

(12) Methanol and methyl alcohol are names for the same organic compound. What is its formula?

(13) The structural formula for propane is 

\[
H-H-H
\]

Draw structural formulas for

\[
H-C-C-C-H
\]

butane, heptane, nonane and octane.

(14) The structural formula for ethanol is 

\[
H-H
\]

What is the structural formula for propanol?

(15) What are the structural formulas for (a) methyl amine, (b) ethyl amine, and (c) propyl amine?
(16) Graphite is a material entirely composed of carbon in the $sp^2$ state.

a. Draw a picture of a piece of the graphite molecular structure.

b. Graphite is an isotropic material; that is, its properties in one direction through the material (electrical conductivity, heat conductivity, etc.) are very different from its properties in another direction. Explain why.

(17) Water has an unusually high molar heat capacity (amount of heat needed to raise the temperature of one mole of water one degree) and boiling point. Knowing the elements in water and the kinds of bonds they form, explain these curious properties.

(18) Draw all of the condensed molecular structures which fit the empirical formulas

a. $C_6H_{14}$

b. $C_4H_8$

c. $C_4H_{10}O$
Apply the IUPAC naming system to the following organic molecules.

a. 3-Methylbutanoic acid

b. 2-Methylbutanal

c. 2-Propanone (acetone)

d. 2-Aminoethanoic acid (glycine or 2-aminoacetic acid)
<table>
<thead>
<tr>
<th>TEXT SECTION</th>
<th>ROUGH TIME ESTIMATES</th>
<th>EXPERIMENTS</th>
<th>DEMONSTRATIONS</th>
<th>TEACHING AIDS</th>
<th>OTHER STUDENT ACTIVITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chap. II Some Chemistry of Simple Carbon Compounds</td>
<td>↑ 1 Day ↓</td>
<td>CHEMS #6 for Natural Gas (optional)</td>
<td>Presence of CO₂ in Natural Gas</td>
<td></td>
<td>Discussion Individual library research</td>
</tr>
</tbody>
</table>

Chap. II Some Chemistry of Simple Carbon Compounds

CHEMS #6 for Natural Gas (optional)
Chapter II: SOME SIMPLE CARBON COMPOUNDS

One of the simplest carbon compounds is methane, CH₄. We consider methane to be uncomplicated because it contains relatively few atoms, because it contains only one type of bonding (sp³) and because the geometry of the bonding (tetrahedral) results in a nicely symmetrical molecule. Thus, with a low molecular weight (few atoms) and little intermolecular attraction (absence of polar bonds, all atoms in the molecule have similar electronegativities, high degree of symmetry) it is not surprising that methane is gaseous under ordinary conditions. Indeed, methane boils at -161°C. (Compared with the rare gases: Argon [bp -189°, At. Wt. 40], Krypton [bp -157°, At. Wt. 84] methane with M.W. of 16 has greater intermolecular forces of attraction than do the rare gases. Compared with simple molecules composed of elements with very different electronegativities, however, it is seen that methane’s intermolecular forces are weak: HF [MW 20, bp +20°C]; NH₃ [MW 17, bp -33°C].) While methane may be considered a simple molecule, the study of methane and its derivatives could lead us up many broad avenues of science including...

One objective of this chapter is to drive home the thought that organic chemistry impinges on our everyday lives in a very substantial way. Even the simplest organic compound, methane, demonstrates this. The lesson could be more elaborate by using examples such as commercial polymere and the natural materials they have replaced with considerable economic turmoil; medicines and increased longevity (the population problem caused by improved drugs while alleviated by better methods of contraception); nerve gases and other organic chemical warfare agents and the moral questions such as why is it allowable to kill with bombs but not with gas, and on...
chemical technology, biology and even economics, politics and other social sciences.

A. ORIGINS OF METHANE

A.1 PRIMORDIAL METHANE

The Russian biochemist A. I. Oparin in 1936 postulated that the primordial atmosphere of the earth was mainly methane, ammonia, hydrogen and water vapor. Interestingly enough, methane, ammonia and hydrogen are probably present now in the atmospheres of Jupiter and Saturn. There is a reasonable basis for expecting methane to be a primordial gas. In the molten surface of the primordial earth the temperature was sufficiently high so that the carbon present was in the form of metal carbides. Direct chemical union of many metals with carbon to form metal carbides occurs at 2200°C and above. Some metal carbides, such as aluminum carbide, Al$_4$C$_3$, and beryllium carbide, Be$_2$C, react with water to yield methane:

$$\text{Al}_4\text{C}_3 + 12 \text{H}_2\text{O} \rightarrow 4\text{Al(OH)}_3 + 3\text{CH}_4$$

Thus, as the earth cooled and water vapor condensed on the surface, conditions became suitable for the production of methane. Calcium carbide yields acetylene with water:

$$\text{CaC}_2 + 2\text{H}_2\text{O} \rightarrow \text{Ca(OH)}_2 + \text{HC}≡\text{CH}$$

Acetylene may react with hydrogen to produce...
ethylene or may break apart, depending on temperature, to yield more methane.

\[ \text{H-C=C-H} + \text{H}_2 \rightarrow \text{H}_2\text{C} = \text{CH}_2 \]

or

\[ \text{H-C=C-H} + 3\text{H}_2 \rightarrow 2\text{CH}_4 \]

The primordial methane is no longer in our atmosphere. Hurray! Where is it?

A.2 METHANE OF BIOLOGICAL ORIGIN

In a wide variety of oxygen-free environments, a certain group of bacteria are capable of living and growing on energy derived from the reduction of carbon dioxide by hydrogen to form methane and water. The detailed mechanism of how these anaerobic bacteria operate is not now known but is the subject of active research. These bacteria thrive in the sediment at the bottom of bodies of still water. The methane produced under these conditions is called marsh gas. In the rumen of cattle methane is produced by the same reaction. Bloated cows belch methane!

One of the purposes of sewage treatment is the removal of organic matter from water. Many sewage plants utilize anaerobic bacteria which convert the organic material to CO\(_2\) and methane. The overall process may be represented by the equation:

\[ \text{C}_6\text{H}_{12}\text{O}_6 + a\text{H}_2\text{O} + b\text{CO}_2 + c\text{CH}_4 \]

Evidence has been published that Methanosarcina barkeri and Methanobacterium formicum, two anaerobic soil bacteria, oxidize CO to CO\(_2\) in the absence of H\(_2\) or reduce CO directly to methane in the presence of H\(_2\). (See Science Vol. 172, p. 1229 (1971).)
In this case not only is organic matter removed from the water but in addition the methane is collected as a valuable by-product.

Methane is often found in coal mines. In this case it is known as fire-damp and has been the cause of destructive fires and explosions. Some coal mines have had to be abandoned because of the fire-damp. It is not certain that bacteria are responsible for fire-damp but they may be.

A.3 METHANE FROM PETROLEUM

Petroleum is a mixture of hydrocarbons which has been trapped in certain geological formations. The proportion of the different possible hydrocarbons differs from one oil field to another or even from one well to the next within a single field. The most valuable material in crude petroleum is the mixture of hydrocarbons used for gasoline. These consist mainly of n-hexane and its isomers, \((C_6H_{14})\), n-heptane and its isomers, \((C_7H_{16})\), and n-octane and its isomers, \((C_8H_{18})\). Simple distillation (refining) of crude oil yields a mixture with a boiling range of about 40° to 225°, which may be used as motor fuel. The bulk of most crude oils does not distill in this range and consists of hydrocarbons with molecular weights too high to be
useful as motor fuels. In order to increase the yield of gasoline the petroleum industry has developed methods for breaking down higher molecular weight hydrocarbons into hydrocarbons suitable for motor fuel. This process is known as catalytic "cracking." Cracking may be caused by high temperature (600-800°C, thermal cracking). In both cases hydrocarbons are broken down into smaller fragments including, among other things, methane (CH₄), hydrogen, ethane (H₂C-CH₃), ethylene (H₂C=CH₂), propylene (CH₃-CH=CH₂) etc.

Petroleum refineries with catalytic crackers are usually combined with petrochemical plants which use these by-products as starting materials. For instance, hydrogen is combined with nitrogen to produce ammonia.

\[ \text{N}_2 + 3\text{H}_2 \rightarrow 2\text{NH}_3 \]

Ethylene and propylene are polymerized to produce polyethylene and polypropylene, respectively. Methane utilization will be discussed later.

Crude petroleum contains methane and the other low molecular weight hydrocarbons which are gases at ordinary temperatures. In addition to these dissolved gases, pockets of natural gas are often found trapped in the same rock formation with petroleum and in direct contact with the crude oil. This gas is called associated
One could speculate on whether or not the outlawing of flaring forced the oil industry to develop the now highly profitable petrochemical industry.

natural gas. These gases are removed from the crude oil. In the past they were simply burned at the oil field in a spectacular flame. This practice, known as flaring, has been outlawed in most of the United States but is still done in the Middle East and in Venezuela.

A.3.a PETROLEUM DERIVATIVE PRODUCTS

These volatile hydrocarbons are now regarded as a valuable resource. The methane has many uses, which we will discuss. The others are used in several ways. For instance, by compressing them until they liquefy a useful product, liquefied petroleum gas (LPG), is obtained. Propane (CH$_3$CH$_2$CH$_3$) and butane (CH$_3$CH$_2$CH$_2$CH$_3$) can be treated with a dehydrogenation catalyst and converted to olefins.

\[
\text{Cat. + high temp. } \quad \text{CH}_3\text{CH}_2\text{CH}_3 \rightarrow \text{H}_2 + \text{CH}_3-\text{C}=\text{CH}_2
\]

Propene

\[
\text{Cat. high temp. } \quad \text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3 \rightarrow \text{H}_2 + \text{CH}_3-\text{CH}_2-\text{CH}=\text{CH}_2
\]

1-butene

Further reaction

\[
\text{CH}_3-\text{CH}_2-\text{CH}=\text{CH}_2 \rightarrow \text{H}_2 + \text{H}_2\text{C}=\text{C}-\text{C}=\text{CH}_2
\]

1,3-butadiene

Butadiene is polymerized to butyl rubber, a synthetic substitute for natural rubber.

By oxidation of the various olefins avail-
able from the dehydrogenation process a vast variety of oxygen-containing derivatives are produced. Included among the simplest of these are the following:

- Ethyl alcohol \( \text{CH}_3\text{CH}_2\text{-OH} \)
- Acetaldehyde \( \text{CH}_3\text{C}=\text{O} \)
- Ethylene Oxide \( \text{H}_2\text{C}=\text{CH}_2 \)
- Acetic acid \( \text{CH}_3\text{-C}=\text{O} \text{-OH} \)
- Ethylene glycol \( \text{HO}-\text{CH}_2\text{-CH}_2\text{-OH} \)
- Isopropyl alcohol \( \text{CH}_3\text{-C}-\text{CH}_3 \)
- Propylene glycol \( \text{CH}_3\text{-C}-\text{C}-\text{H} \)
- Acetone \( \text{CH}_3\text{-C}=\text{CH}_3 \)

How many of these products are you familiar with? What are they used for?

A petroleum refinery and petrochemical operation is necessarily a huge industrial plant with many interconnected processes. These plants are designed so that the end products can be varied as the demand for them shifts. The only Ethyl alcohol, isopropyl alcohol, acetone and propylene glycol are common industrial solvents. Ethylene oxide is a sterilant as well as a chemical intermediate. Ethylene glycol is permanent antifreeze.
thing that is fixed is that the starting material is always crude oil. If the supply of crude oil should fail the entire operation would screech to a halt. The technical problems of stopping and restarting such a complicated operation are so formidable that great pains are taken to insure a continuous supply of crude oil. On more than a few occasions this has involved political deals and commitments at the international level. Another problem that may develop results from the fluctuations in the demand for the main product, gasoline. Sometimes the demand for many of the products may be high while the demand for gasoline is low. It may be economically sound under these conditions to keep operating while attempting to sell more gas. Any "extra" gas that is sold must necessarily come out of the sales of some other oil company. In retaliation price cuts are implemented and price wars develop. By restricting the price cuts to certain geographic areas most of the available gasoline is being sold at normal prices and only the "extra" gasoline is being discounted. Eventually, in the geographic area of the price war, equilibrium is reestablished but at a lower price. Each company maintains
its share of the market and there is no advantage to continue the war. The price rises to the original level, everyone with foresight has a tank full of cheap gas and the company with the surplus gasoline looks for a new locality in which to cut prices and attempt to increase its share of the market.*

A.4 NATURAL GAS

The bulk of the methane now used comes from dry natural gas. This is natural gas trapped in rock formations that is not in contact with crude oil. The adjective "dry" distinguishes such gas from the volatile distillation product of crude oil (also mainly methane) which is known as "wet natural gas." Dry natural gas may be 60 to 90% methane with nitrogen, carbon dioxide, water vapor, helium and other gases present. If the gas contains smelly sulfur compounds or a high proportion of CO₂ it is called sour gas. These contaminants are removed (in part) before sour gas is used. The recovered sulfur constitutes an important source of this element. The recovery of the helium is economically feasible at a concentration as low as 0.3%. Most of the world's supply of helium is obtained from natural gas found in the United

*There are, of course, other reasons for gas wars as well, such as local competition.
States. The U. S. Government has carried out a helium conservation program and maintained a helium research laboratory for many years. There is much fear now that these activities will be stopped for budgetary reasons. Once lost to the atmosphere, helium could not be recovered economically.

The main use of natural gas is for energy. About one-third of the energy required in the U. S. comes from natural gas. The collection of the gas in the Southwest and in Alberta, Canada, uses about 60,000 miles of pipe lines. The high-pressure long-distance transmission of the gas uses 200,000 miles of pipe lines and the low-pressure distribution system accounts for 435,000 miles of pipe line. It is not surprising that the natural gas industry started and waxed rich in the United States. The political stability uniformly encompassing an enormous area of land that included both the source and the consumer made the investment an attractive one. In Europe progress has been slower, but the need for energy is now breaking across political borders. Perhaps this suggests that the need to solve the problems of survival may overcome the trivial obstacles as the survival problems become more acute. One highly imaginative approach
to the problem of supplying Europe's energy needs was implemented in 1964. This involved building a liquefaction plant in Algeria capable of liquefying methane (at -161°C, one atmosphere pressure), several highly insulated tanker ships and a host of terminal facilities and insulated storage tanks. Approximately $200,000,000 was spent and the governments of Algeria, France and England were heavily involved as were at least half a dozen international corporations. All the technical problems were solved and liquefied methane was shipped by boat from Africa to Western Europe. Just as the process proved itself, it was shown that a natural gas field discovered in Holland was large enough (third largest in the world) to supply much of Europe's needs. Following this discovery, natural gas was discovered under the North Sea. Western Europe's energy needs appear to be satisfied for now.

Meanwhile, back in the U.S., the picture is not quite so rosy. Demand for natural gas is soaring, reserves are falling and shortages developing. The gas producers claim that the Federal Power Commission, which regulates the prices on interstate shipments of gas, has set prices so low it doesn't pay to drill new wells.

See American Scientist, Vol. 53, No. 2 and No. 3, 1965 for an account of these events.
Some critics of the industry claim that the producers may be blackmailing consumers for higher prices by hiding reserves. Prices will probably rise some, not enough to satisfy the producers, too much to satisfy the consumers and no one will be happy. (See the Wall Street Journal, April 12, 1971.)

B. USE OF METHANE

B.1 ENERGY SOURCE

The main use of methane is burning it in air (combustion). This reaction releases energy contained in the chemical bonds (potential energy) and gives off heat.

\[ \text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} + \text{Heat} \]

The heat produced is not only used to warm buildings, fry eggs and heat bath water. A significant amount of electricity is produced from methane by using the heat of this reaction to vaporize water to drive turbine generators. Since the only chemical products (under ideal conditions) are carbon dioxide and water, this is an exceedingly clean fuel.

The combustion of pure methane under standard conditions yields 212.8 kilocalories/mole (13.3 kilocalories/gram). Because of the impurities (N\(_2\) and CO\(_2\)) in natural gas, it usually has a slightly lower standard heat of combustion.
approximately 12.5 kilocalories/gram.

Compared to other fuels natural gas is very efficient.

<table>
<thead>
<tr>
<th>Fuel</th>
<th>Kilocalories/gram</th>
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<tbody>
<tr>
<td>Crude Oil</td>
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<tr>
<td>Gasoline</td>
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<td>Kerosene</td>
<td>11.0</td>
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<tr>
<td>Coal</td>
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<td>Charcoal</td>
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<tr>
<td>Beech</td>
<td>4.2</td>
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<tr>
<td>Oak</td>
<td>4.0</td>
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<tr>
<td>Pine</td>
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There are certain advantages (such as cost) in using less efficient fuels, but there are also significant disadvantages as well.

B.2 CHEMICAL INTERMEDIATES

Methane is the starting material for many important products. The simplest of these is plain old carbon.

