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ABSTRACT

This student guide is divided into two sections, "Chemistry of Living Matter" and "Energy Capture and Growth," constituting parts three and four of the third year of the Portland Project, a three-year high school integrated science curriculum. 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 by these chapters: monomers and how they are built; some 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. Reading assignments and experiments for the third year are contained in the student guide. Half of each page is left blank for the purpose of taking notes. (Additional information about the Portland Project in integrated science may be found by seeing SE 013 702 - 705.) (PR)

STUDENT GUIDE

CHEMISTRY OF LIVING MATTER ENERGY CAPTURE & GROWTH

AN INTEGRATED SCIENCE SEQUENCE

1971 EDITION

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Outline: Chemistry of Living Matter

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 build-up, break-down and energy transfer of these bio-molecules. 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. Perhaps 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 multi-armed 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°, brass clusters have five arms and are used to indicate hybridized bond angles of 120°, and 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 hybridisation, and the resulting orbitals are called sp^3 hybrids from a mix of one s and 3 p orbitals.

After this discussion the clusters will be referred to by <u>color</u>, i.e., silver, brass or copper, or by type, such as sp^3.

A.3 HYBRID ORBITALS

A 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 <u>asymmetric</u>. (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 p orbitals. For

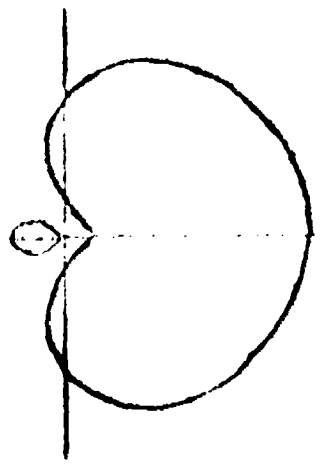


Fig. A.1
A Hybrid Orbital

example, 3 hybrid sp^2 orbitals will be arranged at 120° to each other in a plane, while 3 p orbitals are at 90° 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.

Now the four sp^3 hybrids of carbon are spaced as far apart from each other as are the arms of a tetrahedron, therefore the angles between the bonds are 109° 28'. All sp3 hybrid orbitals have this angl 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^o apart and all lie in the same plane. The non-hybridized p orbital is found at 90^o 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 "σ" or "sigma" bonds and are the covalent bonds that exist in molecules. Figure A.2 shows two p orbitals overlapping to form a σ (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

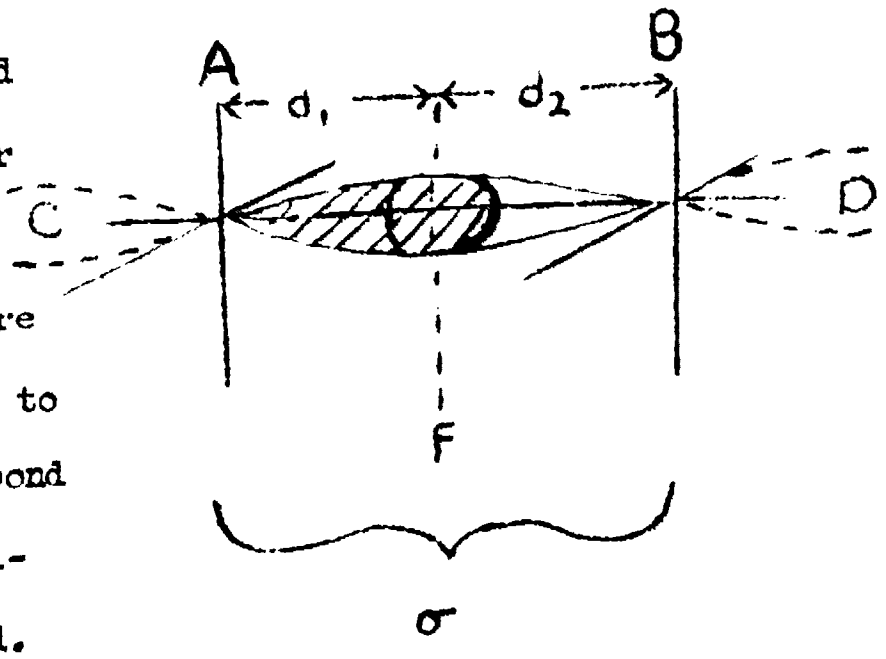


Figure A.2

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 σ 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 towards one atom of the pair. Displacement is towards 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 σ 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 π (pi) bond.

(Illustrations of the orbital overlap in the pi-bond formation may be seen in Figure A.3 and A.6).

Note that the π 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 σ 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)

Atomic Covalent Radii (A°)		
	Single Bond Radii	
C - single 0.77	H	0.30
C - double 0.67	F	0.64
C - triple 0.60	Si	1.17
	P	1.10
N - single 0.74	S	1.04
N - double 0.62	Cl	1.00
N - triple 0.55	Br	1.14
	I	1.33
O - single 0.74		
O - double 0.62		
O - triple 0.55		

Table A.1

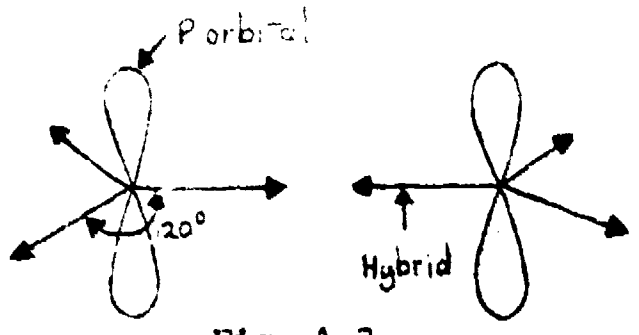


Fig. A.3
Arrows represent hybrid sp^2 orbitals

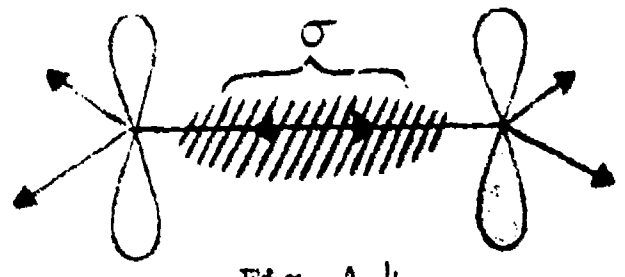


Fig. A.4
Two sp^2 hybrids form σ bond between nuclei.

At the exact same time (σ bond omitted for clarity) distortions of p orbitals leads to overlap and share of electrons, i.e., bond:

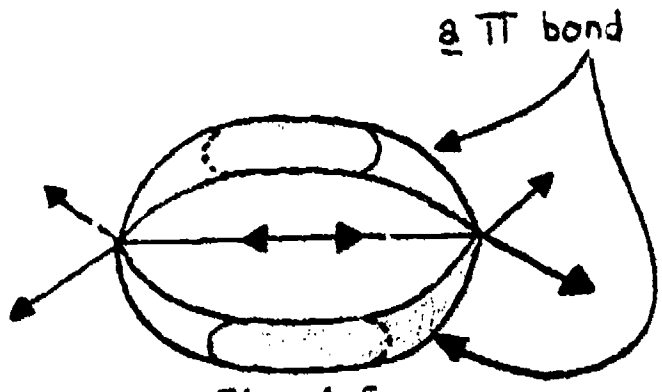
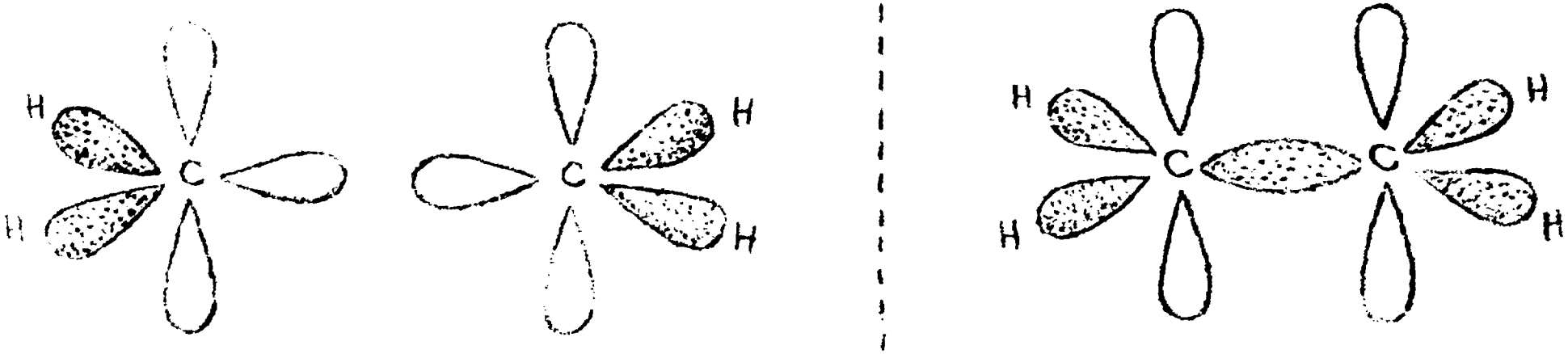
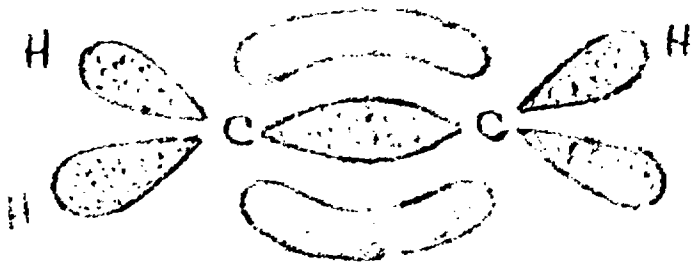


Fig. A.5
This double-armed bond is a pi π bond.

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.





σ bonding

π bonding

Orbital overlap in π-bond formation in ethylene by overlap of p orbitals, as shown in three stages.

Fig. A.6

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.

Bond strength:

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

Morrison and Boyd, Organic Chemistry, Allyn and Bacon.

A.6 TRIPLE BONDS

The copper-colored fastener illustrates the third kind of hybridization of carbon. In this case hybridization involves one s 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 HC≡CH is formed by σ bond formation between sp hybrids and π bonds between the four p orbitals left.

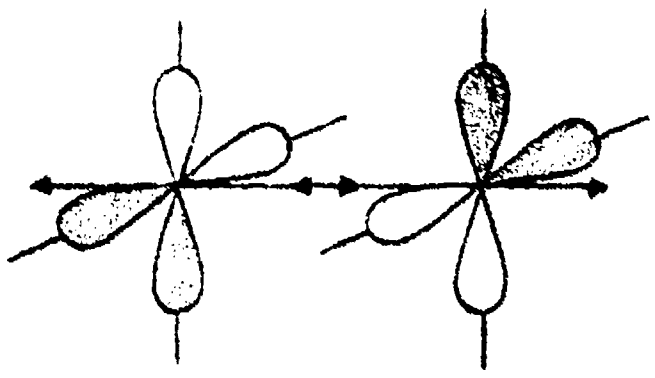


Fig. A.7
'p' orbitals which form π bonds

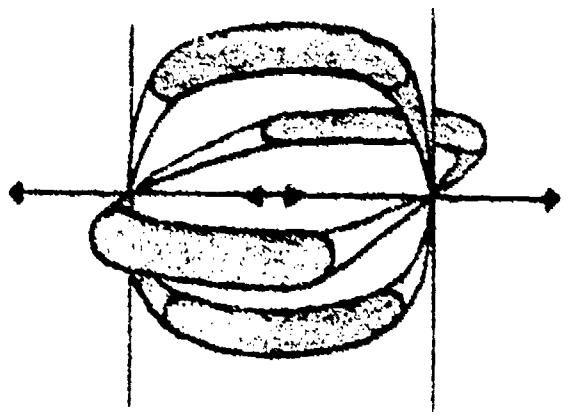


Fig. A.8
π bonds formed

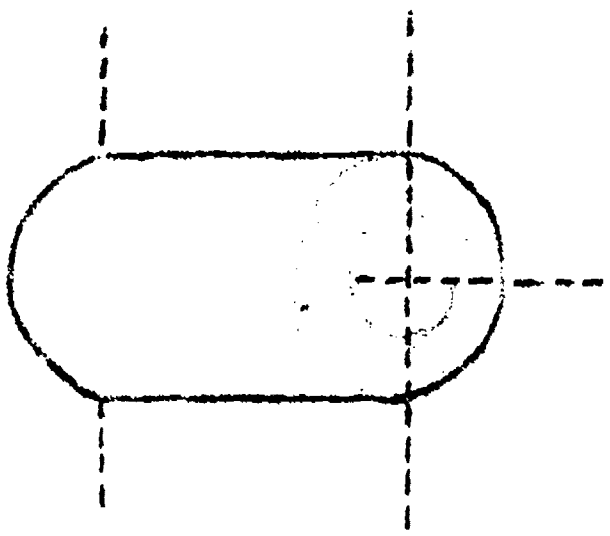


Fig. A.9
Another representation of the tw
π bonds in a triple bond.

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 PIASTIC TUBING

Interatomic distances are measured in Angstrom 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 specific atoms involved in bonding. This is done through a color key found in Table A.2.

Color Coding of Atoms in Kit	
hydrogen-white	bromine-orange
carbon-black	iodine-brown
nitrogen-blue	silicon-light yellow
oxygen-red	phosphorus-violet
fluorine-light green	sulfur-dark yellow
chlorine-dark green	all metals-grey

Table A.2

Current usage is in nanometers:

1 nanometer = 1nm = 10^{-9}m.

∴ 1nm = 10Å.

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.

van der Waals radii in Å									
N	1.50	Sb	2.20	Sc	2.00	F	1.35	I	2.15
P	1.90	O	1.40	Te	2.20	Cl	1.80		
As	2.00	S	1.85	H	1.20	Br	1.95		

Table A.3

The tubing is fastened to the metal clusters by sliding the tu bing 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 atoms. 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 1Å = 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 BONDS 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.

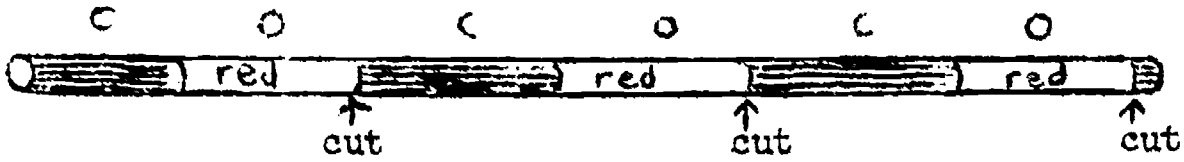


Figure A.10

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 changing from one bond to the next.

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

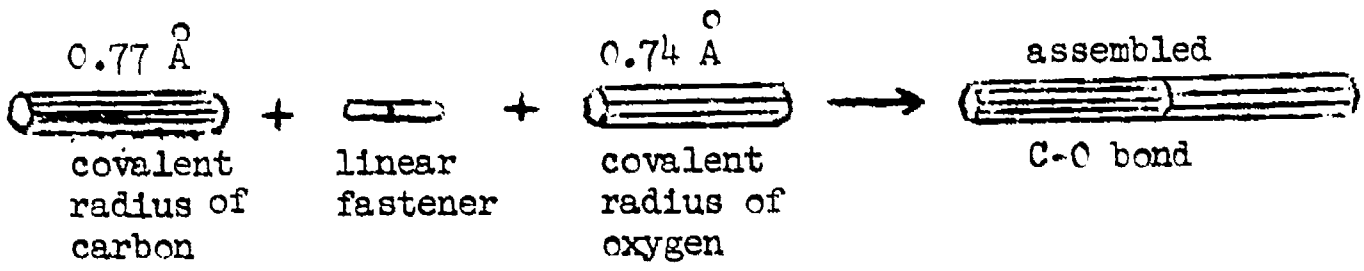


Figure A.11

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,

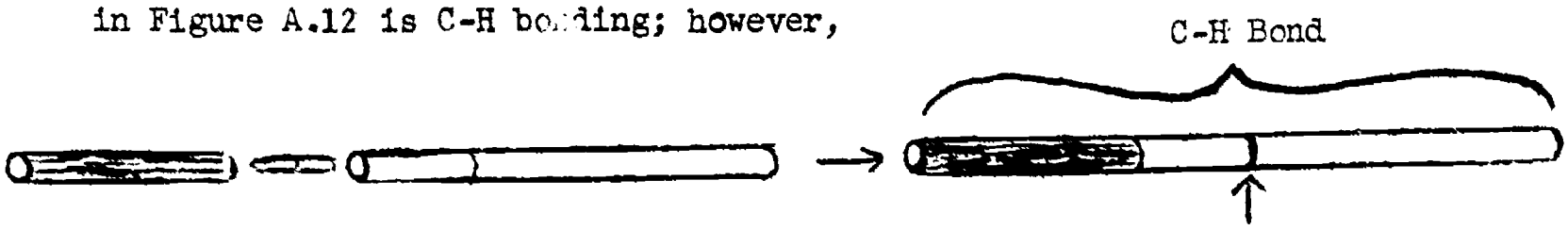


Figure A.12

N-H and O-H bonds are printed (and cut) in the same way.

A.10 ENOUGH TALK, LET'S BUILD MOLECULES

We will start with methane--CH_4. 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.

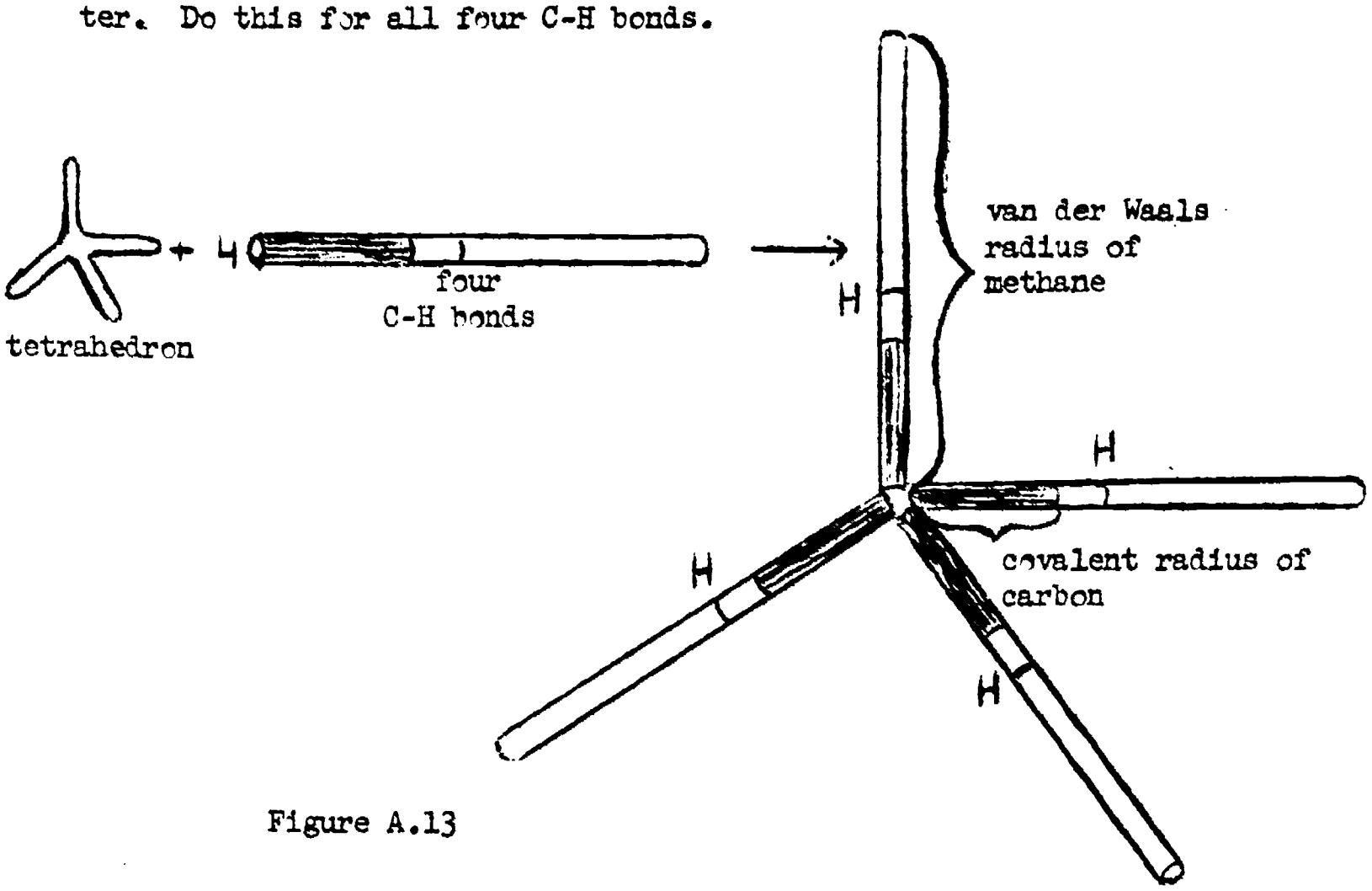


Figure A.13

Next make a 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.

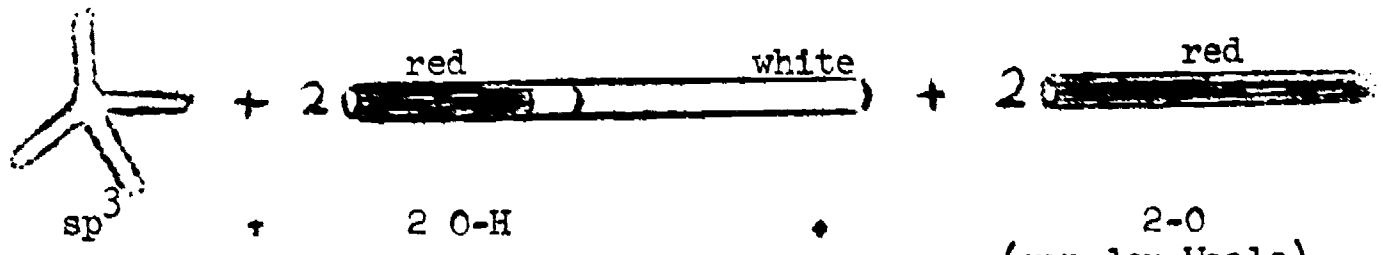


Figure A.14

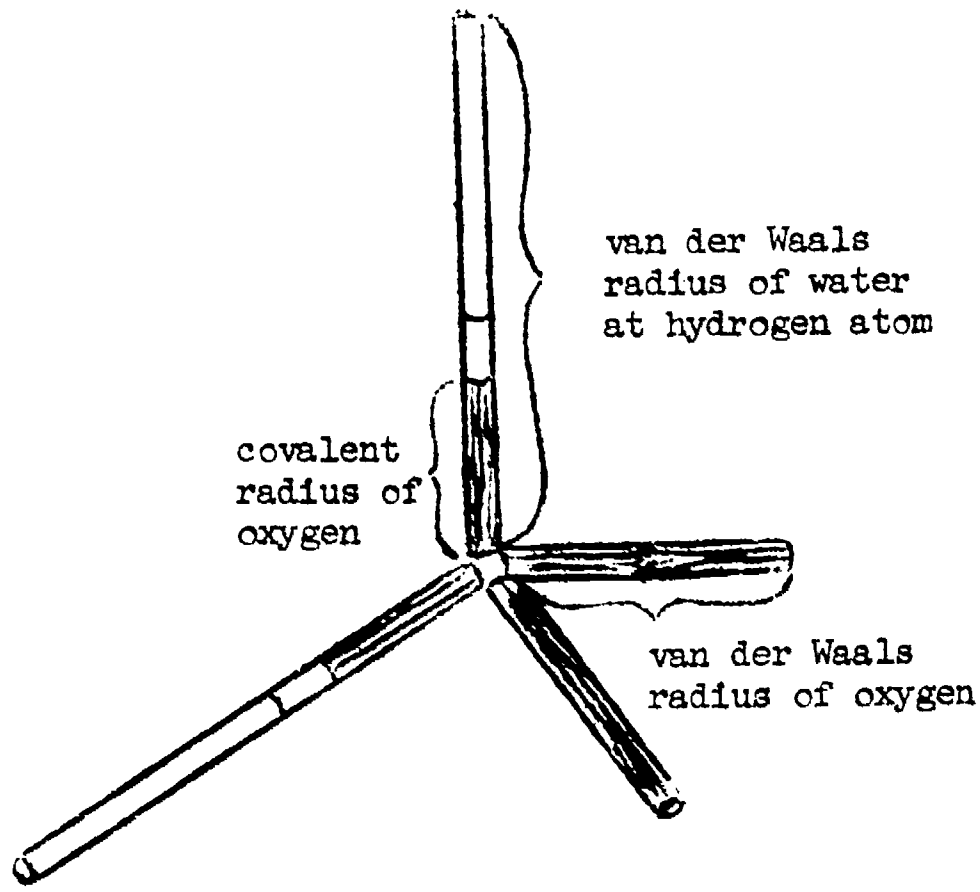
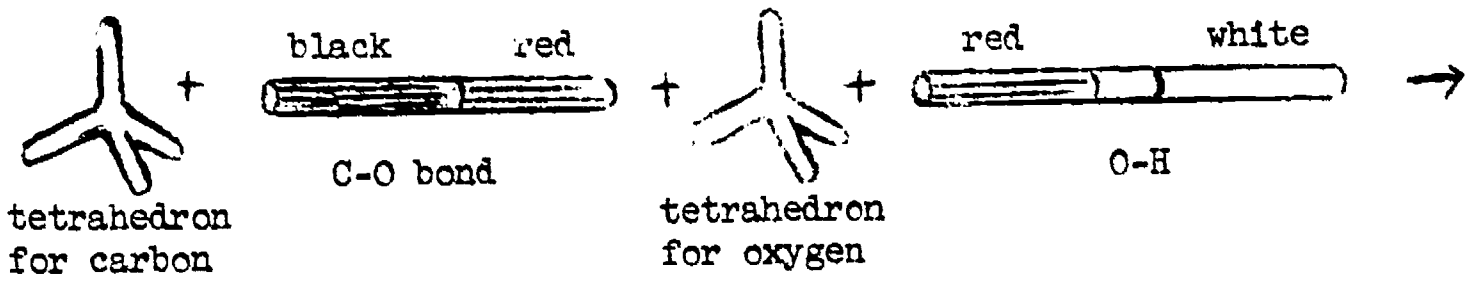


Figure A.15

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.

