Lecture 2 : “Chemistry and Methods”

Chapter 2 Topics

- Fundamental Building Blocks
- Macromolecules
- The Cell

Chapter 3 Topics

- Methods of Culturing Microorganisms
- Microscope

Chapter 2
Fundamental Building Blocks

- Atoms
- Elements
- Molecules and compounds

Atom - unit of an element
- electron - subatomic, negatively charged
- proton - subatomic, positively charged
- neutron - subatomic, uncharged

Ion - atoms in which the number of protons and electrons is unequal
- cation - less electrons than protons
- anion - more electrons than protons

- <u>atomic number</u> reflects the number of protons
- <u>atomic mass number</u> reflects the number of protons **and** neutrons

Isotope - atoms with same atomic number but with varying atomic mass

- radioisotopes are unstable isotopes - used in research and medical applications and in dating fossils and ancient materials

Pure chemical substances composed of **atoms with the same number of protons,** are called

Chemical ELEMENTs

All chemical matter consists of elements.

New elements of higher atomic number are discovered from time to time, usually as products of artificial nuclear reactions.

Different Types of Atoms
Elements and Their Properties

- Changes in numbers of protons, neutrons, and electrons in atoms create **different elements**

  Example: radioisotopic decay ("nuclear fission").

  (this is NOT equal to "atoms interacting chemically with one another")
- Each element has a characteristic atomic structure and predictable chemical behavior
- Each assigned a distinctive name with an abbreviated shorthand symbol
- All elements are organized in the periodic table

Atoms (elements) consist of protons and neutrons (resident in a space called nucleus) and electrons (resident in the shell).

- a **shell** reflects a **period** in the periodic system
- a **valence shell** is the most outer occupied one (usually not complete)
- a **group** includes elements of the same valence

Standard periodic table

Group → / ↓ Period	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H																	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	*	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	**	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Uub	113 Uut	114 Uuq	115 Uup	116 Uuh	117 Uus	118 Uuo

* Lanthanoids	57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
** Actinoids	89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

Element categories in the periodic table

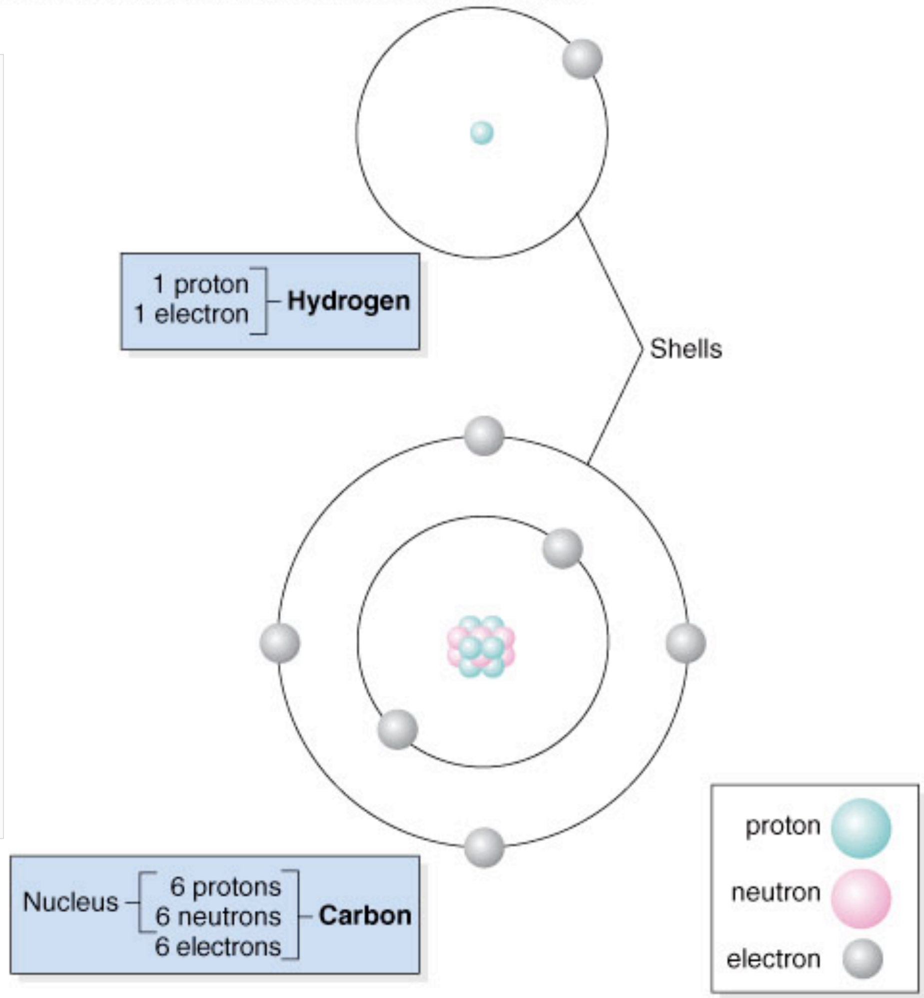
Metals						Metalloids	Nonmetals			Unknown
Alkali metals	Alkaline earth metals	Inner transition elements: Lanthanides	Inner transition elements: Actinides	Transition elements	Other metals	Metalloids	Other nonmetals	Halogens	Noble gases	Unknown

Atomic number colors show state at standard temperature and pressure (0 °C and 1 atm)

Solids	Liquids	Gases	Unknown

Borders show natural occurrence

Primordial	From decay	Synthetic	Undiscovered



(b)

Fig. 2.1 Models of atomic structure

Electrons within a given shell are not all equal and the occupy “preferred spaces” in the shells, also referred to as “**Orbitals**.”

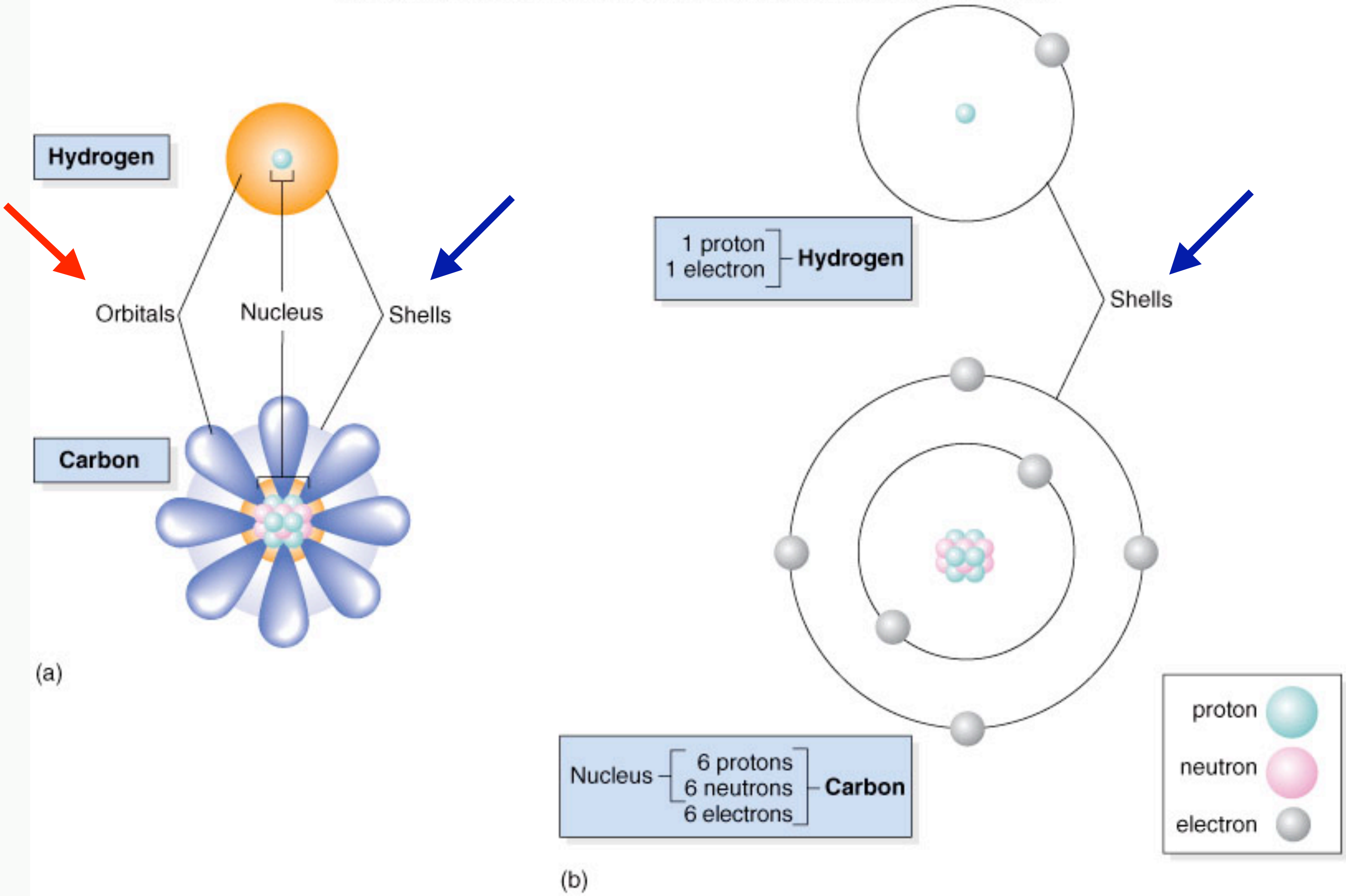


Fig. 2.1 Models of atomic structure

Electron Orbitals and Shells

- An atom can be envisioned as a central nucleus surrounded by a “cloud” of electrons
- Electrons rotate about the nucleus in pathways (“preferred spaces”) called **orbitals** - volumes of space in which an electron is likely to be found
- Electrons occupy energy shells, from lower-energy to higher-energy as they move away from the nucleus
- Electrons fill the orbitals and shells in pairs starting with the shell nearest the nucleus
- Each element, then, has a unique pattern of orbitals and shells

Each element is characterized by a specific "Electron configuration":

2	2	6	2	6	2	10	6	
$1s^2$	$2s^2$	$2p^6$	$3s^2$	$3p^6$	$4s^2$	$4d^{10}$	$4p^6$	……
He	Be	Ne	Mg	Ar	Ca	Zn	Kr	

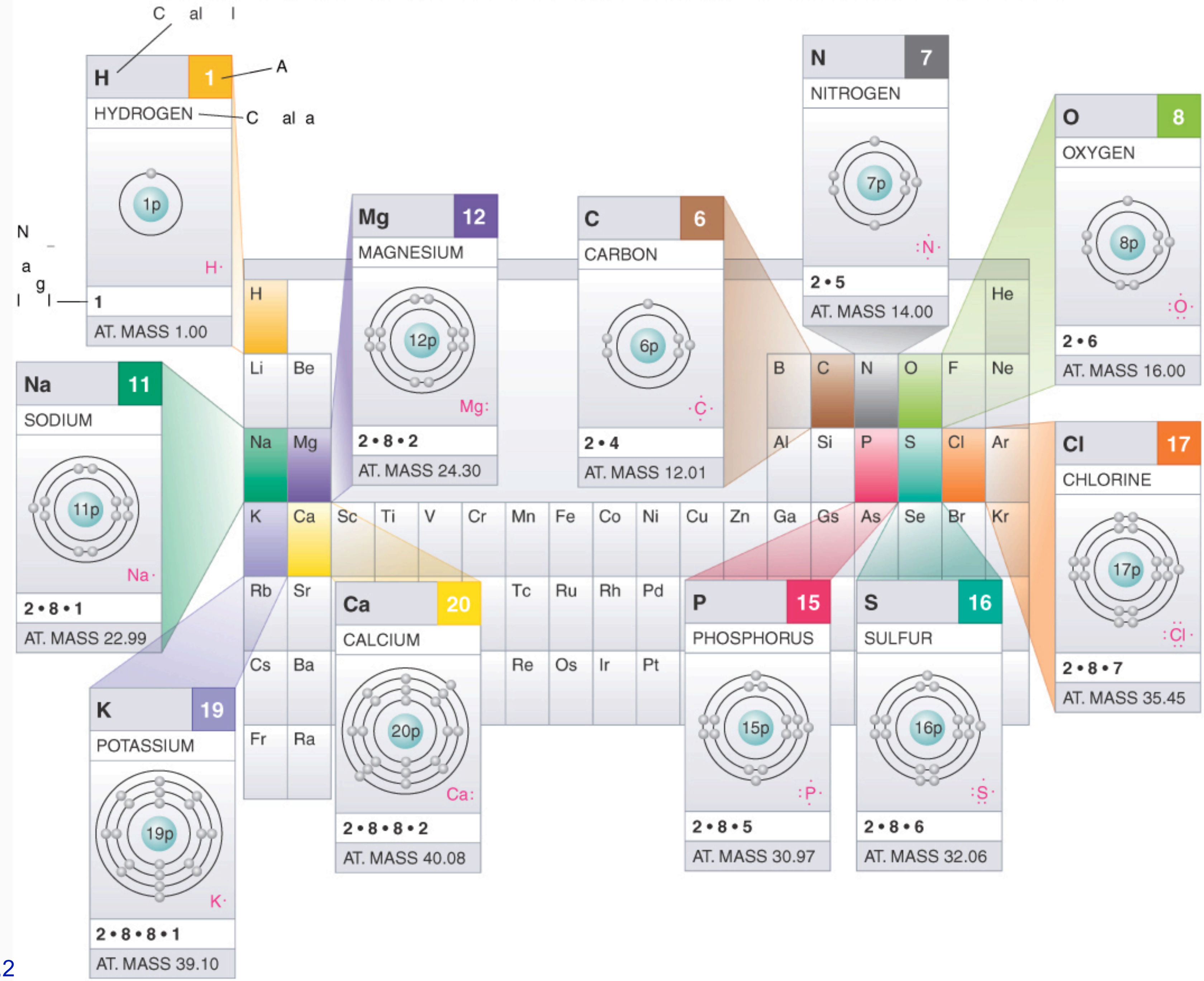

H
1
HYDROGEN
1p
H·
1
AT. MASS 1.00
N
NITROGEN
7
7p
2 • 5
AT. MASS 14.00
O
8
OXYGEN
8p
2 • 6
AT. MASS 16.00
Mg
12
MAGNESIUM
12p
Mg:
2 • 8 • 2
AT. MASS 24.30
C
6
CARBON
6p
2 • 4
AT. MASS 12.01
Na
11
SODIUM
11p
Na·
2 • 8 • 1
AT. MASS 22.99
Cl
17
CHLORINE
17p
2 • 8 • 7
AT. MASS 35.45
K
19
POTASSIUM
19p
K·
2 • 8 • 8 • 1
AT. MASS 39.10
Ca
20
CALCIUM
20p
Ca:
2 • 8 • 8 • 2
AT. MASS 40.08
P
15
PHOSPHORUS
15p
2 • 8 • 5
AT. MASS 30.97
S
16
SULFUR
16p
2 • 8 • 6
AT. MASS 32.06
H He
Li Be B C N O F Ne
Na Mg Al Si P S Cl Ar
K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn Ga Gs As Se Br Kr
Rb Sr Tc Ru Rh Pd
Cs Ba Re Os Ir Pt
Fr Ra

Figure 2.2

Bonds and Molecules

- Most elements do not exist naturally in pure form
- **Molecule** - a distinct chemical substance that results from the combination of two or more atoms (can be two of the same element, such as O_2)
- **Compounds** - molecules that are combinations of two or more different elements (such as CO_2)
- **Chemical Bonds** - When two or more atoms share, donate, or accept electrons
- Types of bonds formed and to which atoms and element bonds are determined by the atom's **valence**

Chemical bonds

- Covalent
- Ionic
- Hydrogen

Chemical bonds involve atoms **sharing**, **donating** or **accepting** electrons

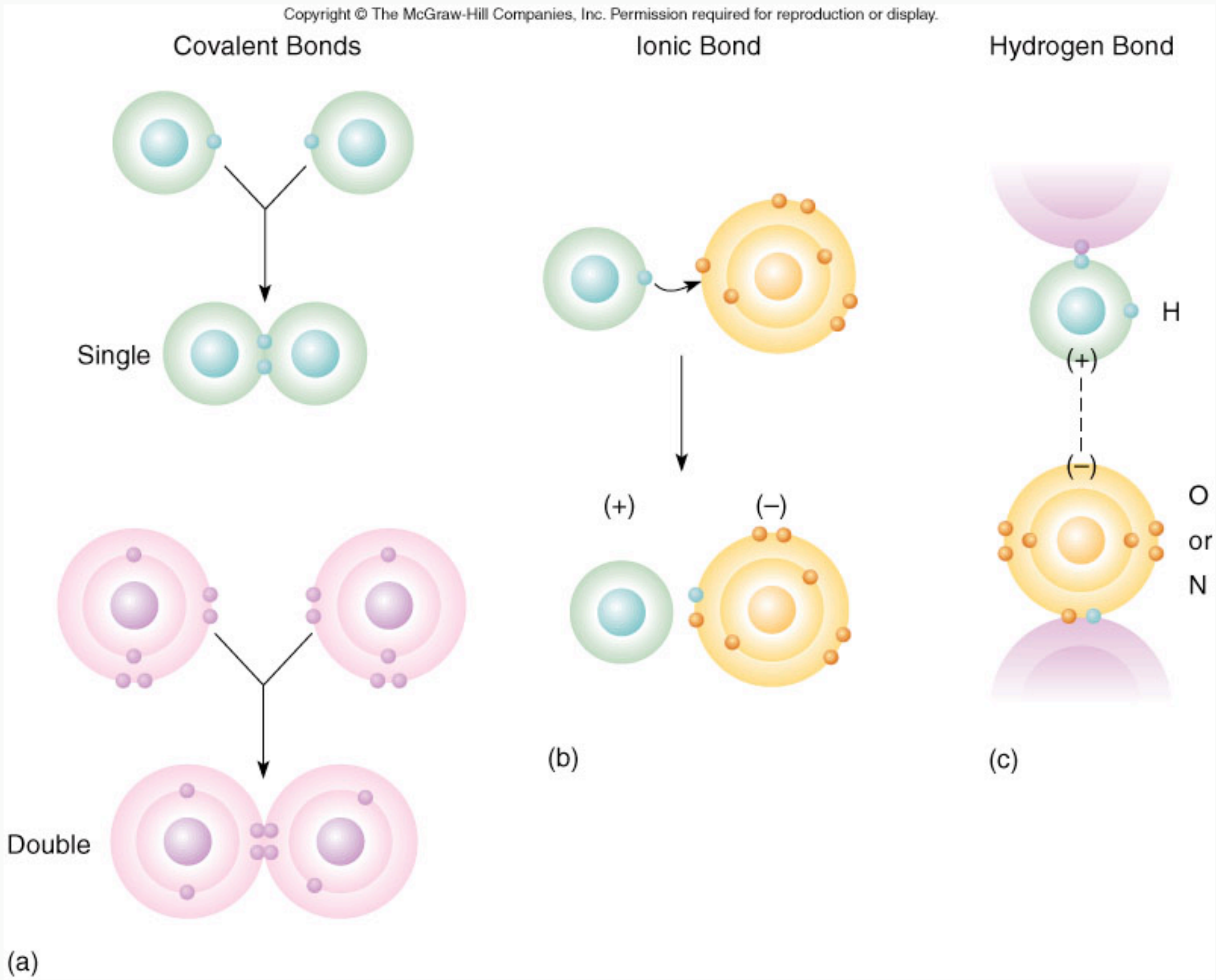


Fig. 2.3 General representation of three types of bonding

Covalent bonds: **shared electron** bond

Usually, the number free valences determines the number of possible covalent bonds.