B.2. A CARBON BLACK

Man discovered at a relatively early point that decoration was desirable and enhanced life. Pigments used for this purpose were developed from the materials available such as various plant products and minerals. The first material used for blackening was probably charcoal ground to a fine powder. By the time of the Greek civilization it was understood that a superior black pigment was obtained from the soot from burning pitch. This was the first carbon black. Fine particles of carbon, approximately 100 to 4,000 Å in diameter, are still

Some class discussion or individual library research projects may be desirable on the comparison of fuels. Topics could include:

Strip Mining
Air Pollution from by-products such as fly ash, mercury, CO, sulfur compounds, oxides of nitrogen
Water Pollution from scrubbing water used to minimize air pollution
Historically the destruction of enormous forests to make charcoal for iron reduction
Energy needs for the future

The formation of CO₂ and H₂O from methane in a burner flame can be demonstrated using lime water to test for CO₂. By limiting the amount of air to the burner CO should form (perhaps Year 1 CO test can confirm its presence) as well as carbon black (soot).
regarded highly - in the U. S. alone, more than two billion pounds are produced annually. And a large share is produced from methane. Several processes are in use but they basically use the same chemical reaction, the thermal decomposition of methane.

\[ \text{CH}_4 + \text{heat} \rightarrow \text{C} + 2\text{H}_2 \]

In one process methane is burned in a large chamber without enough oxygen to burn all the methane in the chamber. The heat generated by the methane that is burned causes the remaining methane to thermally decompose. In the second process an oven is heated by burning methane and then methane is admitted to the oven without oxygen and it undergoes the thermal decomposition reaction. This cycle is repeated continuously.

Most of the carbon black produced is used to strengthen rubber. Automobile and truck tires utilize as much as 90% of the carbon black produced.

Black printing ink is made from carbon black and hydrocarbons such as mineral oil. The necessity for removing the carbon black from old newspapers before they can be reused as newsprint is one of the drawbacks to extensive re-
cycling of newsprint. One newspaper, the Louisville Courier Journal, has made an effort to recycle old newspapers. They find that the cost of collecting and deinking makes the recycled newsprint cost $3 more per ton than newsprint made directly from trees. The avoidance of solid waste disposal problems as well as the saving of trees might outweigh the otherwise unfavorable economics of the situation.

Does your local newspaper use any recycled newsprint? One corporation, Omark, which manufactures chain saws and other equipment used in the pulp wood industry, prints its reports to stockholders on recycled paper. This is a striking example of corporate morality.

B.2.b METHANOL

Methanol (methyl alcohol), the simplest alcohol, CH₂OH, used to be made industrially by decomposing wood. Hence, the common name for this substance is wood alcohol. Now most methanol is made indirectly from methane. The process involves several steps. We will discuss the steps separately. The last step is the reduction of carbon monoxide with hydrogen:

\[
\text{CO} + 2\text{H}_2 \rightarrow \text{CH}_3\text{OH}
\]

\[
\text{200-300 atm. pressure at 250-400°C}
\]

Notice that the mole ratio of 2 moles hydrogen to one mole carbon monoxide balances the
reaction and there are no by-products formed. The real success of this process depends on a means of supplying the starting material in this ratio. As a first attempt the reactions of methane and water at high temperature were used:

1) \( CH_4 + H_2O \rightarrow 3H_2 + CO \)
2) \( CH_4 + 2H_2O \rightarrow 4H_2 + CO_2 \)

These reactions, while useful, yield too much hydrogen for the amount of carbon monoxide produced and, in addition, afford the by-product \( CO_2 \). These difficulties were overcome by the use of a third reaction:

3) \( CO_2 + H_2 \rightarrow CO + H_2O \)

The process is now carried out according to the following equation:

\[
3CH_4 + CO_2 + 2H_2O \xrightarrow{\text{high temp. \\ pressure}} 4CO + 8H_2
\]

Note that the mole ratio of the two products fits the requirements of the methanol reaction perfectly!

The chemical plants used for methanol production are often built so that by changing the starting materials from hydrogen and carbon monoxide to hydrogen and nitrogen, ammonia may be produced instead of methanol. Nearly the same conditions are employed for both syntheses and this added versatility makes the investment
more attractive.

Methanol production in the U. S. is now at an annual rate of nearly 500,000,000 gallons. About half of it is converted to formaldehyde by several processes:

\[
2H_3C{-}OH + O_2 \rightarrow H\stackrel{C=O}{\text{H}} + H_2O
\]

\[
\text{catalyst CH}_3{-}OH \rightarrow H\stackrel{C=O}{\text{H}} + H_2
\]

Formaldehyde is used in making plastics, resins (for plywood for example) and as an intermediate for making still other intermediates. The methanol not used for formaldehyde production either is used as an industrial solvent, an intermediate in the production of plastics, a cheap antifreeze, fuel or in the synthesis of other intermediates.

B.2.c OTHER CHEMICALS

Methane is used as starting material for so many other chemicals we could not attempt to give an exhaustive discussion of them all. The following reactions should serve to demonstrate the scope of products derived from this "simplest" organic compound.

You should be able to find at least several uses for each of the products given.
$1250^\circ$

$2\text{CH}_4 \rightarrow \text{H} - \text{C} = \text{C} - \text{H} + 3\text{H}_2$

- acetylene

$\text{CH}_4 + 4\text{S} \rightarrow \text{CS}_2 + 2\text{H}_2\text{S}$

- carbon disulfide sulfide

$\text{CH}_4 + \text{Cl}_2 \rightarrow \text{CH}_3\text{Cl} + \text{HCl}$

- methylchloride

$\text{CH}_4 + 2\text{Cl}_2 \rightarrow \text{CH}_2\text{Cl}_2 + 2\text{HCl}$

- methylene chloride

$\text{CH}_4 + 3\text{Cl}_2 \rightarrow \text{CHCl}_3 + 3\text{HCl}$

- chloroform

$\text{CH}_4 + 4\text{Cl}_2 \rightarrow \text{CCl}_4 + 4\text{HCl}$

- carbon tetrachloride

$\text{CH}_4 + \text{NH}_3 + 1.5\text{O}_2 \rightarrow \text{HCN} + 3\text{H}_2\text{O}$

- hydrogen cyanide
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<tr>
<th>TEXT SECTION</th>
<th>ROUGH TIME ESTIMATES</th>
<th>EXPERIMENTS</th>
<th>DEMONSTRATIONS</th>
<th>TEACHING AIDS</th>
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Chapter III: POLYMERS OR STRINGING MONOMERS TOGETHER

A.1 BUILDING MODELS OF MONOMERS AND POLYMERS

The molecules which were discussed in Chapter I had molecular weights ranging from 16 for methane to 142 for decane. However, some molecules have molecular weights as high as several billion. (See Table A.1) In living systems such molecules are formed by the joining together of many small molecules in a repeating, chain-like structure of great length. These are called polymers.

<table>
<thead>
<tr>
<th>POLYMER SIZES</th>
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<tbody>
<tr>
<td>Name</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>hemoglobin</td>
</tr>
<tr>
<td>β-galactosidase</td>
</tr>
<tr>
<td>myosin</td>
</tr>
<tr>
<td>E. coli</td>
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<tr>
<td>chromosome</td>
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<td>transfer RNA</td>
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Table A.1

Construct two models of the amino acid

\[ \text{glycine, } H_2N-CH_2-CO \]

Glycine is bifunctional because it has two functional groups, amine and carboxyl. Carboxyl groups can react with amines.
Join the two glycine models by forming an amide bond between them. The product is glycyl-glycine. Does the resulting amide model still have a free carboxyl and amine group?

Yes.

Three amide bonds.

Yes.

Four glycine models.

Even though the amine and carboxyl groups of the original glycine models are part of amide bonds, the number of glycine models used to make the polyamide model can still be counted.

The process of combining small molecules in a repeating fashion, as you did with glycine models, is known as polymerization. The small molecules are monomers. The resulting large molecule is a polymer. Each repeating unit in
the polymer is called a residue.

Glycine is a member of a series of compounds, the α amino acids which have the general structure:

\[
\begin{array}{c}
\text{NH}_2 \\
R-\text{C-COOH}
\end{array} \quad \text{or written} \quad \begin{array}{c}
\text{H} \\
\text{H} \\
\text{O}
\end{array} \quad \begin{array}{c}
\text{N-C-C}
\end{array}
\]

or written

\[
\begin{array}{c}
\text{NH}_2 \\
R-\text{OH}
\end{array} \quad \begin{array}{c}
\text{H} \\
\text{H}
\end{array}
\]

For glycine H-C-COOH the R group represents -H; for the amino acid alanine, R- represents -CH₃:

\[
\begin{array}{c}
\text{NH}_2 \\
\text{H}_3\text{C-C-COOH}
\end{array}
\]

There are 20 amino acids commonly found in living material, each differing only in the composition of the R group. Could you have a polyamide composed of different monomer units? The amide bond is also known as the peptide bond. The terms are synonymous. Therefore, a polyamide is also known as a polypeptide. Proteins are polypeptides (polyamides) having amino acids as the monomers.

It is also possible to have polyamides which do not have amino acids as monomers. If a diamine forms amide bonds with a dicarboxylic acid, then a polyamide will form. Nylon is an example.

Yes, composed of amino acids; the atoms involved in forming the amide bond are identical in all 20 amino acids.
Formation Of Nylon

\[
\begin{align*}
H_2N - (CH_2)_6 - NH_2 + Cl &\rightarrow C - (CH_2)_4 - C - Cl \\
+ H_2N - (CH_2)_6 - NH_2 &\rightarrow C - (CH_2)_4 - C - Cl + HCl
\end{align*}
\]

\[
H_2N - (CH_2)_6 - N - C - (CH_2)_4 - C - N - (CH_2)_6 - NH_2 + HCl
\]

\[
+ Cl - C - (CH_2)_4 - C - Cl
\]

\[
H_2N - (CH_2)_6 - N - C - (CH_2)_4 - C - N - (CH_2)_6 - N - C - (CH_2)_4 - C - Cl + HCl
\]

\[
H_2N - (CH_2)_6 - N - (C - (CH_2)_4 - C - N - (CH_2)_6 - N - C - (CH_2)_4 - C - Cl)
\]

Figure A.1
A.1.a Experiment: PREPARATION OF A POLYAMIDE - NYLON

Work as partners. One partner obtains 25 ml of the dicarboxylic acid chloride solution in a dry 250 ml beaker while the other partner obtains 25 ml of the diamine solution in another 250 ml beaker. Carefully pour the diamine solution down the slanted side of the dicarboxylic acid chloride beaker so that a layer of amine lies under a layer of acid. Using a stirring rod, carefully remove the glob of nylon; raise the polyamide as a rope of continuously forming polymer film to a length of 12 to 15 inches. Cut the polymer at the liquid surface.

A.2 PROTEINS, NUCLEIC ACIDS, CARBOHYDRATES

Study of living systems has shown that there are three large polymers of nearly universal occurrence: (1) protein (a polyamide), (2) nucleic acid (a polyphosphoester) and (3) carbohydrate (a polyacetal or a polyketal).

Figures A.3, A.4 and A.5 show examples of these three polymers. Each of the types of bonds used to join the four types of monomers is shown in Figure A.2, p. 65.

Some chemistry of acetals and ketals, very important in carbohydrate chemistry, is outlined in Appendix A at the end of the chapter, page 81.

The appendix will help in understanding

This preparation cannot proceed unless the diacid is activated. Therefore the actual reactants in this experiment are a diacid chloride like adipoyl chloride or sebacoyl chloride and a diamine.

\[
\text{Cl-C-(CH}_2\text{)}_6\text{-C-CI} \quad \text{adipoyl chloride}
\]

\[
\text{Cl-C-(CH}_2\text{)}_8\text{-C-Cl} \quad \text{sebacoyl chloride}
\]

Adipoyl chloride - 5% volume/volume (v/v) 1 ml/20 ml in benzene. Hexamethylenediamine 5% water/volume (w/v) 1g in 18 ml H$_2$O solution in water with 10 drops of 6M NaOH added to insure that the free amine is present and not amine salt - NH$_3^+$.

For weight/volume and volume/volume solution, the denominator volume is the final, total volume of the solution. Numerator terms (w or v) are the corresponding measures of the pure solute.

Solution

E.g. a 5% (w/v) of NaCl is 5 g of NaCl dissolved in water to a total volume of 100 ml.

Materials and Equipment

(for one student)

2 beakers (250 ml)
1 stirring rod
1 scissors for whole class
5% (w/v) hexamethylene diamine in water (25 ml)
5% (w/v) diacid chloride in benzene (25 ml)

There are solvent alterna-
Figure A.2

These three polymers--protein, nucleic acid and carbohydrate--lead to an important generalization. Monomeric residues of each biological polymer are linked by a specific type of covalent bond. Thus, those chemical or biological reactions which depend upon the making or breaking of polymers depend upon the making or breaking of specific bond types. Knowing this is a great
aid in the study of living systems, because attention may be focused upon the specific bond type of interest to the investigator.

The nature of the bond holding the monomers together is only one factor in the determination of biological activity. It is also possible to influence both the total shape and the biological activity of a polymer by changing the individual monomers in the chain.

Following is an example in which the difference in linkage is important in determining biological activity:

Starch (an α-1-4 linked polymer of glucose)
Cellulose (a \(\beta-1-4\) linked polymer of glucose)

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{H} & \quad \text{OH} & \quad \text{CH}_2\text{OH} \\
\text{ETC.} & \quad \text{H} & \quad \text{OH} & \quad \text{H} & \quad \text{O} & \quad \text{O} & \quad \text{ETC.}
\end{align*}
\]

\(\beta\) CONFIGURATION

The first polymer, starch, is readily digested by humans and is a staple in our diet. The second polymer, cellulose, is a primary constituent of hay, wood and paper. Cows and termites eat cellulose but depend upon certain microorganisms in their digestive tracts to digest the cellulose (break the polymer down into glucose monomers).

An example where it is the difference in the links (monomers) that determines biological activity follows:

LACTOSE

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{H} & \quad \text{OH} & \quad \text{CH}_2\text{OH} \\
\text{ETC.} & \quad \text{H} & \quad \text{OH} & \quad \text{H} & \quad \text{O} & \quad \text{O} & \quad \text{ETC.}
\end{align*}
\]

\(\beta\) CONFIGURATION

GALACTOSE

GLUCOSE

CELLOBIOSE

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{H} & \quad \text{OH} & \quad \text{CH}_2\text{OH} \\
\text{ETC.} & \quad \text{H} & \quad \text{OH} & \quad \text{H} & \quad \text{O} & \quad \text{O} & \quad \text{ETC.}
\end{align*}
\]

\(\beta\) CONFIGURATION

GLUCOSE

GLUCOSE
What is the difference between these two structures? Humans digest lactose, a sugar found in milk, but cannot digest cellobiose, a plant sugar.

In the case of starch, each monomer is a glucose molecule and thus each residue in the polymer is the same (see Figure A.3). However, for protein, in which each monomer is an amino acid, there are many different amino acid residues composing the polymer (see Figure A.4).

Nucleic acids are also polymers. As in proteins, monomers of the nucleic acid polymers are not all the same. (See Figure A.5). The monomeric residues of nucleic acids are nucleotides (see Figure A.6). A nucleotide is composed of a base bonded to either ribose or deoxyribose, bonded to phosphoric acid. RNA (ribonucleic acid) and DNA (deoxyribonucleic acid) are the nucleic acids containing the sugars ribose and deoxyribose respectively. The number of different bases involved in forming a given nucleic acid isolated from living cells is usually four—a much lower number than the twenty different amino acids composing protein.

If a chain consisting of one hundred links or residues is imagined, it should be obvious that all chains with only one type of link
FOOTNOTE TO FIGURES A.3,4,5

From your work with models you know that three dimensions are required to describe molecules. In Figures A.3,4,5 an attempt is made to depict three dimensions by use of darkened wedges. A wedge connecting two atoms means that the two atoms do not lie in the same plane.

For example:

C lies behind the plane of the paper.

D lies in front of the plane of the paper.

A and B are in the same plane - the plane of the paper. (Two atoms joined by a heavy line E-F are both in a single plane in front of the plane of the paper.)
CARBOHYDRATE

Figure A.3

PROTEIN

Figure A.4
Figure A.5
Figura A.6
(residue) and one type of linkage should be identical. Thus, a starch chain of 100 glucose residues should be identical to all other starch chains of 100 glucose residues. However, a protein chain with differing amino acid residues could lead to an enormously large number of different chains. As an example, a protein 100 residues long composed of 20 different amino acids could have $20^{100}$ different possible sequences.

Even when the number of different residues is small, there is still the possibility of an enormous number of different possible residue sequences. For example, a nucleic acid 100 residues long composed of only 4 different nucleotides could have $4^{100}$ different possible sequences.

Polymers 100 residues long are big molecules. One of the easiest ways to demonstrate polymer size is to use porous membranes. Two substances, one large and one small, will be placed in a membranous bag. The pores in the bag are large enough to allow the smaller substance in the solution to pass through the membrane but are small enough to retain the larger substance. The membrane is said to be semipermeable. It is capable of separating
the substances.

A.2.a Experiment: POLYMER SIZE

Fill a 400 ml beaker about three-fourths full with distilled water. Cut off a 45 cm piece of dialysis tubing and put it into the distilled water to soak for several minutes to soften it. Securely tie off one end of the tube. Into the tube pour 10 ml each of salt and starch solutions and tie the top closed. Place the bag in the beaker of distilled water.