Let's make a more complicated molecule, a model of methanol--CH_3OH.

To build this we will need



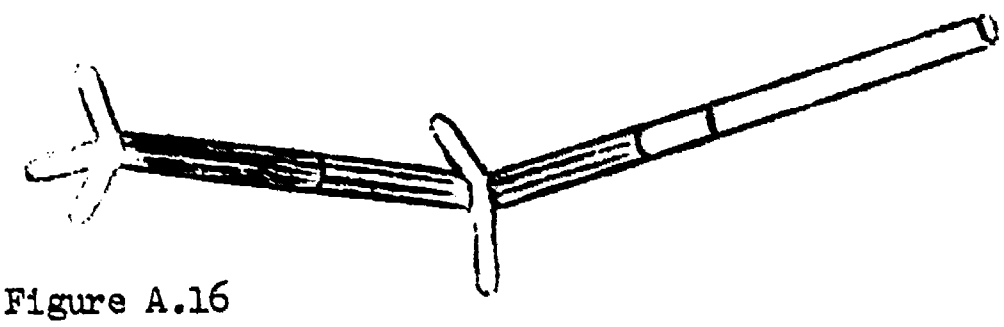


Figure A.16

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.

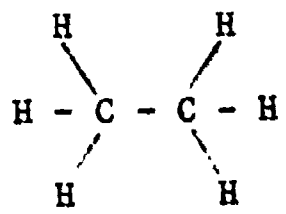
The unbonded electrons occupy a space represented by the van der Waals length only when the unbonded electrons belong to a singly bonded atom.

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 CH_3CH_3 or C_2H_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 tetrahedron, 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 carbon compounds called the alkanes is built in this way. The general formula of this group is C_nH_{2n+2} when "n" is any integer.

After building several of the molecules in the series CH_4, C_2H_6, C_3H_8, C_4H_{10}, C_5H_{12} there are several things you should note. One important factor is that because of the 109° bond

Alkane Series (Saturated Hydrocarbons) First Ten Members
CH_4 - Methane
C_2H_6 -Ethane
C_3H_8 -Propane
C_4H_{10} - Butane
C_5H_{12} - Pentane
C_6H_{14} - Hexane
C_7H_{16} - Heptane
C_8H_{18} - Octane
C_9H_{20} - Nanane
$C_{10}H_{22}$ - Decane

Table A.4

of carbon these molecules twist around and are far from linear. Another point is that there is more than one way to put C_4H_{10} together. In fact the greater the number of carbons, the more possible shapes there are. These different possibilities are called isomers; they have the same empirical formula but are actually different compounds. A good example might be C_2H_6O where one isomer involves C-O-C bonds (an ether) and 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 C_2H_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 several 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 $CH_2=CH_2$ or C_2H_4. To build double bonds correctly, we must be sure we work with double-bond distances, so check Table A.1 on page 14. 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.)

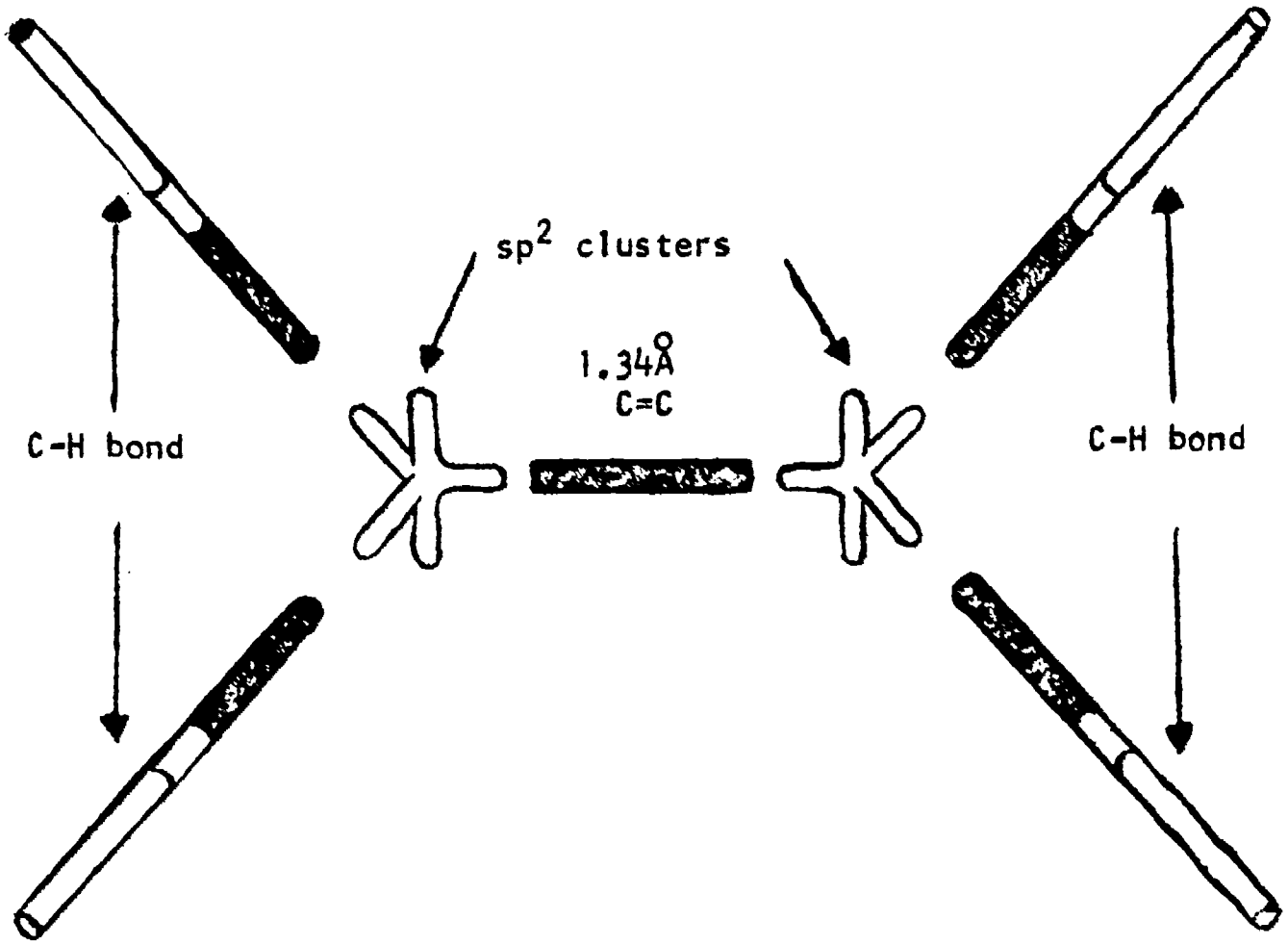


Fig. A.17

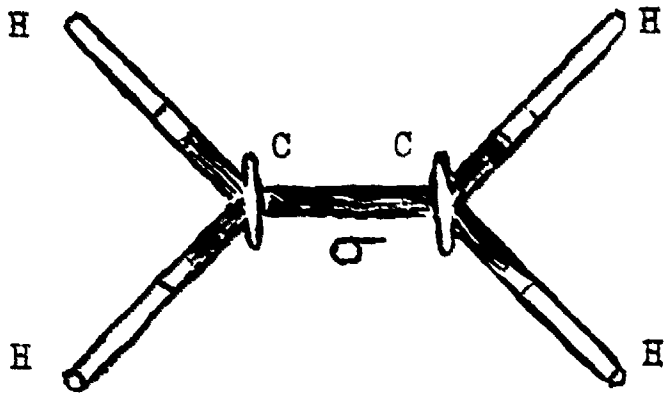


Figure A.18

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:

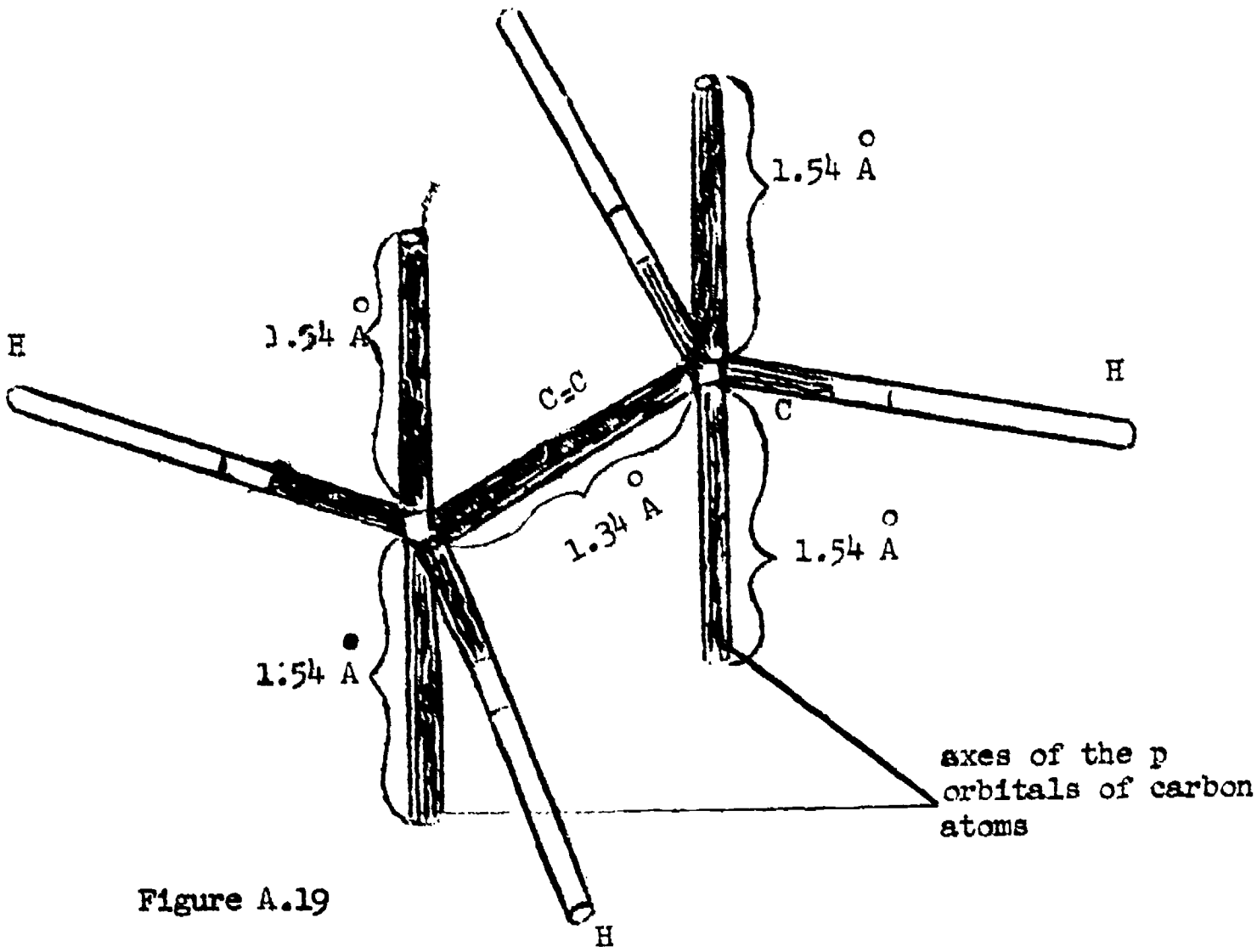


Figure A.19

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:

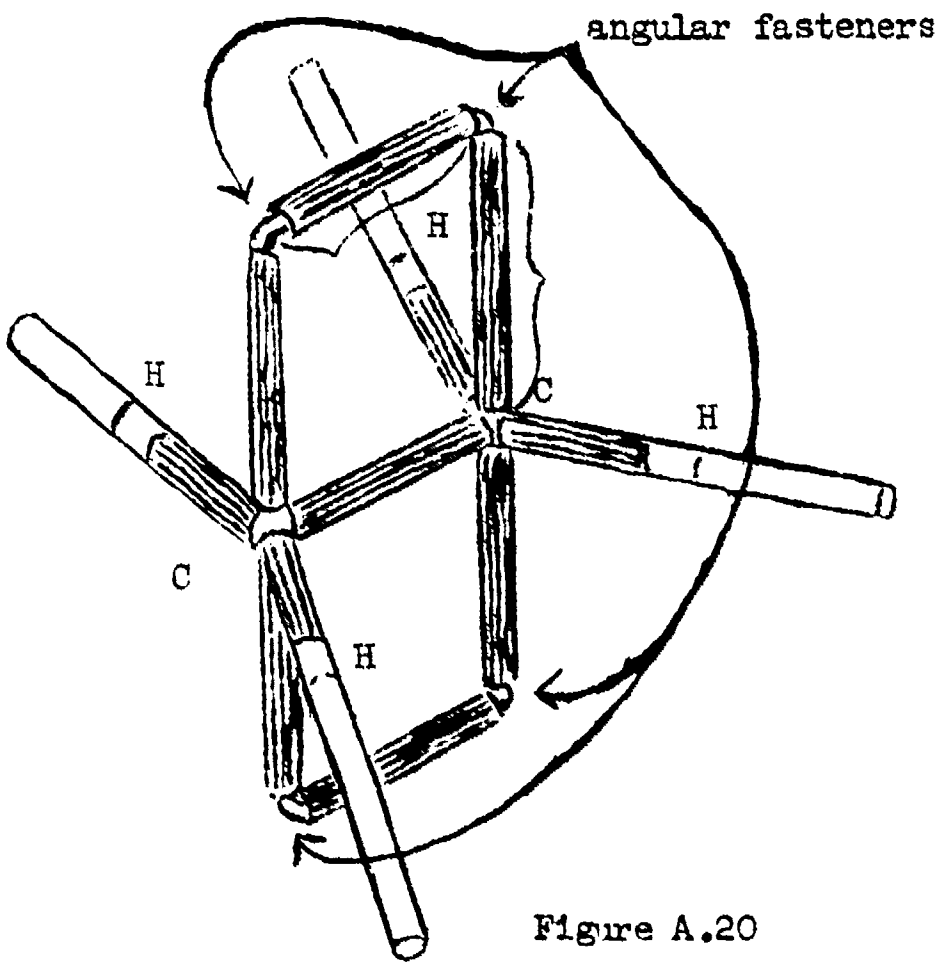


Figure A.20

The reason you used 1.54 inch-long tubing pieces on the axial p orbital is because the actual π bond between carbon atoms is 3.08 Å thick, and therefore this makes an accurate representation of the molecule.

When the π 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=N 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 $\begin{matrix} H \\ H \end{matrix}\!\!>\!C{=}O$.

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.

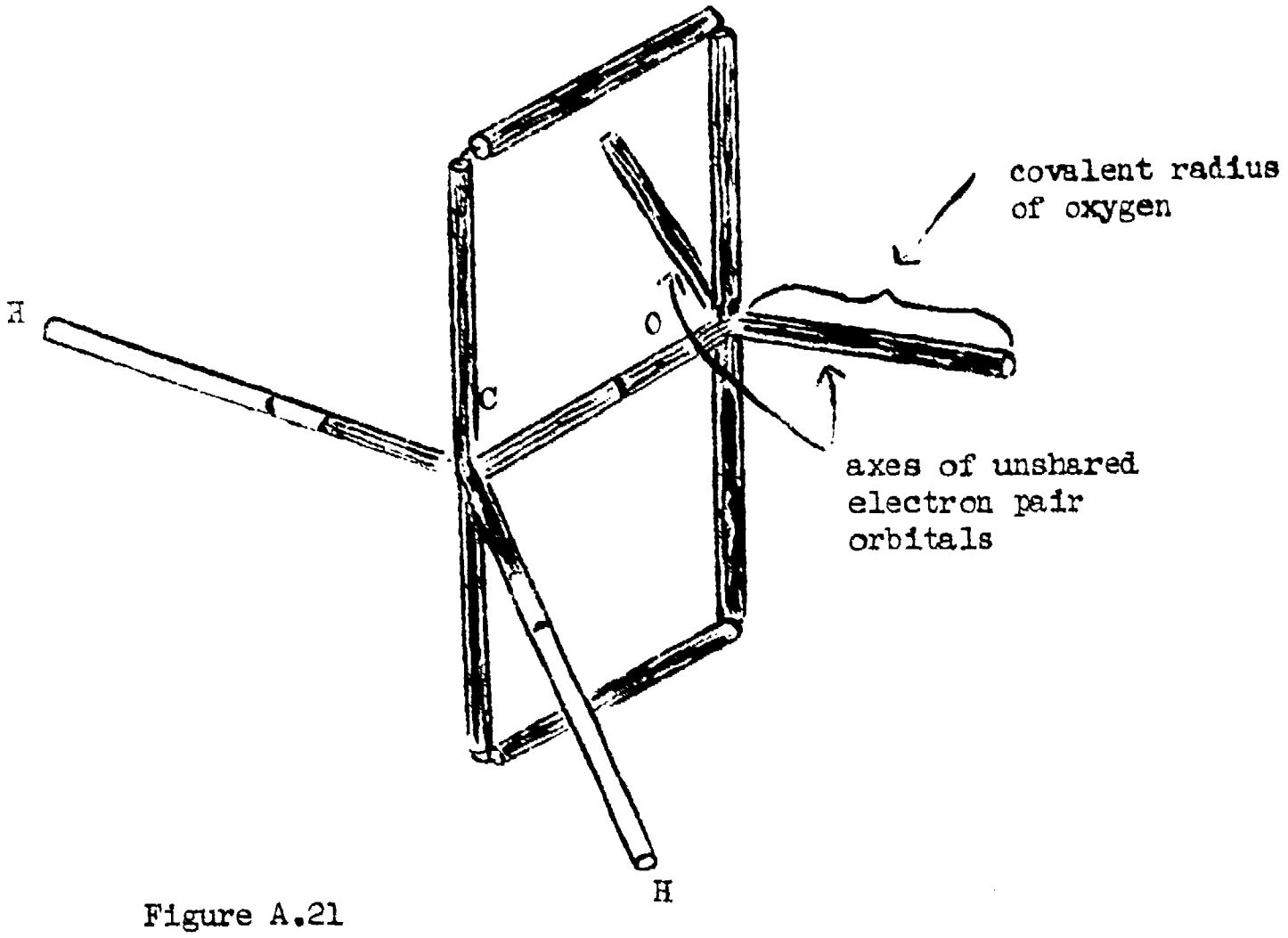


Figure A.21

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 π 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 C_3H_4 and structurally is $\begin{matrix} H \\ H \end{matrix}\!\!>\!C=C=C<\!\!\begin{matrix} H \\ H \end{matrix}$. Here we have

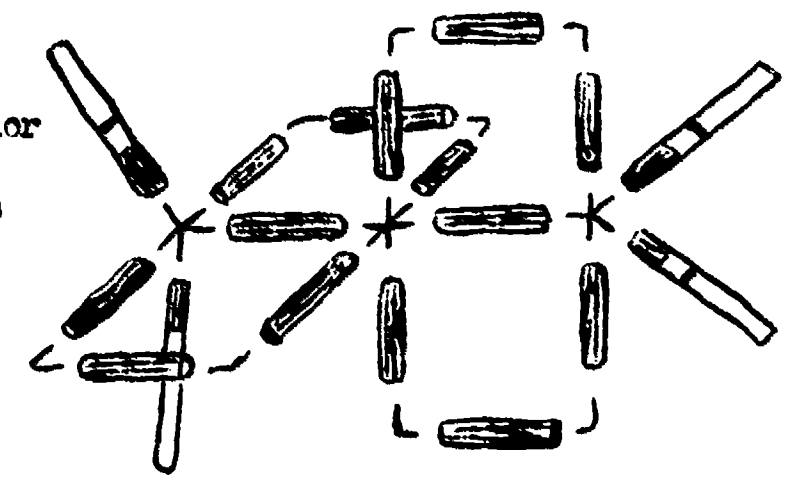


Fig. A.22
Allene-exploded view

one carbon atom, the middle one, double bonded to the other two carbon atoms. This means that the middle carbon atom is π bonded to each of the other carbons. Forming two π bonds is accomplished through sp hybridization. In the kit this is represented by a copper colored cluster. To build allene you will need:

1 copper cluster (sp)

2 brass clusters (sp^2)

6 C=C bonds

4 C-H bonds

8 C-C bonds

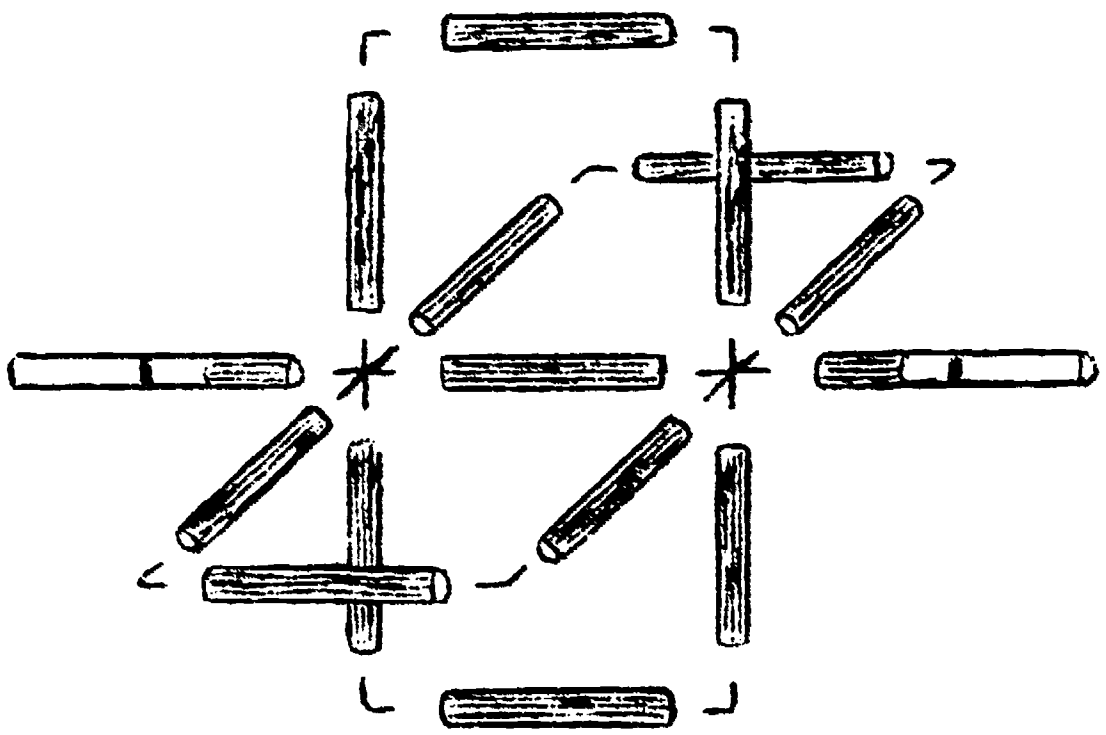
8 angle fasteners

Begin by connecting the sp cluster to an sp^2 cluster by a C=C bond, (σ bond). Now build the π bond between these two. Next connect the other sp^2 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 sp^2 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 C_2H_2 (acetylene) by using the sp clusters and the correct bond lengths from the table.

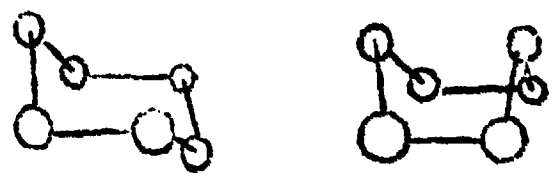


Acetylene--exploded view
Fig. A.23

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 sp^3 clusters and join them together in a ring using 1.54-inch sections of black tubing. You will note that there are two possible positions for this structure:



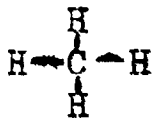
'chair' or 'boat'

Fig. 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

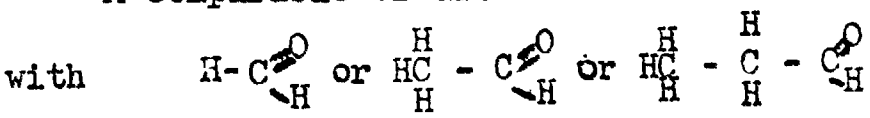


is methane, a carbon atom bonded to four hydrogen atoms. Some other useful conventions follow.