Carbon tends to form only covalent bonds thereby generating an organic compound.

CH_4, CO_2, HCN

The sharing of electrons generates “noble gas electron configurations” (valences are completely occupied); hence, stable bonds.

Hydrogen gas, molecular oxygen, and methane are examples of covalent bonding (atoms sharing electrons).

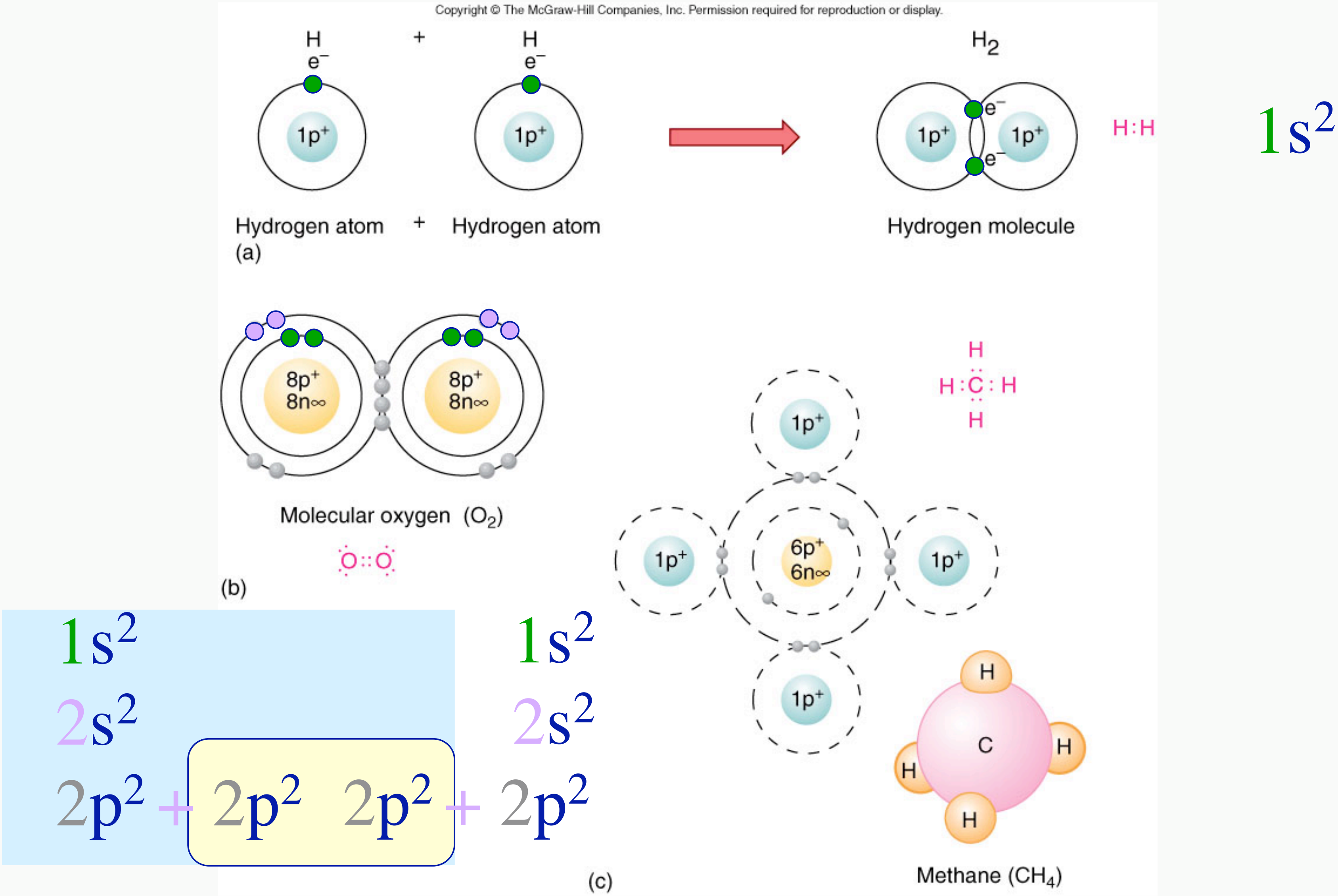


Fig. 2.4 Examples of molecules with covalent bonding

Polar vs. Nonpolar Molecules

- Some covalent bonds result in a **polar** molecule - an unequal distribution of the electrons (charge); example: H_2O.
  - Polarity is a significant property of many large molecules, influences both reactivity and structure

- An electrically neutral molecule is **nonpolar**
- **Van der Waals forces**- weak attractions between molecules with low levels of polarity

Polarity can occur with different types of covalent bonding (ex. H_2O)

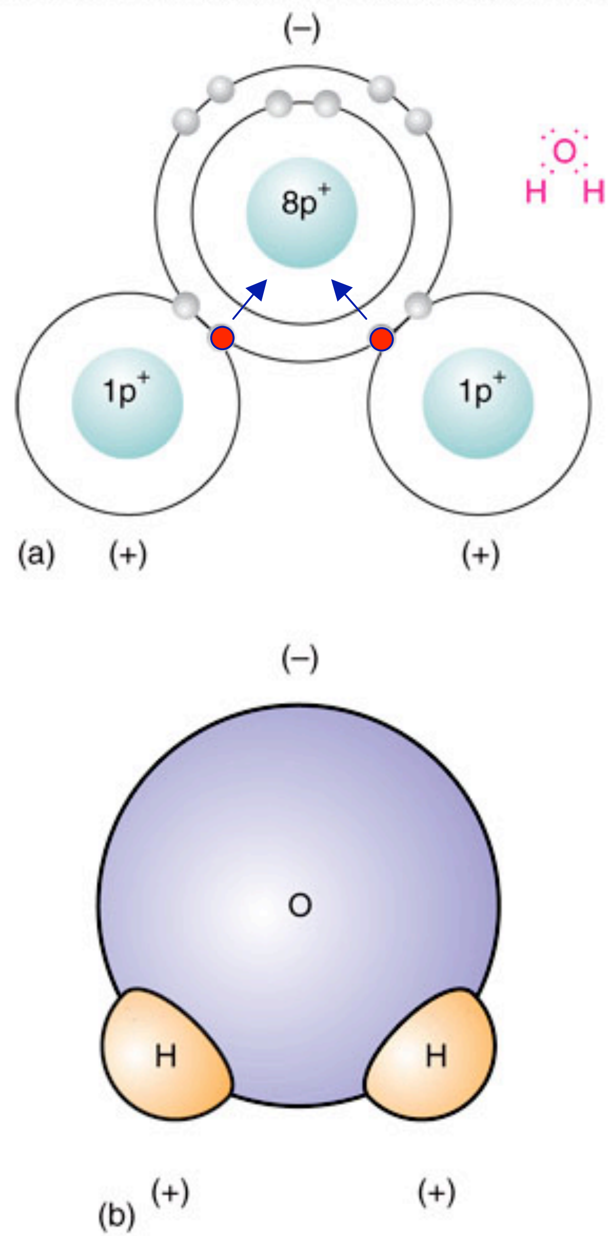


Fig. 2.5 Polar molecule

Ionic Bonds: Electron Transfer Among Atoms

- Electrons transferred completely from one atom to another, **without sharing**, results in an **ionic bond** (ex. NaCl)
- Molecules with ionic bonds, when dissolved in a solvent, can separate in to charged particles called **ions** in a process called **ionization**
- **Cations**- positively charged ions
- **Anions**- negatively charged ions
- These ionic molecules that dissolve to form ions are called **electrolytes**

====>

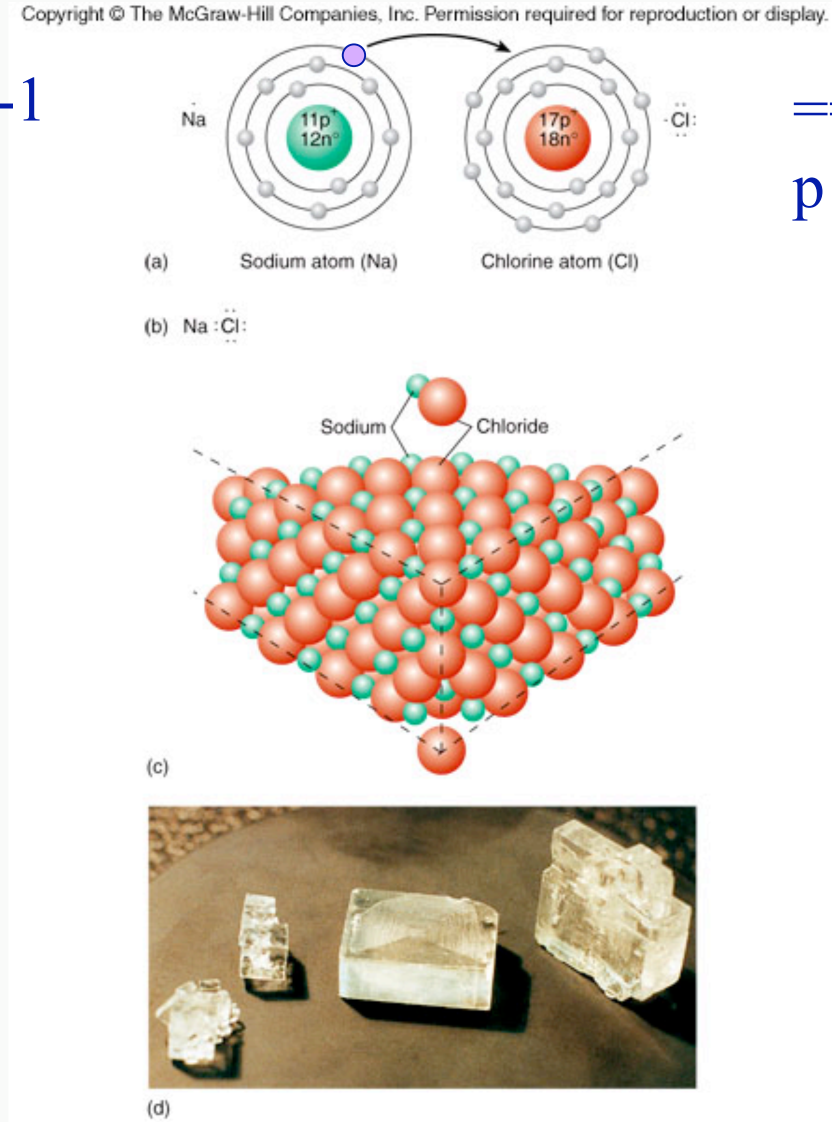
Ionic bonds: electrostatic attraction between oppositely charged ions.

Compounds with ionic bonds (ionic compounds) are usually inorganic and organized in a lattice.

Sodium chloride (table salt) is an example of ionic bonding (electron transfer among atoms or redox reaction).

==> -1

p > e



==> 8

p < e

Fig. 2.6 Ionic bonding between sodium and chlorine

Ionic bonding molecules breakup (ionization) when dissolved in a solvent (water), producing separate positive and negative particles.

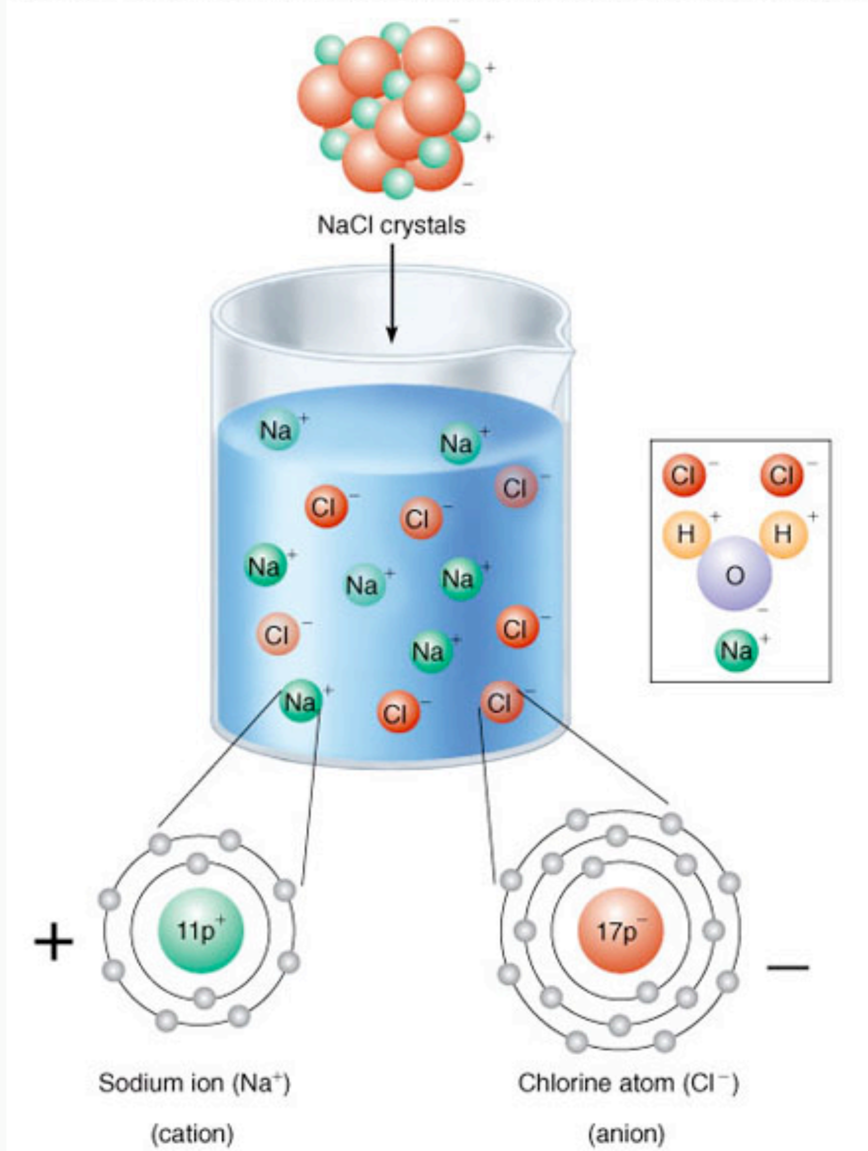


Figure 2.7 Ionization

Hydrogen bonds: interaction of hydrogen atoms (covalently bound to oxygen or nitrogen) with weak opposing electronic charges.

Polarity of the molecule leads to interaction with other polar molecules (water).

Hydrogen bonding is the attraction between the **positive hydrogen ion & a negative atom**. An example would be water molecules.