Prepare two test tubes containing 2.5 ml of starch solution and two more test tubes containing 2.5 ml of salt solution. Add several drops of 0.001 M iodine solution to one tube which contains salt. Repeat with a tube which contains starch. To the remaining starch and salt tubes add several drops of 0.1M silver nitrate. Record your observations for each of the four tubes and save the tubes with their contents as standards.

Near the end of the period, remove 5 ml of water from the beaker and divide it equally between two test tubes. Add several drops of 0.001M iodine solution to one tube and several drops of 0.1M silver nitrate solution to the other tube. Observe and record your observations.

Instruct students to use masking tape to open the tubing.

A good seal is necessary to prevent the solution inside from leaking out. The safest way is to tie two tight overhand knots in each end of the tube.

Materials and Equipment
(for one student)
1 ml AgNO₃ (0.1M)
1 ml I₂ (0.001M)
16 ml NaCl (0.1M)
16 ml Kosher Starch 0.5% w/v
1 beaker 400 ml
1 dialysis tube 45 cm long
4 test tubes 13 x 100 mm

Preparation of Solutions
It is essential for the success of the lab that the starch be Kosher starch, which may be purchased from most grocery stores. This starch is more soluble and gives a better blue color with I₂ than laboratory "soluble starch." Weigh out the starch, add just enough water to make a paste and work the paste until the starch is thoroughly set. Add water and boil the starch "solution" for one minute. Cool to room temperature before using. Be sure to refrigerate if stored because starch is rapidly digested by airborne bacteria. The final "solution" may be cloudy, but it still functions properly in this experiment.

Stock Iodine Solution: 0.001 M Iodine
Potassium Iodide: 0.1 M
Distilled Water to 100 ml
Dilute this solution 1:10 to prepare 0.001M solution.
The soluble ion is $I_3^-$. If stoppered, this solution may be stored for years.

Salt particles must have passed out of the bag because the presence of the $Cl^-$ was indicated by the precipitate formed when $Ag^+$ was added to water removed from the beaker.

$I_3^-$ will pass through the pores in the dialysis bag, turning the starch in the bag blue.

At the beginning of the next day's class again remove 5 ml of water from the beaker and repeat the tests as in the preceding paragraph.

Add several drops of 0.001M iodine solution to the beaker containing the dialysis bag and let it stand for the rest of the period. What happens inside the bag? Explain your observation.

A.3 A CLOSER LOOK AT SIZE

Elemental analysis can be the basis of molecular weight determination. If a chemical compound contains a known element, then the minimum molecular weight can be found by analyzing for the known element. An example is ferrous ammonium sulfate $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$. When the quantity of iron per gram of sample has been determined, it is a simple matter to calculate the grams of sample per mole of iron and thus the molecular weight of the sample. The resulting molecular weight is a minimum molecular weight rather than a true molecular weight because it is expressed on the assumption of a single iron atom per molecule of sample (which is true for ferrous ammonium sulfate). The true molecular weight could be larger by any integral factor,
but it could not be smaller than the determined value.

A.3.a **Experiment: POLYMER SIZE - QUANTITATIVE**

Obtain 2 ml samples of ferrous ammonium sulfate and hemoglobin solutions from your teacher in separate 250 ml beakers. Slowly add 5 ml of concentrated sulfuric acid to each beaker. Swirl until no particles remain (at least one minute). Add 28 ml of water to each beaker followed by 10 ml of 3% hydrogen peroxide. Swirl until thoroughly mixed. Add a pinch of potassium persulfate, producing a clear, straw-colored solution, then add 5 ml of 3M potassium thiocyanate to each beaker. Filter the hemoglobin solution twice using two sheets of filter paper each time.

Following the directions on page 77 for use of the spectrophotometer, a calibration curve of absorbency vs moles of iron/ml is prepared for various dilutions of the ferrous ammonium sulfate-thiocyanate solution. Dilute the dark red solution with 0.1M potassium thiocyanate (carefully record the amount of liquid added) until a spectrophotometric reading of less than 0.7 absorbency is obtained at 480 mm. Do at least two more dilutions for lower absorbency values, taking readings at each dilution. Plot absorbency on the vertical axis and moles of iron/ml...

---

**Materials and Equipment**

(for one student)

- 2 beakers - 250 ml
- 2 test tubes - 13 x 100 mm
- 1% w/v hemoglobin (2 ml)
- 1% w/v Fe(NH₄)₂(SO₄)₂·6H₂O (2 ml)
- 3% v/v hydrogen peroxide (20 ml)
- 3M KSCN (10 ml)
- 0.1M KSCN (50 ml)
- Potassium persulfate (K₂S₂O₈) 1 pinch
- Bausch & Lomb Spectronic 20 (one for the whole class)
(2) Assuming one iron atom per hemoglobin molecule, the quotient obtained in (1) is the minimum molecular weight.

(3) Equals the quotient from (1).

(4) MW hemoglobin $1.6 \times 10^4$, Handbook of Biochemistry. If the hemoglobin solution is turbid because of improper or insufficient filtering, the absorbency value will be too high and consequently the minimum molecular weight will be too low. In trial experiments the writers obtained a value of 5000 with insufficient filtering and a value of 16,000 when filtering was repeated until a clear solution was obtained.

These instructions are for a Bausch & Lomb Spectronic 20.

If any school does not have a spectrophotometer or colorimeter available, then a serial dilution of Fe-SCN complex should be prepared. Simply match the Fe-SCN complex from hemoglobin to one of the tubes of the serial dilution.

By determining the absorbency of twice-filtered hemoglobin solution, moles/ml of iron in this solution can be read from the calibration curve. From the grams of hemoglobin in solution and its total final volume calculate the grams of hemoglobin/ml.

(1) Divide grams hemoglobin/ml by moles iron/ml.

(2) What assumption must you now make in order to obtain the minimum molecular weight for hemoglobin?

(3) What is the minimum molecular weight for hemoglobin?

(4) How does your experimental value compare to literature values for minimum molecular weight?

Students should get detailed instructions on the use of the spectrophotometer from the teacher. Briefly, the general outline is as follows:

(1) Turn on the instrument (left hand knob) and allow five minutes for warm-up.

(2) Set the zero point (left hand knob) on the transmittance scale with nothing in the sample holder.

(3) Fill one of the spectrophotometer
tubes with the solvent being used (to be called the blank) and insert this in the sample holder.

(4) Turn the wave length control to 480 millimicrons and set the absorbency at zero with the light control (right hand knob).

(5) Fill one of the spectrophotometer tubes with sample solution and read the absorbency on the meter.

(6) Repeat step (5) with more or less concentrated sample solutions until enough absorbency values have been collected to span the range from 0.1 to 0.7.

Exercises for Home, Desk and Lab (HDL's)

(1) Draw with structural formulas the reaction of two glycine molecules to produce glycylglycine. Show all atoms and bonds. Designate the amide bond.

(2) Adipic acid is represented by the structural formula

HOOCC-CH2-CH2-CH2-COOH

a. How many functional groups are present?

b. Name the functional group(s).
(3) Draw the structural formula for 1, 6-diamino hexane.

(4) a. Draw the structural formula for the product of the chemical reaction between adipic acid and 1, 6-diamino hexane.

b. What is the type of bond that holds the monomers together?

(5) Draw the functional group

a. for an aldehyde;

b. for an alcohol

(6) Write the structural formula for:

a. ethane

b. ethanol

c. ethanal

d. How many carbon atoms are in each molecule?

(7) What is the ketone functional group?

(8) Write the structural formula for:

a. propane

b. propanol

c. propanone

d. How many carbon atoms are in each molecular formula?
(9) Write the equation for the chemical reaction between two moles of propanol and one of propanone.

(10) What distinguishes an acetal from a ketal functional group?

(11) Use your model kit to prepare the molecule formed in question (9).

(12) Draw the structural formula for phosphoric acid.

(13) Draw the functional group for:
   a. an acid
   b. an ester

(14) What feature distinguishes an acid from an ester?

(15) a. Draw the methyl ester of phosphoric acid.
   b. What is the name of the -O-R bond in the molecule shown in (15) a?

(16) Use your model kit and make the molecule in problem 15.
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<th>EXPERIMENTS</th>
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<td>1 1/2 Days</td>
<td>A.i Polymer shape or permanent wave</td>
<td>Models</td>
<td>1, 3-5</td>
<td>The Double Helix, J. D. Watson Signet 03770 1968</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX A

Acetals are prepared from alcohols and aldehydes:

\[
\begin{align*}
\text{CH}_3\text{C} & + \text{CH}_3\text{CH}_2\text{OH} \\
\text{ethanal} & \quad \text{ethanol} \\
\text{(aldehyde)} & \quad \text{(alcohol)} \\
\end{align*}
\]

\[+ \text{HCl}\]

\[
\begin{align*}
\text{CH}_3\text{C} & \quad \text{CH}_3\text{OCH}_2\text{CH}_3 \\
\text{a hemiacetal} & \\
\end{align*}
\]

\[+ \text{CH}_3\text{CH}_2\text{OH}\]

\[+ \text{HCl}\]

\[
\begin{align*}
\text{H}_2\text{O} & \quad \text{CH}_3\text{OCH}_2\text{CH}_3 \\
\text{an acetal} & \\
\end{align*}
\]
Ketals are prepared in the same way using alcohols and ketones:

\[
\begin{align*}
\text{propanone (ketone)} & \quad \rightleftharpoons \quad \text{HCl} \\
\text{a hemiketal} & \\
\text{a ketal}
\end{align*}
\]

The distinguishing feature of an acetal is the hydrogen attached to the former carbonyl carbon. A ketal has no hydrogen attached to this carbon.

The most common biological instance of polyacetals involves the reactions of a hemiacetal with another hemiacetal.
If the hydroxyl of the hemiacetal or hemiketal is on the same side of the ring as the last carbon (number 6 for hexose, number 5 for pentose), the configuration of the hemiacetal or hemiketal is called $\beta$.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} \\
\text{O} & \quad \text{O} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

$\beta$ CONFIGURATION

The opposite configuration is called $\alpha$.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} \\
\text{O} & \quad \text{O} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

$\alpha$ CONFIGURATION

The same convention is used for acetals and ketals, as well as for other functional groups.
A. POLYMER SHAPE

The long chain-like molecules that have been discussed have specific three-dimensional shapes. It is easy to imagine possible ways of arranging a chain in space: stretched straight; coiled like a spring; randomly tangled; or neatly rolled into a sphere. The number of possible arrangements is enormous.

Factors which determine polymer shape are the possibilities of bonding between various portions of the polymer and the relationship between the polymer and its environment. If bonding is a factor, then there will be a range of bond strengths. This range of bond strengths is given in Table A.1.

<table>
<thead>
<tr>
<th>Type of Bond</th>
<th>Van der Waals</th>
<th>Hydrogen</th>
<th>Covalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcal/mole</td>
<td>1</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

Table A.1
The strongest bond formed is a covalent bond. An important covalent bond in determining protein shape is the disulfide bond (-S-S-) formed between two molecules of the amino acid cysteine (HS-CH₂-CH₄-COOH). Hair is a protein with a high cysteine content. It is built up of two or more polypeptide chains cross-linked through disulfide bonds.

Treatment of hair with permanent wave lotion reduces the disulfide bonds to sulfhydryl (-SH) groups. Destruction of the disulfide cross-link permits stretching and rearrangement of the protein molecules. This is called curling the hair. Following curling, new disulfide bonds are formed by oxidation. Permanent hair curling results from pairing of different sulfur atoms from those which were joined in the original hair.
A.1 Experiment: Polymer Shape or Permanent Wave

Obtain two strands of long, straight hair. Wind each strand of hair around a glass stirring rod. Hold the hair in place upon the stirring rod with rubber bands. Wash the hair by vigorously swirling the hair-wound stirring rod in a beaker of soapy water. Thoroughly rinse the hair under running water. Immerse one rod of hair in permanent wave lotion contained in a test tube. Immerse the other rod of hair in an

Figure A.2

After long, hard thought the writers were unable to come up with a more effective experiment than this inexpensive and graphic demonstration.
equal volume of water in another test tube. Set
the two tubes aside for 15 minutes. After the
15-minute interval, remove the two hair-wrapped
rods and rinse with running tap water until the
odor of lotion cannot be smelled. "Neutralize"
(oxidize) the hair following the directions of
the specific commercial product used.
B. WEAKER BONDS
B.1 H-BONDS BETWEEN BASES
Weaker bonds, for example hydrogen bonds,
were discussed in Chapter 1 of Chemistry of
Living Matter and Chapter 17, CHEM Study. One
important factor in determining the three-dimen-
sional shape of nucleic acids is hydrogen bond-
ing between bases. Two chains of nucleic acid
polymers are held together by hydrogen bonding
between bases. Construct models of the four
bases of DNA: thymine, adenine, cytosine and
guanine. Can these bases hydrogen-bond to each
other? Try it with models.
The most stable arrangement would be the
one with the most hydrogen bonds. Since there
are four different bases, how many different
hydrogen-bonded pairs are possible? Which ar-
rangements of base pairing would you predict to
be most stable? Compare the sizes of the hydro-
gen-bonded pairs in which you find maximum
hydrogen bonding. Pairing of smaller with larger gives a constant-sized pair. Refer to Figures B.2 and B.3.

If the purines and pyrimidines of two separate DNA chains are paired by hydrogen bonding, these bases are most stable, and they are shown in Figures B.2 and B.3.
along their edges, as in Figure B.4, then the two DNA polymers would be held together.

Two DNA chains joined in this fashion are called complementary chains. Every base in one chain must be joined by hydrogen bonding to its unique complementary base in the other chain. Thus DNA is double stranded rather than single stranded because of hydrogen-bonded base pairing.
Remember that $\Delta G = \Delta H - T\Delta S$ where $\Delta H$ is the enthalpy of the system and $\Delta S$ its entropy. $\Delta G$, the Gibbs free energy, is the maximum usable energy in the system. Also recall that $\Delta G = 0$ for a system at equilibrium. For the system illustrated here it is true that in the process of hydrophobic bonding the increase in randomness more than offsets the heat required to melt the "ice-structures" surrounding hydrocarbons. That is to say that when $\Delta G = \Delta H - T\Delta S < 0$, hydrophobic bonding occurs spontaneously, leading to the equilibrium situation.

The DNA backbone, on the other hand, is very soluble in water. The -OH groups on ribose and the charged phosphate groups both react strongly with water.

B.2 HYDROPHOBIC BONDS BETWEEN BASES

Until quite recently there has been a tendency to neglect the role of water in determining three-dimensional shape. The nitrogen and oxygen atoms of amide, acetal and phosphoester bonds can hydrogen-bond to water. However, there is another important role of polar water molecules.

Insertion of hydrocarbons into water has an organizing effect upon the surrounding water molecules. In effect an "ice sheath" is formed around each hydrocarbon molecule, and the water molecules in these sheaths are not as free as those elsewhere in the solution. This leads to a decrease in randomness, thus an increase in usable energy of the system. If the hydrocarbon residues of polymers can fold upon themselves, decreasing the surface area exposed to water, then organization of the surrounding water is reduced. This leads to an increase in randomness of the water molecules, thus a decrease in usable energy; this is, of course, what happens. This process is called "hydrophobic bonding."

The purine and pyrimidine base residues in DNA are quite insoluble in water because their non-polar, large, flat sides will not hydrogen-bond even though they contain polar C=O and
-NH₂ groups on their edges. "Hydrophobic bonding" of DNA's flat purine and pyrimidine residues would cause them to stack like playing cards. We would predict DNA would assume either a zigzag or helical conformation in order to fold up the backbone and bring the base residues close enough to stack.

In this respect the DNA structure is "schizophrenic," half hydrophobic and half hydrophilic, and this is undoubtedly very important in the final structure of the molecule.

![Figure 8.5 - Hydrophobic Bond Formation.](image)

The triangles represent water molecules. As the hydrocarbon residues approach each other some of the water molecules are released from an ordered arrangement close to hydrocarbon to a more disordered state in the solvent.

In fact, DNA molecules have regular helical shapes (like a "slinky"). The stacked base pairs, which are all of equal size, require that the shape of a DNA molecule be a regular helix. This means that the helix has a constant diameter and a constant number of turns per unit length (constant pitch).
The preceding discussion on the shape of DNA is more elegantly and more powerfully presented in the words of James D. Watson. For his description of his discovery of the DNA double helix structure see pages 111-112, The Double Helix (reprinted in the Feb. 1968 Atlantic Monthly).

C. THE IMPORTANCE OF SHAPE

There has been a continual emphasis in this section upon the importance of the three-dimensional shape of biological polymers. Despite the fact that structural determination of large molecules is difficult, the three-dimensional shape of several large molecules has been determined. The three-dimensional representation of the protein lysozyme, taken from the Journal of Biological Chemistry, Volume 243, p. 1664, 1968, should be compared to the representation of the same molecule in Scientific American, November
The deficiencies of such pictures are obvious, leading to the model-building activities of many people engaged in polymer research.