B.1 ALCOHOLS

Consider the series of compounds, methanol (CH_3OH), ethanol (CH_2CH_3OH) and propanol ($CH_3CH_2CH_2OH$). 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 C_2H_5-, C_3H_7-, C_4H_9- and so on.

B.2 OTHER REACTIVE GROUPS

A comparable situation is found with  All of these enter into basically similar reactions, due apparently to .

Similarly

$H - C(=O)OH$, $CH_3 - C(=O)OH$ and $CH_3 - CH_2 - C(=O)OH$

all seem to rely on $-C(=O)OH$

as their source of reactivity. In other words, the molecule is reactive at the site of these different groups. The groups mentioned and others are called <u>functional groups</u>. See Table B.1.

B.3 ALKYL RADICALS

In section B.1 reference was made to use of the symbol <u>R</u> to stand for a group of atoms attached to the reactive group. Examples of these are the alkyl radicals:

CH_3- is methyl as in

CH_3Cl methyl chloride

C_2H_5- is ethyl as in

C_2H_5OH ethyl alcohol

C_3H_7- is propyl and

C_4H_9- is butyl <u>etc</u>.

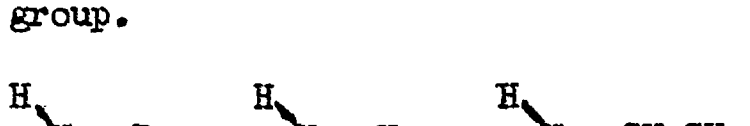
Functional Groups		
R-OH	alcohols	R - OH
R-CHO	aldehydes	R - C(=O)H
R-COOH	carboxylic acids	R - C(=O)OH
R-O-R	ethers	R - O - R
R-CO-R	ketones	R - C(=O) - R
R-COO-R	esters	R - C(=O)O - R
R-O-C(H)(R)-O-R	acetals	R - O - C(H)(R) - O - R

Table B.1

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

B.4 AMINES

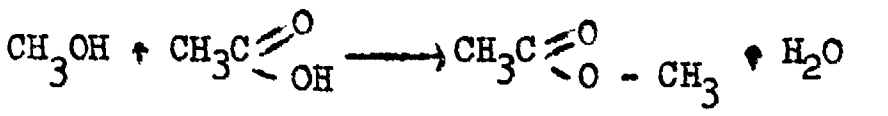
Later you will learn that nitrogen compounds are very important. Some of these include the amine functional group.



an amine ammonia ethylamine

B.5 MODELS OF REACTIONS

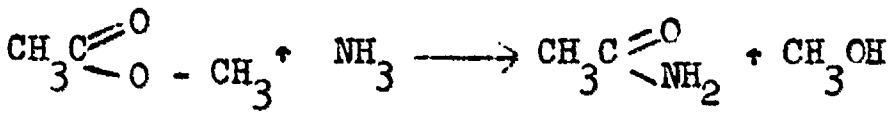
Using the kit you can demonstrate not only molecules but also reactions. After building methyl alcohol and acetic acid, manipulate the models to show this reaction:



$CH_3OH + CH_3C(=O)OH \longrightarrow CH_3C(=O)O - CH_3 + H_2O$

methyl alcohol + acetic acid ⟶ methyl acetate (an ester) + water

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

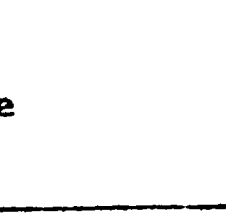
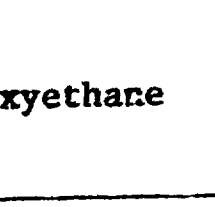
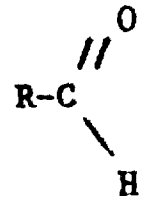
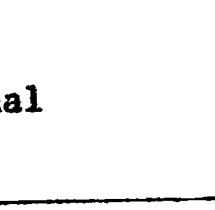
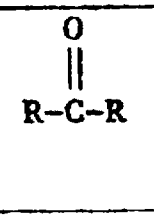
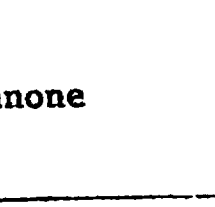


$CH_3C(=O)O - CH_3 + NH_3 \longrightarrow CH_3C(=O)NH_2 + CH_3OH$

methyl acetate + ammonia ⟶ acetamide + methanol.

With the experience you've gained, you should be able to build any structure, given its structural formula.

FUNCTIONAL GROUPS - NOMENCLATURE

Functional Group	Type of Compound	IUPAC* Ending	Example	IUPAC Name	Common Name
	alkane (saturated hydrocarbon)	-ane		ethane	ethane
	alkene (olefin)	-ene		ethene	ethylene
-C≡C-	alkyene (acetylenic)	-yne	H-C≡C-H	ethyne	acetylene
R-OH	alcohol (hydroxyl)	-ol		ethanol	ethyl alcohol
R-O-R	ether	---		methoxyethane	methyl ethyl ether
	aldehyde	-al		ethanal	acetaldehyde
	ketone	-one		propanone	acetone

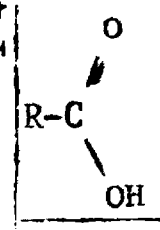
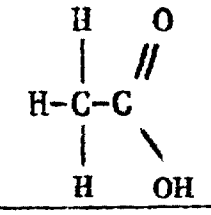
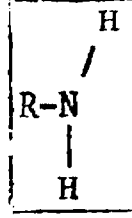
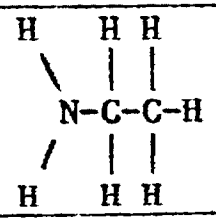
Functional Group	Type of Compound	IUPAC* Ending	Example	IUPAC Name	Common Name
	carboxylic acid	-oic		ethanoic acid	acetic acid
	amine	- - -		2-amino ethane	ethyl amine

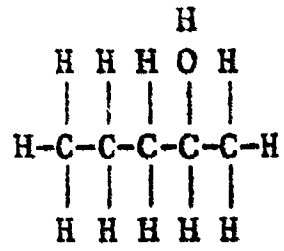
Table B.2

*International Union of Pure and Applied Chemistry

THE IUPAC SYSTEM OF NOMENCLATURE

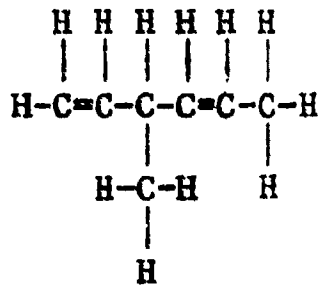
On the following page is a summary of basic IUPAC rules for naming compounds. It would pay to read through them and then go back through the following examples, referring back to the rules as needed.

(1)



		Application	Rule #
a:	pent-	5 carbon atoms	#1
b:	pentan-	saturated chain (all single C-C bonds)	#2
C:	pentanol	ol-alchohol ending	#2
d:	2-pentanol		#3

(2)



a:	hexa-	6 carbon atoms	#1
b:	hexadiene	2 unsaturated bonds di=2 (two)	#2,6
c:	1,4-hexadiene	as opposed to 2,5	#3
d:	3-methyl-1,4-hexadiene	methyl group on #3 carbon	#4

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. (p. 25 Table A.4)

2) Use the appropriate ending to indicate the principal functional group present. (p.41, Table B.1-p. 43, Table B.2) 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 occuring 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 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? 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 sp^3 clusters have 4 legs?

(7) Why does a p orbital have <u>two</u> 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?

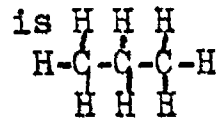
(9) Why is the C=C bond distance less than that of the C-C bond?

(10) Are the bonds as rigid as the clusters?

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

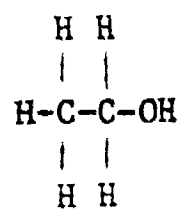
(12) Methanol and methyl alcohol are names for the same organic compound. What is its formula?

(13) The structural formula for propane is



Draw structural formulas for butane, heptane, nonane, and octane.

(14) The structural formula for ethanol is



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 <u>entirely</u> composed of carbon in the sp^2 state.

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

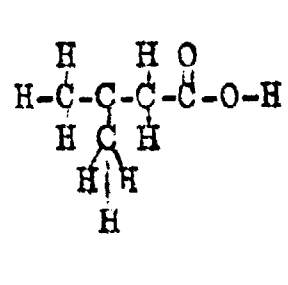
(a) C_6H_{14}

(b) C_4H_8

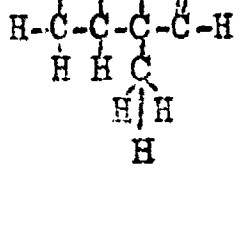
(c) C_4H_8O

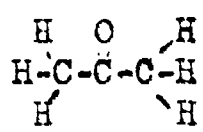
(19) Apply the IUPAC naming system to the following organic molecules.

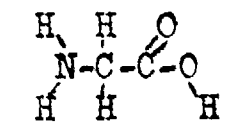
(a)



(b)



c) 

d) 

Chapter II: SOME SIMPLE CARBON COMPOUNDS

One of the simplest carbon compounds is methane, CH_4. We consider methane to be uncomplicated because it contains relatively few atoms, because it contains only one type of bonding (sp^3) 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 $-161^{o}C$. (Compared with the rare gases: Argon bp -189^{o} [At. Wt. 40], Krypton -157^{o} [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]; NH_3 [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 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_4C_3, and beryllium carbide, Be_2C, react with water to yield methane.

$Al_4C_3 + 12\ H_2O \rightarrow 4Al(OH)_3 + 3CH_4$.

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:

$CaC_2 + 2H_2O \rightleftarrows Ca(OH)_2 + HC{\equiv}CH$

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

$H\text{-}C{\equiv}C\text{-}H + H_2 \rightarrow H_2C{=}CH_2$

or

$H\text{-}C{\equiv}C\text{-}H + 3H_2 \rightarrow 2CH_4$

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:

$$C_6H_{12}O_6 + aH_2O \rightarrow bCO_2 + cCH_4$$

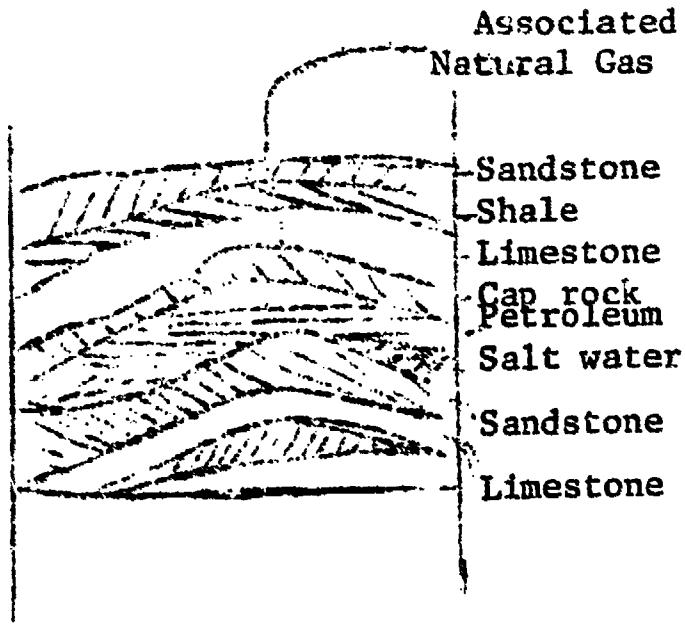
In this case not only is organic matter removed from the water but in addition the methane is collected as a valuable by-product.

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 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 proportions 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^{o} to 225^{o}, 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 (CH_4), hydrogen, ethane (H_3C-CH_3), ethylene ($H_2C=CH_2$), propylene ($Ch_3-CH=CH_2$) etc. Petroleum refineries with catalytic crackers are usually combined with petro-chemical plants which use these by-products as starting materials. For instance, hydrogen is combined with nitrogen to produce ammonia.

$N_2 + 3H_2 \rightleftarrows 2NH_3$.

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 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 In the U. S. 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 ($CH_3CH_2CH_3$) and butane ($CH_3CH_2CH_2CH_3$) can be treated with a dehydrogenation catalyst and converted to olefins.

$$CH_3CH_2CH_3 \xrightarrow{\text{Cat. high temp.}} H_2 + CH_3\text{-}\overset{H}{\overset{|}{C}}\text{=}CH_2$$

Propene

$$CH_3CH_2CH_2\text{-}CH_3 \xrightarrow{\text{Cat., high temp.}} H_2 + CH_3\text{-}CH_2\text{-}CH{=}CH_2$$

1 - butene

$$CH_3\text{-}CH_2\text{-}CH{=}CH_2 \xrightarrow{\text{further reaction}} H_2 + H_2C{=}C(H)\text{-}C(H){=}CH_2$$

1,3-butadiene

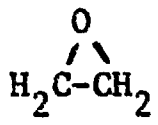
Butadiene is polymerized to butyl rubber, a synthetic substitute for natural rubber.

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

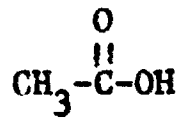
Ethyl alcohol $CH_3CH_2\text{-}OH$

Acetaldehyde $CH_3C(=O)H$

Ethylene Oxide

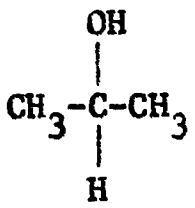


Acetic acid

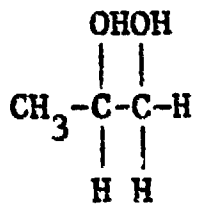


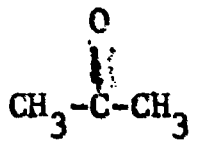
Ethylene glycol $HO\text{-}CH_2\text{-}CH_2\text{-}OH$

Isopropyl alcohol



Propylene glycol



Acetone 

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 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.*

*There are, of course, other reasons for gas wars as well, such as local competition.

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 CO_2 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 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 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. 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. USES 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.

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O + \text{Heat}$

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.

Crude Oil	10.5 Kcal/gram
Gasoline	11.5 Kcal/gram
Kerosene	11.0 Kcal/gram
Coal	6.8 Kcal/gram
Charcoal	8.1 Kcal/gram
Wood	
Beech	4.2 Kcal/gram
Oak	4.0 Kcal/gram
Pine	4.4 Kcal/gram

There are certain advantages (such as cost) in using less efficient fuels, but there are also significant disadvantages as well.

B.2 Chemical Intermediate

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 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.

$$CH_4 + \text{heat} \rightarrow C + 2H_2$$

carbon black

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 recycling 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 overweigh 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, CH_3OH, 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:

$$CO + 2H_2 \xrightarrow[250\text{-}400^{\circ}C]{200\text{-}300 \text{ atm. pressure}} CH_3OH$$

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 materials 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:

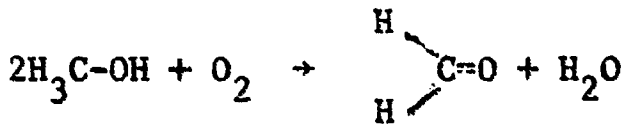
$$3CH_4 + CO_2 + 2H_2O \xrightarrow[\text{pressure}]{\text{high temp. \&}} 4CO + 8H_2$$

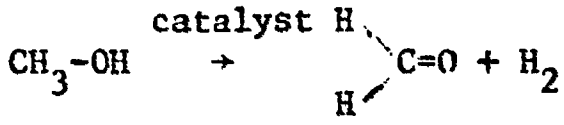
Note that the mole ratio of the two products fits the requirements of the methanol reaction perfectly!

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:





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 anti-freeze, 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.

$$2CH_4 \xrightarrow{1250^{o}} H\text{-}C{\equiv}C\text{-}H + 3H_2$$

acetylene

$$CH_4 + 4S \rightarrow CS_2 + 2H_2S$$

carbon disulfide; hydrogen sulfide

$$CH_4 + Cl_2 \rightarrow Ch_3Cl + HCL$$

methylchloride

$$CH_4 + 2Cl_2 \rightarrow Ch_2Cl_2 + 2HCL$$

methylene chloride

$$CH_4 + 3Cl_2 \rightarrow CHCl_3 + 3HCl$$

chloroform

$$CH_4 + 4Cl_2 \rightarrow CCl_4 + 4HCl$$

carbontetrachloride

$$CH_4 + NH_3 + 1.5\ O_2 \rightarrow HCN + 3H_2O$$

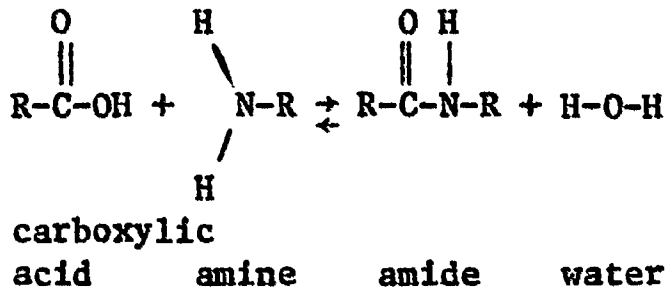
hydrogen cyanide

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.

Construct two models of the amino acid glycine ($H_2N-CH_2-C(=O)-OH$). Glycine is <u>bifunctional</u> because it has two functional groups, amine and carboxyl. Carboxyl groups can react with amines to form amides.



POLYMER SIZES

Name	Type	Molecular Weight
insulin	protein	5.8×10^3
hemoglobin	protein	6.8×10^4
β-galactosidase	protein	5.3×10^5
myosin	protein	6.2×10^5
E. coli chromosome	DNA	2.8×10^9
transfer RNA	RNA	2.5×10^4

an amide bond

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?

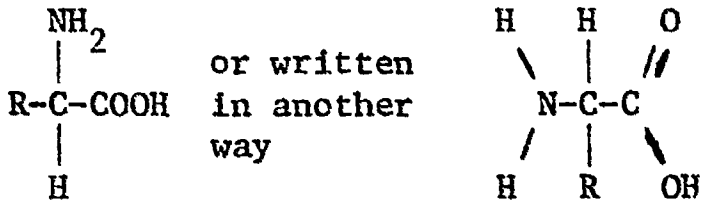
Join your amide model to your neighbor's model. How many amide bonds are represented in the resulting model? Does this model have any free amine or carboxyl groups? How many original glycine models are now involved in this polyamide model?

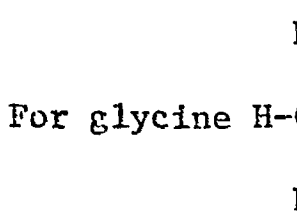
Even though the amine and carboxyl groups of the original glycine models are part of amide bonds, the number of glycine models used to make the polyamide model can still be counted.

The process of combining small molecules in a repeating fashion, as

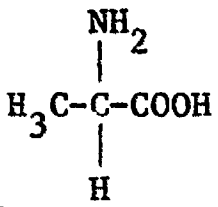
you did with glycine models, is known as polymerization. The small molecules are monomers. The resulting large molecule is a polymer. Each repeating unit in the polymer is called a residue.

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





-H; for the amino acid alanine, R-



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.

Formation Of Nylon

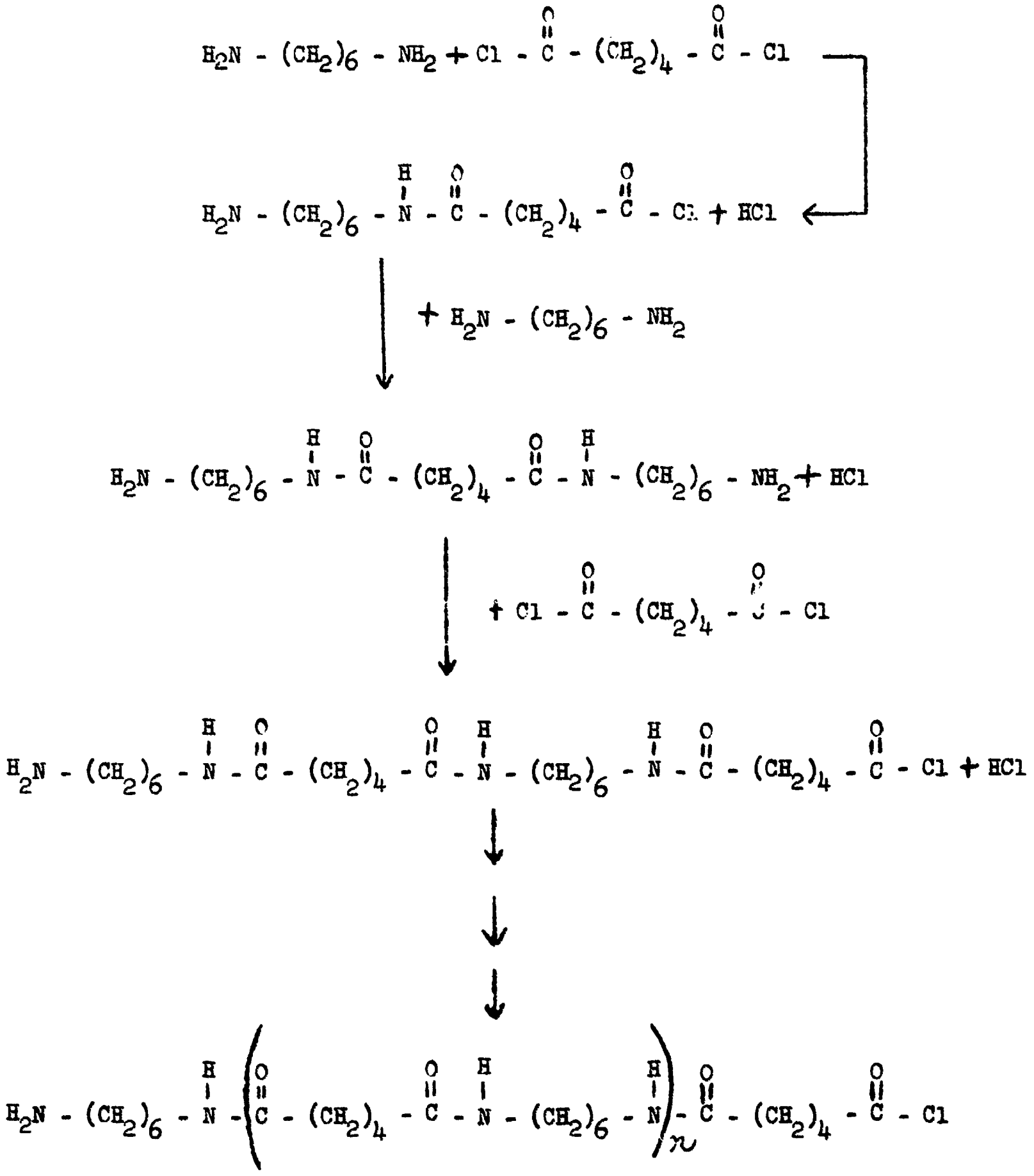


Figure A.1

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

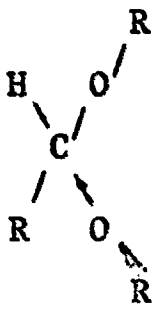
Study of living systems has shown that there are three large polymers of nearly universal occurrence: (1) protein (a polyamide), (2) nucleic acid (a polyphosphoester) and (3) carbohydrate (a

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 are shown in Fig. A.2.

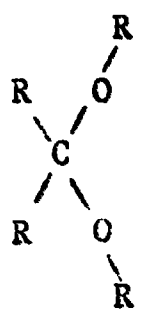
Some chemistry of acetals and ketals, very important in carbohydrate chemistry, is outlined in Appendix A at the end of the chapter, page 98-100. The appendix will help in understanding Fig. A.3 and the discussion of starch and cellulose.

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.

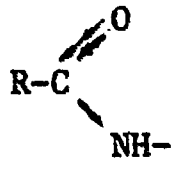
Type of Bond Between Monomers



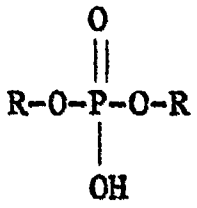
a. acetal bond



b. ketal bond



c. amide bond



d. phosphoester bond

Figure A.2

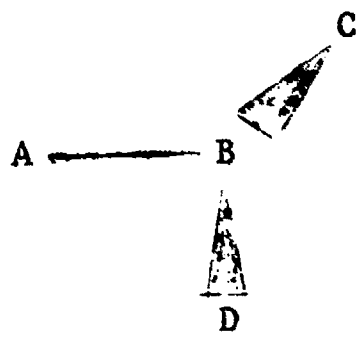
Knowing this is a great aid in the study of living systems, because attention may be focused upon the specific bond type of interest to the investigator.

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.