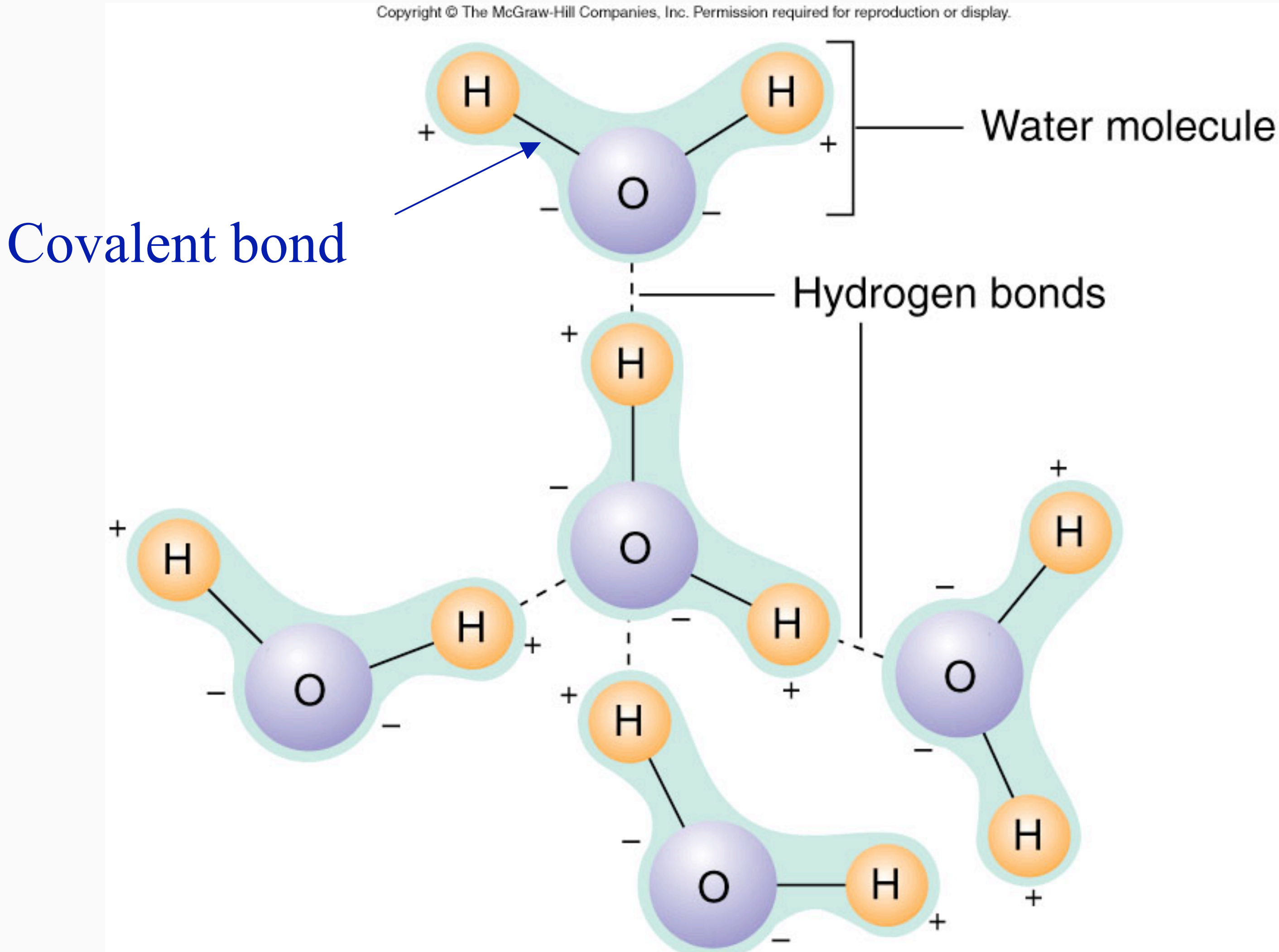


Fig. 2.8 Hydrogen bonding in water

Formulas, Models, and Equations

- **Molecular formula** - gives atomic symbols and the number of elements involved in subscript (H_2O, $C_6H_{12}O_6$).
- Molecular formulas **might not be unique** (i.e., glucose, galactose, and fructose)
- **Structural formulas** illustrate the number of the atoms and the number and types of bonds including individual relationships between Atoms in a molecule/compound

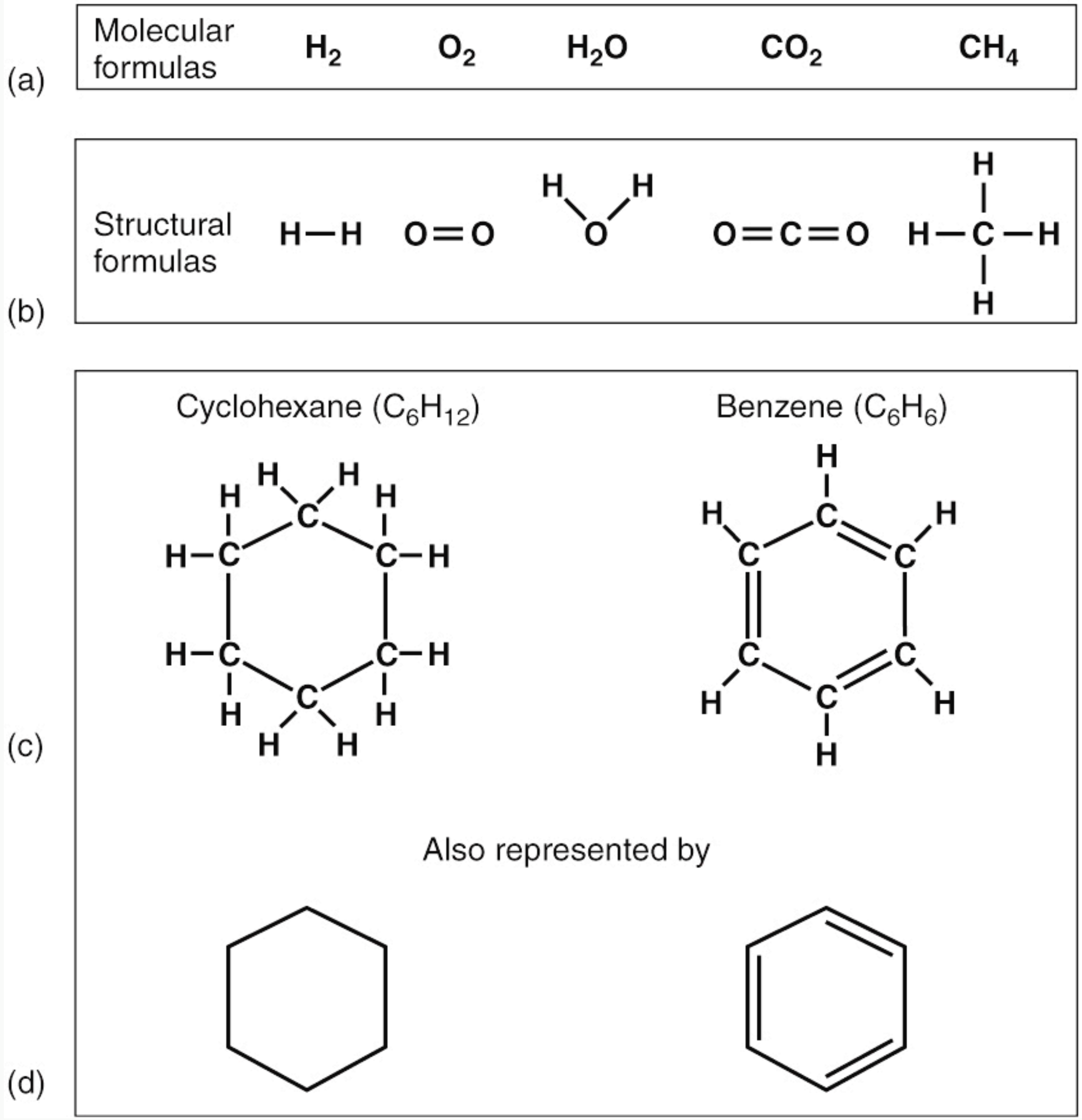

(a)
Molecular formulas
H2
O2
H2O
CO2
CH4
(b)
Structural formulas
(c)
Cyclohexane (C6H12)
Benzene (C6H6)
Also represented by
(d)

Figure 2.9

Chemical Equations

- Balance equations are used to illustrate chemical reactions
  - **Reactants-** Molecules entering (used in) a reaction
  - **Products-** the compounds produced by a reaction

Types of Reactions

- **Synthesis**: **reactants bond** together to form an entirely new molecule
  - A + B <--> AB
  - $S + O_2$ <--> SO_2
  - $2H_2 + O_2$ <--> $2H_2O$ (note that equations must be balanced)

- Decomposition: bonds on a single reactant molecule are permanently broken to release two or more product molecules
  - AB <--> A + B
  - $2H_2O_2$ <--> $2H_2O + O_2$

- **Exchange**: The reactants trade places between each other and release products that are combinations of the two

  AB + XY <-->   AX + BY (reversible reaction)

- **Catalysts (metals or Enzymes)**- increase the rate of the reaction (lower the activation energy)

Solutions: Homogeneous Mixtures of Molecules

- **Solution**- a mixture of one or more **solutes** uniformly dispersed in a **solvent**
- The solute cannot be separated by filtration or settling
- The rule of solubility- “like dissolves like”
- Water- the most common solvent in natural systems because of its special characteristics
  - **Hydrophilic** molecules - attract water to their surface (polar)
  - **Hydrophobic** molecules - repel water (nonpolar)
  - **Amphipathic (amphiphilic)** molecules - have both hydrophilic and hydrophobic properties

Concentration of Solutions

- **Concentration** - the amount of solute dissolved in a certain amount of solvent
  - In biological solutions, commonly expressed as molar concentration or molarity (M)
    - One mole dissolved in 1 L
    - One mole is the molecular mass of a compound in grams

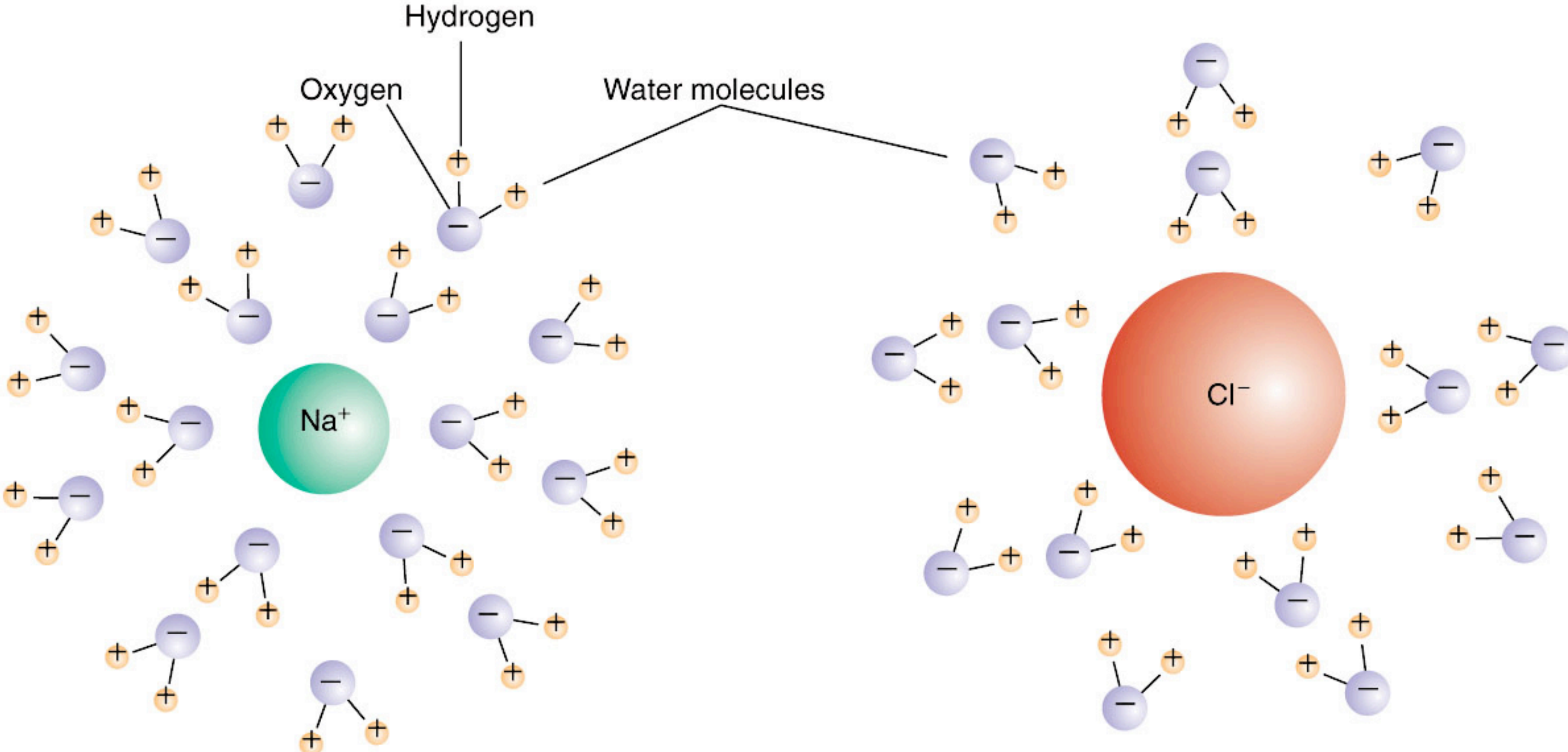


Figure 2.11

Acidity, Alkalinity, and the pH Scale

- **Acidic** solutions - when a compound dissolved in water (acid) releases excess hydrogen ions (H^+)
- **Basic** solutions- when a compound releases excess hydroxide ions (OH^-)

pH scale

- **pH scale-** measures the acid and base concentrations of solutions:
  - Ranges from 0 (most acidic) to 14 (most basic); pH= 7 is neutral (i.e., $[H^+] = [OH^-]$)
  - $pH = -log[H^+]$

TABLE 2.2 Hydrogen Ion and Hydroxide Ion Concentrations at a Given pH

Moles/Liter of Hydrogen Ions	Logarithm	pH	Moles/Liter of OH^-
1.0	10^{-0}	0	10^{-14}
0.1	10^{-1}	1	10^{-13}
0.01	10^{-2}	2	10^{-12}
0.001	10^{-3}	3	10^{-11}
0.0001	10^{-4}	4	10^{-10}
0.00001	10^{-5}	5	10^{-9}
0.000001	10^{-6}	6	10^{-8}
0.0000001	10^{-7}	7	10^{-7}
0.00000001	10^{-8}	8	10^{-6}
0.000000001	10^{-9}	9	10^{-5}
0.0000000001	10^{-10}	10	10^{-4}
0.00000000001	10^{-11}	11	10^{-3}
0.000000000001	10^{-12}	12	10^{-2}
0.0000000000001	10^{-13}	13	10^{-1}
0.00000000000001	10^{-14}	14	10^{-0}

The pH of an environment (exterior or interior of a cell) is important for living systems.

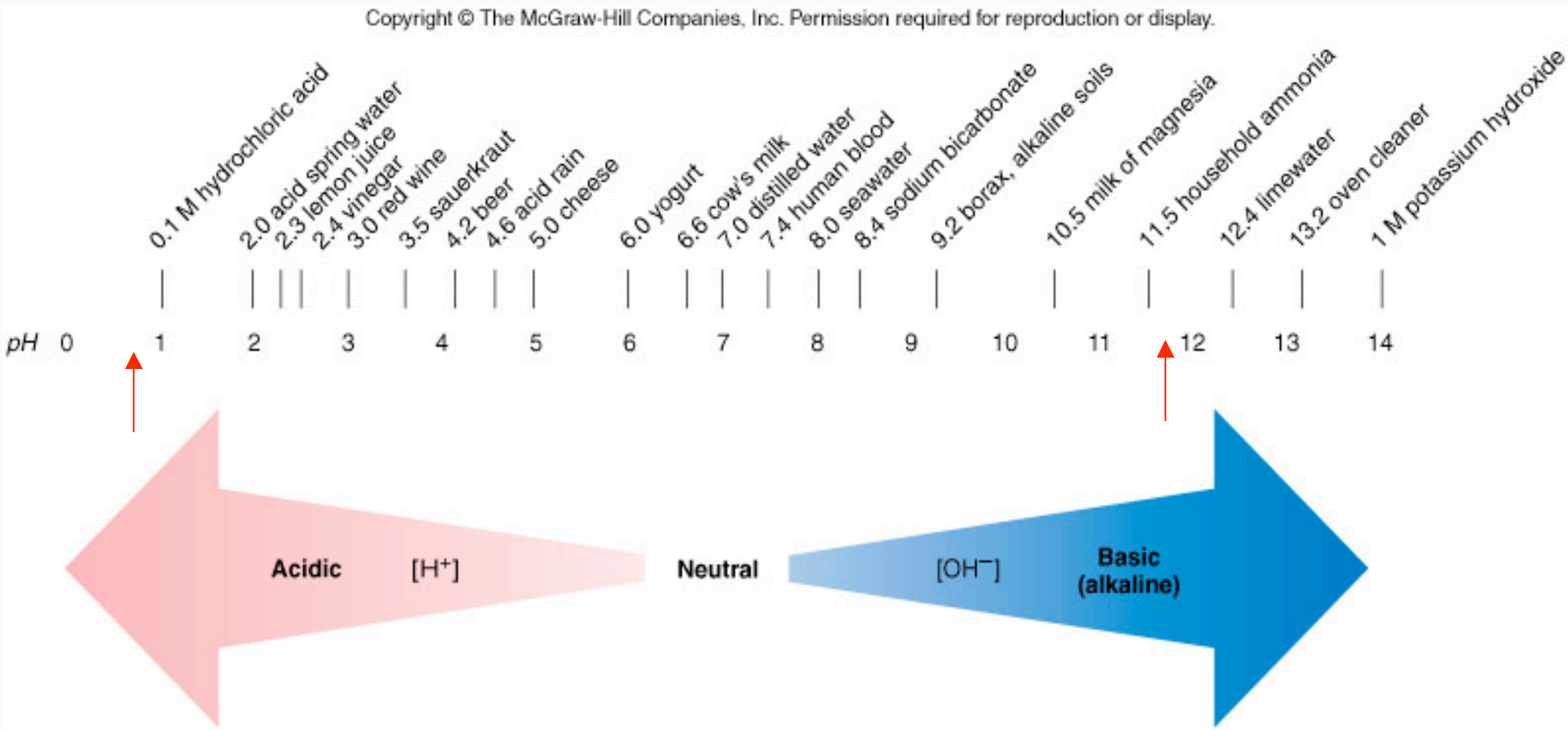


Fig. 2.12 The pH scale

Neutralization Reactions

- **Neutralization** reactions- occur in aqueous solutions containing both acids and bases
- Give rise to water and other neutral by-products
- HCl + NaOH <--> H_2O + NaCl

Molecules

- Can be inorganic and organic molecules.
  - Inorganic: C **or** H is present (ex. CO_2, H_2)
  - Organic: C **and** H are present (ex. CH_3OH)
- Molecules can form complex Compounds

The Chemistry of Carbon and Organic Compounds

- **Inorganic chemicals**: usually do not contain both C and H (ex. NaCl, $CaCO_3$)

- **Organic chemicals**: Carbon compounds with a basic framework of the element carbon bonded to itself and other atoms
  - Most of the chemical reactions and structures of living things involve organic chemicals

Carbon- the Fundamental Element of Life

- Valence makes it an ideal atomic building block
- Forms stable chains containing thousands of C atoms, with bonding sites available
- Can form linear, branched, or ringed molecules
- Can form single, double, or triple bonds
- Most often associates with H, O, N, S, and P