The pictures of DNA in *Scientific American*, October, 1954 (Crick), September, 1957 (Crick), and May, 1967 (Yanofsky), are more adequate than the lysozyme pictures for visualizing structure because double-stranded DNA is more regular in its structural features.

The idea of complementary base pairs can be expanded to the broader idea of complementary surfaces. The idea of complementary surfaces is important in understanding the 3-D shape of polymers. Weak forces are effective only when the interacting surfaces are close. This closeness is possible only when the molecular surfaces have complementary structures.

For instance a bump or a positive charge on the surface of an enzyme must be paired with a hole or negative charge on the substrate surface. Biochemists have used the phrases lock-and-key relationship or template relationship to describe these complementary surfaces. We shall try to establish a connection between complementary surfaces of biological polymers and biological activity.

Hydrophobic bonding is extremely important in the structure of globular proteins such as lysozyme or hemoglobin. Unfortunately proteins lack the simple sequence regularities of DNA and it is extremely difficult --or impossible--to predict a priori exactly how such bonding will influence the resulting protein structure. However, it is a rule of thumb among protein chemists that most globular proteins have a "grease-pit," i.e. a cluster of hydrophobic amino acid residues which form the core of the structure.

While ionic (charge-charge) interactions decrease as $1/r^2$, the weak van der Waals forces decrease approximately as $1/r^6$; obviously close proximity is required for such interactions.
Exercises for Home, Desk and Lab (HDL's)

1. Draw the structural formula for two molecules of cysteine connected by the covalent disulfide bond. Then construct this molecule with your kit.

2. Name five covalent bonds in biomolecular molecules.

3. Name two weak bonds that hold molecules together.

4. What factors determine polymer shape?

5. What role does oxidation play during treatment of hair with a permanent wave lotion?

6. Diagram the structure of the two purine bases and the three pyrimidine bases. Name each diagram.

7. a. Which purines pair with which pyrimidines?
   b. Identify the number of hydrogen bonds between each base pair.
   c. Hydrogen bonds exist between what atoms in each base pair?

8. Hydrophobic bonding describes the situation which exists between hydrocarbons and their water environment. How is water organized in a sheath when hydrocarbons are placed in water?

9. What spatial arrangement would you predict...
for purines and pyrimidines, knowing that such hydrocarbons undergo hydrophobic bonding in a watery environment such as the cell?

(10) The DNA backbone is composed of linkages between what molecules?

(11) Would one expect a single strand of DNA to form a helix or are both strands necessary? Provide the reason for your expectation.

N and N atoms. G-C has hydrogen bonds between two sets of N and O atoms and one set of N and N atoms.

(8) When hydrocarbons are inserted into water the water is organized in a sheath around the nonpolar portion of the hydrocarbon since polar and nonpolar molecules are not compatible.

(9) Hydrophobic bonding between the purines and pyrimidines flat sides (top and bottom) cause the bases to stack like playing cards. This stacking, along with the phosphodiester linkages in the backbone of DNA, causes DNA to develop a helical conformation.

(10) The mononucleotide units are linked together through phosphodiester bridges between the deoxyribose portions of successive nucleotides.

(11) A single strand of DNA could possibly form a helix by itself because of the stacking due to hydrophobic bonding between the flat purine and pyrimidine residues and because of the folding up of the backbone due to phosphodiester bridges.
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Chapter V: WHERE THE ACTION IS--THE ACTIVE SITE

All known biological catalysts are proteins called enzymes. Each enzyme catalyses a specific chemical reaction that is needed by the living organism. The material on which the enzyme operates is called the substrate. Enzymes are often named by adding *ase* to the name of the substrate.

Proteolytic enzymes are those that digest (break down into amino acids) other proteins. These are widespread in nature, being found in bacteria and higher plants as well as in the digestive systems of animals. Some of these proteolytic enzymes have been useful in industry and commerce, and a few are found in common household or kitchen items. Can you think of some such items?

A. LAB ACTIVITIES WITH ENZYMES

A.1 Experiment: PROTEOLYTIC ENZYMES

Bring to class samples of any common product or food item which you think may contain proteolytic enzymes. An easy test for these enzymes is through their digestion of gelatin (the protein used in gelatin desserts). For this test you will use a solution of gelatin which has solidified into a jelly. In this jelly, gelatin molecules form a three-dimensional net-

Usually they will digest themselves as well as other proteins, so that solutions of proteolytic enzymes often will lose their activity in a few hours.

Students should come up quickly with meat tenderizer and enzyme detergents. Less obvious will be such things as rennet or junket-type desserts and such fresh fruits as pineapple or papaya. Students should be encouraged to bring small samples of these items and anything else they wish to test, e.g., saliva and the enzyme urease (to be used in a succeeding experiment).

Gelatin is a soluble form of collagen--a major protein component in bones and cartilage.

**Materials and Equipment**

(for one student)

One to three gelatin plates. Soak 60 gm of Knox’s plain gelatin in 60 to 100 ml of cold water. Add to this 800–850 ml of boiling water and stir to dissolve. Then distribute 15 to 20 ml volumes into little plastic petri dishes (Shaw Surgical Co., Portland) and allow these to set at room temperature. You should obtain
about 50 plates from a liter of gelatin solution.

The holes in the gelatin will probably become obvious in six to eight hours. The teacher may wish to refrigerate the plates at this time until the next class period.

The proteolytic enzymes in some common items are:
- Fresh papaya (papain)
- Meat tenderizer (papain)
- Fresh pineapple (bromelain) 
- Enzyme detergent (bromelain or subtilisin)

Remove or correct previous impart.

In addition, if available from the local slaughterhouse, fresh blood, guava juice and the pancreas all contain proteolytic enzymes.

Materials and Equipment (for one student)
- 4 test tubes
- (12 x 100 mm)
- 1 dropper
- Iodine-KI solution (1 ml)
- 0.1% soluble starch solution (1 ml)
- Meat tenderizer (pinch)
- Phenolphthalein indicator solution
- Iodine-KI solution: Dissolve 0.05 g KI in 10 ml water. Add 0.05 g starch until dissolved. Dilute to 100 ml with water.
- 0.1% soluble starch solution: Boil potato starch in 10 ml water to make a sticky, soluble starch. Add 0.5 g starch to 1 ml of

work with the water trapped in this network. Any enzyme that digests gelatin will break down this network, thereby dissolving the jelly. Try your samples of meat tenderizer, enzyme detergent, etc. Spot a few grains of each onto the surface of a gelatin plate and add a drop of water to each spot. You should also try a drop of saliva and any other materials available. Be sure to label the plate with your name and also (on the back) with the names and location of each material spotted. Let the plate stand at room temperature overnight. Then gently wash the surface of the plate under tap water and gently shake the water off the surface. Have any of your samples "eaten" holes in the gelatin?

A.2 Experiment: SALIVA AND ITS SUBSTRATE

Collect a one milliliter saliva sample in a 13 x 100 mm test tube. Add one drop of starch solution to the saliva and mix the test tube by shaking. Set the tube aside. After at least ten minutes, add one drop of iodine solution to the test tube containing saliva. Shake the tube and record the color of the contents of the tube.

Repeat the above procedure, omitting the ten minute delay. Record the color of the contents of the tube.

Add a pinch of meat tenderizer to a 13 x 100
enzymes. Don't dwell on competitive inhibition when talking to students but rather emphasise the idea. After seeing how urease catalyses the hydrolysis of urea but not thiourea or acetamide ask if the students can explain the lack of reaction in the latter two cases. Based on the film, their answers might be that the incorrect substrate could not fit into the active site or else, once fitted in, the reaction could not take place. Ask them how we might check these two theories. If the substrate would fit into the active site but not react to be hydrolysed, then there would be no active enzyme in the thiourea or acetamide test tubes (provided that an excess of thiourea or acetamide were used). If, however, the molecules would not fit into the active site, the presence of active urease should be capable of detection by the addition of urea to the test tube. The phenolphthalein in the test tube would turn pink due to the liberation of ammonia.

In doing tubes #6, 6 and 7 you should do one at a time, i.e., add nothing to a tube until you are ready to follow the complete procedure. Starting with tube #5, add all ingredients asked for in the protocol except 1% urea, and let the tube rest for 2 minutes. Then add the 1% urea and start timing for the color change as you add it. Follow this procedure for the other two tubes. You should expect times of 8 minutes for #5, 10 minutes for #6, and 0.5 minutes for #7. The inhibition is competitive rather than non-competitive because the tube will eventually (about 10
minutes) turn pink.
Thus the active site is not permanently blocked.

The crucial 3-D shape of urease is determined by hydrogen bonding, van der Waals forces and, most importantly, hydrophobic bonding. At temperatures between 60° and 80° C, hydrophobic bonding is greatly weakened because the increased thermal energy of the water prevents "ice sheath" formation around hydrocarbon residues. This allows the protein to unfold without unfavorable energy changes. Upon return to room temperature, thermodynamics favor refolding of the protein chain, but there are an enormous number of ways to fold which satisfy the thermodynamics. Consequently few, if any, enzyme molecules ever find their way back to the proper 3-D shape. This raises a very interesting point—how proteins manage to fold correctly in the first place, during their synthesis. Protein chemists are still arguing over this point.

Notes on Figure A.1 (Fumarase): This reaction was chosen for illustration because it is one of the simplest reactions catalysed by an enzyme and therefore easiest to show schematically. The substrates fumaric acid and malic acid (for the reverse reaction) are given in the doubly charged anion forms (as these are the species present at the neutral pH where the enzyme functions).
mm test tube containing one milliliter of starch solution. After 10 minutes add 1 drop of iodine solution. Record any color that you observe.

What are your conclusions?

A.3 PRELAB DISCUSSION

The enzyme urease catalyzes the hydrolysis of urea.

\[
\text{enzyme} \quad \text{H}_2\text{N} - \text{C} - \text{NH}_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2
\]

\[
\text{Urease} \quad \text{urea} \quad \text{water} \quad \text{ammonia} \quad \text{carbon dioxide}
\]

Make models!

The following will explore the question of how specific the enzyme urease is. Will urease catalyze the hydrolysis of molecules very similar in structure to urea?

Construct models of urea, thiourea and acetamide.

\[
\begin{array}{c}
\text{H}_2\text{N-C-NH}_2 \\
| \\
\text{0}
\end{array} \quad \begin{array}{c}
\text{H}_2\text{N-C-NH}_2 \\
| \\
\text{S}
\end{array} \quad \begin{array}{c}
\text{H}_2\text{N-C-CH}_3 \\
| \\
\text{0}
\end{array}
\]

urea thiourea acetamide

A.3 Experiment: SPECIFICITY OF UREA

Obtain five milliliters of urease solution from your instructor. Add one drop of phenolphthalein indicator to each of the four 13x100 mm test tubes. Add one milliliter of 1% urea solution to each of test tubes 1 and 2, contain-

water and stir to form a paste. Add 95 ml of boiling water to the paste. Boil two minutes and use.

Discussion

1. Students will generally see no color after adding I\(_3\). They will not usually see the red-brown of I\(_3\) because the solution is too dilute. They will not usually see the blue or purple of the starch -I\(_3\) complex because their spit will hydrolyze the starch. However, there is always the rare student who will not have ptyalin in his spit. This rare student should be encouraged to try the experiment on another day to see if his condition is permanent or temporary. The usual cause is gum chewing.

2. Without any delay to allow for enzymatic action the starch should be intact and therefore a blue or purple complex forms with the I\(_3\).

3. Tenderizer will not catalyze the hydrolysis of starch. The blue starch -I\(_3\) complex will form.

4. Film: "Mechanism of an Organic Reaction" (CHEMS)

Materials and Equipment

(for one student)

7 test tubes (13 x 100 mm)
3 droppers
Phenolphthalein indicator solution
Burner
1% urea (w/v) (3 ml)
Urease solution (5 ml)
4% thiourea (w/v) (2 ml)
4% acetamide (w/v) (2 ml)
Preparation of Urease Solution

Allow 10 grams of Jack Bean Meal (Mathison - Coleman - Bell) to soak in 500 ml of 30% (v/v) ethanol for at least ten minutes. Filter the solution and use the urease solution within one week if not refrigerated. Alternatively dissolve 0.1 g of urease in 500 ml of water.

Discussion

1. The pink color of basic (pH 8-9) phenolphthalein will appear for any substance generating ammonia. Because urea, thiourea, and acetamide are so similar in structure, the concept of complementary surfaces on the enzyme urease should be strongly reinforced. Emphasize that the reaction is an hydrolysis and that the enzyme urease simply speeds the reaction.

2. The urease is quickly denatured by heating. Students have not encountered "denaturation" in this course. Discussion should be along the lines of destroying the complementary surface of the enzyme by breaking the weak bonds maintaining the shape of the surface. Mention the obvious physical and chemical differences between a raw and cooked egg.

3. Film: "Biochemistry and Molecular Structure" (CHEMS) (excellent) This film is a necessary introduction to an extension of the experiment covering competitive inhibition of the indicator. To the third tube add one milliliter of 4% thiourea solution and to the fourth add one milliliter of 4% acetamide solution. Set aside test tube No. 1 containing urea. Add ten drops of urease solution to each of the three remaining test tubes. All of this information is summarized in the Urease Protocol on the following page. Record any color changes that occur before the end of the laboratory period.

Boil the remaining urease for ten minutes. After cooling the urease solution to room temperature, add ten drops of it to test tube No. 1, containing urea. Record the color change.

There seems to be a very sensitive relationship between the molecule hydrolyzed (urea) and the enzyme catalyzing the reaction (urease). Even the relatively mild exposure to a temperature of 100° destroyed the catalyzing ability of urease. The following discussion of polymer shape may help explain this sensitive relationship.

Supposing there is a complementary surface relationship between an enzyme and its substrate (the molecule undergoing reaction in the catalyzed reaction). Thus the enzyme is to the substrate what a lock is to a key. There are locks which operate with many keys and locks which are
Table A.1

*Add this ingredient last; for each tube note and record the number of seconds elapsed from the time of this addition to the color change.
The figure illustrates the reaction catalyzed by fumarase. It should give an idea of the active site of this enzyme and how the substrates fumarate and water fit into the active site, reacting to give malate:

\[
\text{Fumarate} + \text{H}_2\text{O} \rightarrow \text{Malate}
\]

As indicated, the active site probably contains two small, positively-charged cavities at the proper locations to specifically bind fumarate into the active site. The imidazole rings (part of the amino acid histidine) to the right and left are the parts of the active site of the enzyme that catalyze the reaction.
specific for one key. There are enzymes which catalyze many substrates and enzymes which are specific for the catalysis of one substrate.

(See Figure A.1)

Experiment A.3 showed that urease is specific for urea, even though the shapes of thiourea and acetamide are similar. This indicates that the fit between enzyme, urease, and substrate, urea, is a close specific fit. Virtually every chemical reaction which occurs in living systems is catalyzed by a particular enzyme.

Exercises for Home, Desk and Lab (HDL's)

(1) What do you call enzymes which break down themselves as well as other proteins?

(2) Are all enzymes as specific in their behavior as was the enzyme urease on urea? Explain.

(3) What would you hypothesize was the effect of the heat exposure of 100°C upon the enzyme urease?

(1) Proteolytic enzymes

(2) No, all enzymes are not necessarily as specific as urease. There are some enzymes which catalyze many substrates.

(3) The effect of heat upon the enzyme urease was to destroy its catalysing ability. This may have resulted because the temperature increase was able to break up the hydrophobic bonding, which in turn destroyed the three-dimensional folding and thus the activity of the enzyme.

Since the temperature change is not very great, one should expect only the weakest type of bond to be broken in the enzyme. Once

Information on the involvement of imidazole rings as well as other amino acid side chains in the catalytic process can be found in The Mechanism of Enzyme Action by S. A. Bernhard (Benjamin Co.), an excellent paperback on enzymes.
the three-dimensional shape of a protein is destroyed, experiment has shown that the enzyme is not capable of reforming these weak bonds and resuming their activity.

(4) The ending of an enzyme is 'ase.'

(5) Active part

(6) No. Saliva catalyzes starch to sugar. Urease catalyzes urea to NH₃ and CO₂. Hamburger contains protein, which is made up of amino acids.

(4) What ending is used to identify an enzyme when the name of the enzyme is written?

(5) In a hydrolysis reaction does water act as a solvent only, a 'spectator' species only or does it play an active part?

(6) Do you think saliva would hydrolyze hamburger? Would urease? Why?
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<td></td>
<td></td>
<td>Scientific American</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Growth requires that plants be started 2 weeks ahead</td>
<td></td>
<td></td>
<td></td>
<td>Oct 1962</td>
</tr>
<tr>
<td>Activation</td>
<td></td>
<td>A.2 The synthesis and breakdown of starch</td>
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<td></td>
<td></td>
<td></td>
<td>Mar 1963</td>
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<td>3 Days</td>
<td></td>
<td>Models</td>
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<td>2, 8, 9, 10</td>
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<td></td>
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<td></td>
<td></td>
<td>Film: DNA molecule of heredity</td>
<td></td>
<td></td>
<td>12, 13</td>
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</tr>
</tbody>
</table>
Chapter VI: POLYMERS TO POLYMERS

Research on living things was greatly speeded up when it became obvious that all different kinds of organisms are fundamentally alike in their chemistry. For example the mechanism of protein synthesis in man is essentially the same as it is in plants or bacteria. By studying bacteria, which are easier to grow in the laboratory than plants or animals, it is possible to learn much that can be applied to more complex organisms.