FOOTNOTE TO FIG A 3,4,5

From your work with models you know that three dimensions are required to describe molecules. In Figures A. 3,4, and 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 <u>not</u> 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

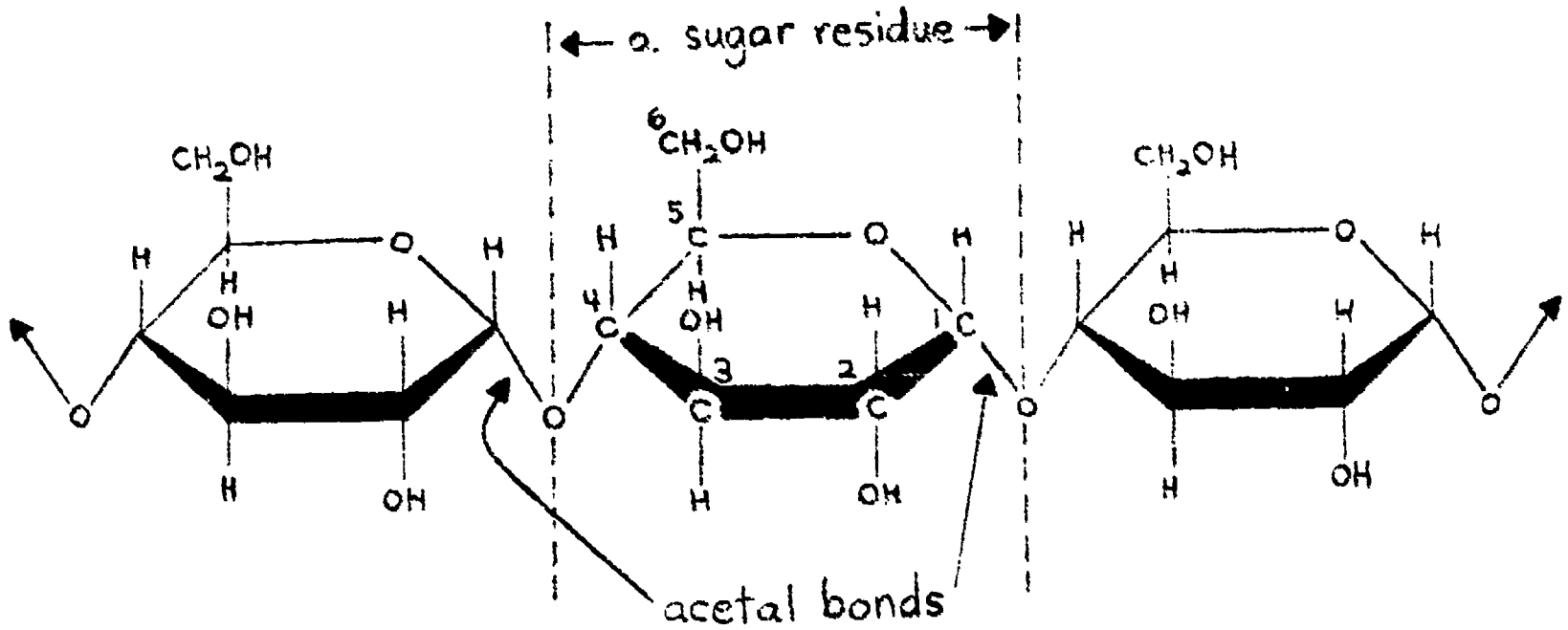


Figure A.3

PROTEIN

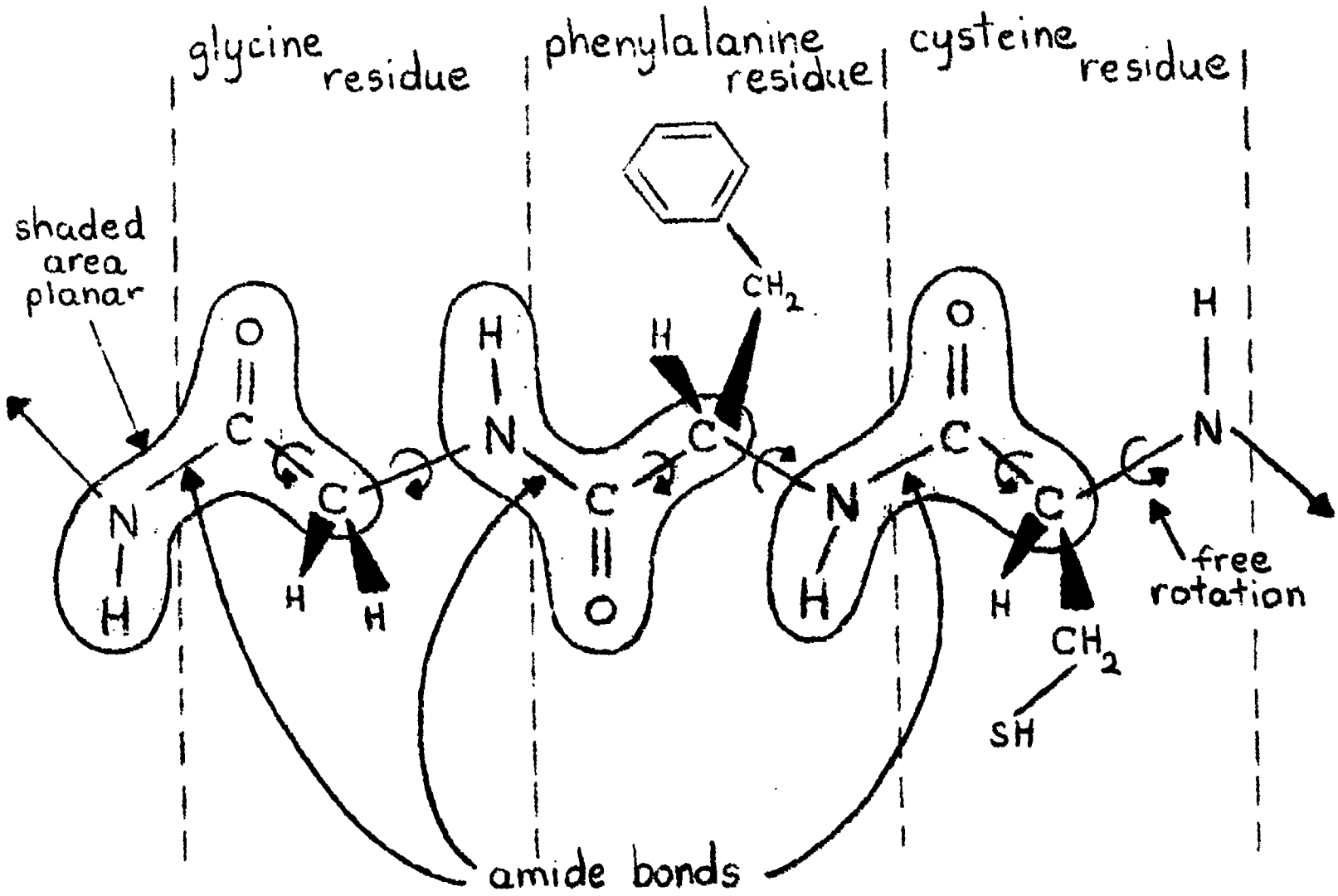
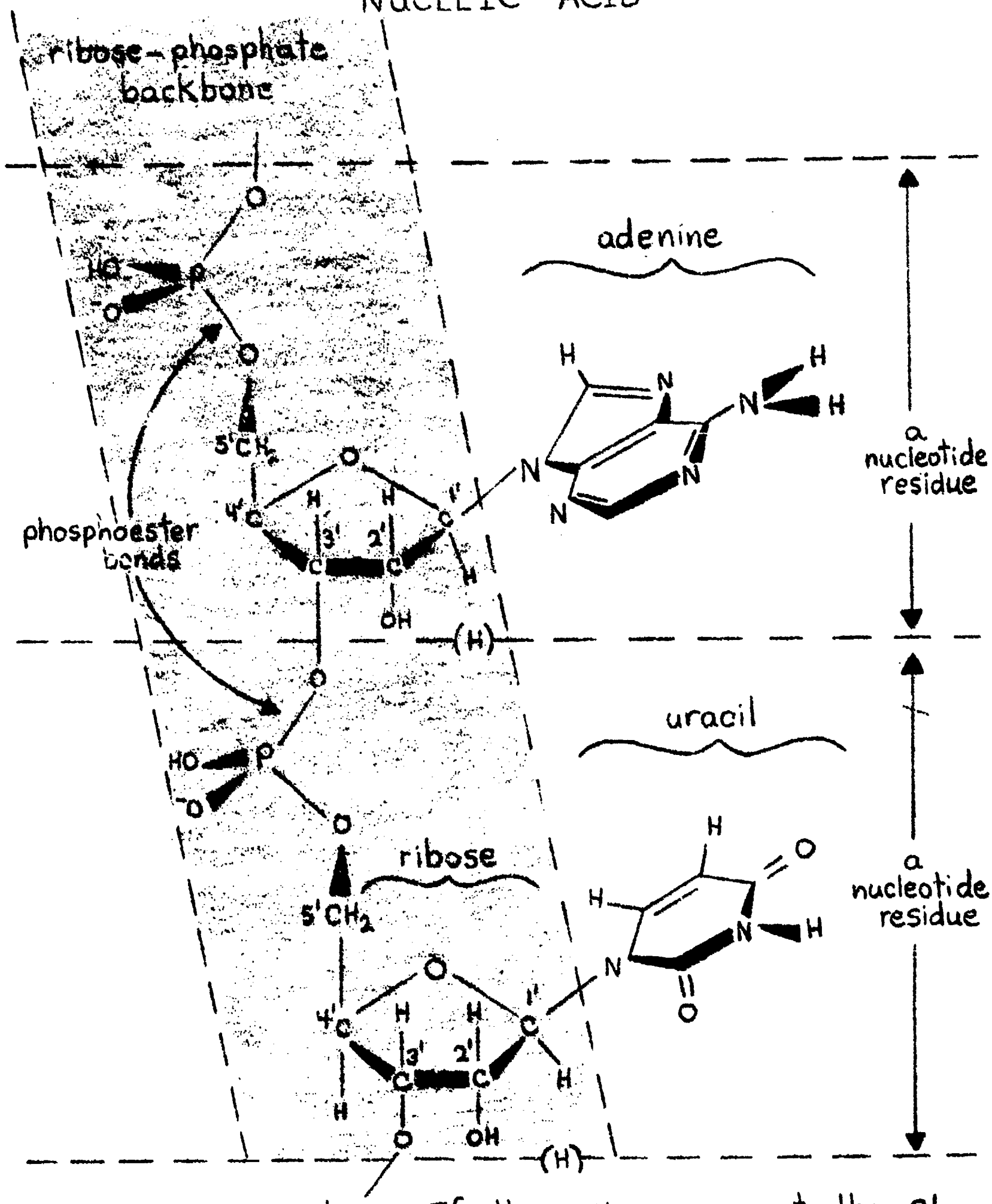


Figure A.4

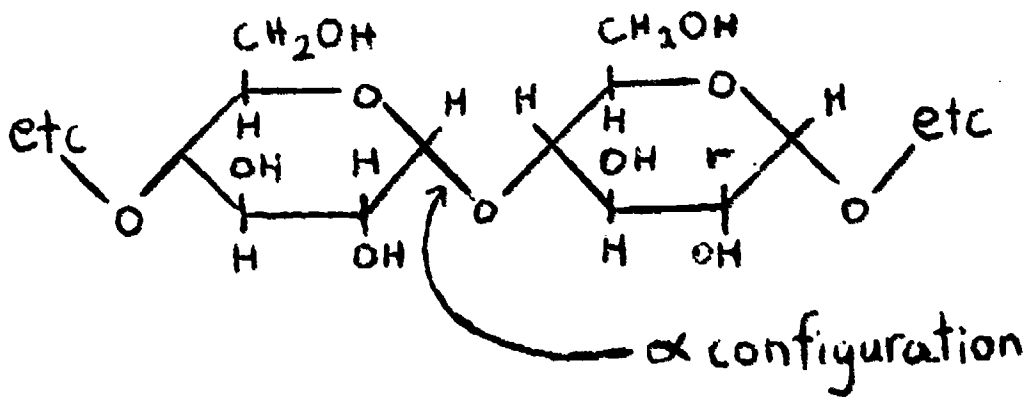


NOTE: RNA is shown. If the -OH groups at the 2' positions on the ribose residues are replaced by H atoms, as indicated, the structure is that of DNA.

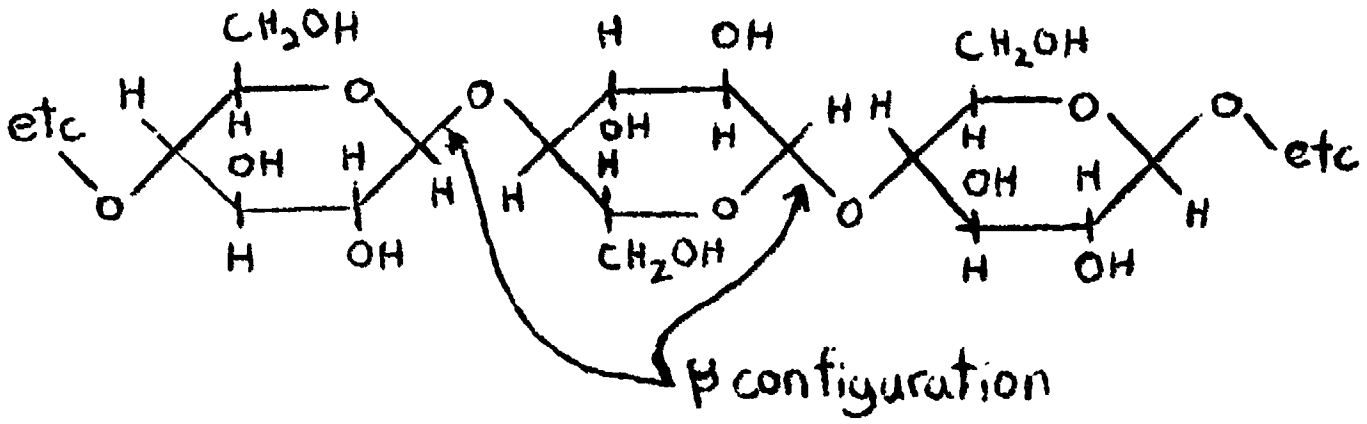
Figure A.5

Following is an example in which the difference in <u>linkage</u> is important in determining biological activity:

<u>Starch</u> (an α-1-4 linked polymer of glucose)



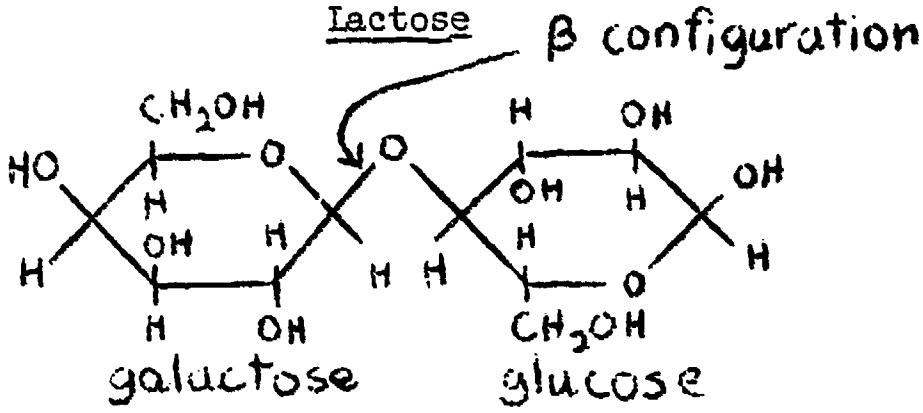
<u>Cellulose</u> (a β-1-4 linked polymer of glucose)



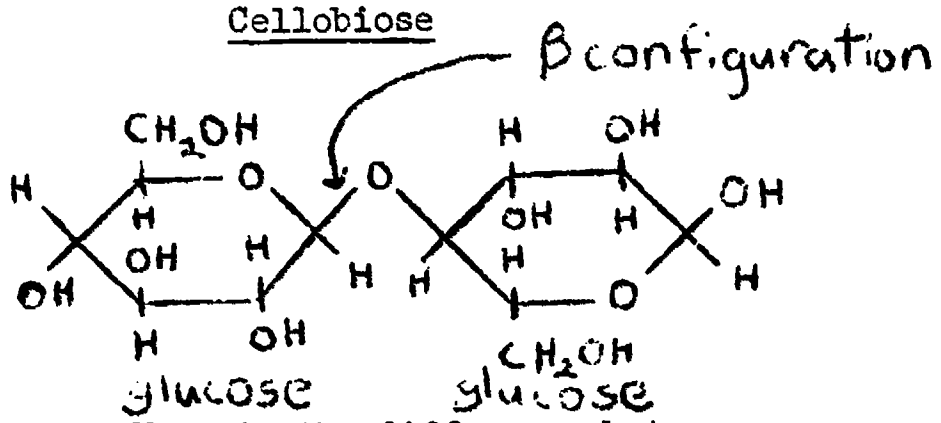
The first polymer, starch, is readily digested by humans and is a staple in our diet. The second polymer, cellulose, is a primary constituent of hay, wood, and paper. Cows and termites eat cel-

lulose but depend upon certain micro-organisms 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:



Cellobiose

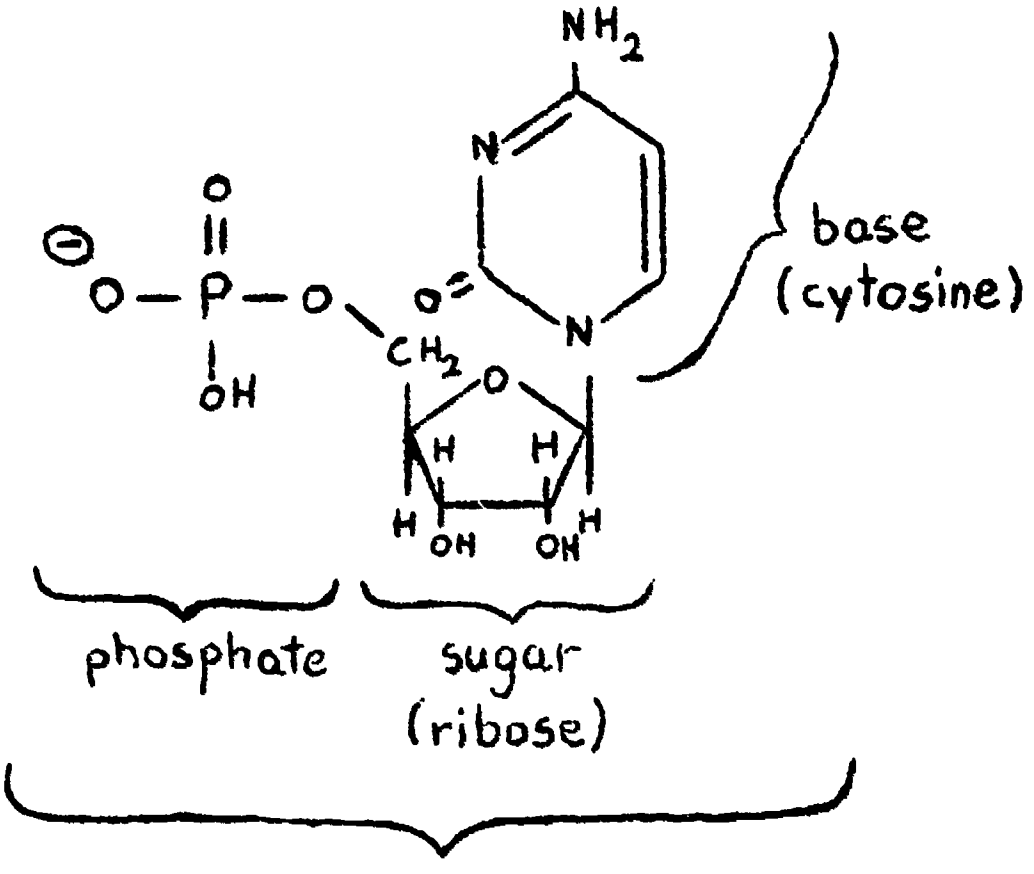


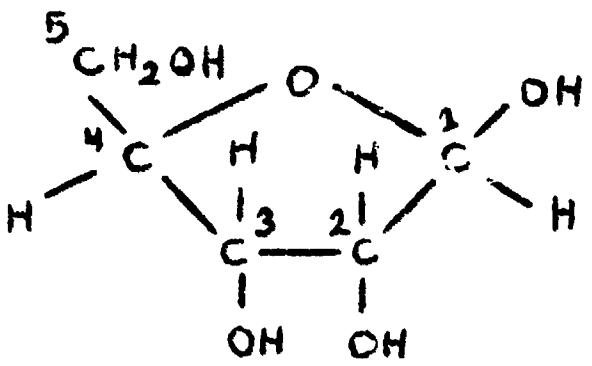
What is the difference between these two structures? Humans digest lactose, a sugar found in milk, but cannot digest cellobiose, a plant sugar.

In the case of starch, each monomer is a glucose molecule and thus

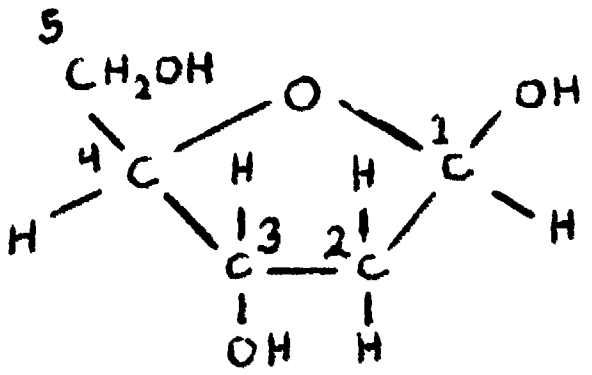
each residue in the polymer is the same (see Figure A.3). However, for protein, in which each monomer is an amino acid, there are many different amino acid residues composing the polymer (see Figure A.4).

Nucleic acids are polymers. Like 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.





ribose



2-deoxyribose

Figure A.6

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 (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.

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 through two sheets of filter paper each time.

Following the directions on page 91 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 on the horizontal axis.

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

$HOOC-CH_2-CH_2-CH_2-COOH$

(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 strucutral formula for:

(a) propane

(b) propanol

(c) propanone

(d) How many carbon atoms are in each molecular formula?

(9) (a) 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?

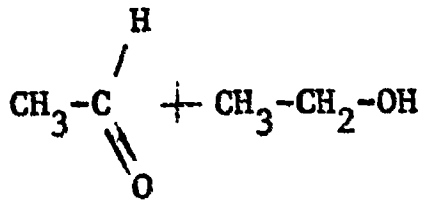
(15) (a) Draw the methyl ester of phosphoric acid.

(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.

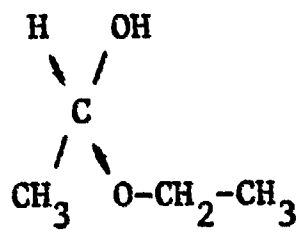
APPENDIX A

Acetals are prepared from alcohols and aldehydes:



ethanal (aldehyde) + ethanol (alcohol)

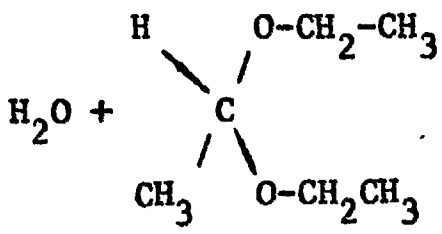
↑↓ HCl



a hemiacetal

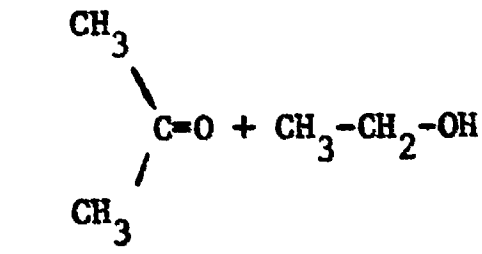
+ CH_3-CH_2-OH

↑↓ HCl

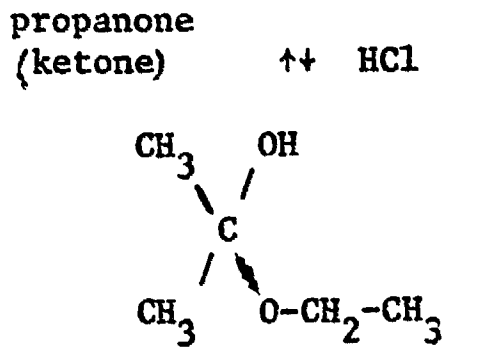


an acetal

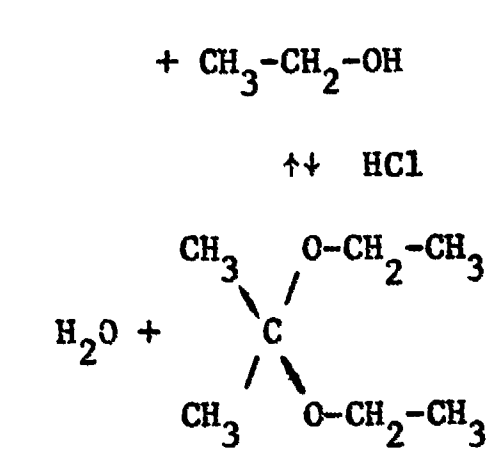
Ketals are prepared in the same way using alcohols and ketones:



propanone
(ketone)



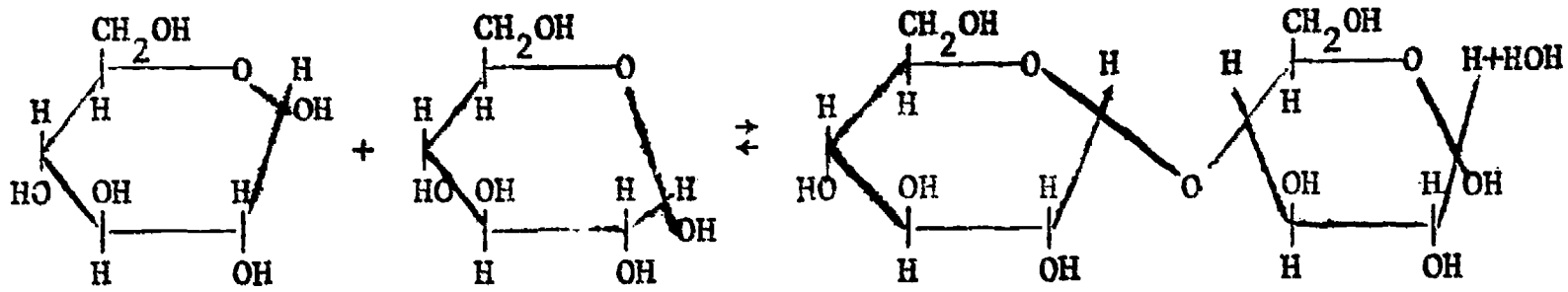
a hemiketal



a ketal

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 case of polyacetals involves the reactions of a hemiacetal with another hemiacetal.