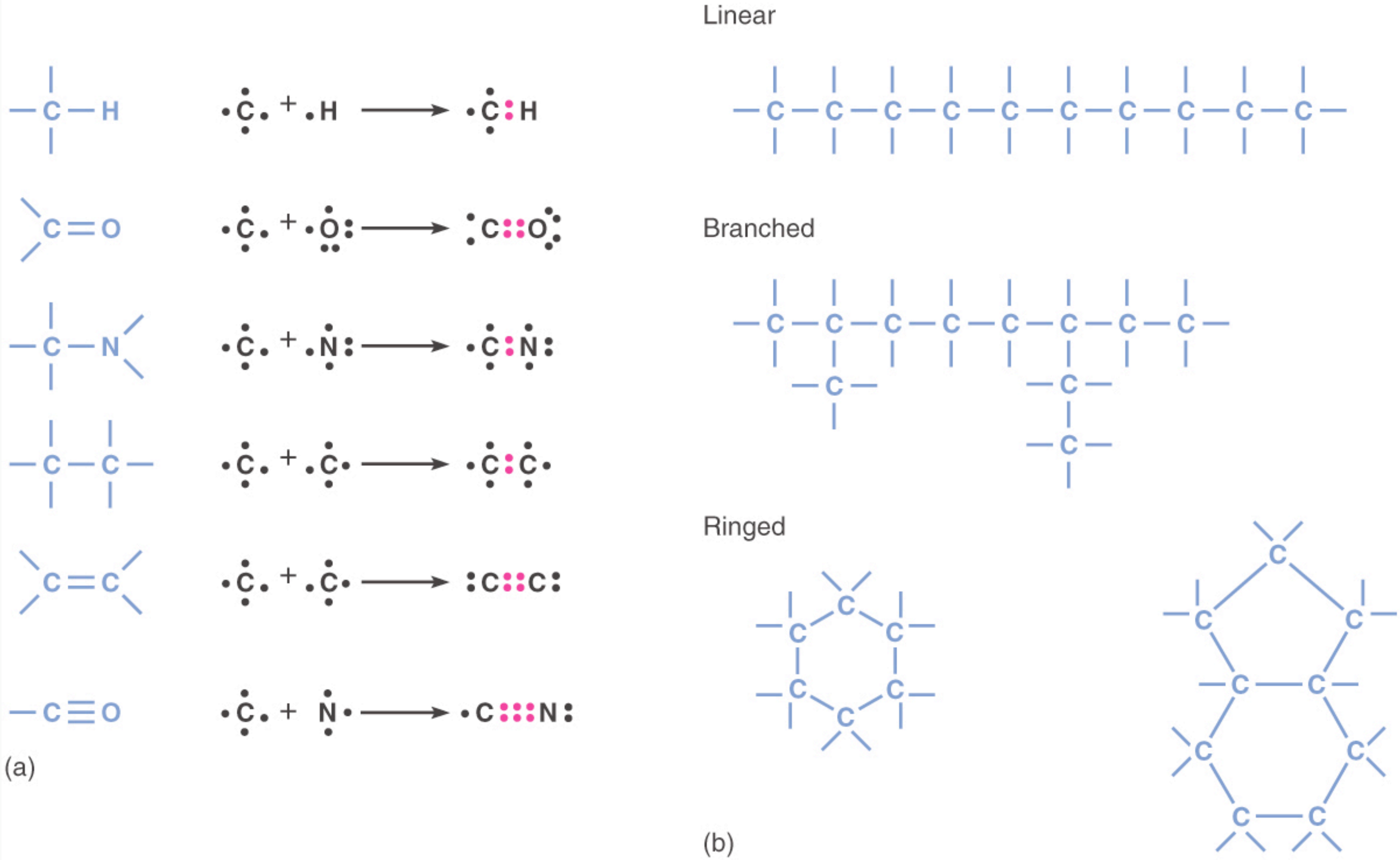


Figure 2.13

Functional Groups of Organic Compounds

- Special molecular groups or accessory molecules that covalently or hydrogen-bond to organic compounds are called **functional groups.**
- FG help define the chemical class of certain groups of organic compounds
- FG give organic compounds unique reactive properties
  - Reactions of an organic compound can be predicted by knowing the kind of functional group or groups it carries

The carbon in inorganic and organic molecules is the basic fundamental element of life. Functional groups (R) bind to these carbons.

TABLE 2.3 Representative Functional Groups and Classes of Organic Compounds

Formula of Functional Group	Name	Class of Compounds
R* — O—H	Hydroxyl	Alcohols, carbohydrates
R — C(=O)—OH	Carboxyl	Fatty acids, proteins, organic acids
R — CH₂ — NH₂ (R—C(H)(H)—NH₂)	Amino	Proteins, nucleic acids
R — C(=O)—O—R	Ester	Lipids
R — C(H)(H) — SH	Sulfhydryl	Cysteine (amino acid), proteins
R — C(=O)—H	Carbonyl, terminal end	Aldehydes, polysaccharides
R — C(=O) — C —	Carbonyl, internal	Ketones, polysaccharides
R — O — P(=O)(OH) — OH	Phosphate	DNA, RNA, ATP

Table 2.3 Representative functional groups and classes of organic compounds

- **Biochemistry**: study of the compounds of life, their synthesis and degradation

- **Biochemicals**: organic compounds produced by (or components of) living things

Biochemicals can be very large and thus called more specifically: **macromolecules.**

2.2 **Macromolecules**

- Polysaccharides (Carbohydrates)
- Lipids
- Proteins
- Nucleic acids

Macromolecules (polymers) are the product of condensation (polymerization) reactions, in which monomers are polymerized thereby forming water.

Carbohydrates

Sugars and Polysaccharides

- Exist in a variety of configurations
  - Sugar (**saccharide**): a simple carbohydrate with a sweet taste
  - **Monosaccharide** usually contains 3-7 carbons
  - **Disaccharide** contains two monosaccharides
  - **Polysaccharide** contains five or more monosaccharides

- Monosaccharides and disaccharides are specified by combining a prefix that describes a characteristic of the sugar with the suffix –ose
  - **Hexoses**- six carbons
  - **Pentoses**- five carbons
  - **Fructose**- for fruit

Carbohydrates

- **Carbohydrates**: Sugars and Polysaccharides

  Most can be represented by the general formula

$$(CH_2O)_n$$

  where n = the number of units of this combination of atoms

Major sugars (monosaccharides) in the cell are glucose, galactose and fructose. Several sugars bonded together are called **polysaccharides**.

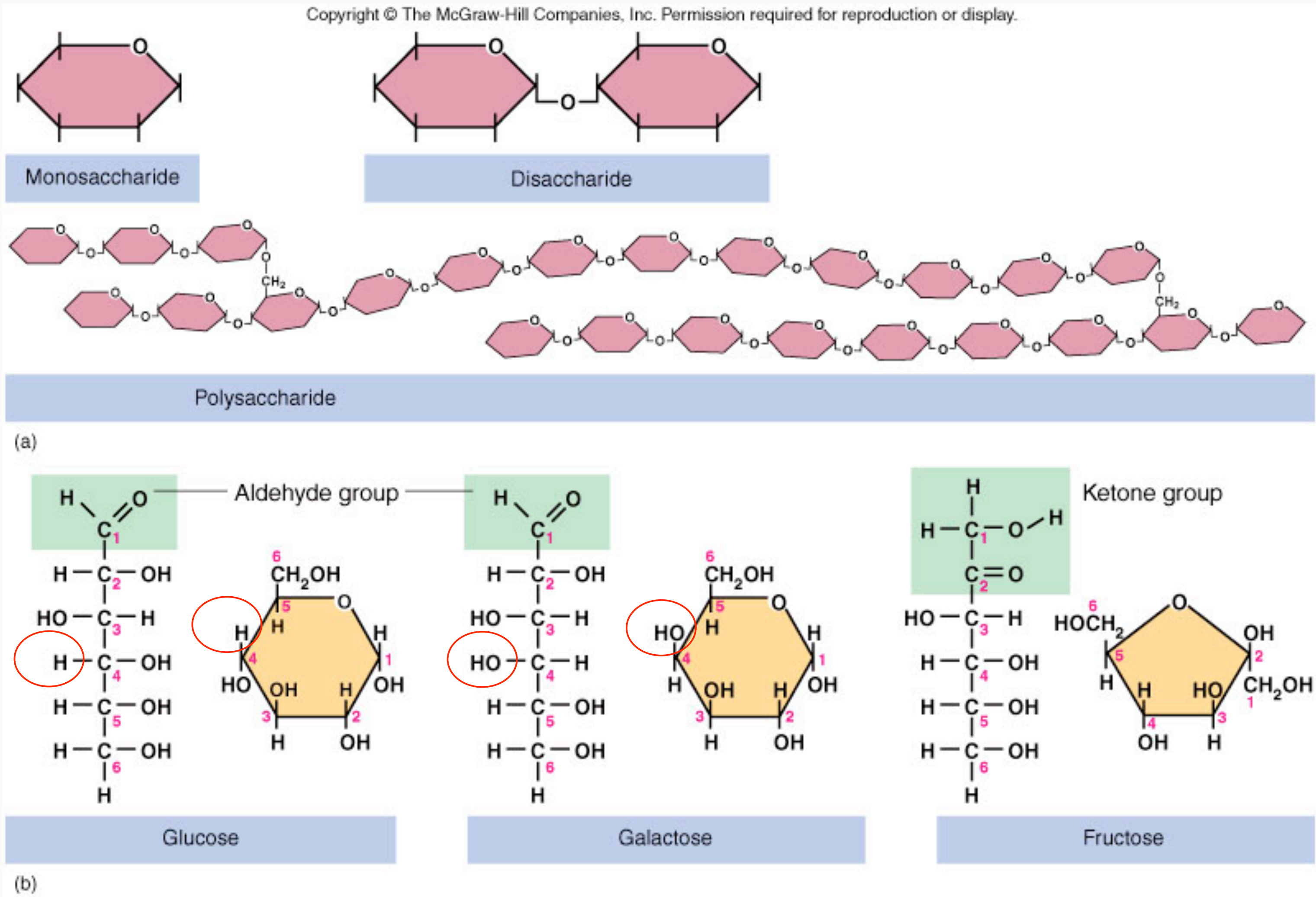


Fig. 2.14 Common classes of carbohydrates

Sugars are bonded by **glycosidic bonds**. Water is released (condensation, dehydration) after the bond is formed.

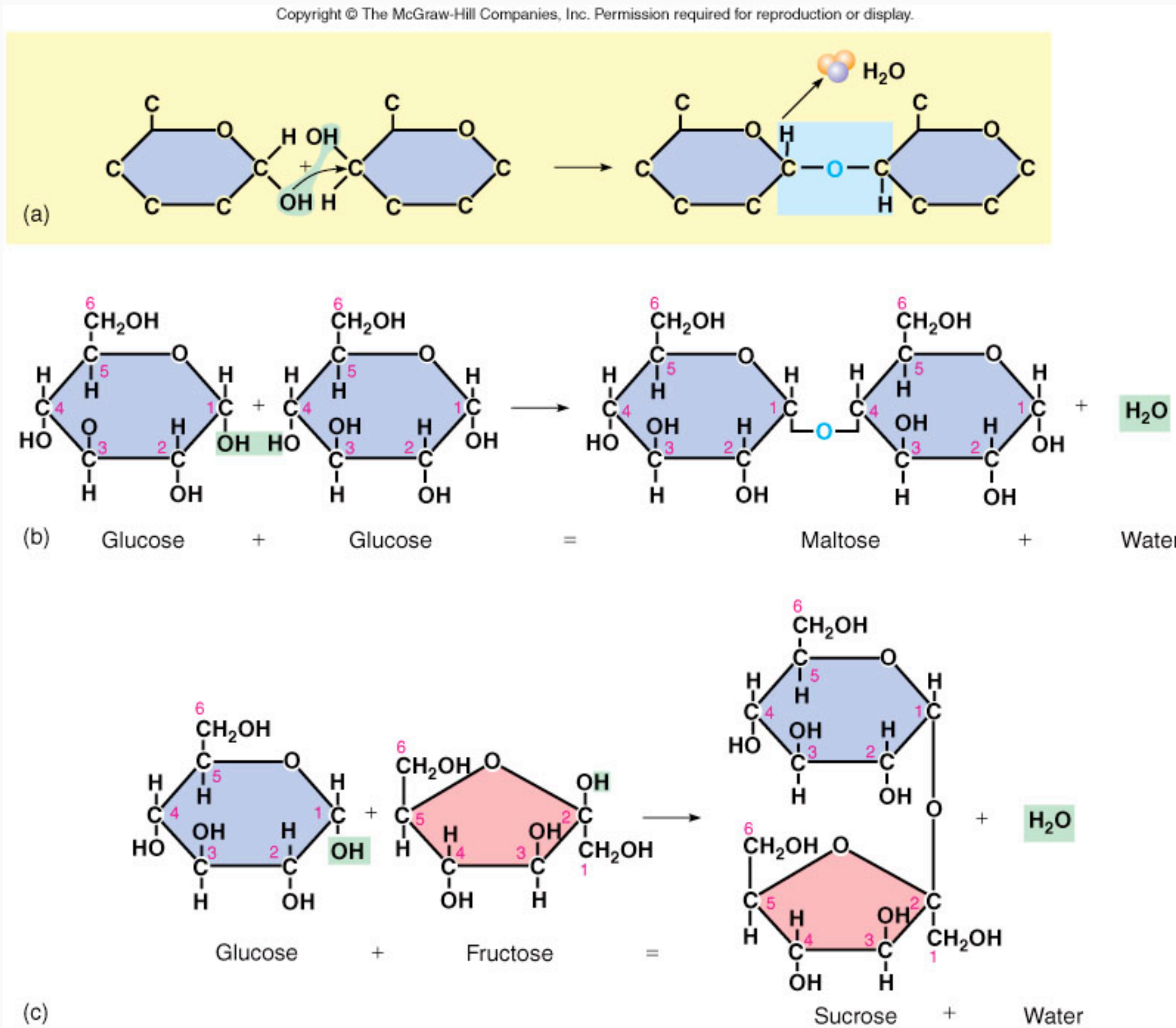
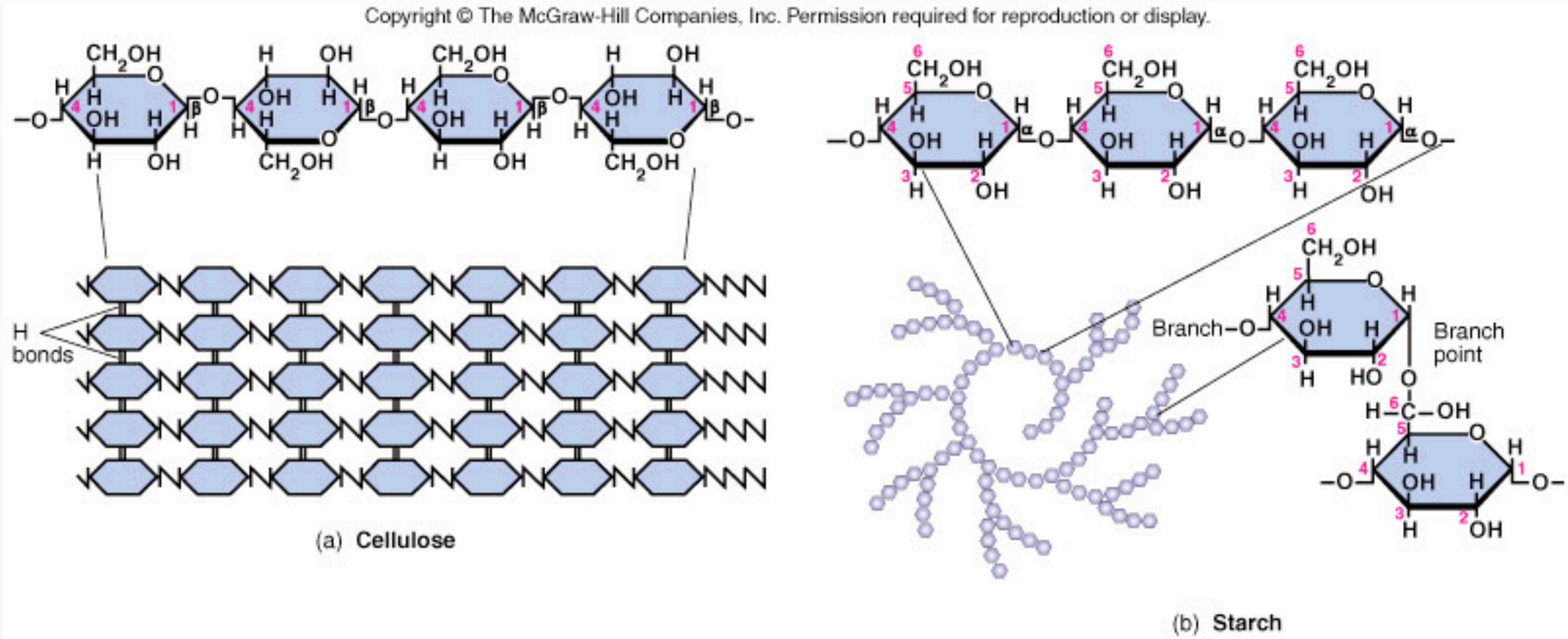


Fig. 2.15 Glycosidic bond

Peptidoglycan in bacteria is an example of a polysaccharide.