The enzyme-catalyzed reactions which cells carry out can be divided into three main types. First, there are reactions which convert food molecules into the various monomers—the 20 amino acids, the sugars and the sub-units of the nucleic acids. Second, there are the reactions which convert either light or chemical bond energy into usable energy. This usable energy is then available to activate the monomers. Third, there are the polymerization reactions: the activated monomers are linked together in the proper sequences to produce the polymers which make up the cell. A great deal is already known about the first topic, the breakdown of food.
This is the reaction: nylon + monomers. \( \Delta H \) is negative and \( \Delta S \) is positive, or:

\[ \Delta G = \Delta H - T \Delta S < 0 \]

Aspirin Synthesis Experiment
(equipment for one student)

- 6 test tubes (13x100 mm)
- 1 bunsen burner with ring stand and wire gauze
- 1 250 ml beaker
- 1% (w/v) salicylic acid solution in 1, 4-dioxane (5 ml)
- 1% (w/v) acetyl-salicylate solution in 1, 4-dioxane (1 ml)
- 0.1% (w/v) FeCl₃ solution in H₂O (6 ml)
- Ventilating hood; three teflon stopcock burets containing acetyl chloride, acetic anhydride and glacial acetic acid respectively. Also, eye-

...into small molecules. Less is known about the polymerization reactions. Much remains to be discovered about the formation of usable energy.

A. THE ACTIVATION PROBLEM

In order to make the monomers of nylon polymerize it was necessary to change one of them to a high energy form. The adipic acid was converted to an activated form (adipoyl chloride) which reacted spontaneously with hexamethylene diamine to give nylon and HCl.

Nylon can be considered to have more chemical energy than an equivalent solution of its monomers. As a consequence nylon will react spontaneously with H₂O to yield the monomers. This reaction, however, is extremely slow in the absence of a catalyst.

Activation of one of the reactants is often required to make a reaction "go." When one of the reactants is a carboxylic acid the usual activated form in the laboratory is an acyl chloride; e.g.

\[
\begin{aligned}
\text{acetic acid} & \quad \text{acetyl chloride} \\
\text{CH}_3-\text{C}^\text{acid} & \quad \text{CH}_3-\text{C}^\text{chloride}
\end{aligned}
\]

This functional group of an acyl chloride contains more chemical energy than the carboxyl group. As a result acyl chlorides will
react vigorously with alcohols to form esters:
\[ R-C_Cl + HO-R' \rightarrow R-C-O-R' + HCl \]

or with amines to form amides:
\[ R-C_Cl + H_2N-R' \rightarrow R-C_NH-R' + HCl \]

Another activated form of carboxylic acids is the acid anhydride:
\[ CH_3-C_0-C-CH_3 \]
acetic anhydride

### A.1 Experiment: ASPIRIN SYNTHESIS

#### Aspirin Synthesis Protocol

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Tube Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid 10 mg/ml in dioxane</td>
<td>1 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl salicylic acid 10 mg/ml in dioxane</td>
<td>--</td>
<td>1 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetyl chloride</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic anhydride</td>
<td>--</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>--</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Heat for ten minutes in boiling water bath before adding FeCl₃

| FeCl₃, 1 mg/ml in water      | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |

Table A.1
V. an

I.M. VibC salicylic acid with acetyl

Acetyl %Alltylt( AL

wicOrin, to which tubavs did you obtAin A purple
color? What combination of compounds is responsible
for the purple color? In which tubes was
aspirin (acetyl salicylic acid) formed?

Cells produce polymers, such as starch
proteins and nucleic acids, which also contain
more chemical energy than their monomers. There-
fore, in order to form polymers cells must con-
vert monomers into higher energy derivatives
which are called activated monomers.

Cells activate monomers in quite different
ways than does the organic chemist. One way is
to attach a phosphate group to the molecule to be
polymerized. For example, glucose can be made
to polymerize to starch if it is first converted
to glucose-1-phosphate. Glucose-1-phosphate
reacts reversibly with a starch molecule to form
a phosphate ion and a starch molecule with one
more glucose residue. The reaction is catalyzed
by an enzyme called phosphorylase and can be written as follows:

This reaction of activated monomers is readily reversible: a glucose subunit can either be added to or removed from the end of a starch molecule. Whether the reaction goes in the direction of elongation or breakdown depends mainly on the relative concentration of glucose-1-
ATP has two phosphoric anhydride bonds.

\[
\text{base} \quad 0 \quad \text{O} \quad 0 \quad \text{O} \\
\downarrow \quad \downarrow \quad \downarrow \quad \downarrow \\
\text{0-P-O} \quad \text{P-O} \quad \text{P-OH} \\
\text{sugar} \quad 0 \quad 0 \quad 0
\]

For more detailed information about ATP and its role in energy transfer, the teacher is referred to Watson, The Molecular Biology of the Gene, Benjamin, 1965, Chapter 5; McElroy and Swanson, Modern Cell Biology, Foundations of Biology Program, Part 2, Prentice-Hall, Chapter 10; Lehninger, A., Bioenergetics, Benjamin, 1965, Chapter 4.

Have a demonstration model of ATP built. You could request that the class build this using FMM kits. Make phosphorus tetrahedral ($sp^3$).

Materials and Equipment
(for one student)

4 test tubes (100x13mm)
1 spot plate, if available
1 beaker
1 universal indicator paper

Solutions: (for class)

(1) substrate solution I
- glucose-1-phosphate 0.2 g
- glycogen [primer] 0.2 g
- water 50 ml

(2) substrate solution II
- glucose 0.2 g
- glycogen [primer] 0.2 g
- water 50 ml

Dissolve the glucose-1-phosphate or glucose in 20 ml water. Using universal indicator paper, adjust the pH phosphate and phosphate ion. If glucose-1-phosphate is high and phosphate ion is low, starch is elongated; if phosphate ion is high and glucose-1-phosphate is low, then starch is broken down. How do cells activate glucose to glucose-1-phosphate? The answer is that they use a high energy organic phosphate compound whose name is usually abbreviated to ATP (adenosine triphosphate). ATP is at a higher energy level so it can easily donate one of its phosphate groups to glucose. A large part of the metabolism of cells is devoted to the formation of ATP. This was referred to earlier as usable energy.

A.2 Experiment: THE SYNTHESIS AND BREAKDOWN OF STARCH BY PHOSPHORYLASE

Place one ml of phosphorylase solution in a test tube labeled B (for boiled) and three ml of phosphorylase solution in a test tube labeled C (for cold). Place tube B in boiling water for ten minutes.

Add 1.0 ml of solution containing glucose-1-phosphate and primer (a starch-like polymer which is too short to give a blue color with iodine) to each of four 13 x 100 mm test tubes. To a fifth tube add 1 ml of glucose and primer solution. The numbering of the tubes is given in the Phosphorylase Protocol (page 113). To the first tube add one ml of boiled phosphorylase
solution B. To the second, third and fifth tubes add one ml of unboiled phosphorylase solution C. Add one ml of distilled water to the fourth tube. At zero time and at three minute intervals thereafter, test each reaction mixture for starch synthesis by adding a drop of iodine solution to a drop of reaction mixture on a spot plate. A blue color indicates the presence of starch. As soon as you have evidence of starch synthesis, carry out the following steps. First, add 1 ml of potassium phosphate solution to all test tubes and continue to test for starch.

Next, inactivate the enzyme in tube 2 by placing the tube in a boiling water bath for 10 minutes. How do you explain the disappearance of starch in tube 3 but not in tube 2? Did you expect any starch to be formed in tube 5?

to 6.1-6.3 with HCl and dilute to 25 ml with water.

Dissolve the glycogen in 25 ml water. Boil, filter and mix the glycogen solution with the glucose-1-phosphate (or glucose) solution.

(3) phosphorylase solution

Peel a small potato and cut it into small cubes. Place these in a blender, add 40 ml of 0.01M sodium fluoride and homogenize for 30 seconds. The purpose of the sodium fluoride is to inhibit the potato enzyme phosphatase, which would otherwise hasten the hydrolysis of glucose-1-phosphate to glucose and phosphate.

Filter the homogenate through a double layer of cheesecloth into a beaker. Squeeze out as much of the liquid as you can. Centrifuge the suspension for 3 minutes, then decant and keep the supernatant. Take a small portion of this extract and see that it tests negative to the iodine test for starch. This should be enough enzyme for the whole class and should retain its activity for nearly a week if carefully refrigerated. The enzyme preparation is used at full strength in the experiment.

Because potatoes do contain some short-chain starch (primer), it is not absolutely necessary to add primer in the experiment. However, if glycogen (primer) is added, then starch will appear in less than five minutes. If glycogen (primer) is not added, then it will be about fifteen minutes before starch
appears. Thus if lab time is short in your school, add primer to the glucose-1-phosphate solution. If there is ample lab time in your school, omit primer from the glucose-1-phosphate solution.

(4) potassium phosphate
Make 50 ml of 1 M potassium or sodium dihydrogenphosphate (KH$_2$PO$_4$ or NaH$_2$PO$_4$). Add 13.9 ml of 1 M sodium hydroxide. Dilute the mixture to 100 ml with water.

(5) iodine solution
Dissolve 1.5 g KI in 25 ml water. Add 0.3 g I$_2$; stir until dissolved. Dilute to 150 ml with water.

Starch disappeared from tube 3 because the enzyme phosphorylase catalyzed the phosphorolysis of starch by the excess phosphate ion. This is a simple example of LeChatelier's Principle.

\[
\text{starch} + P_i \rightarrow \text{glucose-1-P}
\]

Tube 2 did not catalyze this reaction because the enzyme phosphorylase had been inactivated by heat.

The starch-I$_2^-$ blue color is due to a clathrate complex (cage complex) in which a section of the helical starch molecule "wraps" around a string of iodine atoms. There is a minimum required length for the starch helix to give the blue color. A shorter length gives a red or purple color. Shorter lengths than this give no color.
**PHOSPHORYLASE PROTOCOL**

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Table A.2
B. THE SEQUENCE PROBLEM OR HOW CELLS MAKE RNA (RIBONUCLEIC ACID)

In Chapter II you saw that RNA is made of four different kinds of monomers linked together to form long chains. The monomers (residues) found in RNA include the bases adenine, guanine, cytosine and uracil (abbreviated A, G, C and U).

Remember that the monomer is the small molecule by itself and the residue is what is left of this when it is in the polymer. The structures of these four bases as well as that of thymine, found in DNA, are shown in Figure B.1 of Chapter IV.

RNA molecules vary from 70 residues to over 10,000 residues in length. Since each sequence of monomers gives a different RNA molecule, the number of possible RNA molecules is so vast as to be beyond comprehension. For example, there are four possible choices (bases) for each position in the sequence of an RNA molecule: For molecules four bases long there are, therefore, \(4^4\) or 256 possible different sequences. How many different sequences are possible for RNA molecules 10 bases long? This means that if the monomers of RNA were polymerized at random no two molecules of RNA could be expected to be alike. However cells do not produce random RNA molecules; rather they produce several thousand
specific types of RNA molecules. Each kind of cell has its characteristic set of RNA molecules and it can produce many copies of each kind.

Thus the cell is faced with two problems in synthesizing RNA. It must activate the monomers of RNA so that polymerization will be favored, and it must direct the sequence of polymerization so that the correct RNA molecules are formed. The first task requires ATP, which donates phosphate groups to the other monomers to form triphosphates. ATP is itself an activated monomer of RNA, but its formation is an extremely complex process.

\[
\text{base} \quad \text{sugar} \quad \text{phosphate}
\]

\[
\text{adenine} \quad \text{ribose} \quad \text{phosphate}
\]

\[
\text{adenosine monophosphate (AMP)}
\]

\[
\text{adenine} \quad \text{ribose} \quad \text{phosphate} \quad \text{phosphate}
\]

\[
\text{adenosine diphosphate (ADP)}
\]
The activation of the other monomers occurs by means of the following types of reactions. We will use the activation of uridine monophosphate (UMP) as an example.

1. $\text{UMP} + \text{ATP} \rightleftharpoons \text{UDP} + \text{ADP}$
2. $\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$

Here U stands for the base-ribose combination, uracil-ribose, called uridine. Thus the activation of UMP requires the "expenditure" of two ATP's. These ATP's are replenished by means of the energy-capturing reactions of the cell.

The second task requires a molecule which will align the monomers in proper sequence for polymerization. This molecule is DNA. A common term for this type of alignment device is template; DNA is a template molecule.

The polymerization reaction may be expressed as follows:

See Part IV, Chapter I for a presentation of energy capture to form ATP.

Dihydrogen pyrophosphate is:

\[
\begin{align*}
\text{HO}_2&\text{-PO}_2\text{-OH} \\
\text{O} & \text{O}
\end{align*}
\]

formed by buffering action of cell medium.
Uracil and thymine have the same base pairing properties.

where P-P stands for the inorganic ion, pyrophosphate. The RNA formed has the same base sequence as one of the DNA strands except that the base uracil is substituted for the base thymine of DNA. Precisely how the enzyme RNA polymerase and template DNA cooperate to specify the RNA base sequence is not known. Almost certainly hydrogen bond base pairing between the incoming activated monomers and the template DNA strand is an essential factor.

Figure B.1
C. THE SEQUENCE PROBLEM CONTINUED--HOW CELLS MAKE PROTEINS

The character and individuality of cells arise from the protein molecules they are able to synthesize. As enzymes these molecules determine the pattern of reactions the cell can carry out as well as the shape and size of cells (structural proteins and regulatory proteins). As you have seen, protein molecules can assume great varieties of shapes, depending primarily on their sequence of amino acids. Because of this, life itself has enormous diversity, versatility and adaptability. Proteins are the vehicles of expression of the hereditary traits.

As in the case of RNA the number of different protein molecules possible is enormous, yet a given cell makes only a limited set of protein molecules. Again as in the case of RNA, the cell must both activate the amino acid monomers and polymerize the activated amino acids in proper sequence. This turns out to be a more complex process than the production of RNA.

The amino acids are activated by attachment to a special type of RNA called transfer RNA or tRNA for short. There are about 40 different tRNA molecules, each about 70 residues in length. Each tRNA can attach to one and only one amino acid. (Since there are only 20 different amino
The attachment of an amino acid to its transfer RNA goes in two steps:

1. **Amino acid** + **ATP** + activating enzyme → amino acid-AMP-enzyme + P-P

   or

   \[ R-C-C_2 + \text{ATP} + \text{activating enzyme} \rightarrow \text{NH}_2 \]

   \[ H-O \]

   \[ R-C-C_2 + \text{ATP} + \text{activating enzyme} \rightarrow \text{NH}_2 \]

   \[ H-O \]

2. Amino acid-AMP-enzyme + tRNA → amino acid-tRNA-enzyme + AMP

   or

   \[ R-C-C_2 + \text{AMP} + \text{tRNA} \rightarrow \text{NH}_2 \]

   \[ H-O \]

   \[ R-C-C_2 + \text{AMP} + \text{tRNA} \rightarrow \text{NH}_2 \]

   \[ H-O \]

Note activation of amino acid by anhydride formation.

Acids in proteins, some amino acids must have more than one corresponding tRNA. The linking of a given amino acid to its tRNA requires ATP and a special enzyme.

The sequence of amino acids in a protein is determined by the sequence of bases in an RNA template called messenger RNA or mRNA. You will remember that a DNA strand acts as a template for RNA by forming hydrogen bonds with the activated RNA monomers. However amino acids by themselves can't hydrogen-bond to DNA or RNA. Therefore, in order for mRNA to act as a template for amino acid polymerization it is necessary for the amino acids to be linked to adaptor molecules that can hydrogen-bond to the bases of the mRNA. This is precisely what the tRNA molecules do. When tRNA molecules linked to their amino acids align themselves on mRNA, the amino acids are brought close together, and since they are in an activated state they can polymerize (in the presence of an enzyme). Thus the tRNA has two roles: it is an activator and it enables the base sequence of an mRNA molecule to be "translated" into the amino acid sequence of a protein molecule.

What are the rules for this translation - in other words, what is the 'code' by which the
"sentences" of mRNA are translated into "sentences" embodied in the structure of proteins? In the past 10 years a great deal of work has been done on the coding problem, using isolated protein-synthesizing systems from many types of cells. It has been possible to use chemically synthesized mRNA of known base sequence to direct amino acid polymerization and then to analyze the protein formed for its amino acid sequence.

The genetic code is now completely cracked. It is a triplet code—that is, it takes a sequence of three consecutive nucleotides in the mRNA to specify one amino acid. In protein synthesis the mRNA is "read" three nucleotides at a time, starting from one end of the molecule and proceeding to the other without skips. Each triplet of nucleotides is called a codon. There are 64 possible codons—that is, there are $4^3$ (64) possible sequences of A, G, C and U taken three at a time. There are only 20 amino acids in proteins, and it turns out that several codons can specify the same amino acid. For example, the amino acid glycine is specified by any one of the following codons: GCU, GCC, GCA, GCG. All but three of the 64 codons have been assigned to a specific amino acid. The remaining three
codons are probably used to end a protein molecule; that is, they serve the same purpose as a period does for a sentence.