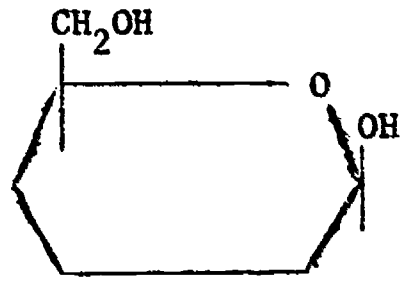


hemiacetal
(glucose)

hemiacetal
(glucose)

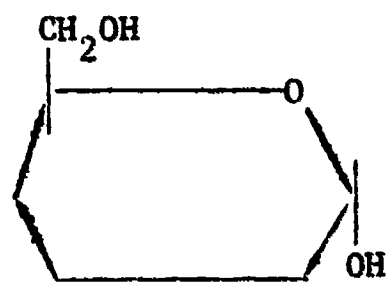
acetal
(maltose)

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 β.



β configuration

The opposite configuration is called α.



α configuration

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

Chapter IV: POLYMERS IN 3D OR THE SHAPE OF THINGS TO COME

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.

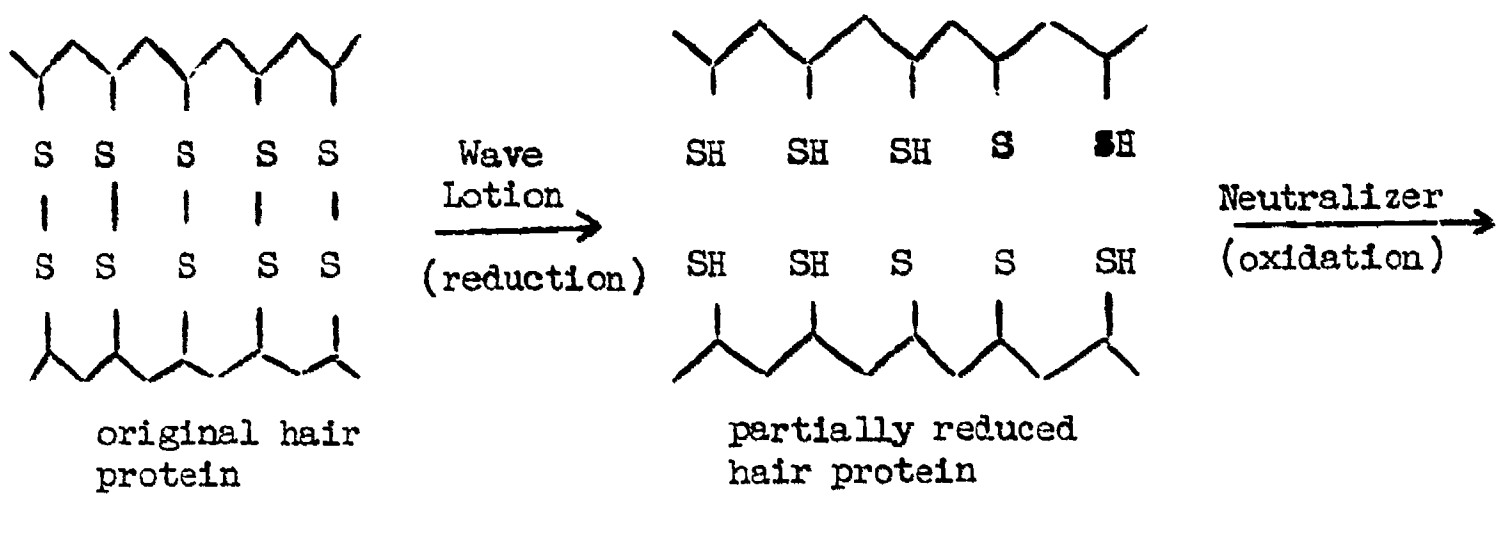
Relative Bond Strengths

Type of Bond	Van der Waals	:	Hydrogen	:	Covalent
Kcal/mole	1	:	10	:	100

Table A.1

The strongest bond listed 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 ($HS-CH_2-\underset{NH_2}{CH}-COOH$). 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.



original hair protein

partially reduced hair protein

curled hair protein with new disulfide bonds

Figure A.2

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. Im-

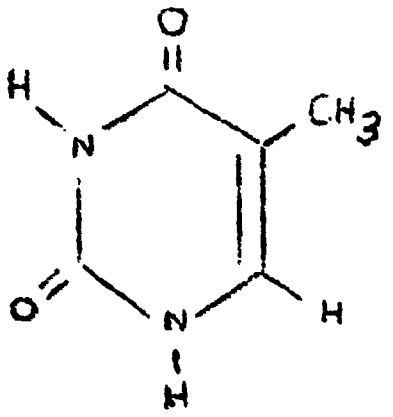
merse the other rod of hair in an equal volume of water in another test tube. Set the two tubes aside for 15 minutes. After the 15-minute interval, remove the two hair-wrapped rods and rinse with running tap water until the odor of lotion cannot be smelled. "Neutralize" (oxidize) the hair following the directions of the specific commercial product used.

B. WEAKER BONDS

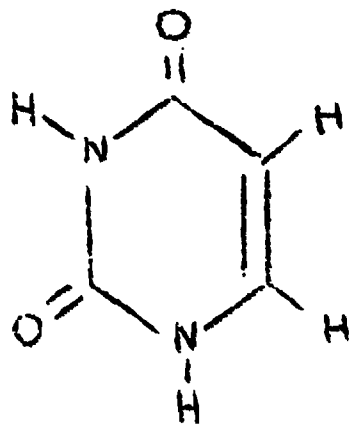
B.1 H-BONDS BETWEEN BASES

Weaker bonds, for example hydrogen bonds, were discussed in Chapter I of Chemistry of Living Matter and Chapter 17, CHEM Study. One important factor in determining the three-dimensional shape of nucleic acids is hydrogen bonding 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.

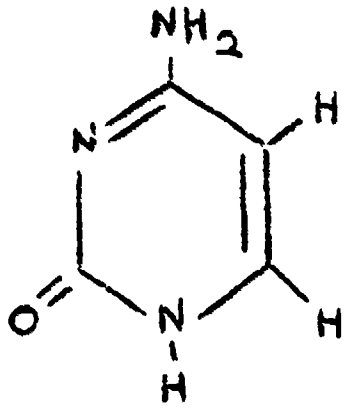
Bases of Nucleic Acids



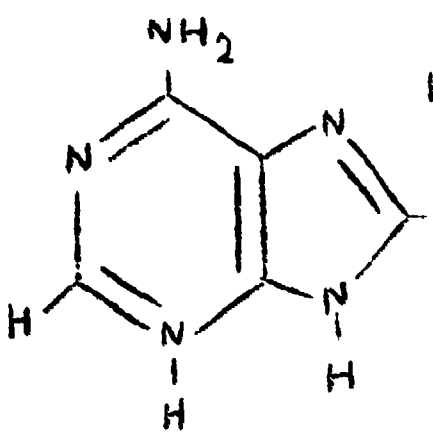
THYMINE (in DNA)



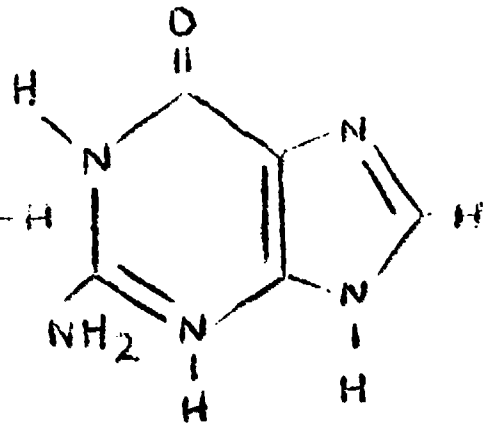
URACIL (in RNA)



CYTOSINE



ADENINE



GUANINE

These bases, each with one ring, are called pyrimidines.

These two-ring bases are called purines.

Figure B.1

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,

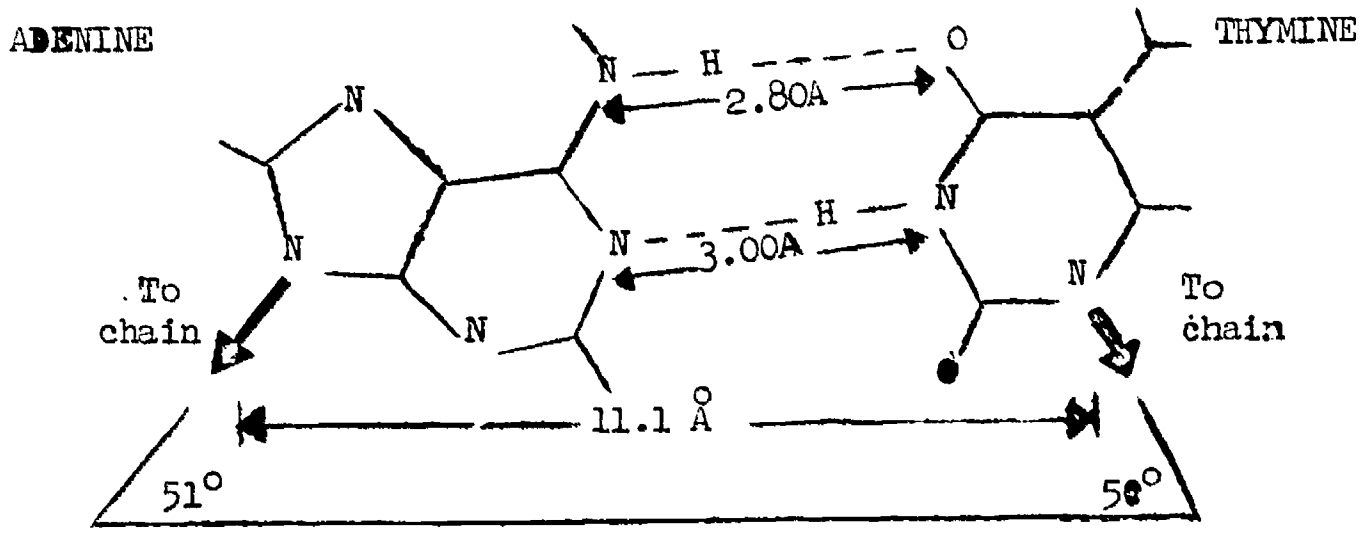


Figure B.2

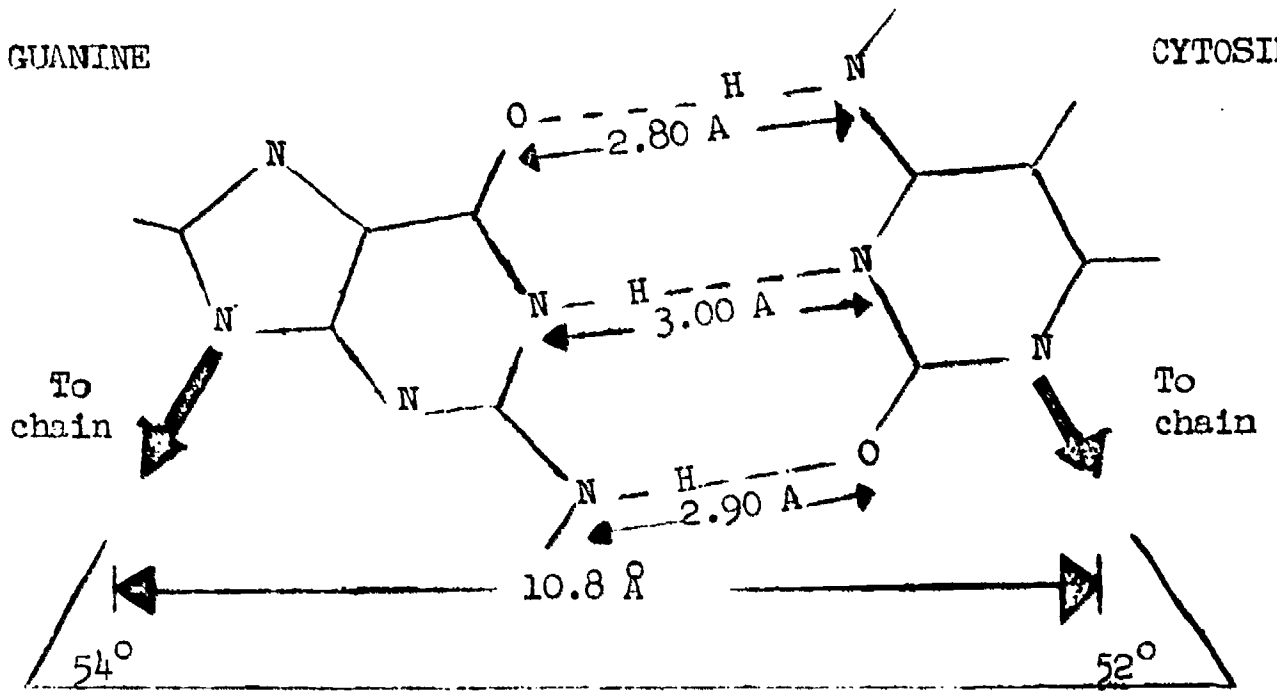


Figure B.3

how many different hydrogen-bonded pairs are possible? Which arrangements of base pairing would you predict to be most stable? Compare the sizes of the hydrogen-bnnded pairs in which you find maximum hydrogen bonding. Pairing of smaller with larger gives a oonstant-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 along their edges, as in Figure B.4, then the two DNA polymers would be held together.

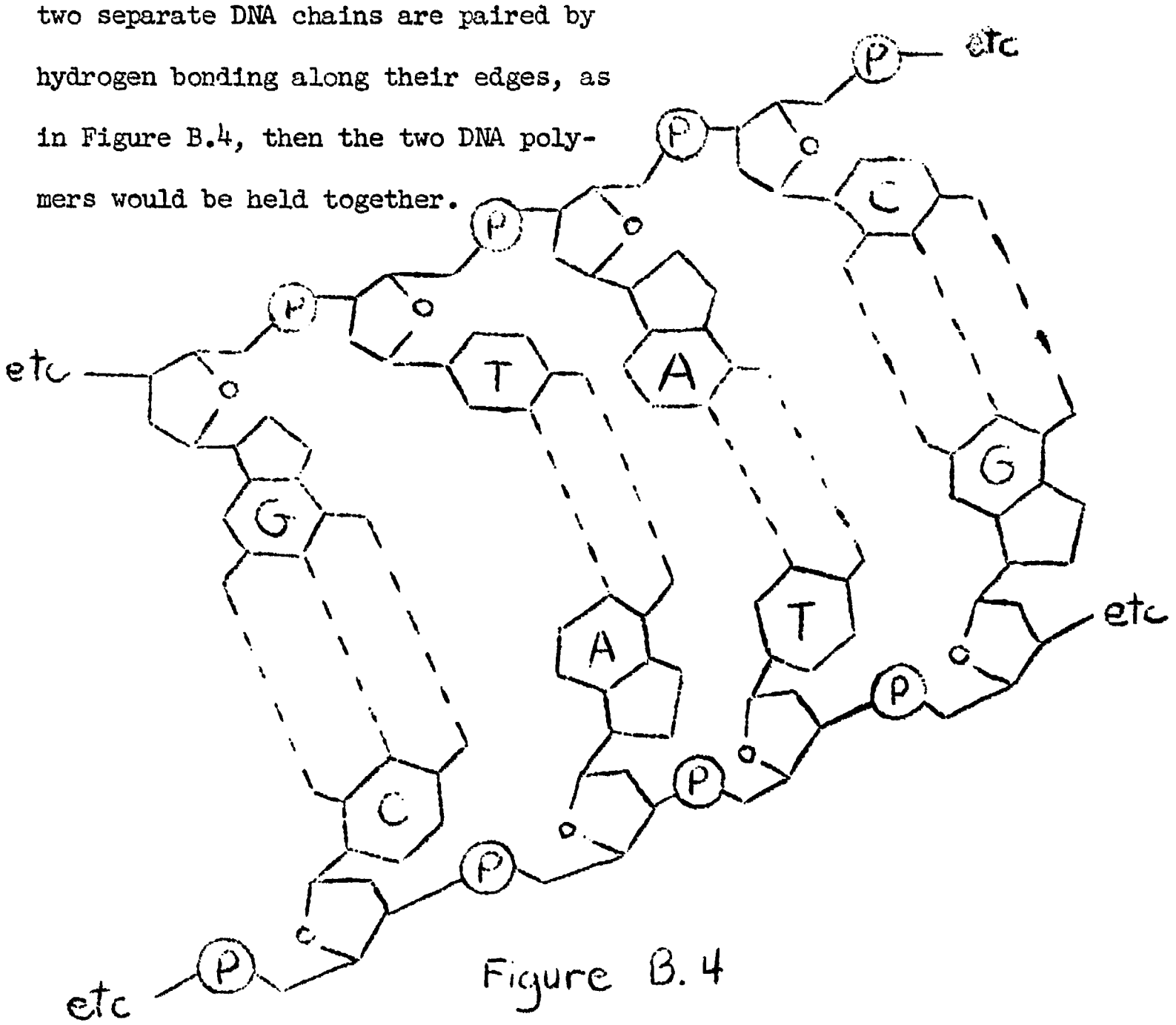


Figure B.4

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.

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."

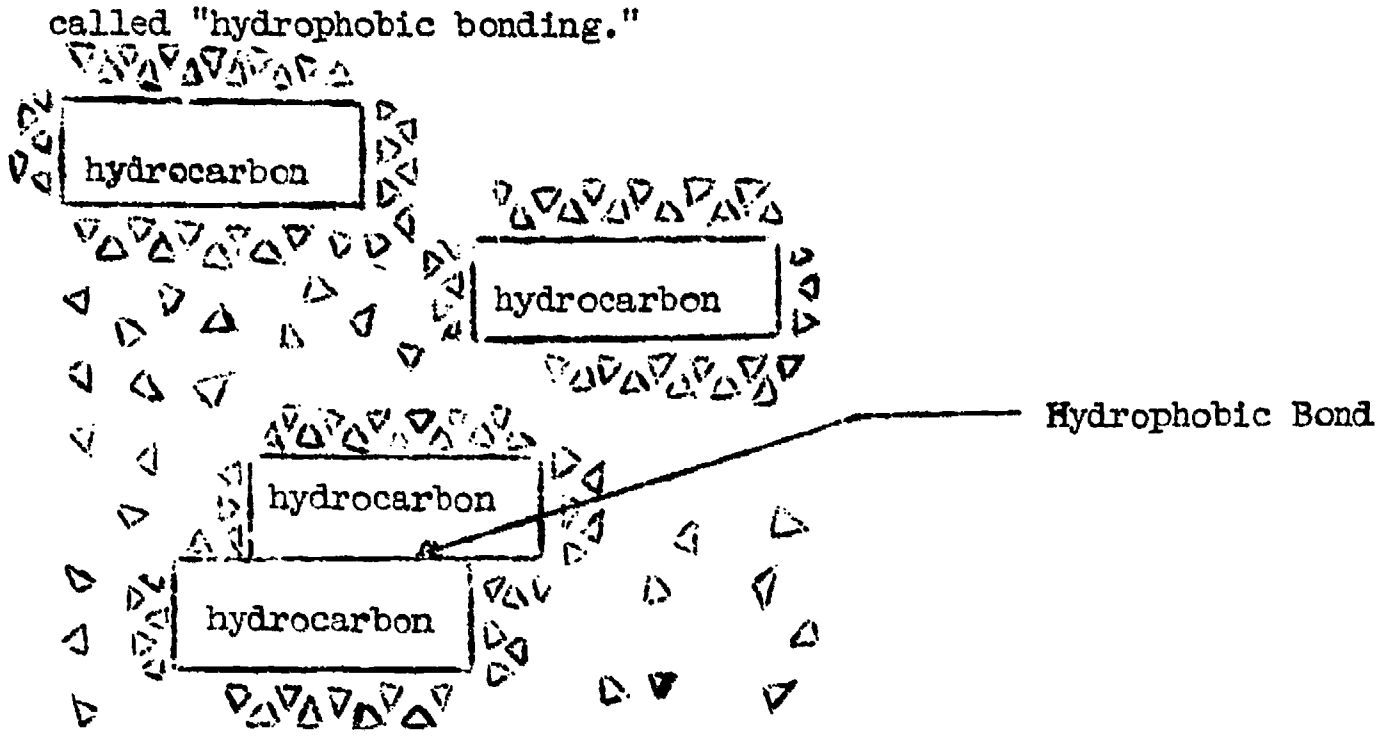


Figure B.5 - Hydrophobic Bond Formation. The triangles represent water molecules. As the hydrocarbon residues approach each other some of the water molecules are released from an ordered arrangement close to hydrocarbon to a more disordered state in the solvent.

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 $-NH_2$ 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 zig-zag or helical conformation in order to fold up the backbone and bring the base residues close enough to stack.

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).

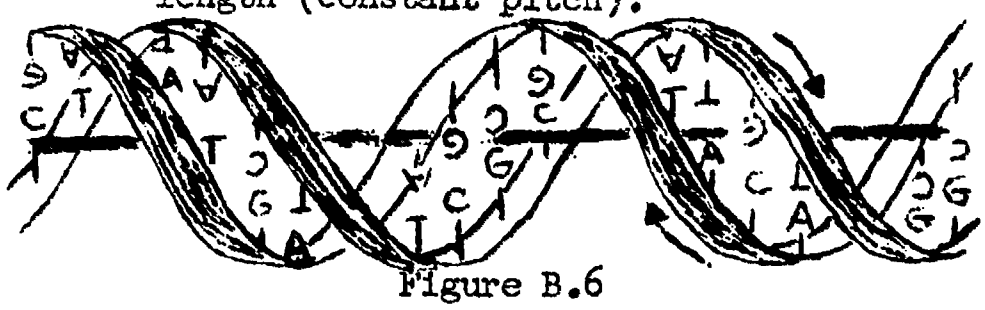


Figure B.6

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 1966, page 78. The deficiencies of such pictures are ob-

vious, 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.

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--a priori to predict 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.

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.

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 found 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 two 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.

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 network with the water trapped in this network. Any enzyme that digests gelatin will breakdown 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 13x100 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 13x100 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.

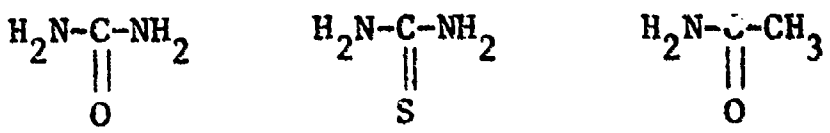
$$H_2N - \underset{\underset{O}{\|}}{C} - NH_2 + H_2O \xrightarrow[\text{urease}]{\text{enzyme}} 2NH_3 + CO_2$$

urea water ammonia 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.



urea thiourea acetamide

A.3 Experiment: SPECIFICITY OF UREASE

Obtain five milliliters of urease solution from your instructor. Add one drop of phenolphthalein indicator to each of the four 13 x 100 mm test tubes. Add one milliliter of 1% urea solution to each of test tubes 1 and 2, containing 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°C destroyed the catalyzing ability of urease. The following discussion of polymer shape may help explain this sensitive relationship.

Suppose there is a complementary surface relationship between an enzyme and its substrate (the molecule undergoing reaction in the catalyzed reaction).