Poly-cellobiose = cellulose

Starch: (1,4)-bonded (linear) and
Glycogen: (1,6) branching starch

Fig. 2.16 Polysaccharides

Lipids:

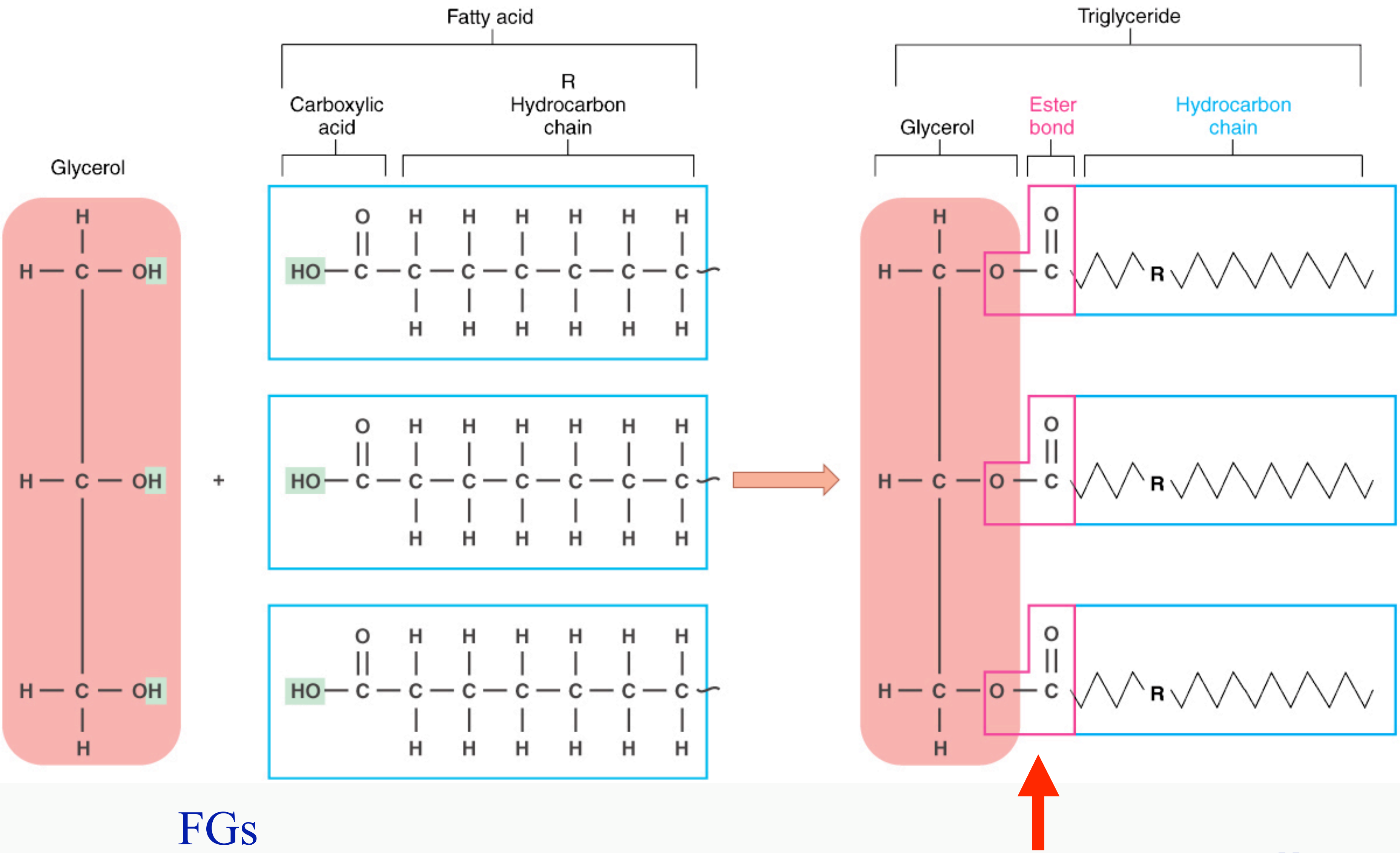
Fats & Oils, Phospholipids, and Waxes

- **Lipids**- a variety of substances that are not soluble in polar solvents
- Building blocks: **Alcohol and fatty acids**
- Will dissolve in non-polar solvents
- Main groups of lipids:
    - Triglycerides (Fats & Oils)
    - Phospholipids
    - Miscellaneous lipids: Steroids & Waxes

Lipids

- **Triglycerides** (Includes fats and oils) :

  A single molecule of the poly-alcohol glycerol covalently bound to three fatty acids



FGs

Figure 2.17

Phospholipids

- **Phospholipids** - Contain
  - glycerol with
  - two fatty acids attached to
  - a phosphate group on the third glycerol binding site

- **Phospholipids** serve as a <u>major structural component of cell membranes</u>.

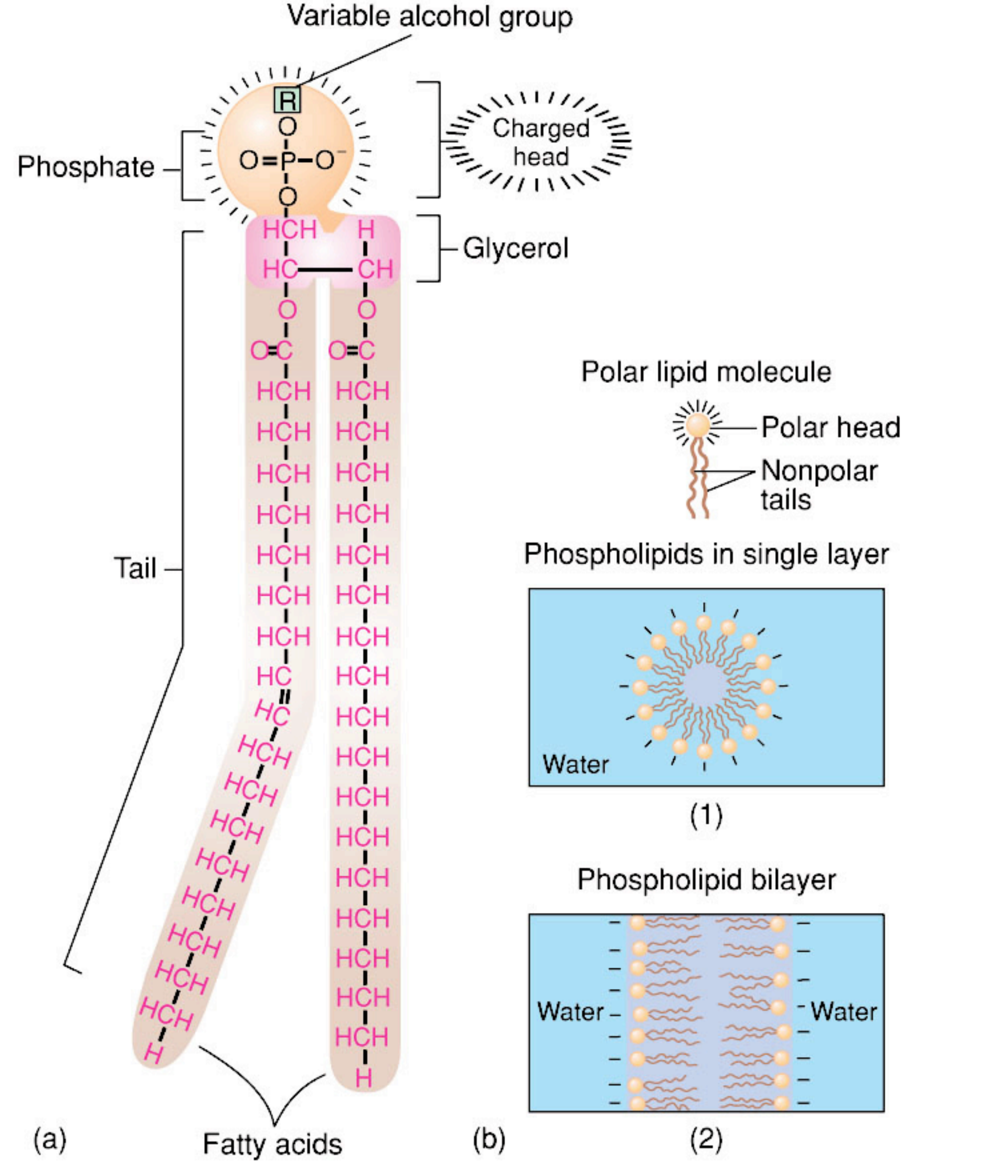


Figure 2.18

Miscellaneous Lipids

- **Steroids**: complex ringed compounds commonly found in cell membranes and as animal hormones
  - Best known: **cholesterol**

- **Waxes**: esters formed between a long-chain alcohol and a saturated fatty acid

Cholesterols are associated with cell membranes of some cells. They bind to the fatty acid of a lipid.

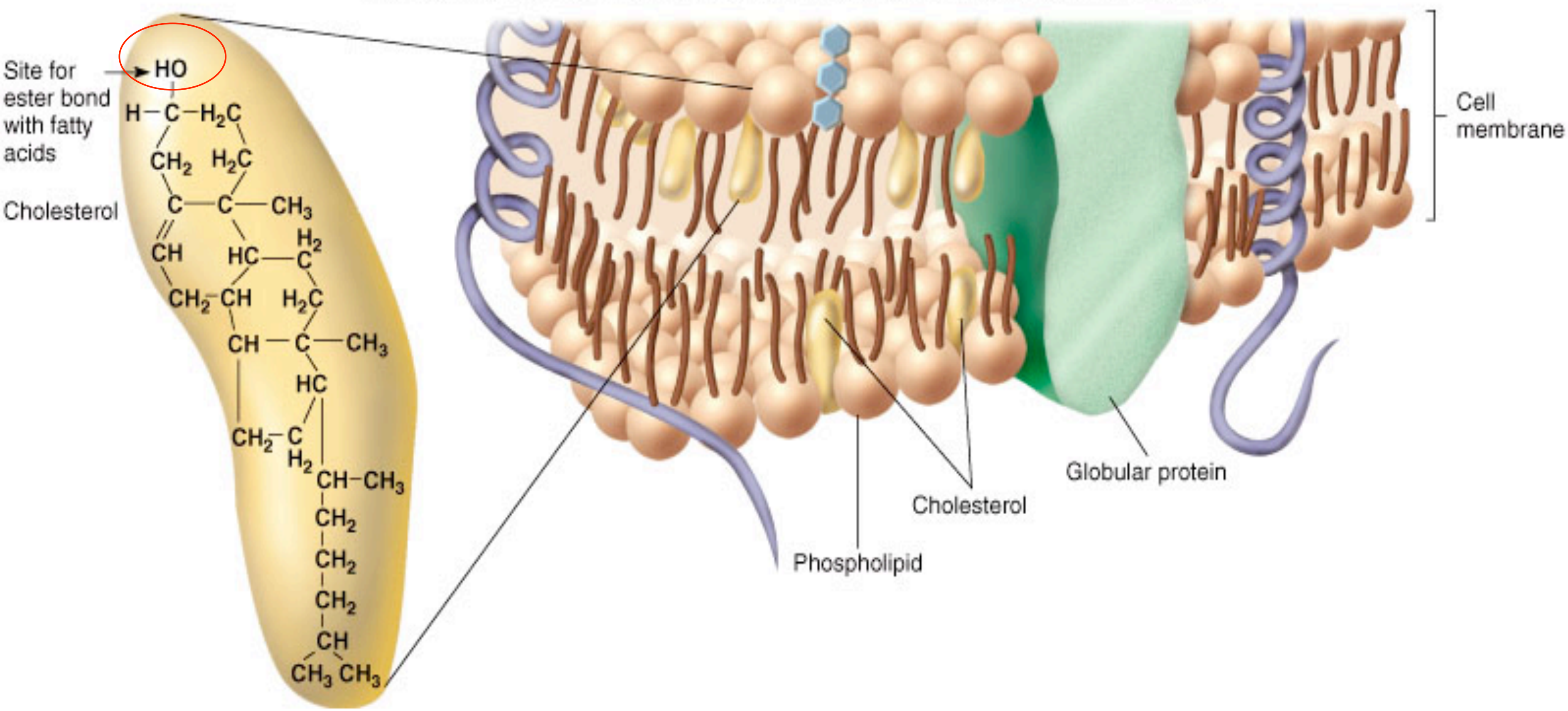


Fig. 2.19 Formula for cholesterol.

Proteins

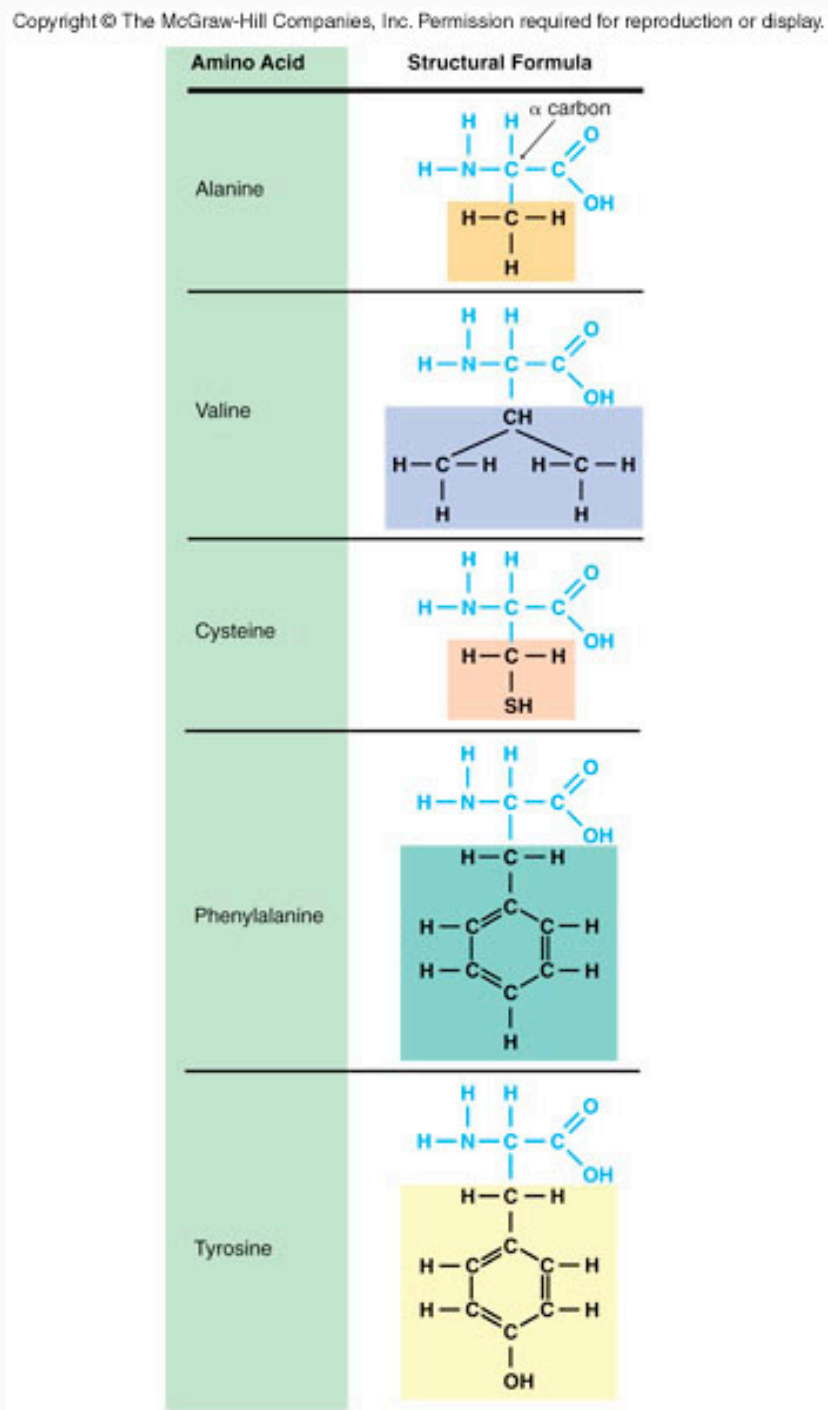
- Proteins are the predominant organic molecule in cells (58% of dry mass)
- Building blocks (monomers): **amino acids**
- Proteins consist of a series of amino acids (ex. Peptides, polypeptides)

- Examples: enzymes, immunoglobulins, etc.

Proteins: “Shapers of Life”

- Building blocks- **amino acids**
  - 20 different naturally occurring forms
  - Basic skeleton- a carbon (the α carbon) linked to an amino group (NH_2), a carboxyl group (COOH), a hydrogen atom (H), and a variable R group

Amino acids vary based on their reactive (R) groups present.



Ala

Val

Cys

Phe

Tyr

Fig. 2.20 Structural formula of selected amino acids

TABLE 2.5 Twenty Amino Acids and Their Abbreviations

Acid	Abbreviation	Characteristic of R Groups*
Alanine	Ala	NP
Arginine	Arg	+
Asparagine	Asn	P
Aspartic acid	Asp	−
Cysteine	Cys	P
Glutamic acid	Glu	−
Glutamine	Gln	P
Glycine	Gly	P
Histidine	His	+
Isoleucine	Ile	NP
Leucine	Leu	NP
Lysine	Lys	+
Methionine	Met	NP
Phenylalanine	Phe	NP
Proline	Pro	NP
Serine	Ser	P
Threonine	Thr	P
Tryptophan	Trp	NP
Tyrosine	Tyr	P
Valine	Val	NP

*NP, nonpolar; P, polar; +, positively charged; −, negatively charged.

Similar

Table 2.5 Twenty natural occurring amino acids and their abbreviations

A covalent peptide bond forms between the amino group on one amino acid and the carboxyl group on another amino acid.

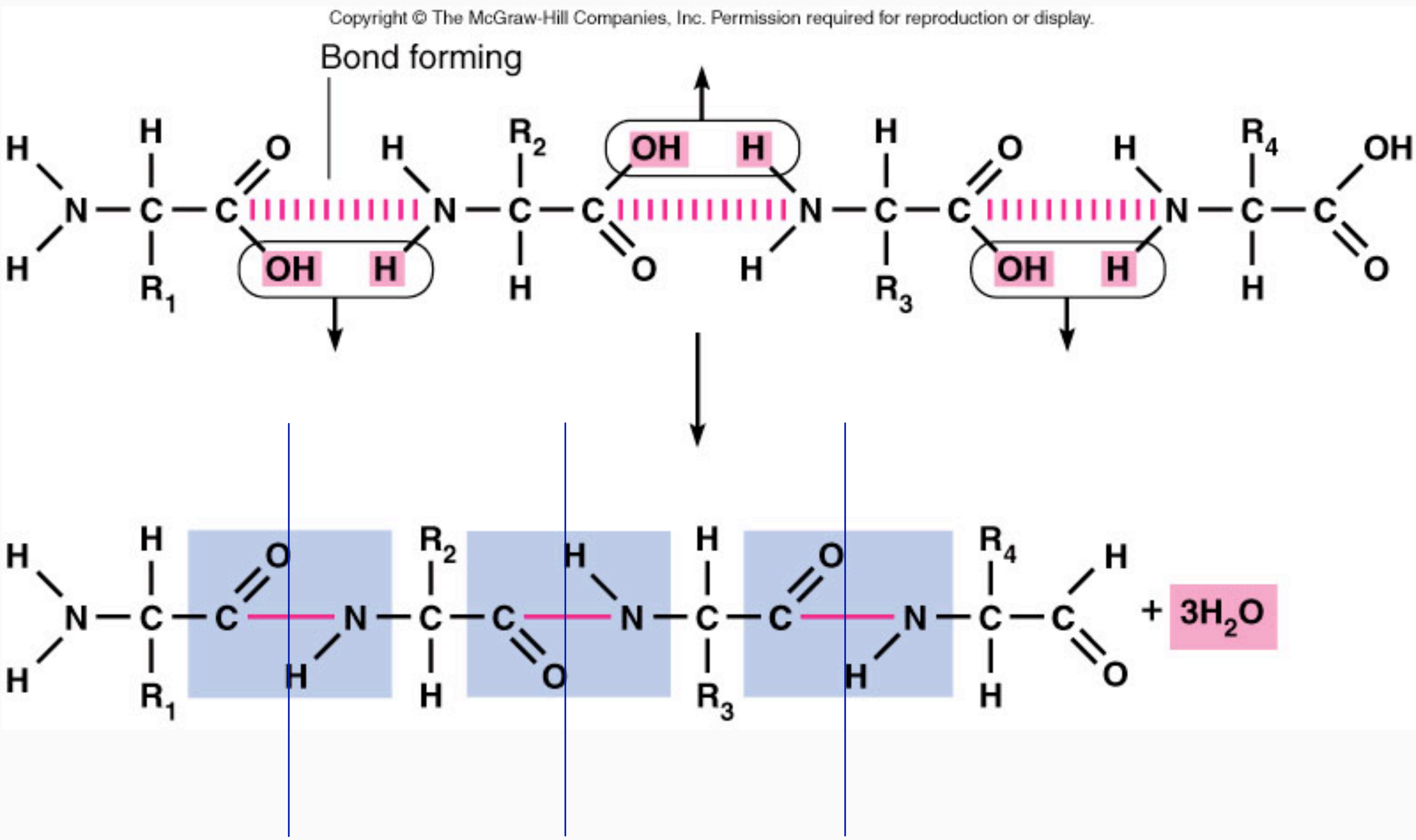


Fig. 2.21 The formation of a peptide bonds in a tetrapeptide

Proteins take on a variety of shapes, which enables specific interactions (function) with other molecules.