Since each amino acid to be polymerized is aligned on the mRNA, it follows that each kind of tRNA must have a specific "anti-codon"—a sequence of bases which can hydrogen-bond to the codon of the mRNA. For example, if the codon on the mRNA is UUU then the anticodon on the tRNA is AAA.

The mRNA and the various tRNA-amino acid combinations are brought together in or on the ribosomes. Ribosomes are particles found in the cytoplasm of cells. (See Figure C.2.) They are composed of RNA and protein, and it is here that the actual polymerization of amino acids takes place. The process of protein synthesis can be diagrammed as in Figure C.1.
Figure 6.1
Figure 6.7 An Idealized Animal Cell
D. THE SEQUENCE PROBLEM COMPLETED OR THE MASTER MOLECULE

You have seen that the polymerization of amino acids is not a random polymerization, because the insertion of amino acid subunits is directed by the sequences of bases in mRNA. The polymerization of RNA, in turn, is controlled by the sequence of base pairs in DNA. Thus the structures of all proteins in the cell are controlled indirectly by the base pair sequence of DNA.

When DNA is synthesized, where are the instructions for its polymerization? The answer is: in the pre-existing DNA molecule. Thus DNA combines two types of instructions in its base sequence—one for RNA and one for its own replication. It is this dual role of DNA which makes it unique—the master molecule. Because of the specific pairing that exists between the bases, the two strands of DNA are complements of each other. This means that each strand of the double helix can act as a template for the synthesis of the other.

As in the case of RNA synthesis, DNA synthesis requires activation of monomers to the triphosphate level and the presence of a specific enzyme or enzymes. One of the enzymes involved in DNA replication, called DNA polymerase, has


Actually DNA only contains one type of instruction or information—namely its sequence. This sequence is replicated into an RNA molecule by one enzyme and into a new DNA molecule by another enzyme. Note: for RNA transcription only one of the two DNA strands is read, while both are read in making a DNA copy.
been obtained in pure form and used to study DNA replication in the test tube. Not all aspects of DNA replication have been worked out, but it is known that replication starts at one end of the molecule and proceeds to the other and that each of the two daughter molecules consists of one old strand and one newly-synthesized strand. This is what would be expected if replication occurs as diagrammed on the following page.
Figure D.1

DNA REPLICATION
7.5 cm of DNA would be around \(2 \times 10^8\) base pairs in length. Assuming that most protein chains are 500 amino acids in length, the figure of 100,000 different proteins is obtained.

Symbiosis is biological cooperation between two different kinds of organisms.

It turns out that DNA molecules are the longest of the biopolymers. Probably each chromosome of the cell contains only one huge molecule of DNA. In the case of human chromosomes this means that single DNA molecules would be as much as 7.5 cm in length. Each such DNA molecule could code for up to 100,000 different proteins.

E. MOLECULAR SYMBIOSIS

The polymers of the cell interact in a way that might be described as symbiosis on the molecular level. Each kind of polymer contributes to the synthesis of the other kinds. DNA acts as a storehouse of information for the synthesis of the thousands of protein molecules of the cell; RNA serves as a part of the translation machinery; and the proteins themselves are the universal catalysts and building blocks of the cell. Without proteins, nucleic acids could not be synthesized.
Exercises for Home, Desk and Lab (HDL's)

(1) In the polymerization of glucose to starch, the glucose first must be activated. What is the activated form of glucose?

(2) In the formation of RNA what are the activated monomers?

(3) When amino acids are polymerized to proteins, how are the amino acid monomers activated?

(4) Itemize the reagents needed by a cell to produce protein containing ten different amino acids.

(5) What are the general types of reactions which are catalyzed by enzymes in cells?

(6) a. What are two activated forms of acetic acid?
   b. What is the structure of these two activated forms?
   c. Write out two reactions which may occur with one of the activated forms.

(1) Glucose - 1 - phosphate
(2) ATP  GTP
     CTP  UTP
(3) With transfer RNA (tRNA)
(4) Ten amino acids
   1 tRNA for each amino acid (10)
   1 ATP to attach each tRNA to its amino acid (10)
   1 enzyme to catalyze each tRNA-amino acid attachment
   1 mRNA for template
   Enzymes to polymerize the activated amino acids on template in RNA
   If we go back a step and make the DNA from which the RNA is formed, the list becomes enormous.

(5) a. Foods (polymers) are converted into monomers (amino acids).
   b. Chemical bond energy is used to activate monomer.
   c. Polymerization reactions, linking the activated monomers in the proper sequence.

(6) a. Acetyl chloride and acetic anhydride are two activated forms of acetic acid.
   b. CH₃-C\(\text{Cl} \equiv \text{O}\)  CH₃-C\(\text{O} \equiv \text{C} \equiv \text{O}\)  C\(-\text{CH}_3\)
   c. Possibilities:
      CH₃-C\(\text{Cl} \equiv \text{O}\)  +  HO-R  \(\rightarrow\)  CH₃-C\(\text{O} \equiv \text{C} \equiv \text{O}\)  +  HCl
      an ester
      CH-C\(\text{Cl} \equiv \text{O}\)  +  H₂N-R  \(\rightarrow\)  CH₃-C\(\text{NH} \equiv \text{R}\)  +  HCl
      an amide
(7) a. phosphorylase
b. The starch polymer is elongated if glucose - 1 - phosphate ion concentration is high and phosphate ion concentration is low.
c. If the phosphate ion concentration is high and glucose - 1 - phosphate is low, then starch is broken down.

(8) The cell must activate the monomers of RNA so that polymerization will be favored, and it must direct the sequence of polymerization so that the correct RNA molecules are formed.

(9) DNA

(10) RNA differs from DNA in that the pyrimidine, uracil, has replaced the pyrimidine thymine. In addition, RNA is based upon ribose (a pentose sugar) while DNA is based upon desoxyribose.

(11) The sequence of amino acids in a protein is determined by the sequence of bases in an RNA template called messenger RNA, labelled mRNA.

(12) DNA combines two types of instructions in its base sequence—ones for RNA and thus proteins and one for its own replication.

(13) In or on ribosomes.

(7) Cells may activate the monomer glucose by adding a phosphate group.
a. What enzyme polymerizes the activated monomer into the glucose primer?
b. Under what conditions does the reaction proceed to elongate the primer?
c. Under what condition does the reaction proceed to shorten the elongated polymer?

(8) What are two problems a cell is faced with in synthesizing RNA?

(9) What is the template molecule for RNA?

(10) In what way does RNA differ from DNA?

(11) How is the sequence of amino acids in a protein determined?

(12) DNA in its unique role as a master molecule carries two types of instructions for the molecules of a cell. What are they?

(13) Where does the cell manufacture proteins?
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A. MISTAKES IN GENETIC MATERIAL

In order for cell duplication to be precise, it is necessary that the genetic material (DNA) be replicated exactly and distributed to the daughter cells. The fidelity of DNA replication is extremely high, but it is not perfect. There is a small but significant chance of mistakes whenever a DNA molecule is replicated. Mistakes can be of several kinds. The simplest and most frequent is the random insertion of the wrong monomer, leading ultimately to the change of a base pair. Other kinds of accidents may involve addition or deletion of base pairs or other gross rearrangements of the DNA base sequence. Often such errors are caused by radiation or by certain chemicals. Whatever the nature of the accident, once made it is replicated with the same high fidelity as the original sequence.

Figure A.1

A base change mutation can come about as follows:

![Diagram of DNA replication and mutation process]
Two points: 1. Mutation can show up (be expressed in the phenotype) after either the first or second round of replication. Remember that only one strand of the DNA is used as a template for mRNA synthesis. We will call this the "sense" strand and the complementary strand the "anti-sense" strand. If the incorrect base is put into the "sense" strand, immediately all mRNA and protein derived therefrom are altered. If the mutation is in the "anti-sense" strand, obviously a "sense" strand (with the mistake) must be copied before the mutation is expressed.

2. Only one of the four granddaughter DNA molecules is mutant.

Examples of mistakes where protein alterations do not occur are:

1. a base pair change of mRNA codon to a synonym, e.g. GCU to GCC, both of which designate glycine; or

2. a base pair change in a segment of the DNA which codes for ribosomal RNA, i.e., does not specify a protein.

Show the PKU film "PKU Detection" available from the Oregon State Dept. of Health.

A prepared slice showing sickle cells can be obtained from Carolina Biological Supply, Cat. #PH-1015 Sickle Cell Anemia - $1.25 per slide. The base pair change would be from A- T to T - A corresponding to an mRNA codon change from GAA to GUA.

This ability of the genetic material to perpetuate sequence changes is a very important property of living things. It is the source of the enormous diversity found in the biological realm. Any inherited alteration of DNA is called a mutation. Many of these change the sequence of an mRNA molecule and thus the sequence of a protein molecule.

3. EFFECTS OF MISTAKES

A change of even one amino acid in the amino acid sequence of a protein can have profound effects. A classical example of this is provided by sickle cell anemia. Certain people carry a mutant gene controlling the amino acid sequence of part of the hemoglobin molecule. The mutant hemoglobin sequence differs from normal hemoglobin by only one amino acid out of a total of about 140. Position six of the mutant hemoglobin is occupied by an amino acid called valine, while normal hemoglobin has an amino acid called glutamic acid at this position. In all other respects the two sequences are identical. Yet because of this single amino acid difference the mutant hemoglobin is less soluble than normal hemoglobin. It tends to crystallize within the red blood cells, especially when the oxygen concentration is low. The hemoglobin
stals distort the red cells into odd cres-
shapes--hence the name sickle cell. The
sickle cells are rapidly destroyed by the body,
apparently because they are damaged. For this
reason the afflicted individual develops severe
anemia and usually dies by the age of two.

This whole train of events is probably the
result of the change of a single base pair in
the DNA of the hemoglobin gene. There are many
examples in which the change of a single amino
acid in the structure of an enzyme strongly af-
facts the catalytic activity of the enzyme.
The most frequent effects are complete loss or
drastic reduction in catalytic activity. This
should not be surprising, since natural selec-
tion has been refining the catalytic efficiency
of most enzymes for many millions of years.

C. CAUSING MISTAKES

If some mutations are the result of base
pair changes causing amino acid changes, it
should be possible to find chemicals which in-
crease the possibility of mistakes in DNA rep-
lication and which therefore increase the fre-
quency of mutation. One such chemical is
bromouracil (BU). This compound has a struc-
ture which is identical to thymine except that
a bromine atom is substituted for the methyl
group of thymine.

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BU Tautomers

BU exists as an equilibrium mixture of two tautomeric forms—the keto and the enol tautomers. The keto form is the predominant one.

Bromouracil is able to "fool" DNA polymerase. Cells grown in the presence of BU incorporate it into their DNA. However, the pairing properties of BU are not as precise as those of thymine: BU usually pairs with adenine, but sometimes it exists in a form which pairs with guanine. Because of this imprecise pairing behavior, DNA containing BU is much more likely to make mistakes during replication. Therefore, BU greatly increases the frequency of mutations.

Each gene (section of DNA which specifies a protein) has a characteristic probability of detectable error during each replication. For most genes this spontaneous mutation rate is somewhere around 1 mutation for every $10^7$ to $10^8$ replications. The use of a mutagen (mutation inducing agent) such as BU may increase the probability of mutation by a factor of 100 or 1000, but even so we are dealing with a very small fraction of mutational events (1 in $10^5$
replications). In studying the effects of mutagens, therefore, it is necessary to use large populations. Microorganisms are especially suited for this because very large populations can be grown easily and very rapidly.

It is convenient, in determining whether a compound is a mutagen, to choose a test organism which is already a mutant and therefore is deficient in some enzymic activity. It determines whether the presumed mutagen can revert the original mutation, thereby restoring the missing enzymic activity.

**Exercises for Home, Desk and Lab (MDL's):**

1. Three types of mistakes possible in DNA replication are:
   - a. Random insertion of wrong monomers
   - b. Addition of base pairs
   - c. Deletion of base pairs

2. A mutant DNA molecule can transfer a change in sequence down to the proteins. Trace this transfer.

3. What evidence do we have in this chapter that the enzyme DNA polymerase is not specific?

4. What are sections of DNA which specify a particular protein called?

5. If a mutant bacterial strain lacks the ability to synthesize the amino acid alanine, under what conditions might this strain be healthily grown?

---

**Error of Incorporation:**

- Cytosine → Thymine (rather than guanine)

**Error of Direction:**
- Cytosine → Thymine (rather than guanine)

- Many species of bacteria can double every 20 minutes in a rich medium.

---

- a. Random insertion of wrong monomers
- b. Addition of base pairs
- c. Deletion of base pairs

2. The mutant DNA would transfer the error to mRNA, thus affecting the sequence of a protein molecule.

3. Bromouracil is able to fool DNA polymerase in cells grown in the presence of bromouracil so that it is incorporated into their DNA.

4. Genes

5. The mutant bacterial strain which lacks the amino acid alanine could be grown if that amino acid was supplied by an outside source, such as being supplied in the bacterial nutrient agar.
(6) 2AP is a mutagen. Base sequence would show a mutation if 2AP rather than T is incorporated into protein.

(7) No. It would be a phenotypic change only. It would not be inherited.

(6) The compound 2-aminopurine or 2AP can be mistakenly incorporated into DNA in place of adenine (A). During replication 2AP usually pairs with thymine (T), but sometimes with cytosine (C). Is 2AP a mutagen? If so, how could it induce a change in base sequence?

(7) Suppose that a medicine was developed for sickle-cell anemia which acted by directly changing the valine at position 6 in the mutant hemoglobin back to glutamic acid, so that the mutant hemoglobin is converted to normal hemoglobin. Would this induced change in the hemoglobin be a mutation?
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2. See page 154, T.G. for D.1.
3. Re-do Ex. B.1, standardizing the effects of heat on the manometer gas Ex. B.2
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</table>
Chapter I: ENERGY CAPTURE

A. INTRODUCTION

Life requires energy. There is no alternative; energy is required for all the things a living system must do. Most animals, including humans, get this energy and the raw materials for growth by eating other animals and plants. Since the process of converting food into living material is less than perfectly efficient (recall the second law of thermodynamics), each step down the food chain sustains a smaller amount of life. Without an outside source of energy it is clear that the living community would soon starve itself. Ultimately there must be an energy input from a non-living source. That source is, of course, the sun, and its energy is converted into living material by photosynthesis. On land and sea this process takes place predominately in green plants. Therefore, they are the ultimate food producers for the entire living community.

Most photosynthesis takes place in the oceans by single-celled organisms called algae or phytoplankton. The chemical reactions which occur during marine photosynthesis are almost

For example it takes many pounds of feed to make one pound of cow, while the more efficient consumer, the bacteria E. coli, will make almost half a gram dry weight of E. coli from one gram of glucose.

The sun is also the ultimate source of coal, oil and hydroelectric power.

At least 60% of all photosynthesis takes place in the top layers of the ocean.

Only about 0.1% of the total incident light energy from the sun is captured via photosynthesis.
Materials and Equipment

Bean seeds (40 per squad of students) Plant seeds at least two weeks prior to anticipated time of analysis

Planters or a number of \( \frac{1}{2} \) gallon milk cartons. (Cut the cartons down to a height of approximately six inches.)

The two groups of leaves should give a significant amount of photosynthesis so that a difference in the weight of the groups becomes apparent.

The student should obtain results that clearly demonstrate a weight difference between plants growing in light that actively photosynthesize and those plants growing in the dark that do not photosynthesize. These results should indicate the necessity of light for the photosynthetic process.

exactly the same as occur in land plants.

In both cases the plants use light as the energy source to build up the complicated polymers of which they are made. This very complex process we can investigate partially. For a start we can ask if light is necessary for plant growth.

A.1 Experiment: LIGHT AND PLANT GROWTH

Weigh two groups of bean seeds, each group containing 20 seeds. Plant the seeds in planters (20 in each) or in one-half gallon milk cartons. Since each carton should contain 5 seeds four cartons will be equal to one planter (group of twenty). One group of 20 seeds will be grown in the dark and the other group of 20 will be grown in a lighted area. When the second cluster of leaves is apparent on those plants growing in the light, remove both groups of plants (those growing in the dark and those growing in the light) and compare the relative growth rates by comparing the lengths. Then remove the soil, rinse away all of the soil debris and dry the plants (by groups) in an oven at approximately 100 degrees Celsius. After drying, weigh each set of bean plants and make an analysis based on the following:
If there is no drying room available, the plants may be dried using an infrared or heat lamp.

If students question the non-drying of the original bean seeds, let some member of the class dry the bean seeds. The weight loss is about 10%.

1. Students should compare the average masses of the light and the dark groups. They may also wish to construct histograms.

2. Growth = increase in dry mass.

4. If the students feel that air "quality" is an important factor in the difference, then have them devise a set-up that circulates the same air by both sets of seedlings.

Light will have many effects on plant development: the main point for the student to get is that light, as measured by the increase in dry mass of the seedlings over to dry mass of the seeds, occurs only in the light.