UREASE PROTOCOL

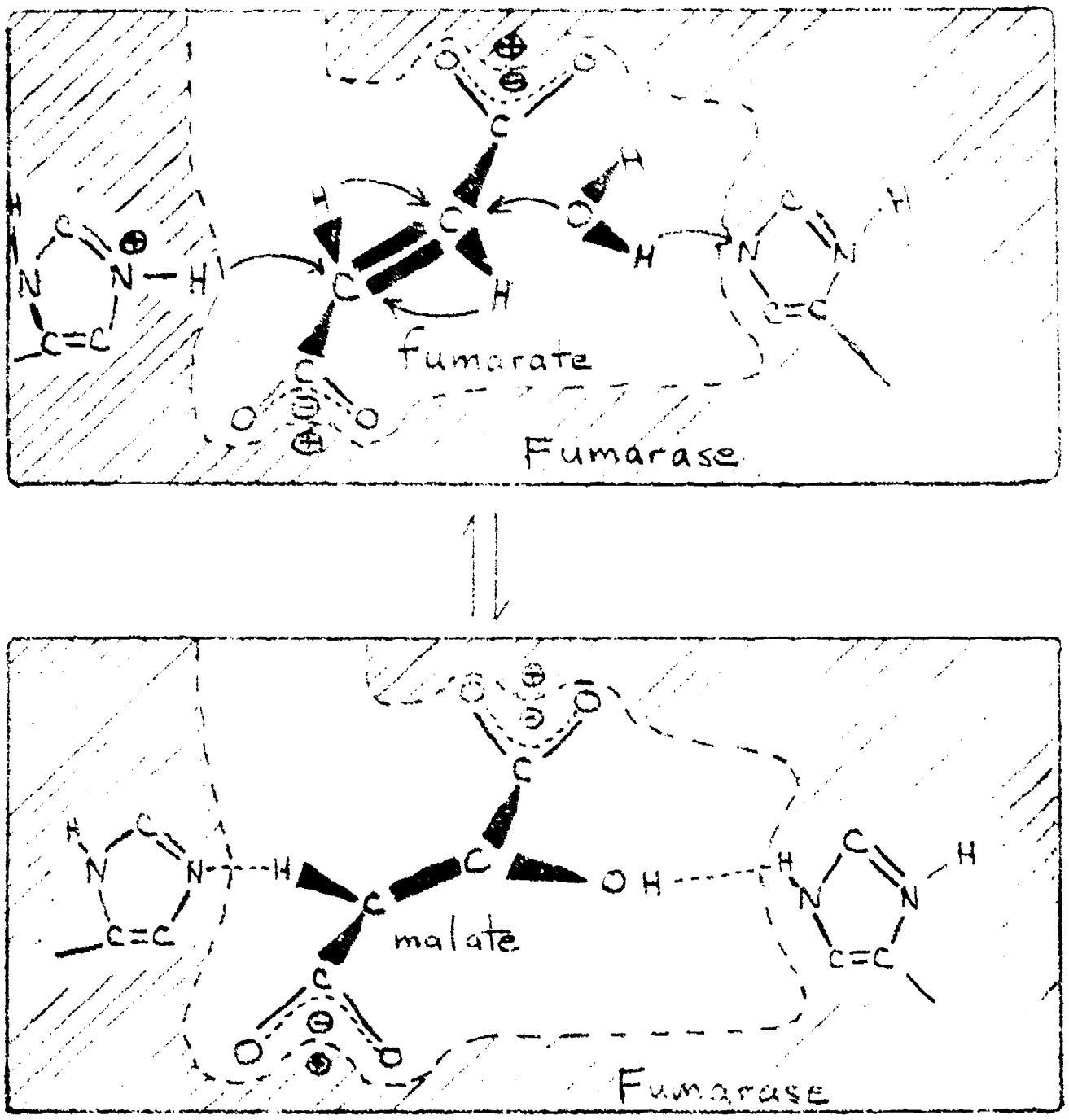
reagent \ Test tube #	Specificity of urease				Substrate competition		
	1	2	3	4	5	6	7
Phenolphthalein	one drop	one drop	one drop	one drop	one drop	one drop	one drop
1% urea solution	1 ml	1 ml	—	—	4* drops	4* drops	4* drops
4% thiourea solution	—	—	1 ml	—	1 ml	—	—
4% acetamide solution	—	—	—	1 ml	—	1 ml	—
urease solution	—	10 drops	10 drops	10 drops	10 drops	10 drops	10 drops
boiled urease solution	10 drops	—	—	—	—	—	—
H_2O	—	—	—	—	—	—	1 ml

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.

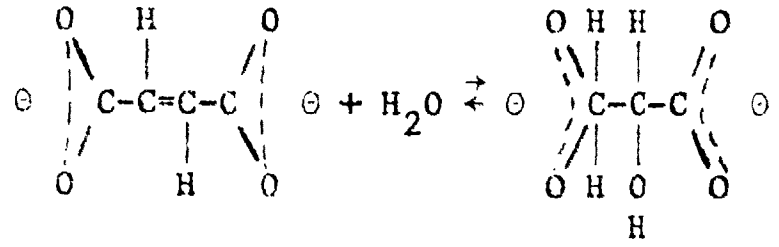
Thus the enzyme is to the substrate what a lock is to a key. There are locks which operate with many keys and locks which are specific for one key. There are enzymes which catalyze many substrates and enzymes which are specific for the catalysis of one substrate. (See Figure A.1.)

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.



Mechanism of Fumaruse Action

The figure illustrates the reaction catalyzed by fumarase and should give an idea of the active site of this enzyme and how the substrates fumarate and water fit into the active site and react to give malate:



(fumarate) malate

Figure A.1

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.

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?

(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?

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

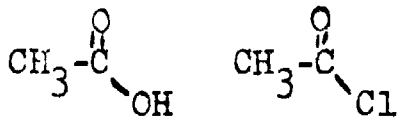
A. THE ACTIVATION PROBLEM

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.

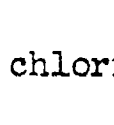
Nylon can be considered to have more chemical energy than an equivalent solution of its monomers. As a consequence, nylon will react spontaneously with H_2O to yield the monomers. This

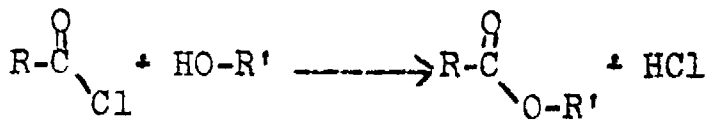
reaction, however, is extremely slow in the absence of a catayst.

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.

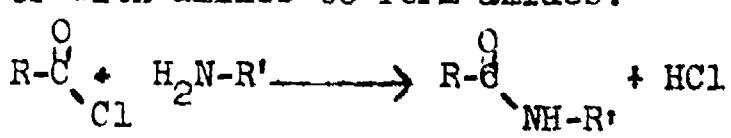


acetic acid acetyl chloride (an acyl chloride)

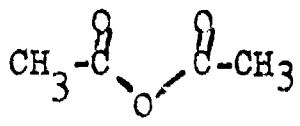
This functional group of an acyl chloride () contains more chemical energy than the carboxyl group. As a result, acyl chlorides will react vigorously with alcohols to form esters:



or with amines to form amides:



Another activated form of carboxylic acids is the acid anhydride:



acetic anhydride

A.1 Experiment: ASPIRIN SYNTHESIS

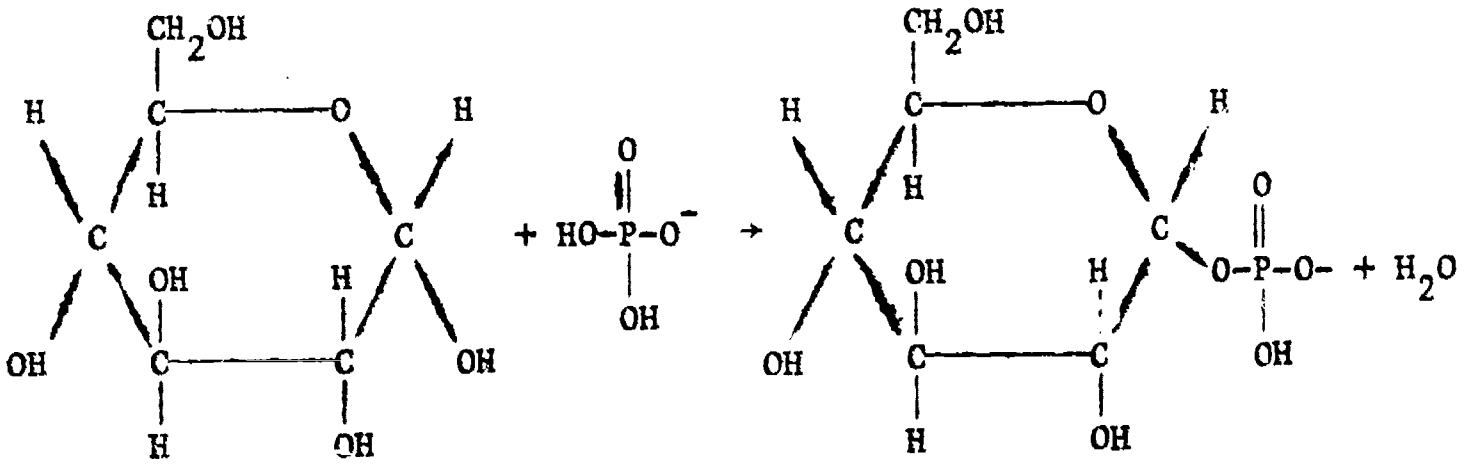
Aspirin Synthesis Protocol						
Reagent	Tube Number 1	2	3	4	5	6
Salicylic acid; 10 mg/ml in dioxane	1 ml	—	1 ml	1 ml	1 ml	1 ml
Acetyl salicylic acid; 10 mg/ml in dioxane	—	1 ml	—	—	—	—
Acetyl chloride	—	—	0.5 ml	—	—	—
Acetic anhydride	—	—	—	0.5ml	1 ml	—
Glacial acetic acid	—	—	—	—	—	1 ml
Heat for ten minutes in boiling water bath before adding $FeCl_3$						
$FeCl_3$; 1 mg/ml in water	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

Table A.1

We will now use salicylic acid with acetyl chloride and acetic anhydride to make an ester, acetyl salicylic acid, which is usually called aspirin. In which tubes 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?

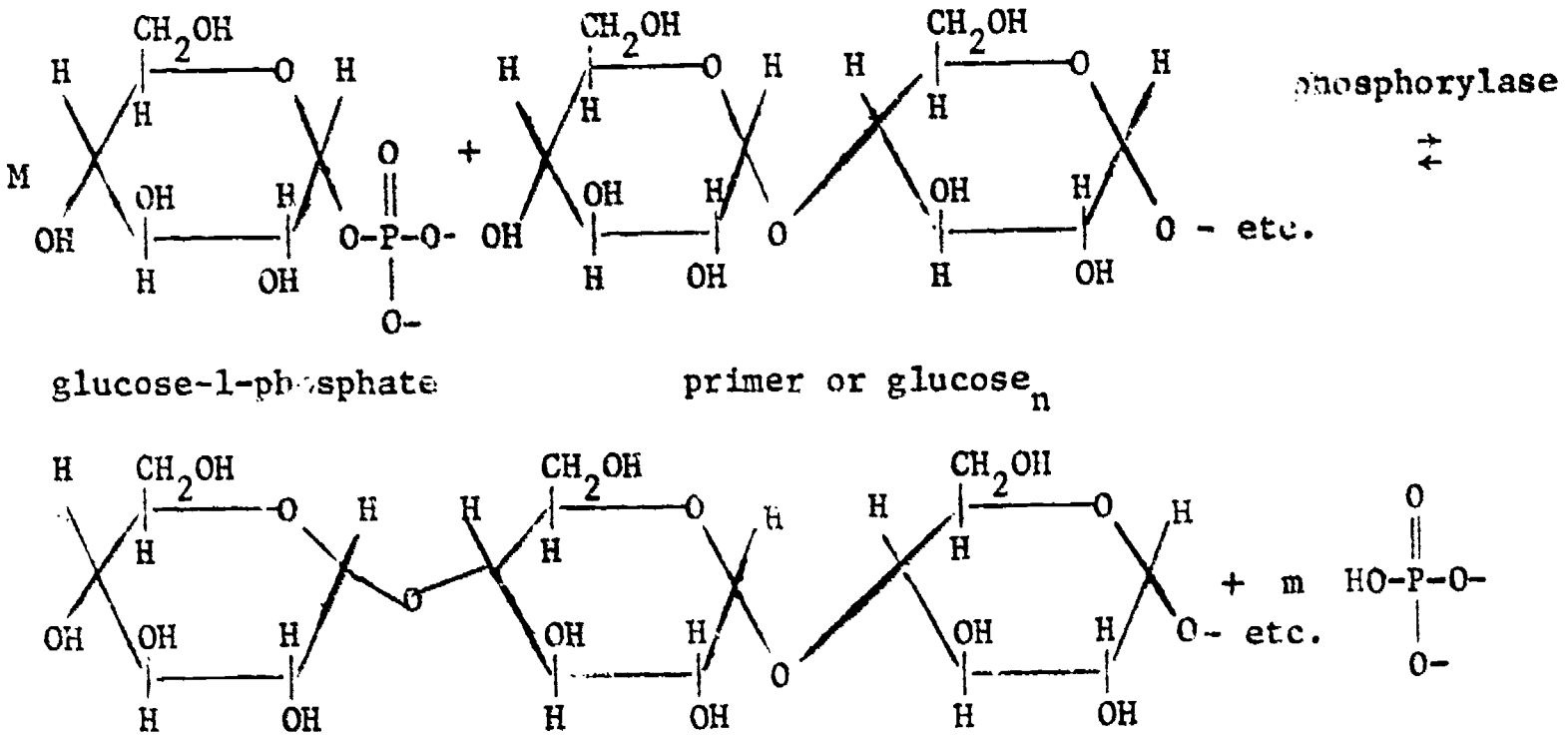
The polymers produced by cells such as starch, proteins and nucleic acids also contain more chemical energy than their monomers. Therefore, in order to form polymers cells must convert monomers into higher energy derivatives which are called activated monomers.

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 phosphate glucose-1-phosphate (ionized form)

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-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 labelled B (for boiled) and three ml of phosphorylase solution in a test tube labelled 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 giver in the Phosphorylase Protocol (Page 107). 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?

PHOSPHORYLASE PROTOCOL

Reagent \ Test Tube #	1	2	3	4	5
glucose-1-phosphate and primer solution	1 ml	1 ml	1 ml	1 ml	———
glucose and primer solution	———	———	———	———	1 ml
boiled phosphorylase (B)	1 ml	———	———	———	———
phosphorylase (C)	———	1 ml	1 ml	———	1 ml
Distilled H_2O	———	———	———	1 ml	———
Test plate iodine solution	1 drop	1 drop	1 drop	1 drop	1 drop
Color observed					

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).

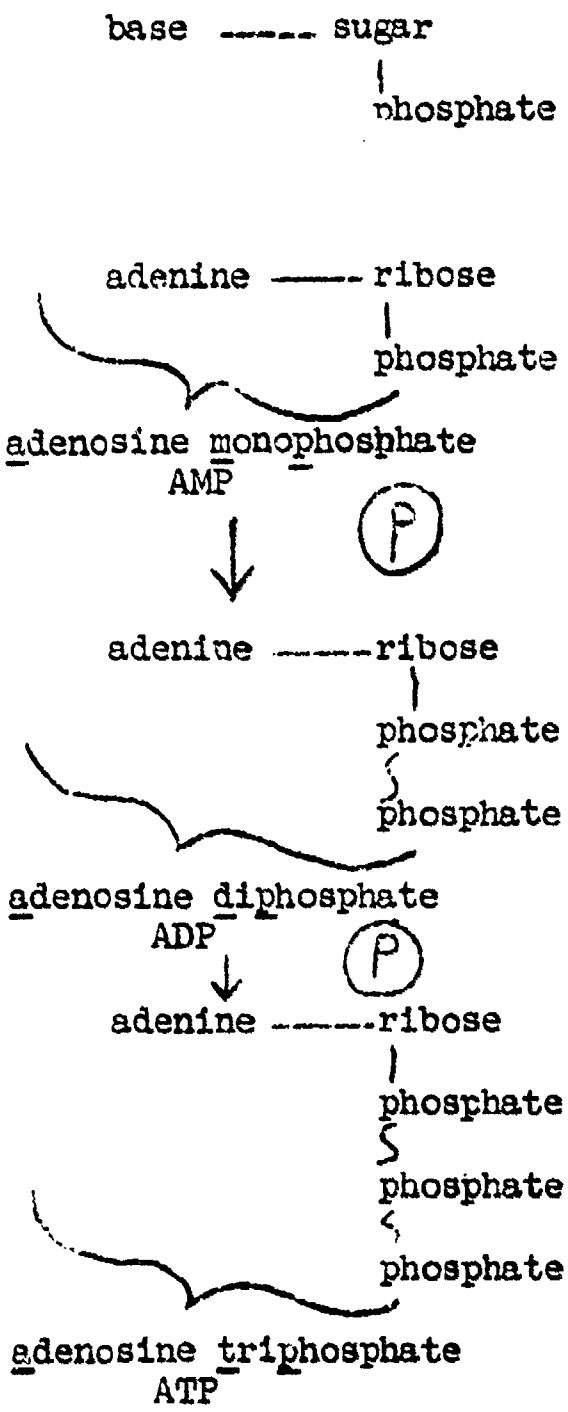
Remember that the monomer is the small molecule by itself and the residue is what is left of this when it is in the polymer. The structures of these four bases as well as that of thymine, found in DNA, are shown in Figure B.1 of Chapter III.

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.



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. UMP + ATP $\xrightarrow{\text{enzyme}}$ UDP + ADP

2. UDP + ATP $\xrightarrow{\text{enzyme}}$ UTP + 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:

$$n \begin{Bmatrix} \text{ATP} \\ \text{GTP} \\ \text{CTP} \\ \text{UTP} \end{Bmatrix} \xrightarrow[\text{RNA polymerase}]{\text{DNA (template)}} \text{RNA} + 4n(\text{P-P})$$

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.

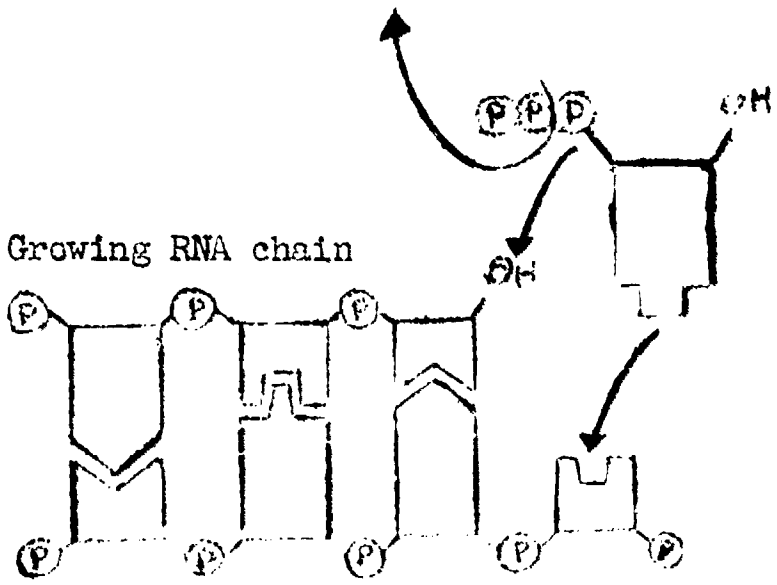


Figure B.1

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 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

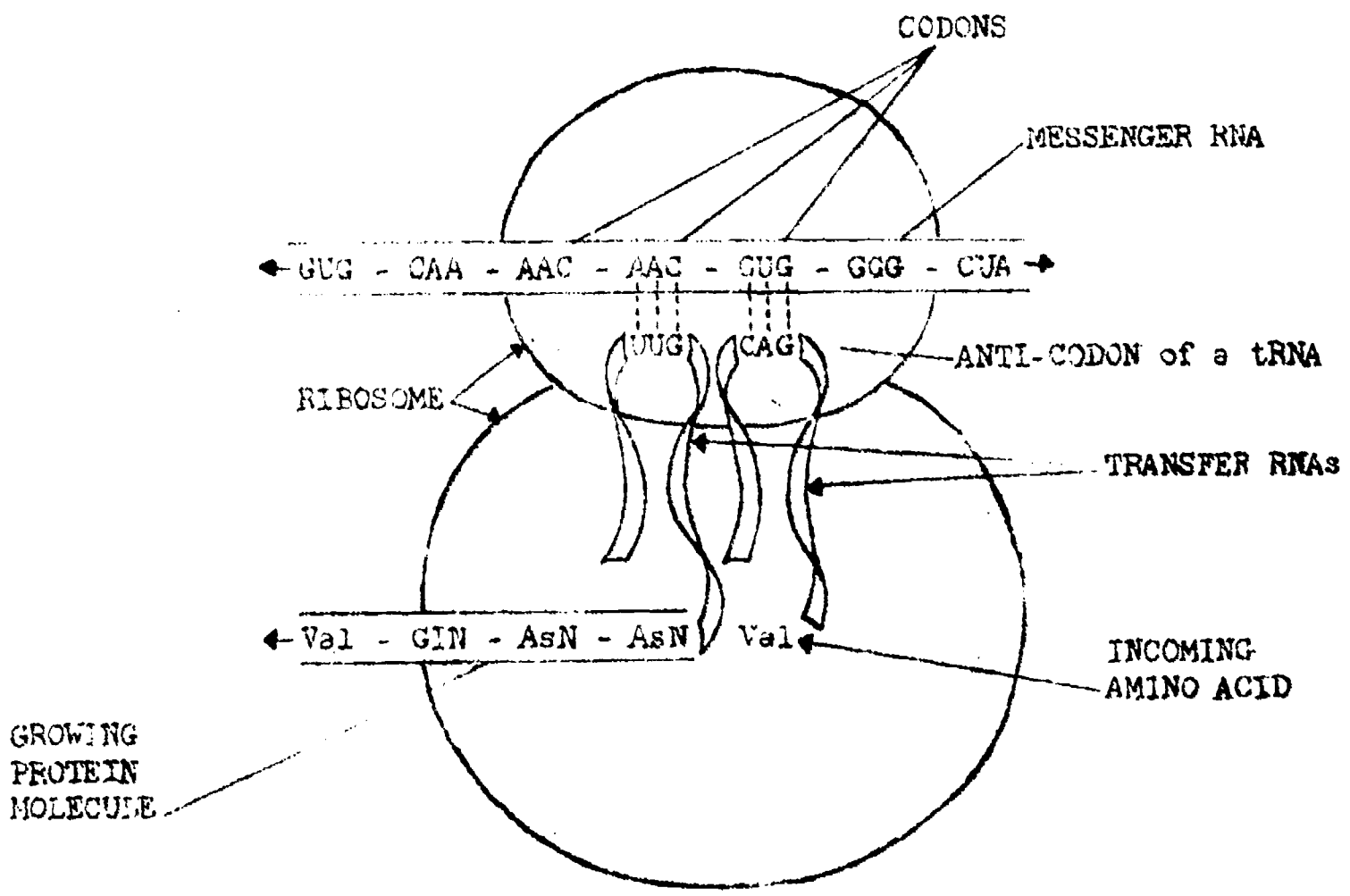
been done on the coding problem, using isolated protein-synthesizing systems from many types of cells. It has been possible to use chemically synthesized mRNA of known base sequence to direct amino acid polymerization and then to analyze the protein formed for its amino acid sequence.

The genetic code is now almost 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 anti-codon 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 on next page. 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 follows.