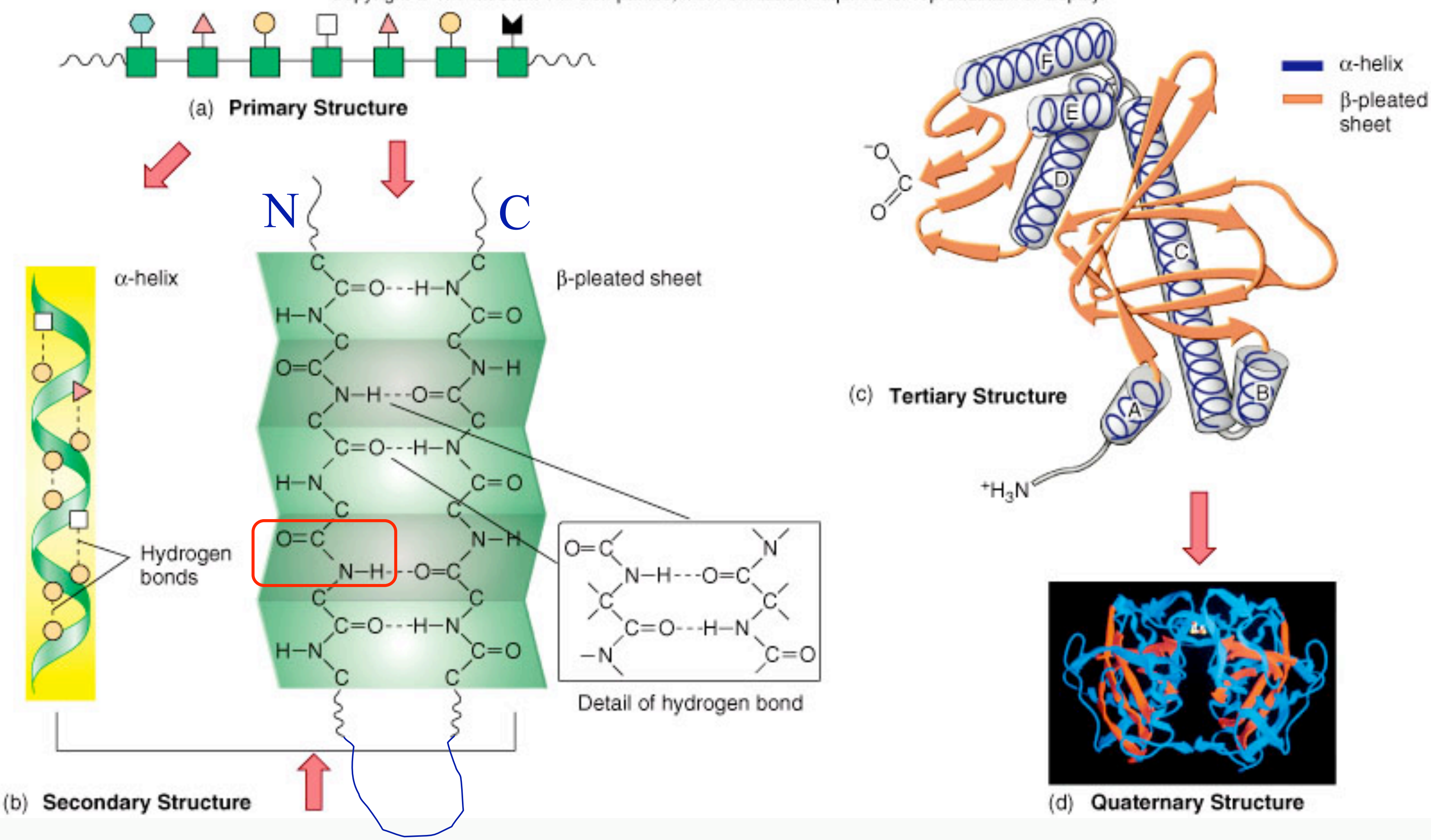


Fig. 2.22 Stages in the formation of a functioning protein

Nucleic acids

- Deoxy-ribonucleic acid (DNA)
- Ribonucleic acid (RNA)
- DNA contains genetic information which is captured (transcribed) into RNA
- The information stored in RNA can be translated into the primary sequence of proteins

Nucleic acids are polymers of monomers called nucleotides.

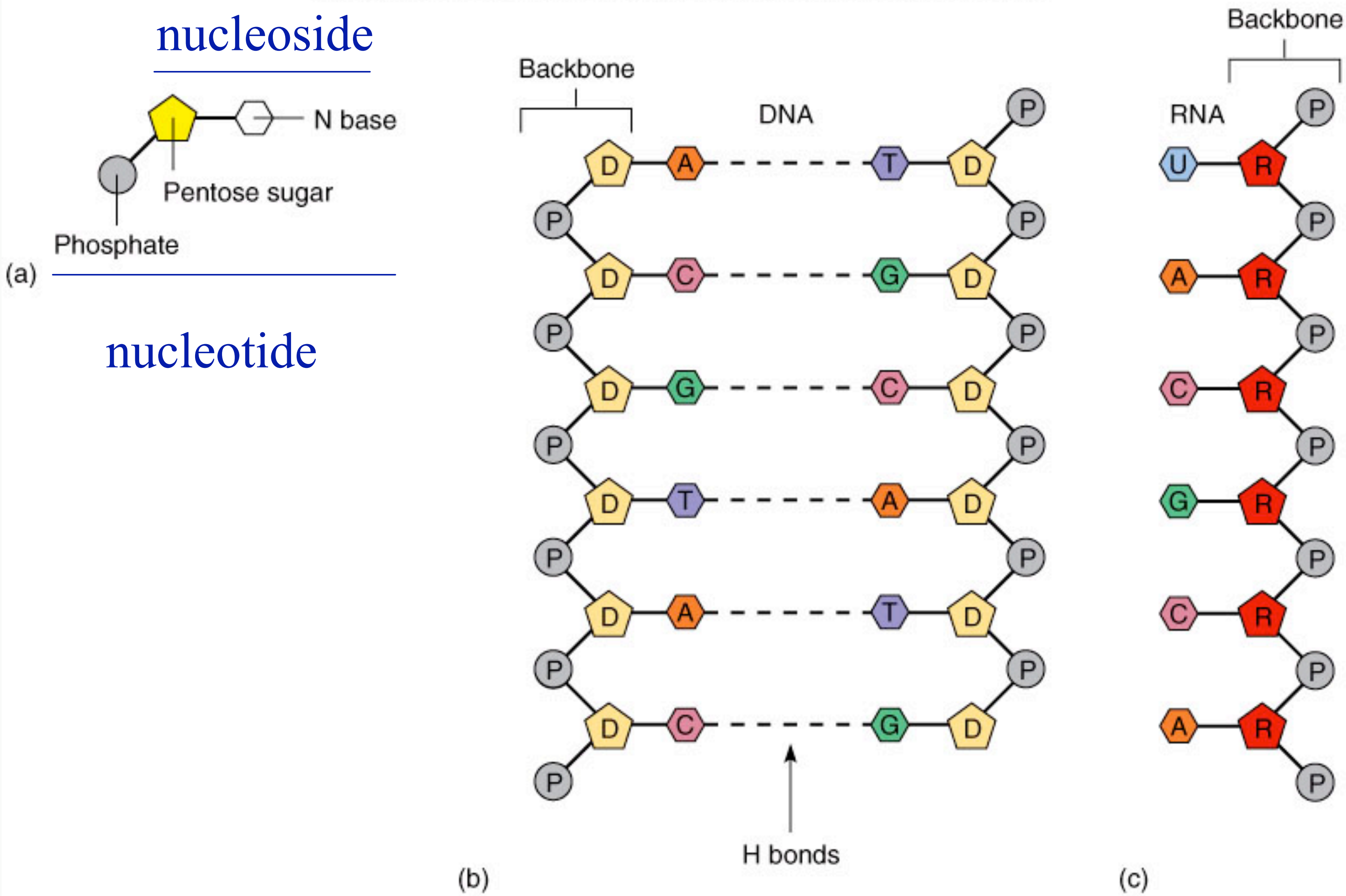


Fig. 2.23 The general structure of nucleic acids

The pentose sugars and nitrogen bases determine whether a molecule will be DNA or RNA.

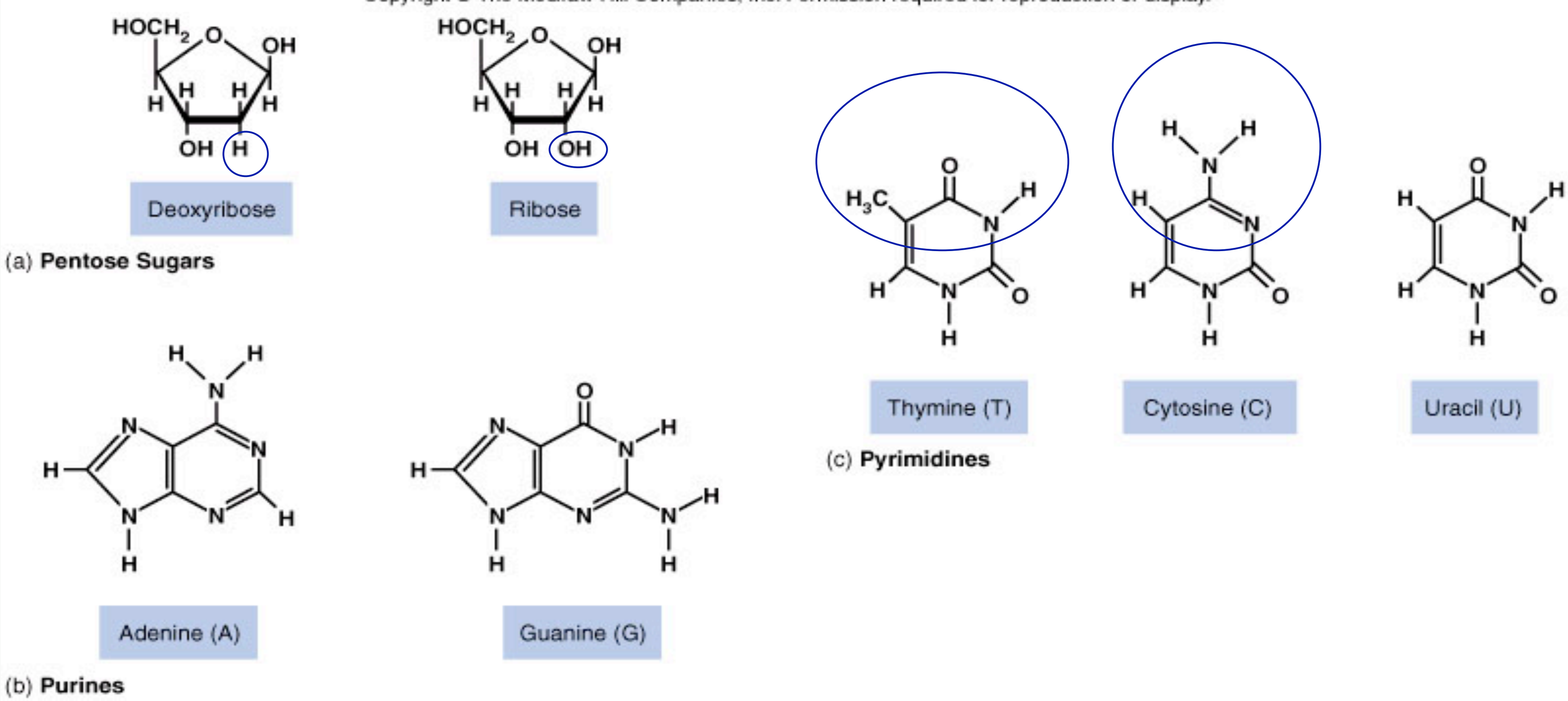


Fig. 2.24 The sugars and nitrogen bases that make up DNA and RNA.

The DNA configuration is a double helix.

Fig. 2.25 A structural representation of the double helix of DNA

DNA serves as a universal template for the synthesis of new DNA, mRNA, tRNA and rRNA.

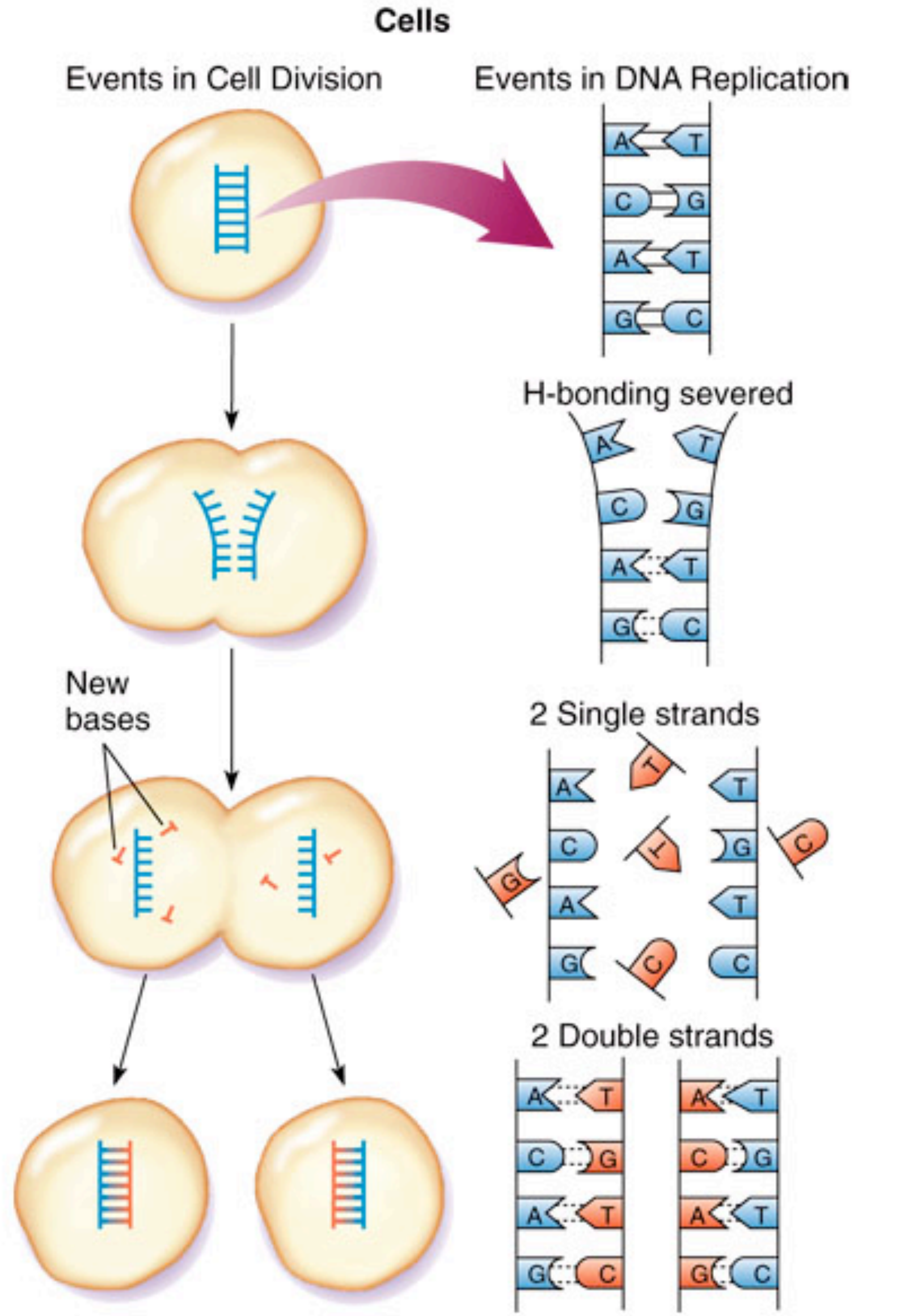


Fig. 2.26 Simplified view of DNA replication in cells.