The main elements are C, N, O, P and H, with a little S. C constitutes about 50% of the dry mass, N about 14%, O 25%, and H about 6%. P, S and salts

1. Compare the dry mass of the plants growing in the light with those that have been in the dark.

2. Does the relative growth rate of bean plants seem to be affected by dark or light environment?

3. Using the information gathered in this experiment, define growth.

4. If the bean seeds kept in the dark do not grow, how does one account for this relative period of no growth?

If it is found that light is necessary for growth, we can ask two questions:

1. What environmental materials does the plant incorporate in order to grow?

2. What colors of light promote plant growth?

Answering the first question involves making some guesses and then testing them. From your
In what chemical forms were these elements available in the environment during your experiment "Light and Plant Growth?"

A.2 THE SOURCES OF CARBON AND HYDROGEN USED IN PHOTOSYNTHESIS

Our guess is that plants obtain their carbon from CO₂ and their hydrogen from H₂O. Let's test these hypotheses by measuring the uptake of radioactive carbon dioxide [¹⁴CO₂] and the evolution of a gas (O₂) during photosynthesis.

A.2.a Experiment: CARBON DIOXIDE UPTAKE DURING PHOTOSYNTHESIS

Place a 15-centimeter sprig of Elodea in each of two (25 x 150 mm) test tubes. Completely cover one with aluminum foil so that light cannot reach the Elodea. Add enough 0.3% bicarbonate solution to each test tube to completely cover the plant. Take the test tubes up to the instructor, who will add several drops of radioactive bicarbonate solution to each test tube. Allow both test tubes to remain in a brightly lighted place for at least 40 minutes.

After a period of 40 minutes, wearing plastic gloves, pour the radioactive bicarbonate reading in Chemistry of Living Matter what kinds of elements must the plant take up in order to make proteins, carbohydrates and nucleic acids? Let's concentrate on C and H for now. In what chemical forms were these elements available in the environment?
solution from the test tube into a sink with running water. The Elodea should be rinsed thoroughly, placed on a paper towel and gently blotted. Wash the contents of the test tube down the sink and then rinse the test tubes carefully and thoroughly.

Place the Elodea back in the test tubes (be sure to label correctly) and dry overnight in a 100°C oven.

Prepare planchets carrying the dried sprigs according to your teacher's instructions. Determine the radioactivity of each sprig by ascertaining the counts per minute of $^{14}$C. Save the dried sprig which was illuminated for experiment E.i.

1. Are both sprigs of Elodea significantly radioactive?
2. Is carbon incorporated into either Elodea sprig?
3. Is light necessary for the uptake of carbon?

An additional experiment involves collecting considerable gas from a large amount of Elodea photosynthesizing for perhaps 24 hours and then using the glowing splint test to show the gas is O$_2$.

With a little luck you have just shown that with light the plant converts $^{14}$CO$_2$ into some-

Plastic gloves
Geiger-Mueller counter or radiation level scaler
0.3% NaHCO$_3$ solution
Test tubes 25 x 150 mm.

Fresh, green, young sprigs of Elodea must be dark adapted (kept in the dark for 24 hours) or they will not perform at maximum efficiency. The "food" stored in the Elodea plant must be used up in the dark so that maximum photosynthesis can occur when exposed to light.

The teacher should have the student bring his or her test tube containing Elodea covered with 0.3% bicarbonate solution up to a central place where he will then add 1.0 microcurie of $^{14}$C bicarbonate solution to each tube. To obtain a convenient working volume the teacher must dilute the radioactive bicarbonate solution with non-radioactive 0.3% NaHCO$_3$ solution to a concentration of about 5 µc/ml.

Before counting the $^{14}$CO$_2$ uptake in the leaves the student should first determine the background count per minute for a period of three minutes. One background count for the entire class is sufficient. The background count should be recorded on a data table in an appropriate column labeled CPM (counts per minute). Broiler-grade aluminum foil is suitable for making planchets. The latter are simply discs of foil of a diameter that will fit your counting set-up. These can be cut out with a razor blade or scissors. The dried Elodea sprig is very fragile and light.
Pulverize the dried Elodea against the bottom of the test tubes with a long stirring rod. Push the aluminum foil planchet over the mouth of the test tube forming a cap. Remove the cap, smear the inner side of the cap with Elmer's glue, place the cap back on the test tube and invert the test tube so that all of the Elodea pieces will fall into the cap. The cap (planchet) is now ready to be counted.

Results:

The tube covered with aluminum should show only a small increase in CPM's, if any, whereas the tube not covered should indicate an increase in CPM's of about 200. These results are presumptive for the uptake of carbon dioxide in the photosynthetic process. As bicarbonate is the only tagged material the interpretations are obvious.
thing in the plant and that a gas is given off in the process (try splint test). The \( \text{C}_2 \) probably comes from the splitting of \( \text{H}_2\text{O} \).

\[
2\text{H}_2\text{O} \xrightarrow{\text{hv}} 2[\text{H}^+] + 2\text{H}^+ + \text{O}_2
\]

But did your experiment prove that \( \text{H}_2\text{O} \) was the source of oxygen? What the \( ^{14}\text{CO}_2 \) is converted into is still another mystery which we will try to solve in another experiment.

First, however, let's look more closely at the light involved. For a start we will try to find out about light and photosynthesis, varying first light intensity and then wavelength.

B. QUANTITY OF LIGHT IN PHOTOSYNTHESIS

B.i Experiment: LIGHT INTENSITY AND OXYGEN EVOLUTION

Set up the equipment as shown in Figure B.1. Place Elodea and sodium bicarbonate solution in the test tube with the solution exactly half filling the test tube. With the clamp removed, carefully insert the stopper as tightly as possible. Attach the long rubber tubing to the manometer and place the test tube in the Erlenmeyer flask with water.

Place the lamp at a distance of 40 cm from Elodea. Be certain to measure the distance between the test tube and the light bulb and not the base of the lamp. Allow the apparatus to stand for five minutes before placing the clamp.

Use the apparatus illustrated in Fig. B.1, modified by replacing the manometer by a test tube filled with water.

The exact chemical nature of the hydrogen \( /\text{H}^+ / \) produced is complicated, but essentially it is in the form of a strong reducing agent.

No. It could have come from the \( \text{CO}_2 \) or somewhere else. Experiments have shown that the source was \( \text{H}_2\text{O} \), by using water labeled with the heavy isotope \( ^{18}\text{O} \) and showing that the \( \text{O}_2 \) evolved was \( ^{18}\text{O}_2 \).
on the short rubber tube. This is done in order to allow the solution to become saturated with oxygen. Fasten the clamp. Tap the test tube before each reading in order to free any trapped bubbles of oxygen.

Immediately observe and record the reading of the manometer. The reading may be made at either end of the manometer tube, but all readings must be made thereafter at the same end of the manometer tube.

Take manometer readings at two-minute intervals until the rate has been stabilized, that is, until the same amount of oxygen is evolved during at least three consecutive two-minute intervals.

Once the rate of oxygen evolution has been stabilized, record the rate. Move the light source to a distance of 20 cm from the test tube and repeat steps 3 and 4 of the above procedure.

When the rate has been stabilized at a distance of 20 cm, record the rate. Move the light source to a distance of 10 cm from the test tube. Repeat the above procedure and record the rate at 10 cm.

Plot the data, using the distances traveled (mm) by the colored solution in the manometer as the vertical axis and time in minutes as the horizontal axis.

1% sodium bicarbonate 100 ml
Meter stick
Lamp with 100 watt bulb
Masking tape
Test tube, 25 mm x 150 mm
1 two-holed stopper to fit test tube
2 pieces of glass tubing each about 6 cm long
1 piece of rubber tubing 5 cm long
1 clamp
1 piece rubber tubing 10-15 cm long
1 manometer tube (glass) bent to form a "U" with each arm 20-24 cm long (inside diameter of manometer tube 2-3 mm)
Colored water or food coloring to put in manometer to height of 3-4 cm in each arm (add several drops of detergent solution)
1 piece of cardboard, approximately 25 x 10 cm, which is stapled to a sheet of millimeter-ruled graph paper as background for the manometer tube.

NOTE: Oxygen production is proportional to light intensity only at low light intensity. If the light intensity gets too high, then oxygen production is limited by other factors like CO₂ availability, etc.
1. mm of O₂/unit time

2. To allow for equilibration of O₂ and temperature (water has to become O₂-saturated before O₂ gas production is constant).

B.2 Optional Experiment: RELATIONSHIP OF CO₂ CONCENTRATION TO RATE OF PHOTOSYNTHESIS

Set up the apparatus as in Experiment B.1 with the light 10 cm from the Elodea. Submerge the Elodea consecutively in the following concentrations of sodium bicarbonate solution and measure the rate of oxygen evolution in each: 0%, 0.25%, 0.5%, 0.75%, and 1.0%.

One sprig of Elodea usually will "wear out" before all concentrations are used. There are several ways to circumvent this problem, such as using several sprigs of Elodea. You could discuss this with the class and elicit the various methods of standardization which could be applied to normalize results from different sprigs.

Construct a graph showing the relationship of rate of oxygen production (vertical axis) and carbon dioxide concentration (horizontal axis).

Remember:
\[ E = h\nu \]
so which color of light has the most energy? The least energy?

1. How did you measure the rate of photosynthesis?

2. Why is it necessary to wait five minutes before taking readings on the manometer?

C. LIGHT QUALITY AND PHOTOSYNTHESIS

The effect of light quality on photosynthesis can be investigated if we separate light into its component colors. Then we could illuminate different plants with different colors of light and determine which grow and which don't. A prism separates white light into its colors. The colors and their wave lengths are:

<table>
<thead>
<tr>
<th>Color</th>
<th>Wavelength Range λ</th>
<th>Frequency Range ν</th>
</tr>
</thead>
<tbody>
<tr>
<td>violet</td>
<td>3800-4200 Å</td>
<td>8 x 10¹⁴ waves/sec.</td>
</tr>
<tr>
<td>blue</td>
<td>4200-4900 Å</td>
<td></td>
</tr>
<tr>
<td>green</td>
<td>4900-5350 Å</td>
<td></td>
</tr>
<tr>
<td>yellow</td>
<td>5350-5900 Å</td>
<td></td>
</tr>
<tr>
<td>orange</td>
<td>5900-6400 Å</td>
<td></td>
</tr>
<tr>
<td>red</td>
<td>6400-7000 Å</td>
<td>4 x 10¹⁴ waves/sec.</td>
</tr>
</tbody>
</table>

Light of wavelengths shorter than 3800 Å or longer than 7000 Å is invisible to the human eye; the first is called ultraviolet radiation and the second infrared radiation. Plants use light in the visible spectrum for photosynthesis. A practical way of separating out the color wanted is
to use colored cellophane. For example red cellophane looks red because it allows only light in the wavelength region 6400-7000 Å to pass through. Thus we could wrap our plants in various colors of cellophane and expect to get only the color of light transmitted by the cellophane. Then we could measure either total growth as before, or we could follow CO₂ uptake or O₂ evolution as in the second experiment; these latter measurements would be faster than total growth.

C.1 Experiment: LIGHT COLOR AND PHOTOSYNTHESIS

Set up the apparatus as in Experiment B.1. Wrap colored cellophane around the Elodea test tubes. Place the Elodea at a distance from the light given by your teacher.

Measure the rate of oxygen evolution. Construct a graph showing the relationship of oxygen production (vertical axis) and light color (horizontal axis).

The experiment shows us that some wavelength ranges are much more effective than others in causing photosynthesis and in particular that green light is most ineffective. Perhaps this is why most photosynthesizing plants are green. They don't use the green light, and therefore it is not absorbed. Let's now turn the argument around. Light that is effective in photosynthesis...
calibrated relative to each other, as to the amounts of light energy transmitted.

When the student runs the experiment, they should place their cellophane-covered test tubes at the calibrated distances.

Because of the time involved each team of students should use cellophane of only one color. Place their data on the board. Have each student plot all of the class data. It is therefore important that each team use an Elodea sprig of the same size.

Materials and Equipment
(for one student)

Elodea (15 cm sprig)
Acetone (20 ml)
Mortar and pestle
Test tubes (13 x 100 mm)

C.2 Experiment: ABSORPTION SPECTRUM OF GREEN PLANT PIGMENTS

Place a 15 cm sprig of Elodea in a mortar, add to it 10 ml of acetone and grind the Elodea using a pestle. Save the supernatant solution for this experiment and the next one. Take readings as indicated below at successive 20 millimicron intervals on the spectrophotometer. (See instructions below.)

A spectrophotometer can be used to make precise measurements of pigment absorption of specific light wavelengths over the entire visible spectrum. From these data a plot of the absorption spectrum can be prepared. Because of the variation in the composition of pigments from plant to plant, as well as differences in different measuring instruments, the plot you are to prepare will perhaps differ from the one you will see in general textbooks.

The student should plot the spectrum and get a curve showing a primary absorption peak in the blue-green, green region and a secondary
instructor. Briefly, the general outline is as follows:

1. Turn on the instrument and allow five minutes for warm-up.
2. Set the zero point on the transmittance scale with nothing in the sample holder.
3. Fill one of the special spectrophotometer tubes with the acetone solvent being used (to be called the blank) and insert this in the sample holder. Turn the wavelength control to 430 millimicrons, and set the absorbency at zero with the light control. (You will have to repeat this blank setting for each new wavelength measurement, since the solvent itself has its own absorption spectrum for which compensation must be made, and because the different wavelengths emitted by the lamp have different intensities.)
4. Prepare a very dilute sample of your extract by adding a few drops to a spectrophotometer tube half-filled with the solvent. Adjust this sample by addition of solvent or extract so that it has an absorbency of about 0.7 at 430 millimicrons.

These instructions are for a Bausch & Lomb Spectronic 20 Spectrophotometer. More complete instructions for use of the instrument are given on pp. 202 ff of Arditti and Dunn's *Experiment Plant Physiology.*
Materials and Equipment
(for one student squad)

Acetone extract from C.2
1 beaker (250 ml)
1 chromatostrip
Benzene 7 ml
Acetone 3 ml
1 TLC plate Baker-flex silica gel 1B. Use a piece 10 cm x 24 cm,

(5) Now set the wavelength at 380 millimicrons. Using the blank solvent tube, set the scale at zero absorbency. Insert the pigment extract sample and read the absorbency on the meter. Repeat these steps, taking successive readings at 20 millimicron intervals up to 700 millimicrons. Before changing to each new wavelength setting, turn the light control down (counterclockwise) to avoid overloading the photocell.

The data should be reported on a report sheet. Graph the results with light absorption on the vertical axis and wavelength on the horizontal axis.

On the same piece of graph paper, plot the results of Experiment C.1: LIGHT COLOR AND PHOTOSYNTHESIS. Explain the relationship between the two sets of data.

C.3 Optional Experiment:
CHROMATOGRAPHY OF GREEN PLANT PIGMENTS

The acetone extract left over from Experiment C.2 should be concentrated by evaporating the acetone by means of a hot water bath until only about 1 ml is left.

Place 1 drop of the leaf pigment extract 1.5 cm from one end of a chromatostrip. Place
the strip vertically with the spot end down in a 250 ml beaker containing a 1 cm layer of benzene: acetone, 7:3 v/v. Cover with a watch glass or aluminum foil.

The solvent rapidly rises through the thin layer of adsorbent. Separation of the pigments is apparent almost immediately. Allow the solvent to rise until it reaches 1 cm from the top of the strip. Remove the strip from the solvent and observe the pigments.

Lower percentages of acetone in the development solvent can be used for better separation of chlorophyll a and b. If the pigment spots are too dim, extract a greater quantity of pigment with the same amount of solvent as the original extract.

You should note that a yellow band (carotenes) moves about as fast as the solvent front. Lower on the paper will be one or more yellow (xanthophyll) bands, a bluish-green (chlorophyll a) band and a yellowish-green (chlorophyll b) band, in that order. Identify and outline these pigment bands with pencil and save your chromatograms for later discussion.

The green bands on your chromatograms are the chlorophylls a and b. Both your action spectrum for photosynthesis and your absorption spectrum for the leaf pigments will have large bumps which is ¼ of a full sheet.

J.T. Baker Chemical Boker-Flex Silica Cel 1B Flexible TLC Sheet (5 x 20 cm Box of 50 $13.50)
Also used in Exp. E.1

The accessory pigments (carotenes and possibly xanthophylls) also function in the photosynthetic process. They may help trap light energy at wavelengths at which the chlorophylls don’t absorb. They may also function in the transfer of the energy to the site(s) of the photochemical reactions (see following section).
A.2 Experiment: GLUCOSE METABOLISM BY YEAST JUICE

(1) Work in squads as indicated by your teacher. Blend 1 ounce (4 packets or about 28 g) of dry yeast in a blender with 80 ml of 0.1 M potassium bicarbonate for 1 minute. Allow the blended material to stand for five minutes and then blend again for 1/2 minute.

(2) Pour the blended liquid into centrifuge tubes, make sure that the centrifuge is balanced and centrifuge at a maximum speed for 15 minutes. Then carefully siphon off the cloudy liquid layer with a pipet. It is not necessary that you get all the liquid, and it is better if you don't pick up any of the solid material. Put all of the liquid - the yeast juice - into a 125 ml Erlenmeyer flask.