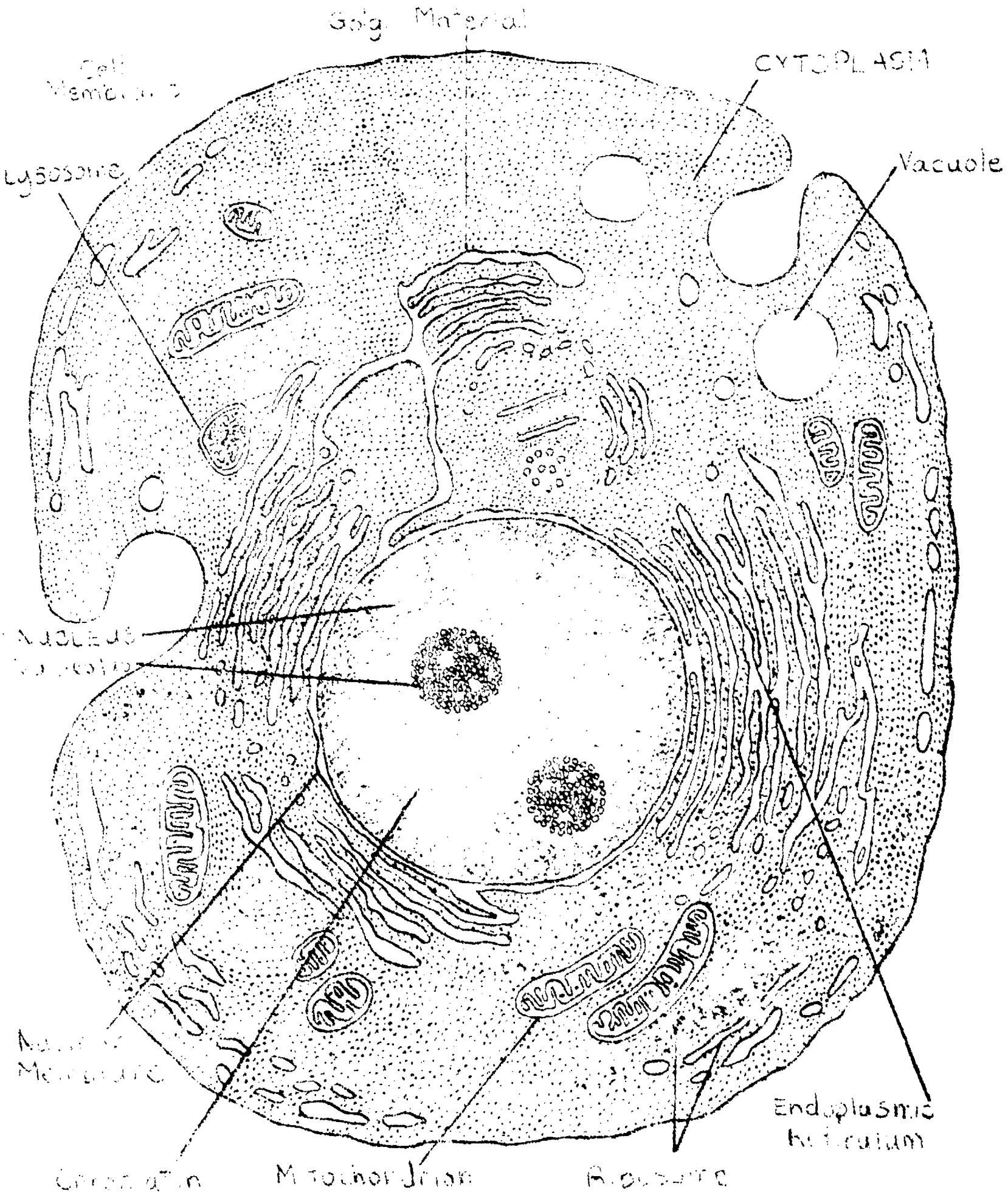


Figure 6.2 An Idealized Animal Cell

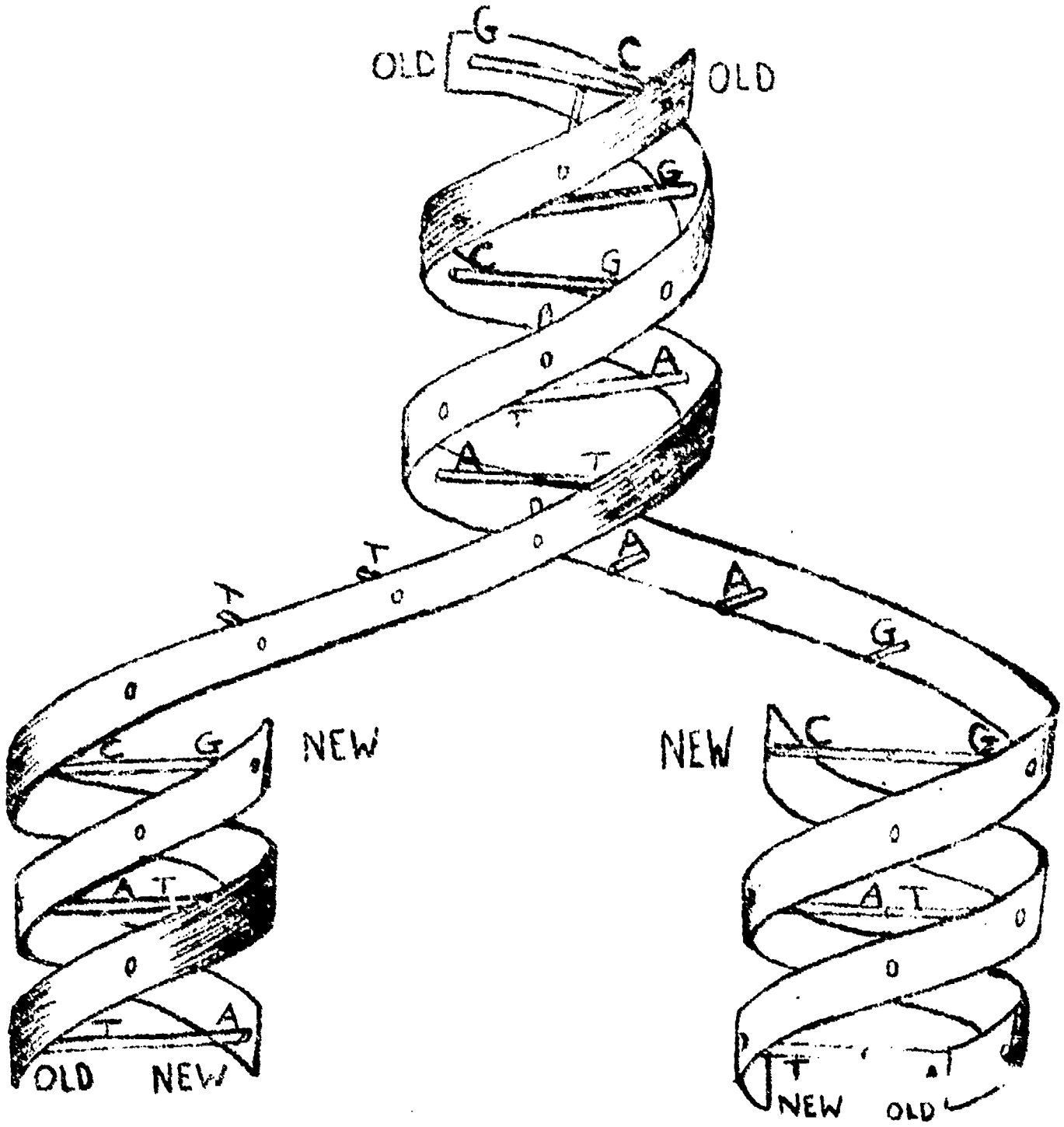
D. THE SEQUENCE PROBLEM COMPLETED OR THE MASTER MOLECULE

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 <u>two</u> types of instructions in its base sequence--one for RNA and one for its <u>own</u> 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 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.



DNA Replication

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 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.

(7) Cells may activate the monomer glucose by adding a phosphate group.

(a) What enzyme polymerizes the activated monomer into the $glucose_n$ primer?

(b) Under what condition 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?

Chapter VII: GENES, PROTEINS AND MUTATIONS

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.

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.

B. 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,

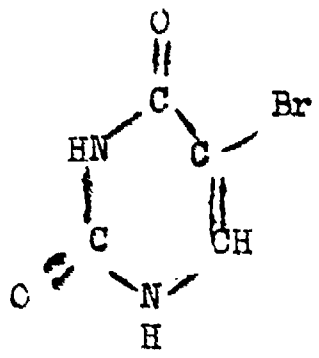
while normal hemoglobin has an amino acid called glutamic acid at this position. In all other respects the two sequences are identical. Yet because of this single amino acid difference the mutant hemoglobin is less soluble than normal hemoglobin. It tends to crystallize within the red blood cells, especially when the oxygen concentration is low. The hemoglobin crystals distort the red cells into odd crescent 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 assingle amino acid in the structure of an enzyme strongly affects the catalytic

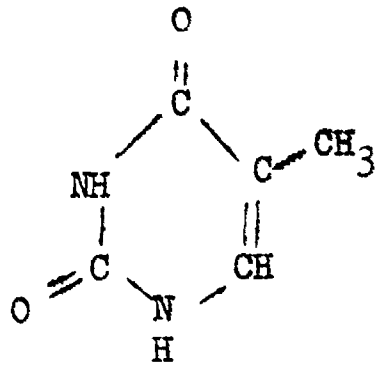
activity of the enzyme. The most frequent effects are complete loss or drastic reduction in catalytic activity. This should not be surprising, since natural selection has been refining the catalytic efficiency of most enzymes for many millions of years.

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 increase the possibility of mistakes in DNA replication and which therefore increase the frequency of mutation. One such chemical is bromouracil (BU). This compound has a structure which is identical to thymine except that a bromine atom is substituted for the methyl group of thymine.



bromouracil



thymine = methyluracil

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. One determines whether the presumed mutagen can revert the original mutation, thereby restoring the missing enzymic activity. In the present experiment the test organism will be a bacterial strai..

which has lost the ability to synthesize the amino acid tryptophan because of a previous mutation in one of the genes controlling the enzymes of tryptophan synthesis. Therefore, this bacterial strain cannot grow unless tryptophan is supplied in the environment. We will look for mutations which restore the ability to synthesize tryptophan in the cells. Such cells will be able to grow without external tryptophan. They can be identified and counted by spreading the population on to a solid growth medium which lacks tryptophan. The cells which have the ability to synthesize tryptophan will grow to form visible colonies; the other cells can't grow. Even a very small proportion of tryptophan-synthesizing cells can be detected in this way.

C.1 Experiment: INDUCTION OF MUTATIONS BY BROMOURACIL

Obtain two petri plates and a spreader. Add two drops of the bac-

terial suspension to each plate, using an eye dropper. Pass the spreader several times (quickly) through a bunsen burner flame, cool for a few seconds in air and then use it to spread the drop of suspension evenly over the surface of the plate. Mark one plate "BU" and the other"T". Drop a few crystals of bromouracil onto the surface of the BU plate and a few crystals of tryptophan onto the surface of the T plate. Be sure to write your name(s) on the plates. Incubate in a warm place (30-40°C) for several days. Examine the plates daily for signs of bacterial growth. Can you conclude that BU is a mutagen? Why not? What is the purpose of the T petri dish? Using the broad ends of sterile toothpicks, pick lightly into two colonies on the BU petri dish. Streak a new plate (no tryptophan) with the same end of the toothpick. Repeat this process using the T petri dish. Are the colonies picked from the BU petri dish able to grow without tryptophan? Can you now conclude

that BU is a mutagen?

There may be common household chemicals (aspirin, coffee, horseradish, pepper, etc.) which are mutagens. Try them if the time and plates are available.

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

(1) Three types of mistakes possible in DNA replication are:

(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?

(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?

ENERGY CAPTURE AND GROWTH

Outline: Energy Capture and Growth

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 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:

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?

Having 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 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 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 CO_2 and their hydrogen from H_2O. Let's test these hypotheses by measuring the uptake of radioactive carbon dioxide $[{}^{14}CO_2]$ and the evolution of a gas (O_2) during photosynthesis.

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

Place a 15-centimeter sprig of Elodea in each of two (25 x 100 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 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.1.

1. Are both sprigs of Elodea significantly redioactive?
2. Is carbon incorporated into either

<u>Elodea</u> sprig?

3. Is light necessary for the uptake of carbon?

An additional experiment involves collecting considerable gas from a large amount of <u>Elodea</u> 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 something in the plant and that a gas is given off in the process (try splint test). The O_2 probably comes from the splitting of H_2O.

$$2H_2O \xrightarrow[h\nu]{} 2\,[H] + 2H^+ + O_2$$

But did your experiment prove that H_2O was the source of oxygen? What the $^{14}CO_2$ 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.1 - 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 on the short rubber tube. This is done in order to allow the solution to become saturated with oxygen. Fasten

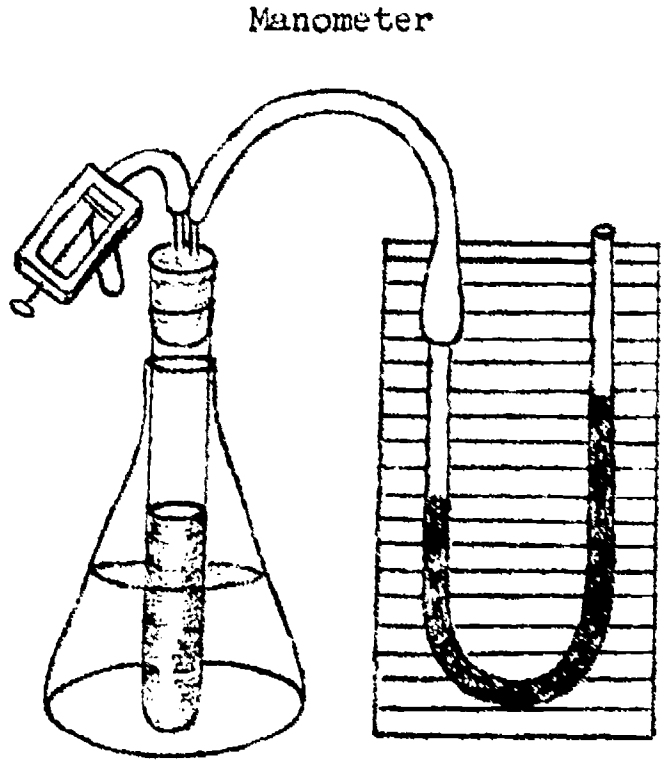


Figure B.1

Materials and Equipment

(for each squad)

1 sprig of Elodea
about 15 cm long
1 erlenmeyer flask (250 ml
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

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 distance traveled (mm) by the colored solution in the manometer as the vertical axis and time in minutes as the horizontal axis.

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:

Color	Wavelength Range λ	Frequency Range ν
violet	3800-4200 Å	8×10^{14} waves/sec.
blue	4200-4900 Å	
green	4900-5350 Å	
yellow	5350-5900 Å	
orange	5900-6400 Å	
red	6400-7000 Å	4×10^{14} waves/sec.

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 CO_2 uptake or O_2 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 abosrbed.

Let's now turn the argument around. Light that is effective in photosynthesis must be absorbed by something in tne plant. Absorption means the capture and conversion of the energy that is the light. We might guess that the capturing agent is some kind of molecule or molecules. If so we should be able to separate it out of the plant material using chemical techniques.

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 get detailed instructions on the use of the spectrophotometer from the 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 omitted 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.

(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.2.a Optional Experiment:
CHROMATOGRAPHY OF GREEN PLANT PIGMENTS

The acetone extract left over from the 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 Elodea 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 or peaks in the wavelength regions 4000-4800 Å (blue) and 6200-6800 Å (red). If you had chromatographed enough pigments you could have recovered the pure chlorophylls from the chrom-

atogram and done light absorption spectra on them. These spectra would have shown peaks in the same two wavelength regions. Still, even if there is a suspicious resemblance between action spectrum of the plant and the absorption spectrum of the chlorophyll, we can't really say that the pigment is necessary for photosynthesis. In fact, many other experiments do show that chlorophylls are the primary photosynthetic pigments; that is, they are the agents which capture light energy for photosynthesis.

D. THE CELL POWERHOUSE

All of the photosynthetic pigments are confined in tiny particles within the plant cell. These particles are called chloroplasts. They are the powerhouse for the cell, capturing light energy and using it in two reactions.

(1) $2H_2O \xrightarrow[\text{chloroplasts}]{h\nu} 2[H] + 2H^+ + O_2$

where $2[H]$ is "reducing power."

(2) $ADP + H_3PO_4 \xrightarrow[\text{chloroplasts}]{h\nu} ATP + H_2O$

where ATP, as you already know, is "activating power."

These reactions are often called the light reactions of photosynthesis. The reducing and activating chemicals produced in the light are then used in "dark" or light-independent reactions to chemically convert CO_2 into cellular materials.

Chloroplasts can be easily seen in plant cells under the microscope, especially if the plants are single-celled algae.

D.1 Observation: CHLOROPLASTS IN PLANT CELLS

Place a small drop of algae culture on a microscope slide and lay a coverslip onto the drop of liquid: observe through a microscope first at low power (100 - 150 x), then at higher power (250 - 400 x). Is the green color spread throughout the cells or localized? Try observing chloroplasts in leaves. Can you slice the leaves with

a razor blade so as to obtain a single layer of cells?

It is quite easy to isolate chloroplasts from cells. If the isolation is done gently and quickly, the particles will carry out ATP generation and evolve O_2 when exposed to the light. Purified chlorophyll will not do these things. It appears that in addition to chlorophyll, some part of the chloroplast structure is necessary for the light reactions.

E. PATH OF CARBON IN PHOTOSYNTHESIS

The first product made from CO_2 during photosynthesis is 3-phosphoglyceric acid. As will be explained later (see diagram of [illegible] pathway, page 210) this compound can be metabolized in several ways, so as to ultimately make amino acids (for proteins) and glucose (for starch) as well as the monomers for nucleic acids. For the moment let's simply determine if radioactivity from $^{14}CO_2$ gets into

amino acids.

E.1 Experiment: PHOTOSYNTHETIC INCORPORATION OF $^{14}CO_2$ INTO AMINO ACIDS

Place the dried Elodea from Experiment A.2.a in a test tube. Add enough 80% ethanol to the test tube to cover the Elodea and place the tube in a boiling water bath for two minutes.

Obtain a piece of thin layer chromatography (TLC) material from your teacher. Draw a light pencil line about 2 cm from the end of the TLC plate. Wearing plastic gloves and using a micropipet, draw up some of the Elodea extract and make a thin streak of pigment along the pencil line. Allow this to dry. Repeat this step several times until a dark green color appears on the plate. It is important to get a strong concentration of pigments in one neat streak. On another TLC plate, streak the casamino acids solution in the same way as you did the Elodea extract. This will be a comparison

plate which will show the locations of all the amino acids.

Place enough development solvent in a 250 ml beaker to just cover the bottom of the beaker but not to cover the pigment streak on the TLC plate. Place the TLC plates in the beaker with the streak of green or amino acids at the bottom and the TLC plates leaning against the side of the beaker. Cover the beaker with aluminum foil or a watch glass. Allow the development solvent to rise up the TLC plates to within 1 cm of the top of the plate. Remove the TLC plates. Allow to dry until there is no odor of ammonia. Spray or very quickly dip the plates in ninhydrin solution (wear gloves!). Dry the plates in an oven at 90° until purple streaks appear. Each purple streak that appears is due to an amino acid. Cut out the streak(s) from the Elodea extract plate, place each streak on a separate planchet, and count with a

radioactivity counting device.

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

(1.) The sun, which is the ultimate source of energy, has its energy converted into living material by photosynthesis. Where does most photosynthesis occur?

(2.) (a) Proteins are made up of what kinds of elements?

(b) Carbohydrates are composed of what elements?

(3.) What is the source of carbon and hydrogen in plants?

(4.) In Experiment B.1: Light Intensity and Oxygen Evolution

(a) What effect might the lamp at a distance of 10 cm from the test tube have on the temperature of the sodium bicarbonate solution during the length of that one trial?

(b) What then would the effect be on the gas trapped in the top of the test tube and manometer?

(c) Is this compensated for during your experiment?

(d) What would have to be done in order to account for this amount of error?

(5.) (a) What colors of light are effective in photosynthesis?

(b) What color of light is least effective in photosynthesis?

(6.) Paper chromatography shows four color bands due to the solvent elution.

(a) List the band color and the corresponding pigment molecule, starting from the top of the chromatography paper.

(b) If you are provided with the given information that small molecules are carried at a faster rate than large molecules, which of the four molecules listed above is the largest?

(7.) Where are photosynthetic pigments located within the cell?

(8.) Can plants convert CO_2 into cellular materials during the night? Explain.

Chapter II: ENERGY CONSUMPTION AND METABOLISM

Consumer organisms are those that feed directly on other organisms. Animals, of course, are all consumers. They convert either plant material or other animals into raw materials for their own use. Also they obtain their energy from the chemical energy stored in their food. This chapter is concerned with some of the details of these processes.

The first problem of the consumer is digestion of polymers down to monomers. This requires special enzymes. You will recall there are proteolytic enzymes that split proteins to amino acids, and there is an enzyme in saliva that converts starch to glucose. There are also enzymes which convert nucleic acids to nucleotides. There are several reasons why polymers must be broken down before they can be used.

First, remember that proteins and nucleic acids of every kind of organism

are specific - different from those in any other living thing. Second, such large molecules as proteins and nucleic acids cannot pass through the cell membrane. If cell membranes permitted such molecules to get in easily they could also get out easily, and that would be a disaster for the cell (and the organism).

All the chemical and physical processes continuously going on in living cells taken together constitute metabolism. Not only are polymers being broken down, but new monomers and polymers are being synthesized. In addition, another very important process involving the release of usable energy from glucose in the form of ATP takes place. Glucose is one of the best foods for almost all living things. It is quickly and easily converted to usable energy and also yields necessary raw materials for cell growth.

A. GLUCOSE METABOLISM

We will concentrate our laboratory work on glucose metabolism.

A.1 Experiment: GROWTH OF YEAST ON GLUCOSE

Add a very small pinch of dried yeast to a 25 x 150 ml test tube containing 21 ml of solution A. Allow the dried yeast to soak for a few minutes. Place one drop of the soaked yeast upon a microscope slide and examine with the high power objective of a microscope. If there are too many yeast organisms in the field of vision dilute the yeast culture with solution A until there are 20 to 40 yeast cells in the high power field of view of a single drop of diluted culture.

Use this diluted yeast culture to prepare your experimental cultures as indicated in the protocol table Yeast Growth.

Leave these yeast cultures at room temperature overnight. Examine one drop

of each culture under the microscope. You will have to use a long eyedropper to obtain a drop of culture from culture 4. Relative cell densities should be about

100 (3) : 10 (4) : 1 (1 & 2).

Note the differences in cell density between the cultures when started and one day later. Try to obtain estimates of the relative cell densities in each culture; for example culture 3 may be ten times as dense as culture 4. Is there any obvious indication of growth (cell multiplication) in cultures 1 or 2?

To each of the NaOH solution tubes taken from your cultures add 4 ml of $BaCl_2$ solution. What do you observe in each case?

Transfer 3 ml of each culture to a numbered centrifuge tube and centrifuge down the yeast cells. Carefully pour off the clear supernatants and add one drop of each to 3 ml of alcohol test solution. Also try a drop of the 10%

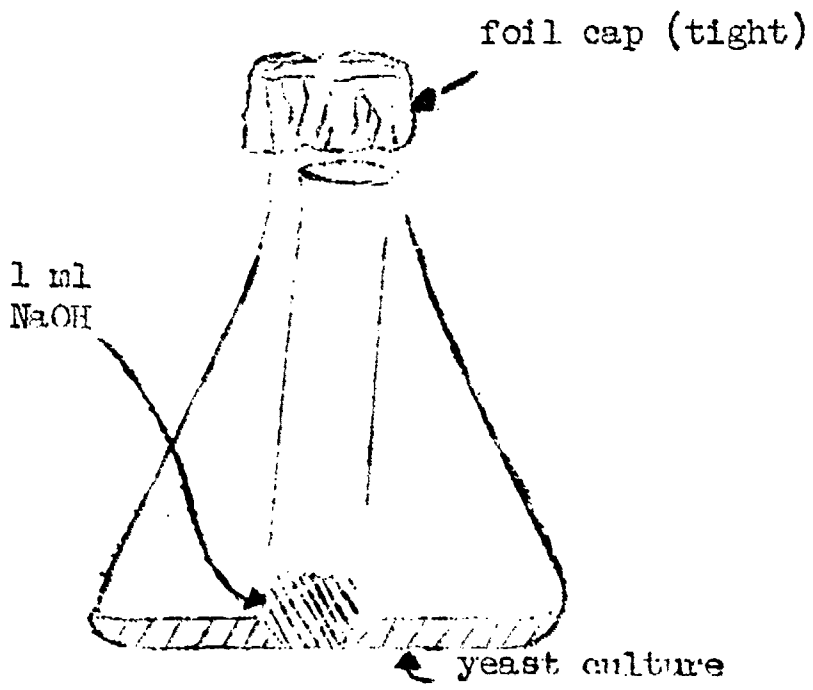
Protocol: Yeast Growth

Reagent \ Culture #	1	2	3	4
Diluted Yeast in solution A	5 ml	5 ml	5 ml	5 ml
10% Glucose solution	one drop	——	one drop	one drop
1M Phosphate solution	——	one drop	one drop	one drop
Container to be used for this culture	foil capped 125 ml erlenmeyer See diagram (1) below	as for culture #1	as for culture #1	see diagram (2) below

Table A.1

Diagram 1

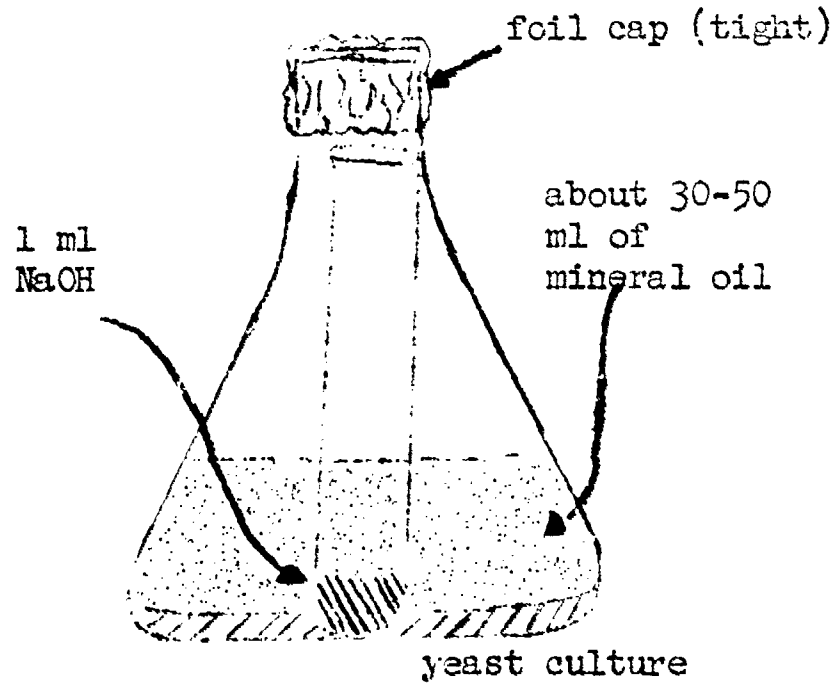
After the yeast culture is in a flask, carefully put into the flask a 10 x 75 mm test tube containing 1 ml of 1 M NaOH solution as indicated below



Cultures 1, 2 and 3

Diagram 2

Prepare culture 4 exactly like 1-3, but before putting in the NaOH test tube carefully fill flask with mineral oil as indicated



Culture 4

standard ethyl alcohol solution provided by the teacher. A color change from yellow to red indicates the presence of ethanol. Which of your yeast cultures made ethanol?