The Cell

- **Fundamental components**:
  - Genetic element
  - Membrane
  - Ribosome

- **Fundamental characteristics**
  - Reproduction
  - Metabolism
  - Motility (Response to molecules)
  - Protection and Storage (Cell wall or membrane)
  - Nutrient transport

Chapter 3

Topics

- Methods of Culturing Microorganisms
- Microscope

Methods of Culturing Microorganisms

- Five basic techniques
- Media
- Microbial growth

Five basic techniques

1. Isolation
2. Inoculate
3. Incubate
4. Inspection
5. Identification

Inoculation and Isolation

- **Isolation**: separating one species from another
  - Separating a single bacterial cell from other cells and providing it space on a nutrient surface will allow that cell to grow in to a mound of cells (a **colony**).
  - If formed from a single cell, the colony contains cells from just that species.
- **Inoculation**: producing a culture
  - Introduce a tiny sample (the inoculum) into a container with nutrient **medium**

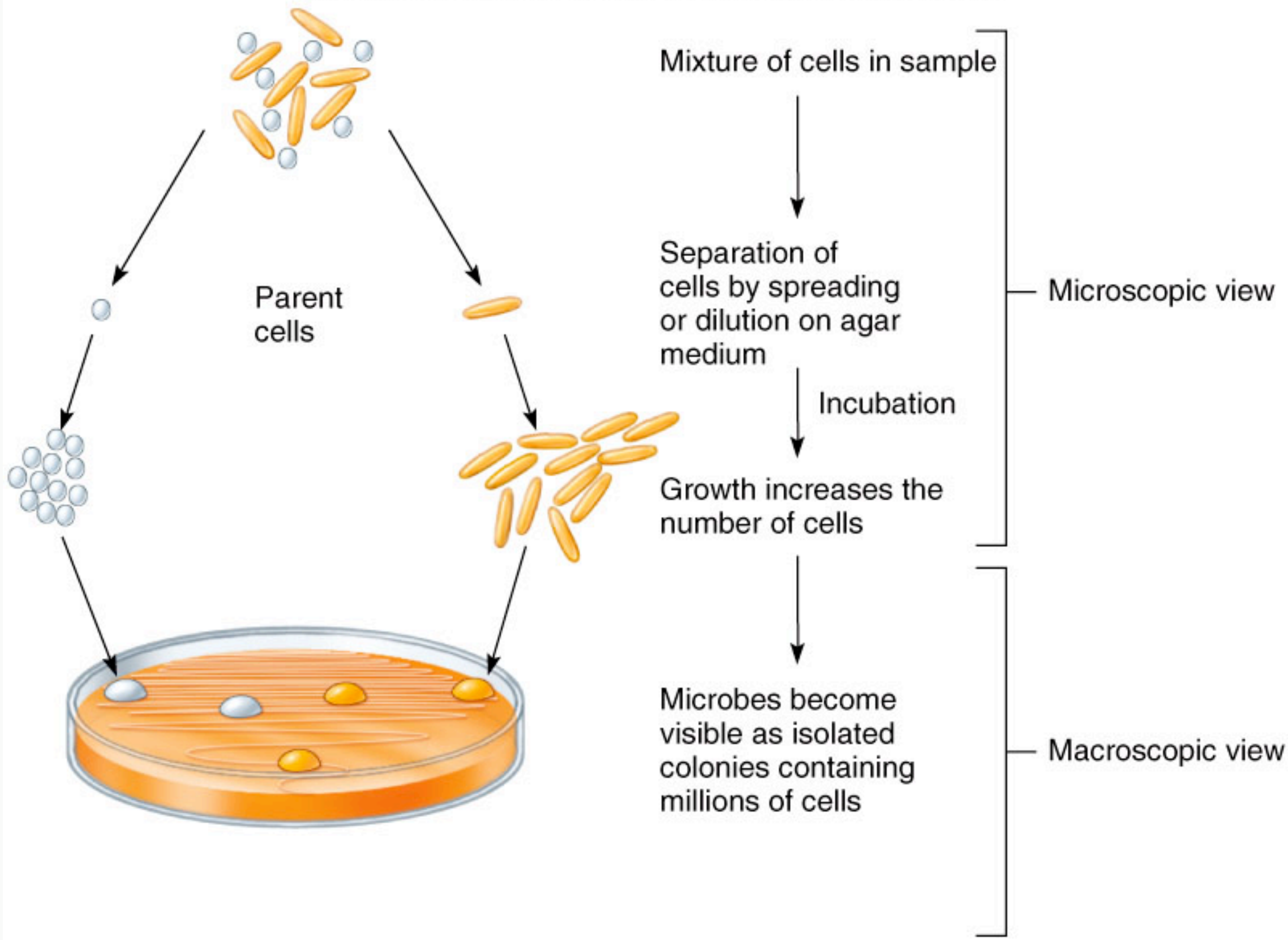
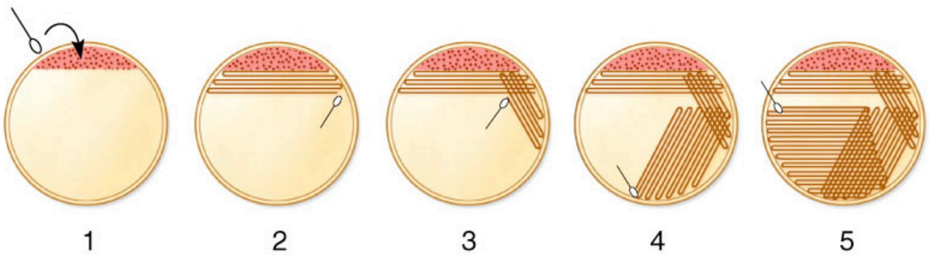
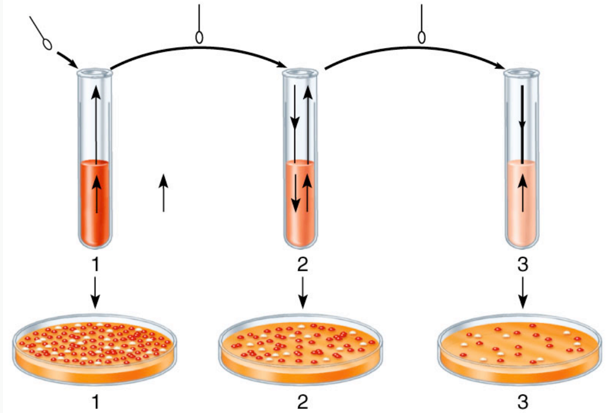


Fig. 3.2 Isolation technique

Note: This method only works if the spreading tool (usually an inoculating loop) is resterilized after each of steps 1–4.



(a) **Steps in a Streak Plate**



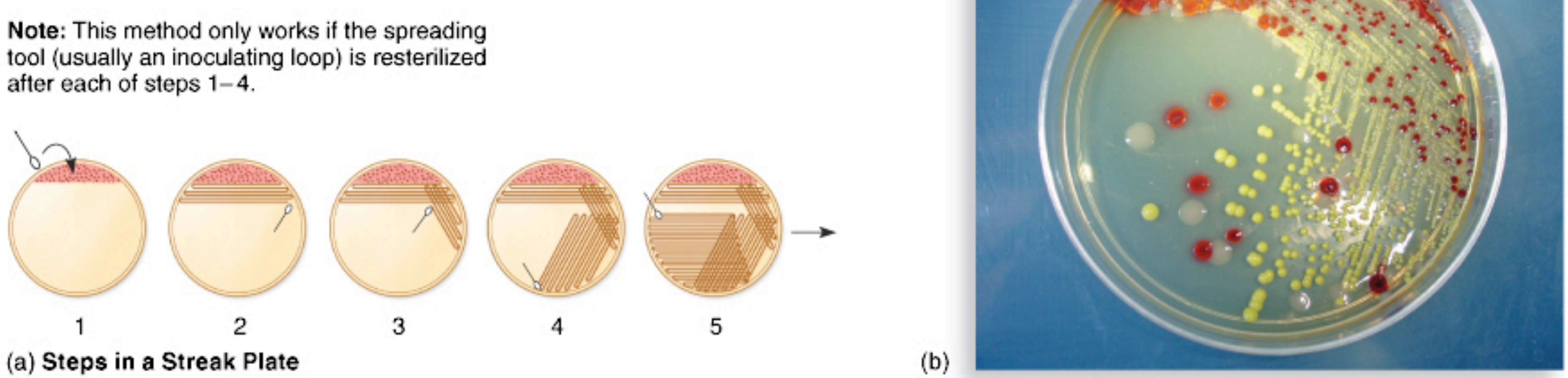
(c) **Steps in Loop Dilution**

(e) **Steps in a Spread Plate**

Fig. 3.3 Three basic methods for isolating bacteria.

Streak Plate Method

- Streak plate method- small droplet of culture or sample spread over surface of the medium with an inoculating loop
  - Uses a pattern that thins out the sample and separates the cells

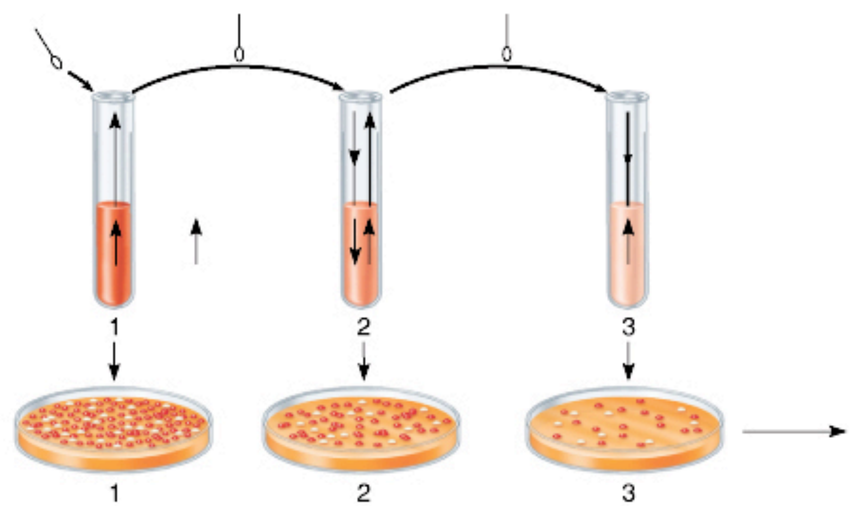


(a) **Steps in a Streak Plate** (b)

Figure 3.3 a,b

Loop Dilation Method

- Loop dilation, or pour plate, method- sample inoculated serially in to a series of liquid agar tues to dilute the number of cells in each successive tubes
  - Tubes are then poured in to sterile Petri dishes and allowed to solidify



(c) Steps in Loop Dilution

(d)

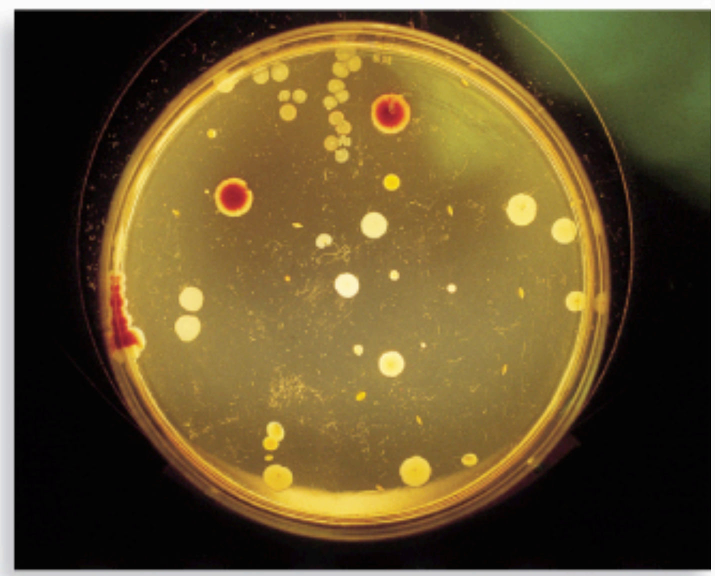
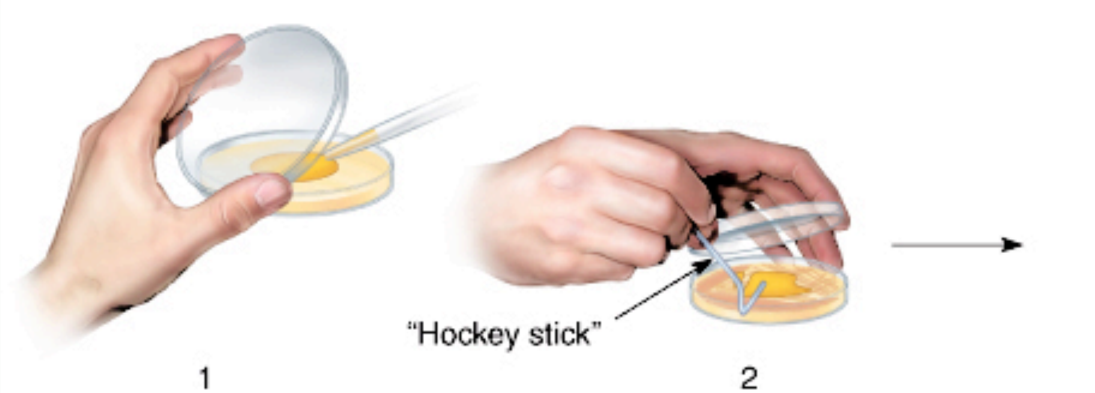


Figure 3.3 c,d

Spread Plate Method

- Spread plate method- small volume of liquid, diluted sample pipette on to surface of the medium and spread around evenly by a sterile spreading tool



(e) **Steps in a Spread Plate**

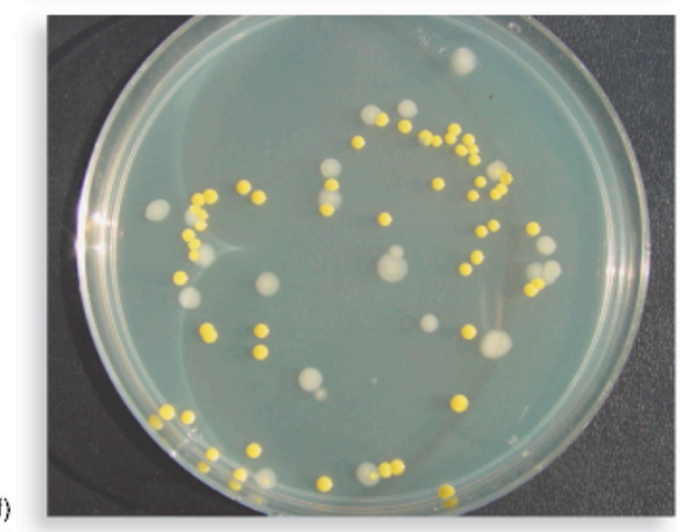

(f)

Figure 3.3 e,f

Media: Providing Nutrients in the Laboratory

- At least 500 different types
- Contained in test tubes, flasks, or Petri dishes
- Inoculated by loops, needles, pipettes, and swabs
- Aseptic technique necessary
- Classification of media
  - Physical state
  - Chemical composition
  - Functional type

TABLE 3.1 Three Categories of Media Classification

Physical State (Medium's Normal Consistency)	Chemical Composition (Type of Chemicals Medium Contains)	Functional Type (Purpose of Medium)*
1. Liquid 2. Semisolid 3. Solid (can be converted to liquid) 4. Solid (cannot be liquefied)	1. Synthetic (chemically defined) 2. Nonsynthetic (complex; not chemically defined)	1. General purpose 2. Enriched 3. Selective 4. Differential 5. Anaerobic growth 6. Specimen transport 7. Assay 8. Enumeration

*Some media can serve more than one function. For example, a medium such as brain-heart infusion is general purpose and enriched; mannitol salt agar is both selective and differential; and blood agar is both enriched and differential.

Classification of Media by Physical State

- **Liquid media**: water-based solutions, do not solidify at temperatures above freezing, flow freely when container is tilted
  - Broths, milks, or infusions
  - Growth seen as cloudiness or particulates
- **Semisolid media**: clotlike consistency at room temperature
  - Used to determine motility and to localize reactions at a specific site
- **Solid media**: a firm surface on which cells can form discrete colonies
  - Liquefiable and non-liquefiable
  - Useful for isolating and culturing bacteria and fungi

Liquid media are water-based solutions that are generally termed **broths, milks and infusions**.