(3) To the yeast juice add 8 ml of 1 M glucose and 10 ml of 0.2 M fructose-1, 6-diphosphate. Incubate this mixture by placing the flask in a large beaker of warm (35-40°C) water. Keep it there for 30 minutes or until it begins to bubble vigorously (CO₂ gas). Then store the juice in a refrigerator if class time is up.

(4) Using this preincubated yeast juice, set up 4 manometer tubes like that shown in...
Figure 8.1, Chapter I (Energy Capture). Set up these manometers as indicated in Protocol: Yeast Juice. A very light film of grease (lanolin or vaseline) should be applied to the stoppers to ensure an airtight seal. If the apparatus leaks it is of no value.

(5) After assembling the entire apparatus, place the test tubes in a rack in a room temperature water bath (a plastic dishpan will do). Let them reach temperature equilibrium with the water bath (3 to 5 minutes) and then close the clamps and start manometer readings.

(6) Each manometer should be read at 5 minute intervals. Between readings, the test tubes should be shaken gently every 30 to 60 seconds to release all CO₂ from the liquid. Record your readings in a table something like this:

<table>
<thead>
<tr>
<th>Time</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:35</td>
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<td>1:36</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:37</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:38</td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
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<td>13</td>
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<tr>
<td>1:41</td>
<td></td>
<td>14</td>
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<tr>
<td>1:42</td>
<td></td>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>1:43</td>
<td></td>
<td></td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inorganic phosphate present in the yeast juice, so that the requirement for same in glucose metabolism can be demonstrated.

Sources for special materials:

1. Schwarz Bioresearch, Inc. Orangeburg, N.Y. 10962
   #4214-23, Fructose-1, 6-diphosphate
   Monomagnesium salt at Tech grade $8/25 g

2. Sigma Chemical Co.
   3500 De Kalb St. Louis, Mo. 63118
   FLE-250, buffered Firefly Lantern Extract; extract from 250 mg lanterns, $9.

The main points for the student to grasp are:

1. That an intact living cell is not needed to catalyse glucose breakdown;
2. Inorganic phosphate is needed in stoichiometric quantities.
## Protocol: Yeast Juice

<table>
<thead>
<tr>
<th>Reagent</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preincubated Yeast Juice</td>
<td>5.0 ml</td>
<td>5.0 ml</td>
<td>5.0 ml</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>1.0 M potassium phosphate pH 7.0</td>
<td>—</td>
<td>0.3 ml</td>
<td>0.05 ml</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>1.0 M glucose</td>
<td>1.0 ml</td>
<td>—</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>0.3 ml</td>
<td>1.0 ml</td>
<td>0.3 ml</td>
<td>—</td>
</tr>
</tbody>
</table>

Table A.2
Read each manometer for at least 30 minutes.

In which is CO₂ production greatest; least?

(7) Disassemble the manometers and save the tubes and their contents. (Number the tubes!) Obtain two ml sample of firefly lantern solution from the teacher. Take this and your manometer solutions into a dark room. Place 10 drop samples of the firefly lantern solution into four wells of a spot-plate and when your eyes have adjusted to the dark, a single drop of juice from each manometer tube into one of the wells.

All light in the room should now be extinguished. Do any of your mixtures of juice and firefly solution glow in the dark?

A.3 Experiment: GLUCOSE METABOLISM BY BOILED YEAST JUICE

Repeat steps 1, 2 and 3 of Experiment A.2. Then divide the yeast juice equally between two test tubes. Place one in a boiling water bath for four minutes. Save this boiled juice in a refrigerator. Place the other half of the juice in dialysis tubing contained in a beaker of distilled water (500 ml water). Dialyze overnight in a refrigerator.

Now set up three manometers. All receive glucose and phosphate as indicated for manometer #4 in the last experiment. No. 1 receives 5 ml.

CO₂ production should be greatest in #4, with considerable production in #3. With the teacher's help students may be able to calculate how many moles of CO₂ were produced. For this they will need to estimate (or measure) the volume(s) of their manometers. They will need to know the value of 1 atmosphere pressure in mm of water and the value of the universal gas constant in the appropriate units. If the reaction had been allowed to go to completion, between 2 and 3 moles of CO₂ would have been produced per mole of glucose, with the consumption of 2 moles of inorganic phosphate.

Firefly lantern solution: Add 25 ml of distilled H₂O to vial of powdered lantern extract. Mix well to make a cloudy solution. Use within a few hours.

The juice from manometer #4 should glow most brightly, indicating the highest concentration of ATP, resulting from glucose fermentation.

Because A.3 repeats A.2, chemicals and materials are the same except for dialysis tubing. There are 3 conditions: boiled juice in which enzymes are denatured but small molecule cofactors are present and active, dialyzed juice containing active enzymes but little of the cofactors, and the mixture of the two (which should metabolize glucose).

A major dialyzable cofactor is ADP. Without this no glucose breakdown can occur.
of boiled yeast juice, No. 2 receives 5 ml of dialyzed yeast juice and No. 3 receives 2.5 ml of each, boiled and dialyzed yeast juices. Repeat steps 4, 5 and 6 of Exp. A.2 with these manometers. In which do you get CO₂ production?

The analysis of glucose breakdown by yeast juice and also by muscle-juice (the processes are almost identical) occupied biochemists for 40 years. Gradually, a step at a time, they were able to identify the individual steps in the reaction pathway (metabolic pathway) and in every case to isolate and purify the enzyme responsible. This pathway, called the Embden-Meyerhof-Parnas (EMP) pathway is basically the same in yeast, man, trees and every other living thing. This pathway is shown in Figure A.2 on the next page, leading from one molecule of glucose to two molecules of pyruvate. In fermenting yeast the pyruvate is converted to ethanol in two steps.

\[
\begin{align*}
\text{CH}_3 - C - COO^- + CO_2 + H+ & \rightarrow \text{CH}_3 - C - OH \\
\text{pyruvate} & \rightarrow \text{acetaldehyde} \rightarrow \text{ethanol}
\end{align*}
\]

In the muscles of an athlete running the 100 yard dash pyruvate is converted to lactate in one step.
Figure A.2

The EMP pathway diagram shows the metabolic conversion of glucose to pyruvate, including reactions involving ADP, ATP, and reducing power. The pathway includes steps like the conversion of glucose to glucose-6-phosphate, subsequent reactions leading to the formation of pyruvate, and the overall process of generating ATP and reducing power. Each step is represented with chemical formulas and structural diagrams.
In both cases, but especially for the alcohol, the temporary product, ethanol or lactate, is completely burned up by another pathway when \( O_2 \) becomes available. This is why you can run now and breathe later.

It is not important that you remember the details of this scheme. But some things you should see and understand. For example the importance of phosphate and how it is used. First you can see from the EMP diagram that the cell has to spend two molecules of ATP to convert glucose into the right chemical form (fructose -1, 6-diphosphate) for further reactions. Then it can earn, from further reactions, four molecules of ATP. Thus the net gain is two molecules of ATP from ADP and phosphate. (Do you see now why the yeast juice requires added phosphate to ferment glucose?)

This pathway can be run in the reverse direction with a few alterations. Thus it can be used to synthesize glucose and then starch from simpler molecules. If you remember 3-phosphoglyceric acid (PGA) as the initial product of
CO₂ fixation in photosynthesis, then running this reaction scheme backwards makes sense. It is undoubtedly how starch is made in plants. Starch is then stored energy which the plant uses in the dark and at other times when photosynthesis is impossible.

A couple of large questions might bother you at this point. For instance, how come yeast grows so much better with O₂ than without it? And how do we get from any of the chemicals in the EMP pathway to any and all of the amino acids and nucleotides? After all, these latter chemicals are the important ones in growth.

The answer to the first question is that there is still another pathway for the complete combustion of pyruvate when O₂ is available. This is called the tricarboxylic acid cycle (TCA cycle) and it produces, indirectly, 18 times as much ATP as the EMP pathway. For a diagram of the TCA cycle see Figure A.3.

Once again there are some important points to see:
1. The three carbon atoms in pyruvate are all converted to CO₂.
2. For every two molecules of pyruvate that are combusted and one molecule of oxaloacetate is regenerated. Obviously some

*Also called the **Krebs Cycle** or **Citric Acid Cycle.***
CH₃-C-S-Coenzyme A is an activated form of acetate (acetic acid) analogous to other activated forms of carboxylic acids. This activation permits the reaction with oxaloacetate to proceed.
of the reactions are used to "get into position" for other reactions which actually produce usable energy. You may ask how all of the ATP's are produced? The answer is that the reducing power of [H] is used to do this. This is done in another pathway which is still largely a mystery. We do know that the reducing power is transferred step by step down a chain of stronger and stronger oxidizing agents until ultimately it is used to reduce O₂ to two molecules of H₂O. In some of these oxidation-reduction steps, ATP is produced. Overall this pathway makes 3 molecules of ATP for each pair of protons which make the trip.

The second question referred to the formation of amino acids, nucleotides and other essential compounds. It has been shown that the two pathways just considered are the "core" of the metabolism of almost every living thing. There are "side streets" leading off at various points to all of the amino acids and other monomers which the cell needs. If we schematize the EMP and TCA pathways, some of the side streets can be visualized.

What is really amazing is the economy in the number of steps used by living things to produce all the chemicals they need. The whole
Figure A.4
metabolic scheme is a little like the road map of
a well-designed city. There are the freeways
branching off into main streets branching off into
side streets. The main pathways produce usable
energy (ATP and reducing power) which are then
consumed in the branch pathways. (See Figure
A.4.)

Exercises for Home, Desk and Lab (HDL's)

(1) Proteolytic enzymes are capable of digest-
ing what kind of polymers down to what
kind of monomers?

(2) An enzyme in saliva can convert the starch
polymer into what monomers?

(3) a. Under what two circumstances may
     glucose be degraded by yeast?
     b. Write the balanced equation for each.

   c. Which reaction appears to be the
      reverse of photosynthesis?

(4) a. When glucose is converted to pyruvate
     the reaction requires how many mole-
cules of ATP?

(1) Proteolytic enzymes split
proteins to amino acids.

(2) This enzyme converts
starch to glucose.

(3) a. Glucose may be de-
graded by yeast
    either with or with-
    out the presence of
    oxygen. Aerobic deg-
radiation occurs in
    the presence of oxy-
gen. Anaerobic deg-
radiation occurs in
    the absence of oxy-
gen.
    b. \[ \text{C}_6\text{H}_12\text{O}_6 + 6\text{O}_2 \rightarrow \]
       \[ 6\text{CO}_2 + 6\text{H}_2\text{O} + \]
       usable energy

    c. \[ \text{C}_6\text{H}_12\text{O}_6 \rightarrow \]
       \[ 2\text{C}_2\text{H}_5\text{OH} + \text{CO}_2 + \]
       usable energy

   c. The aerobic degrada-
tion looks like pho-
tosynthesis run in
   reverse.

(4) a. Two molecules of ATP
     are required for the
     conversion of glucose
to pyruvate.
b. Four molecules of ATP are produced when glucose is converted to pyruvate, which leaves the cell with a net increase of two molecules of ATP.

(5) a. This pathway is called the Embden-Meyerhof-Parnas pathway.

b. Yeast is able, in two steps, to convert it to ethanol. Man's muscles are able to convert it to lactate.

(6) Yeast is able to grow better in air because there is an additional pathway, called the tricarboxylic acid cycle, for the complete combustion of pyruvate when O₂ is available.

(7) a. 18 amino acids are shown as resulting from this scheme.

b. Tryptophan, tryosine, phenylalanine, arginine, proline, alanine, serine and glycine.

(8) a. No.

b. Yes, the larger the amount of phosphate, the greater the rate of production of CO₂.
Chapter III: METABOLISM AND GENES

Many small molecules other than glucose can serve consumers as total sources of usable energy and of monomers. These include other kinds of sugars as well as alcohols, carboxylic acids and amino acids. Each of these potential foodstuffs requires special enzymes for its breakdown. These special catabolic enzymes are useless unless the particular substrate molecule is present in the environment. One would expect, therefore, that a special enzyme would not be produced unless its substrate were available. In fact, this is what is found for many consumer organisms.

The breakdown of lactose (the sugar found in milk) by the bacterium *E. coli* (*Escherichia coli*) is a good example of catabolism. Lactose is a disaccharide; that is, it is made up of two simpler sugar residues, one glucose and one galactose.

Catabolic enzymes break down or convert foodstuff molecules into usable energy and at the same time synthesize the starting material for essential monomers.
Mammals must perform the same hydrolysis of lactose in order to utilize it.

Lactose and certain other β galactosides induce E. coli to synthesize β galactosidase (step 1).

Here galactose is in the β form. It is bonded via the one-carbon oxygen of galactose (in the β configuration) to the four position of glucose. Hence it is often called a β-galactoside.

In order for E. coli to utilize lactose it must first hydrolyze or split lactose into its two monomeric components, galactose and glucose, utilizing a special catabolic enzyme.

\[
\text{enzyme} \\
\text{lactose + H}_2\text{O} \rightarrow \text{galactose} + \text{glucose.}
\]

The glucose can then be used directly via the EMP pathway, while the galactose must first be converted into glucose. This requires another three enzymes. Overall the total conversion of lactose to glucose is:
The enzyme that performs step 1 is called β-galactosidase. \textit{E. coli} makes it only when presented with lactose or a similar β-galactoside. β-galactosidase is specific for the β-galactose half of the molecule and will therefore hydrolyze or split almost any molecule containing this sugar.

There are a number of important points in regard to this metabolic pathway. One is that \textit{E. coli} makes only a tiny amount of β-galactosidase unless lactose or some other β-galactose is present. Thus this very simple organism has the ability to turn on and off one of its genes. Another is that β-galactosidase is the only pathway of lactose utilization available.

Actually there are two genes involved in lactose uptake and splitting. One, called the Z gene, specifies β-galactosidase and the other, called the Y gene, specifies the permease protein. This latter protein transports lactose across the cell membrane into the cell. Without it lactose cannot enter. Some mutants can make β-galactosidase but cannot eat lactose. These are permease-negative mutants, unable to pump lactose into the cell. Both of these genes (Z and Y) are turned on by lactose or some related galactoside in the environment. Another gene (the

Steps 2, 3, and 4 are catalyzed by a set of enzymes induced by galactose, not lactose. Both in \textit{E. coli} and in humans a genetic defect causing loss of function of the enzyme catalyzing step three leads to a disease called galactosemia. For the bacteria this disease is fatal, and for human infants this defect leads to feeble-mindedness if the infant is fed normal lactose-containing milk.

Each \textit{E. Coli} cell can contain as many as 3000 molecules of β-galactosidase.

There are probably hundreds of its genes which \textit{E. Coli} can turn on and off. The same enzyme is found in the lining of the human intestine. Why is it there?
These active sites are binding sites, not catalytic sites (such as when urease occupies one site, distorting the other binding site, i.e. pulling it out of shape).

Mutations in the i gene stop the synthesis of active repressor. Hence the Z and Y genes are turned on all of the time in such mutants. The repressor was only a hypothesis in the minds of geneticists until recently, when it was finally isolated and purified. This was a very difficult task as there are only tiny amounts of repressor—about 10 molecules per cell.

E. coli can eat about 50 different sugars, alcohols and organic acids. With a few exceptions all these compounds are metabolized by induced enzymes, that is, enzymes made only in the presence of the compound.

i gene) is involved in this control of Z and Y function. The i gene specifies another protein, called the repressor, which specifically binds to a small region of the DNA near the Z gene, thereby preventing any messenger RNA synthesis for the Z or Y genes. The repressor (R) is a double-active-site enzyme. One site combines with the specific DNA segment, the other site with lactose. These two binding activities are mutually antagonistic such that when there is sufficient lactose around all of the repressor is "pulled" off the DNA.

\[
\begin{align*}
R + DNA & \rightarrow R-DNA \\
+ \text{lactose} & \rightarrow \text{R-lactose (Z and Y turned on)}
\end{align*}
\]

The lactose genes Z and Y constitute what is called an inducible system, that is, a set of genes which are induced or turned on by the presence of the right chemical in the environment. Almost all energy-producing (catabolic) systems are inducible. Why does this make sense for the organism?

On the other hand the amino acid synthesizing pathways (sets of enzymes) are repressible. That is, the presence of the particular amino acid in the environment turns off the synthesis of the
enzymes responsible for production of that amino acid. This means that _E. coli_ will not make this particular amino acid from glucose as long as it can get it "free." Does this make sense?

This sort of control of gene activity takes place in all living things. For more complicated organisms it is a more complicated process but still essentially the same. Every cell in every organism is using only part of its genes at any time. When this control breaks down the consequences can be very bad. For example many scientists believe that cancer cells are cells which have lost the ability to control certain genes.

Some genes, such as those specifying the enzymes in the EMP and TCA pathways, are always turned on because the cell always needs these pathways. Nevertheless these genes are also controlled, since the cell may need more or less energy under differing conditions.

_E. coli_ is probably not using more than 1/2 (about 1500) of its genes at any one time.