You will have found that in the presence of O_2 (culture 3) glucose is almost completely consumed, the products being more yeast cells and CO_2. In the absence of oxygen (culture 4) the glucose is much less efficiently used. That is, fewer yeast cells are made and the glucose is partly converted to ethanol (CH_3CH_2OH or C_2H_5CH). The overall reactions are:

aerobic degradation (with O_2):

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O +$ usable energy

(glucose)

anaerobic degradation (without O_2):

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 +$ usable energy

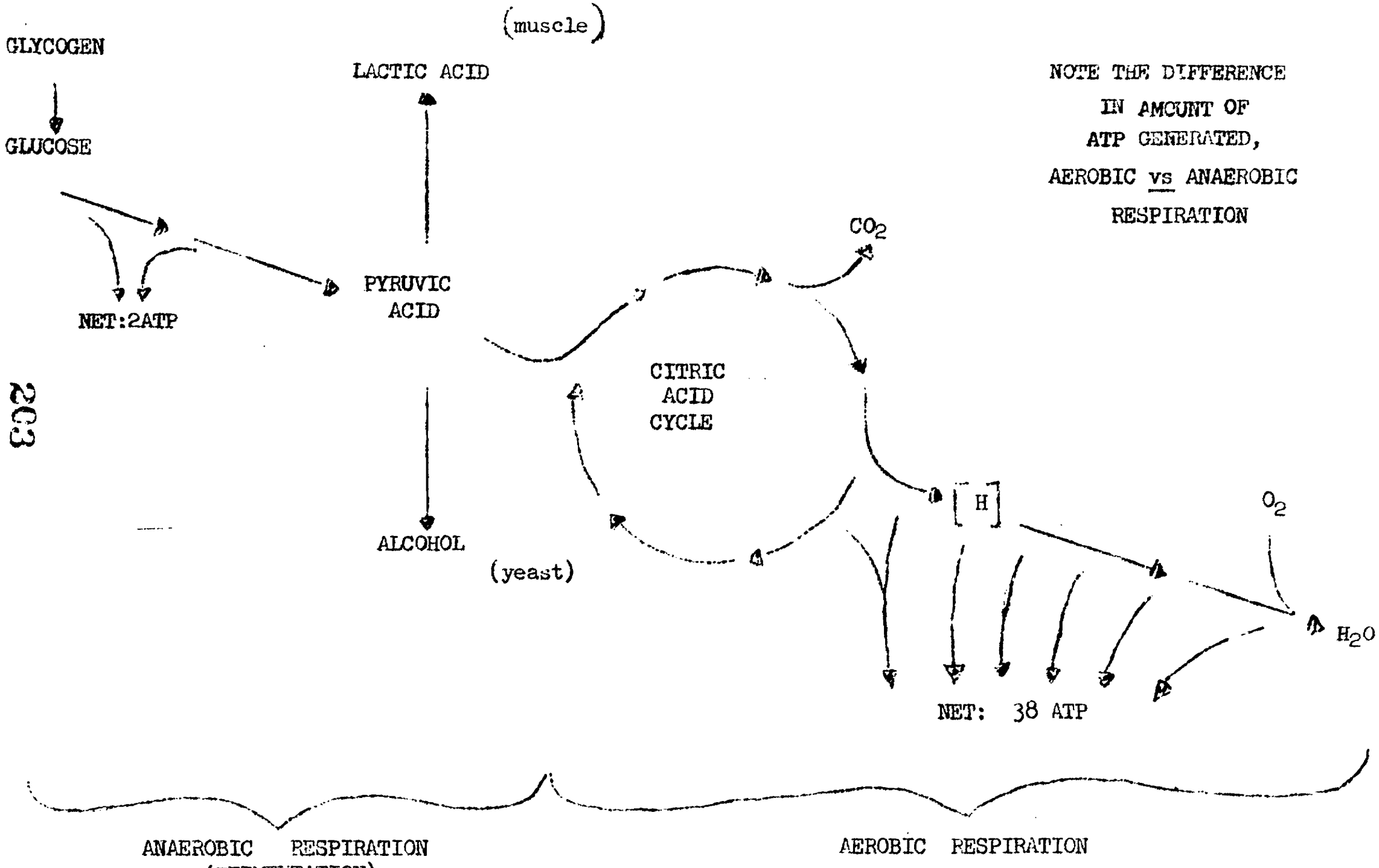
(glucose) (ethanol)

Aerobic degradation looks very much like photosynthesis run in reverse. Photosynthetic energy stored in glucose

is converted into usable energy (ATP) as the glucose is broken down into CO_2 and H_2O. You will remember that photosynthesis starts with these simple molecules and by adding light energy converts them into glucose, other monomers, and O_2. This chemical and energy interdependence of producers and consumers is vital to life as we know it. Thus, the chemical elements composing life are continually reused and recycled in the overall conversion of light energy into life.

These summary equations show only the input (glucose) and the final products. They don't show the many chemical steps in between. Also they account only for the glucose catabolized to provide energy, although obviously part of the glucose was converted into more yeast cells.

The degradation of glucose by yeast in the absence of oxygen is a very important process which has the name <u>fermentation</u>. It is the process used to make wine and



NOTE THE DIFFERENCE IN AMOUNT OF ATP GENERATED, AEROBIC vs ANAEROBIC RESPIRATION

Figure A.1

beer; also it is used in making bread. What product of glucose fermentation is important in making wine or beer? In "raising" bread dough?

It was a great surprise to scientists when they found that they could completely grind up yeast cells and that the "juice" obtained would also ferment glucose. This discovery was the beginning of classical biochemistry. Classical biochemistry is primarily concerned with discovering and understanding the intermediate chemical steps of metabolism and their interrelationships. We will now try to study some of these intermediate chemical steps.

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 ½ 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.

(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 (CO_2 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 B.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 CO_2 from the

Protocol: Yeast Juice

Reagent \ Manometer #	1	2	3	4
Preincubated Yeast Juice	5.0 ml	5.0 ml	5.0 ml	5.0 ml
1.0 M potassium phosphate pH 7.0	——	0.3 ml (6 drops)	0.05 ml (1 drop)	0.3 ml
1.0 M glucose	1.0 ml (20 drops)	——	1.0 ml	1.0 ml
Distilled H_2O	0.3 ml (6 drops)	1.0 ml	0.3 ml	——

Table A.2

liquid. Record your readings in a table something like this:

Manometer Readings

Time	#1	#2	#3	#4
1:35	10			
1:36		12		
1:37			9	
1:38				13
1:40	13			
1:41		14		
1:42			13	
1:43				21
		etc.		

Read each manometer for at least 30 minutes. In which is CO_2 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

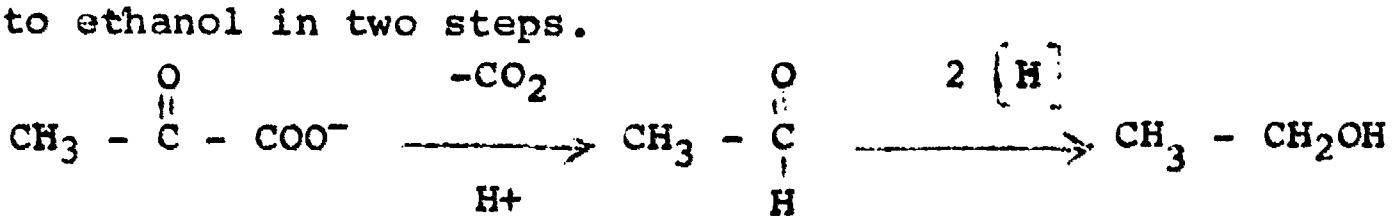
into one of the wells. All light in the room should now be extinguished. Do any of your mixtures of juice and firefly solution glow in the dark?

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 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 CO_2 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.



pyruvate acetaldehyde ethanol

EMP PATHWAY

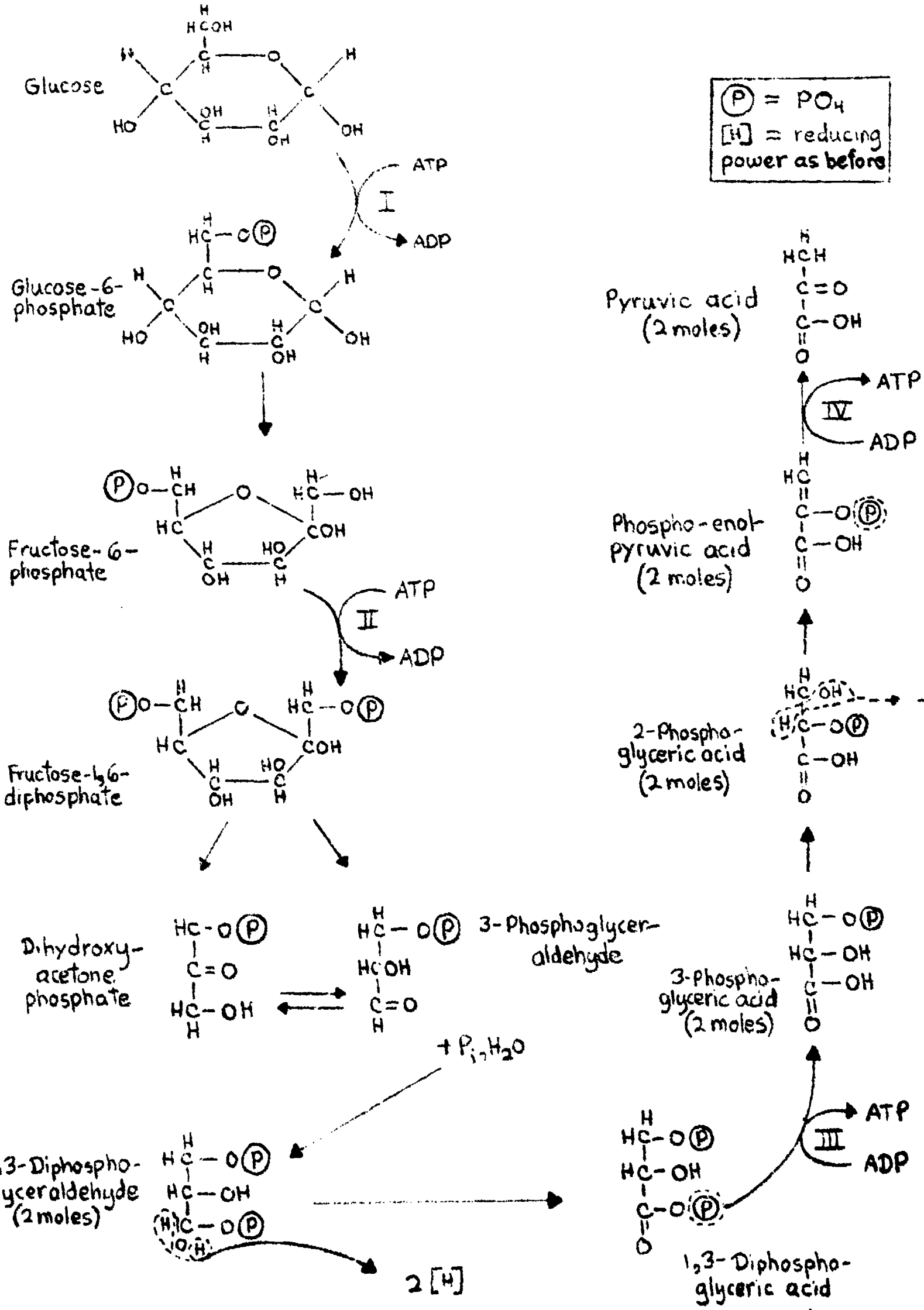
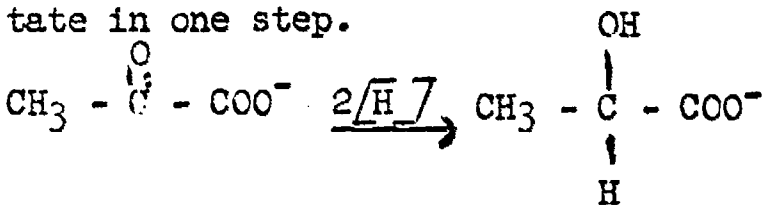


Figure A.2

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



pyruvate lactate

In both cases, but especially for the athlete, the temporary product, ethanol or lactate, is completely burned up by another pathway when O_2 becomes available. This is why you can run <u>now</u> and breathe hard <u>later</u>.

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 <u>spend</u> two molecules of ATP to convert glucose into the right chemical form (fructose-1, 6-diphosphate) for further reactions. <u>Then</u> 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 CO_2 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 O_2 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 O_2 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 the next page.

Once again there are some important points to see:

1. The three carbon atoms in pyruvate are all converted to CO_2,
2. For every turn of the cycle one molecule of pyruvate is combusted

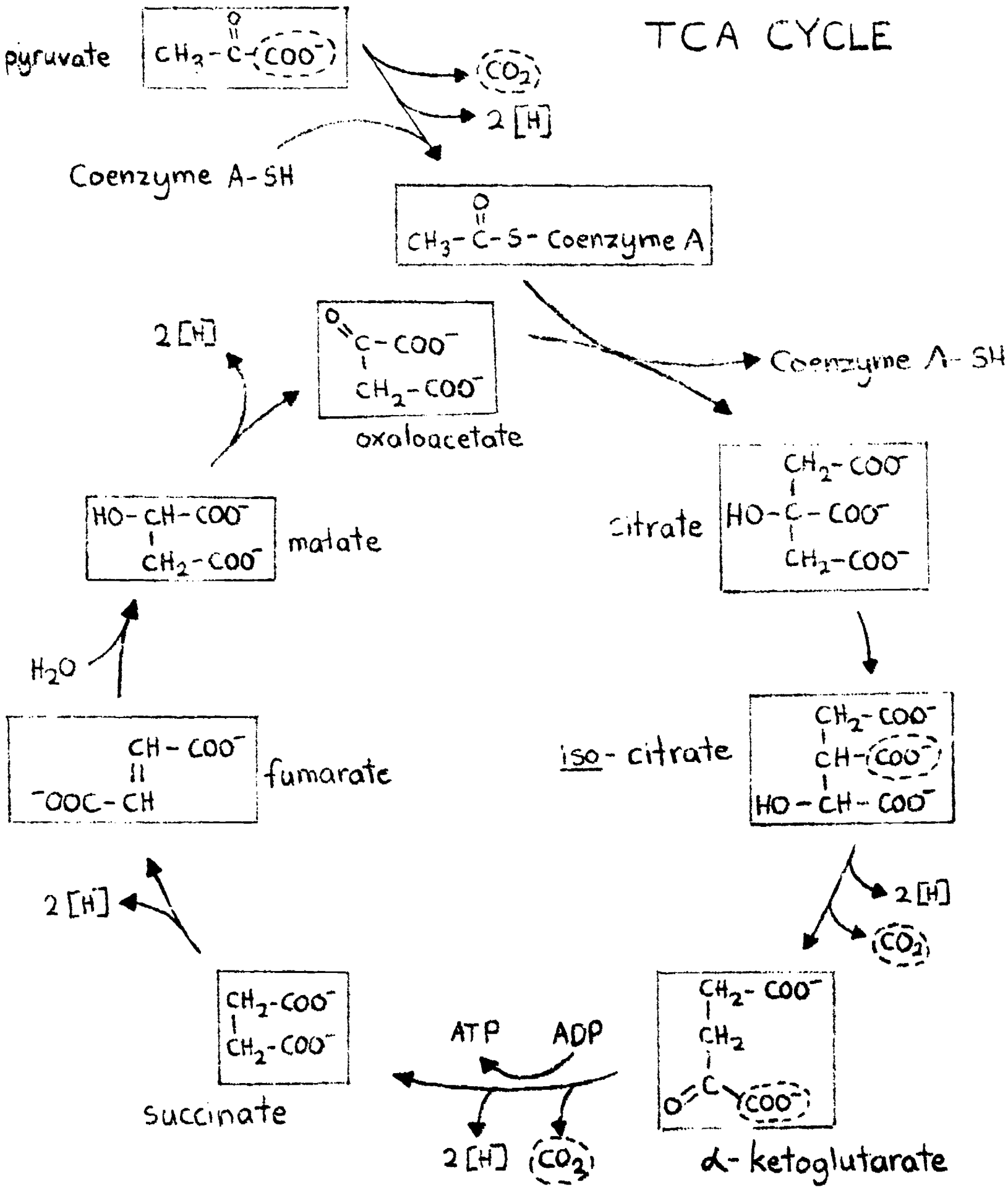


Figure A.3

TCA CYCLE

CH_3-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.

and one molecule of oxalacetate is regenerated. Obviously some 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 O_2 to two molecules of H_2O. 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 answer to the second question is 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 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.)

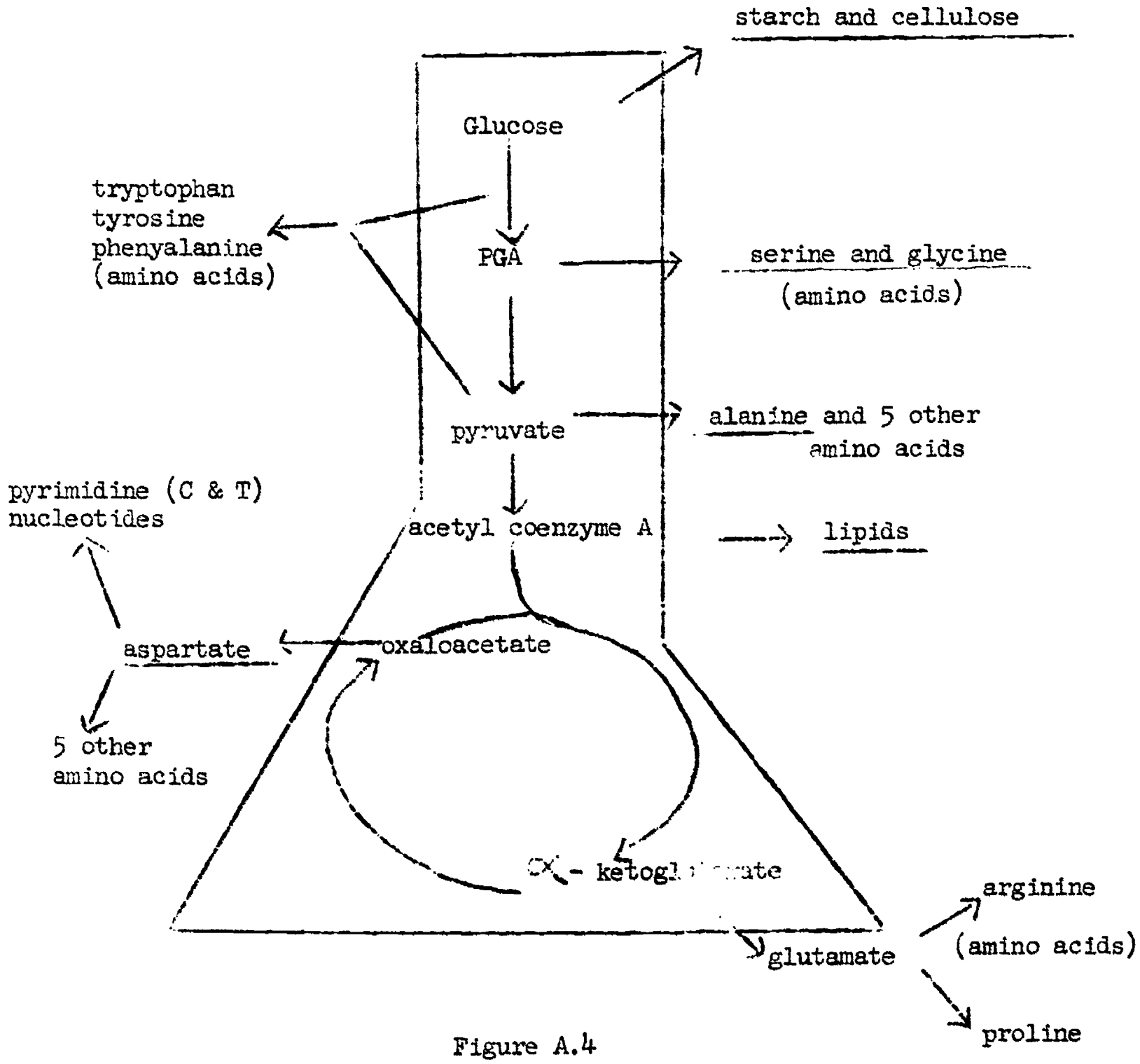

starch and cellulose
Glucose
tryptophan
tyrosine
phenyalanine
(amino acids)
PGA
serine and glycine
(amino acids)
pyruvate
alanine and 5 other
amino acids
pyrimidine (C & T)
nucleotides
acetyl coenzyme A
lipids
aspartate
oxaloacetate
5 other
amino acids
α - ketoglutarate
arginine
(amino acids)
glutamate
proline

Figure A.4

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

(1.) Proteolytic enzymes are capable of digesting 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 molecules of ATP?

(b) How many molecules of ATP are produced during this series of reactions?

(5.) (a) The metabolic pathway which is basically the same in yeast, man, trees and other living things is known by what name?

(b) The pyruvate produced by this pathway can be converted by yeast and man to what?

(6.) Why is yeast able to grow better in air than without air?

(7.) In the schematized EMP and TCA pathways you are shown some of the side streets which lead to the production of amino acids needed by the body.

(a) How many amino acids are shown to be produced?

(b) List the names of specific amino acids shown in the scheme.

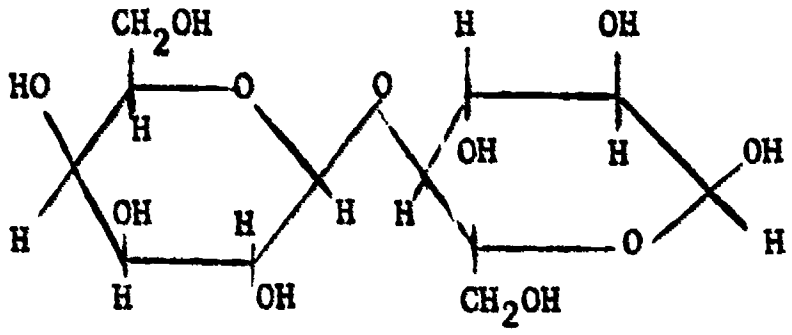
(8.) (a) Are whole, live yeast cells required to break down glucose?

(b) Does the amount of phosphate present in a solution effect the metabolism rate? Explain.

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.



(galactose residue) (glucose residue)

lactose

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 of 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.

$$\text{lactose} + H_2O \xrightarrow{\text{enzyme}} \text{galactose} + \text{glucose}.$$

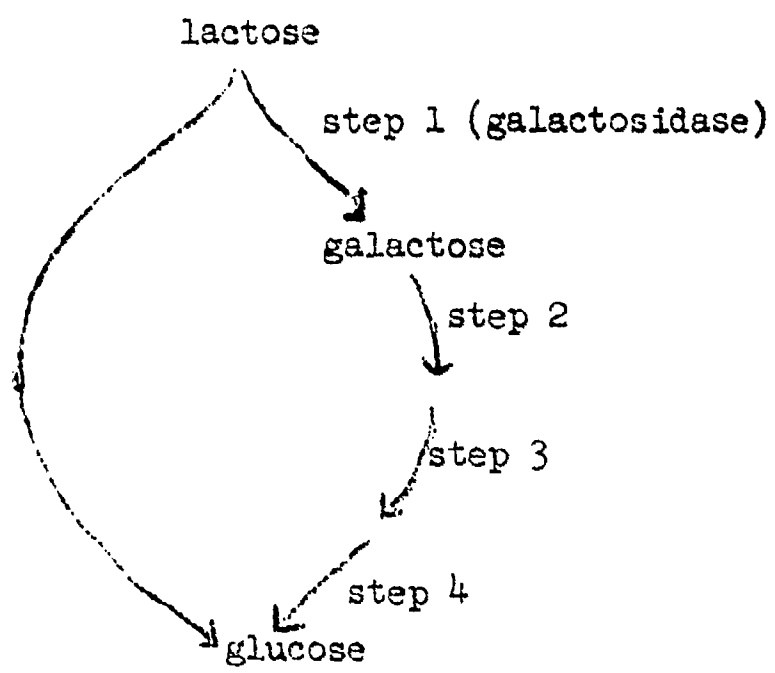
The glucose can then be used directly via the EMP pathway, while the galactose

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-galactose. E. coli makes

it only when presented with lactose

or a similar β-galactoside. β-galac-

tosidase is specific for the galactose

half of the molecule and will therefore

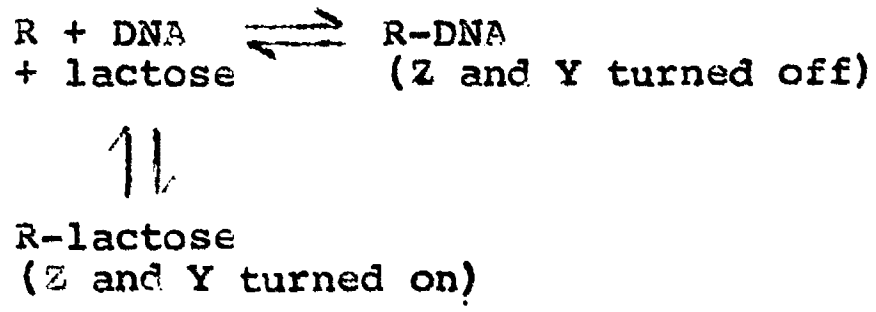
hydrolyze or split almost any molecule

containing this sugar.

There are a number of important points in regard to this metabolic pathway. One is that 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 G-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 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 protein. 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.



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.