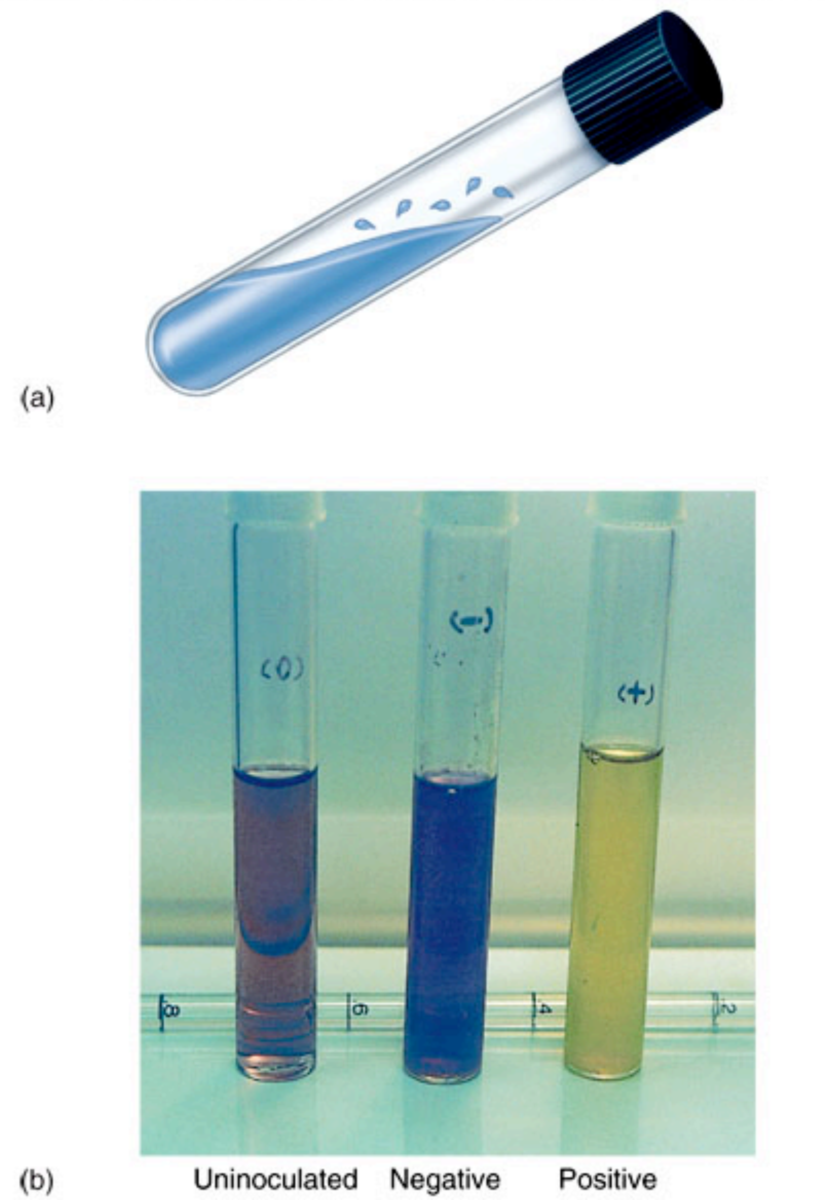
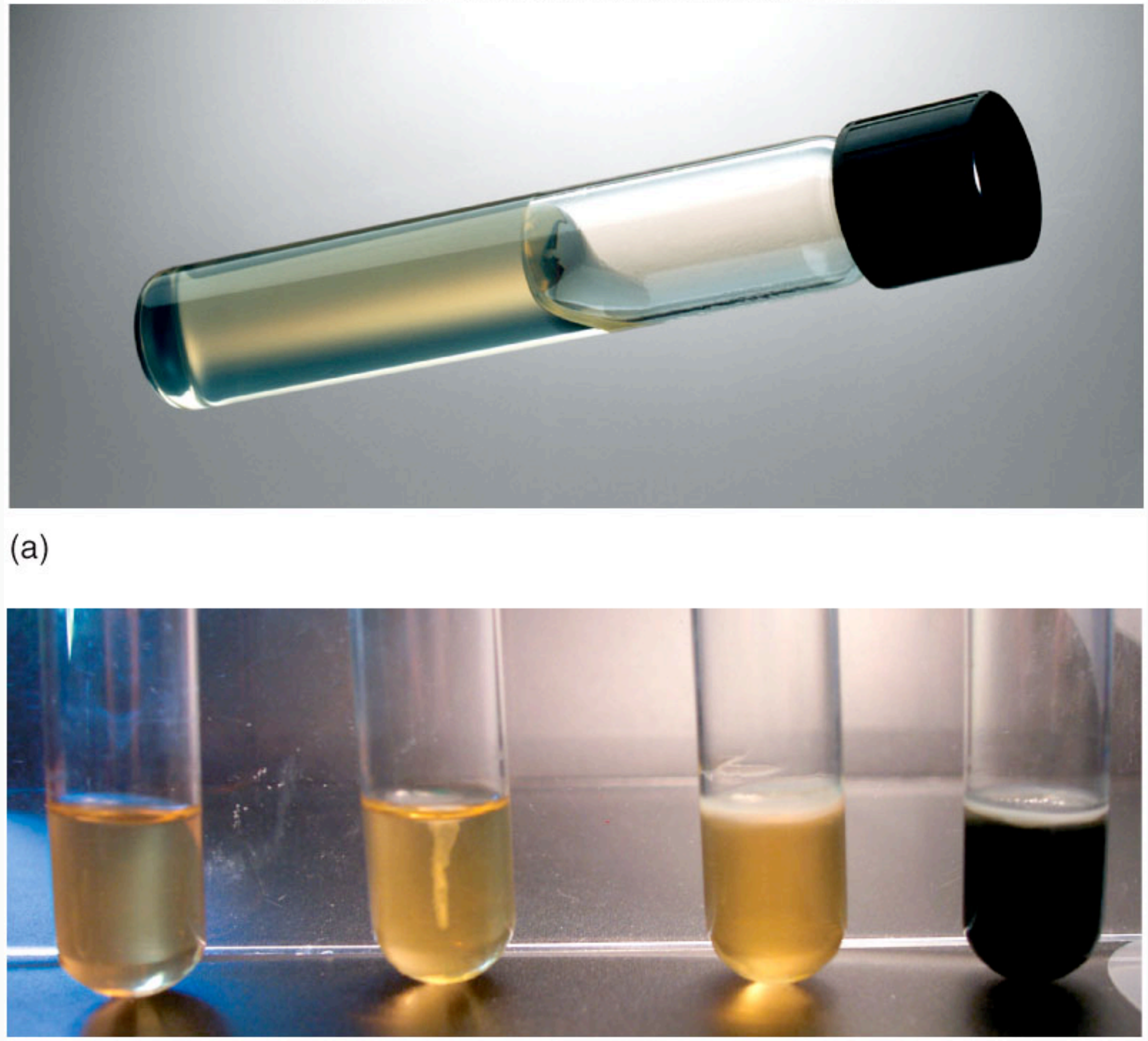


Fig. 3.4 Sample liquid media

Semi-solid media contain a low percentage (<1%) of agar, which can be used for motility testing.



(a)

(b)

Fig. 3.5 Sample semisolid media

Solid media contain a high percent (1-5%) of agar, which enables the formation of discrete colonies.

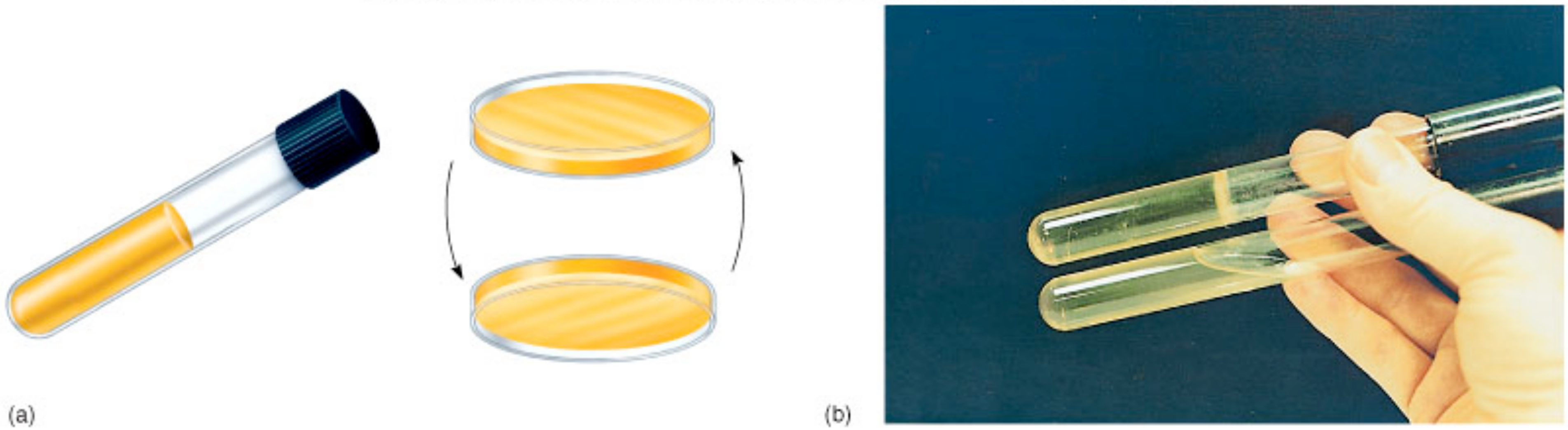


Fig. 3.6 Solid media that are reversible to liquids

Classification of Media by Chemical Content

- Synthetic media- compositions are precisely chemically **defined**
- **Complex** (nonsynthetic) media- if even just one component is not chemically definable

Synthetic media contain pure organic and inorganic compounds that are chemically defined (i.e. known molecular formula).

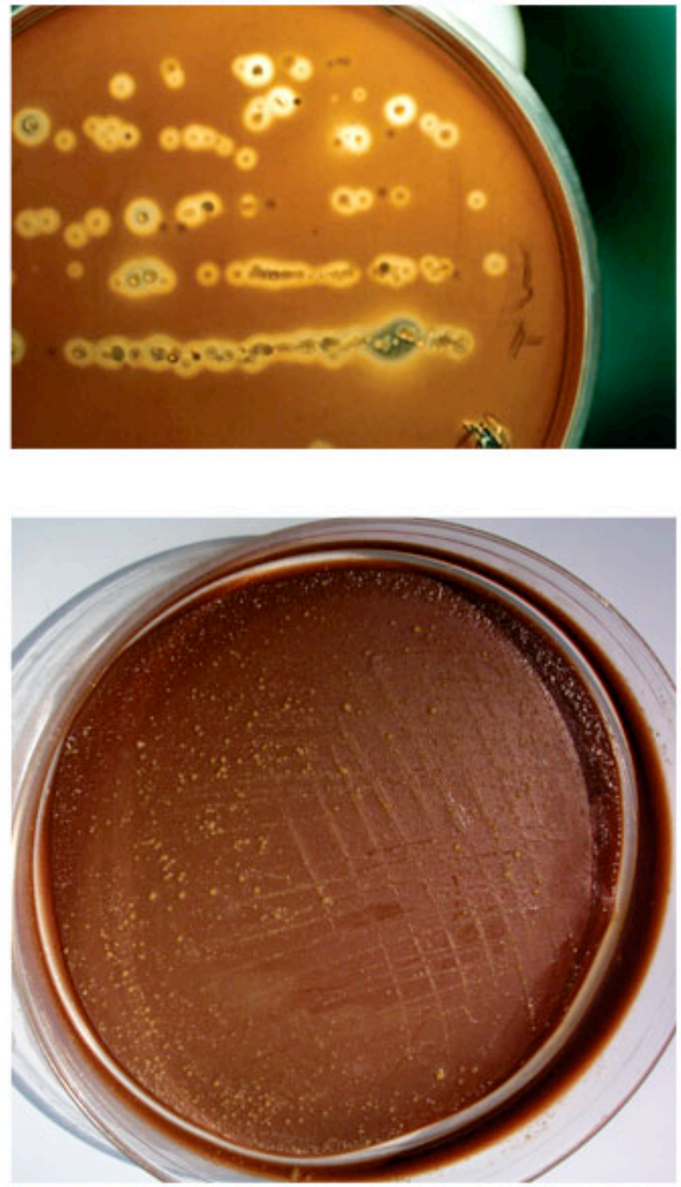
TABLE 3.2 Medium for the Growth and Maintenance of the Green Alga *Euglena*

Glutamic acid (aa)	6 g
Aspartic acid (aa)	4 g
Glycine (aa)	5 g
Sucrose (c)	30 g
Malic acid (oa)	2 g
Succinic acid (oa)	1.04 g
Boric acid	1.04 mg
Thiamine hydrochloride (v)	12 mg
Monopotassium phosphate	0.6 g
Magnesium sulfate	0.8 g
Calcium carbonate	0.16 g
Ammonium carbonate	0.72 g
Ferric chloride	60 mg
Zinc sulfate	40 mg
Manganese sulfate	6 mg
Copper sulfate	0.62 mg
Cobalt sulfate	5 mg
Ammonium molybdate	1.34 mg

Note: These ingredients are dissolved in 1,000 ml of water.
aa, amino acid; c, carbohydrate; oa, organic acid; v, vitamin; g, gram; mg, milligram.

Table 3.2 Medium for the growth and maintenance of the Green Alga Euglena

Complex or enriched media contain ingredients that are not chemically defined or pure (i.e. animal extracts).



(a)

(b)

Fig. 3.7 Examples of enriched media

Classification of Media by Function

- **General purpose media**: to grow as broad a spectrum of microbes as possible
  - Usually nonsynthetic
  - Contain a mixture of nutrients to support a variety of microbes
  - Examples: nutrient agar and broth, brain-heart infusion, trypticase soy agar (TSA).

Functional types of growth media

- Enriched media
- Selective media
- Differential media

Enriched Media

- **Enriched media**- contain complex organic substances (for example blood, serum, **growth factors**) to support the growth of **fastidious bacteria**. Examples: blood agar, Thayer-Martin medium (chocolate agar)

Selective media enables one type of bacteria to grow, while differential media allows bacteria to show different reactions (i.e. colony color).

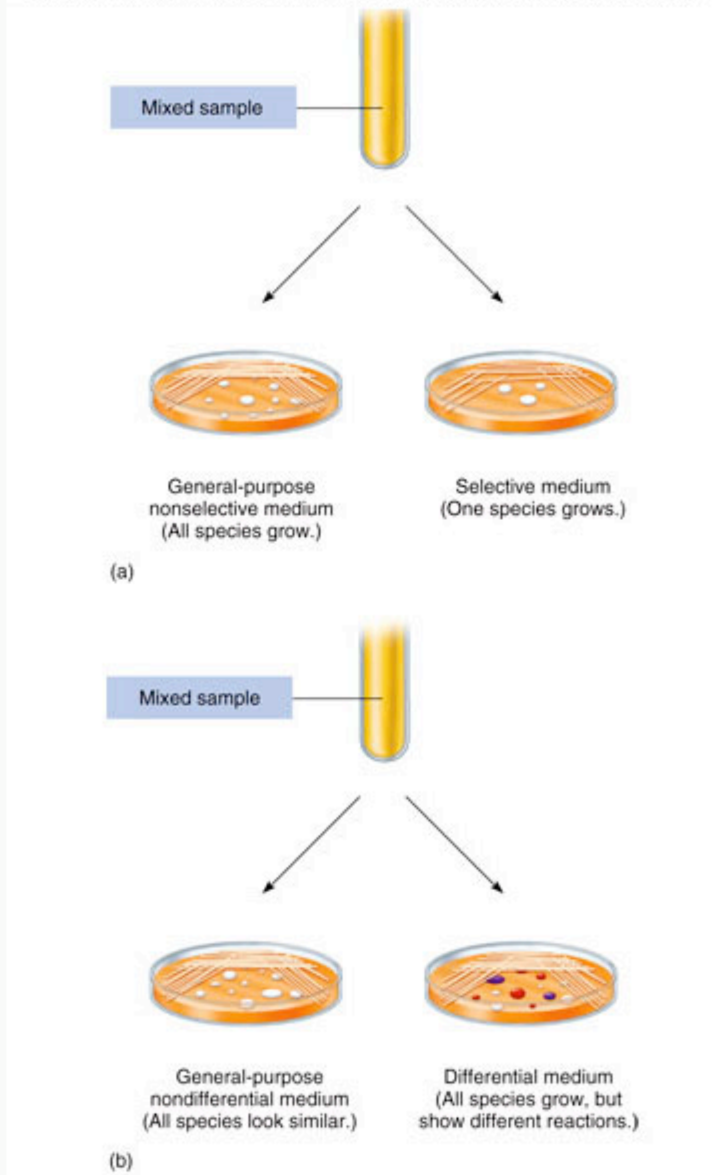


Fig. 3.8 Comparison of selective and different Media with general-purpose media.

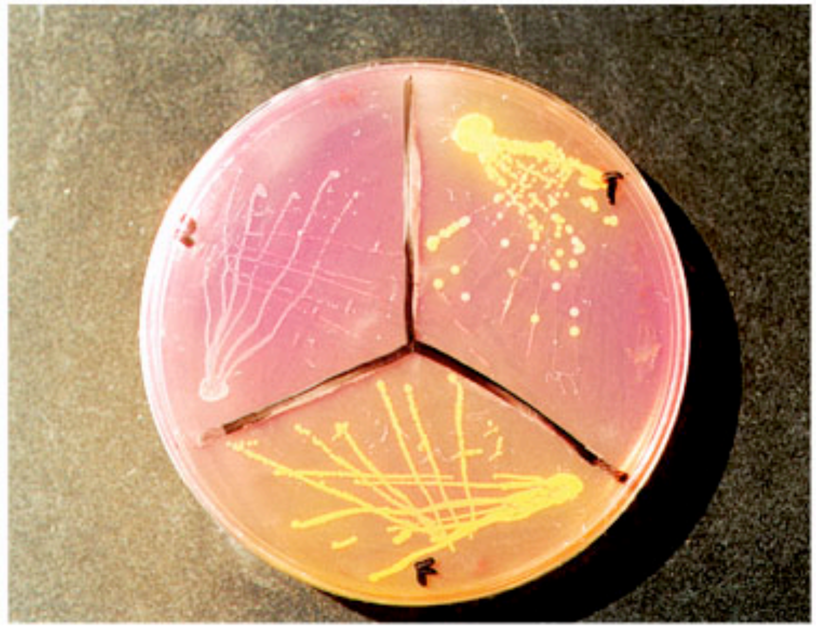
Examples of differential media.

TABLE 3.4 Differential Media

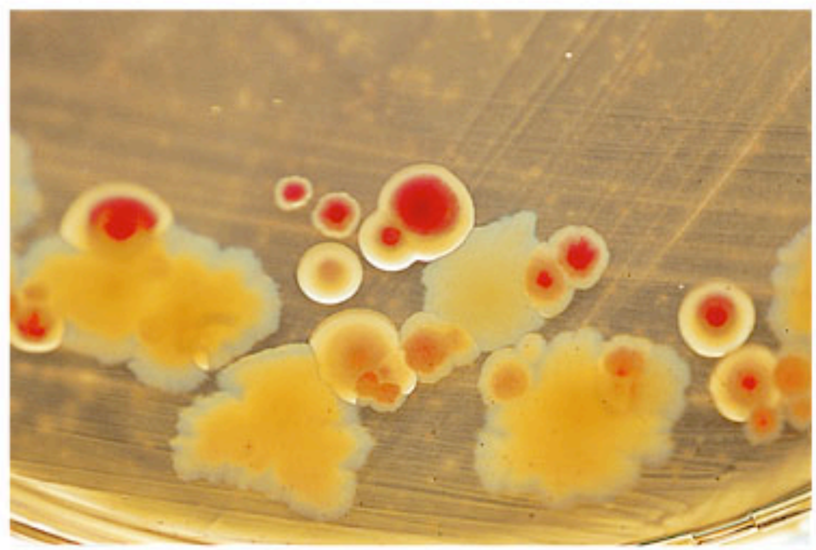
Medium	Substances That Facilitate Differentiation	Differentiates Between
Blood agar	Intact red blood cells	Types of hemolysis
Mannitol salt agar	Mannitol, phenol red, and 7.5% NaCl	Species of *Staphylococcus* NaCl also inhibits the salt-sensitive species
Hektoen enteric (HE) agar	Brom thymol blue, acid fuchsin, sucrose, salicin, thiosulfate, ferric ammonium citrate, and bile	*Salmonella, Shigella*, other lactose fermenters from nonfermenters Dyes and bile also inhibit gram-positive bacteria
MacConkey agar	Lactose, neutral red	Bacteria that ferment lactose (lowering the pH) from those that do not
Urea broth	Urea, phenol red	Bacteria that hydrolyze urea to ammonia
Sulfur indole motility (SIM)	Thiosulfate, iron	H_2S gas producers from nonproducers
Triple-sugar iron agar (TSIA)	Triple sugars, iron, and phenol red dye	Fermentation of sugars, H_2S production
XLD agar	Lysine, xylose, iron, thiosulfate, phenol red	*Enterobacter, Escherichia, Proteus, Providencia, Salmonella,* and *Shigella*
Birdseed agar	Seeds from thistle plant	*Cryptococcus neoformans* and other fungi

Table 3.4
Differential media

Mannitol salt agar is a type of selective media, and MacConkey agar is a type of differential media.



(a)



(b)

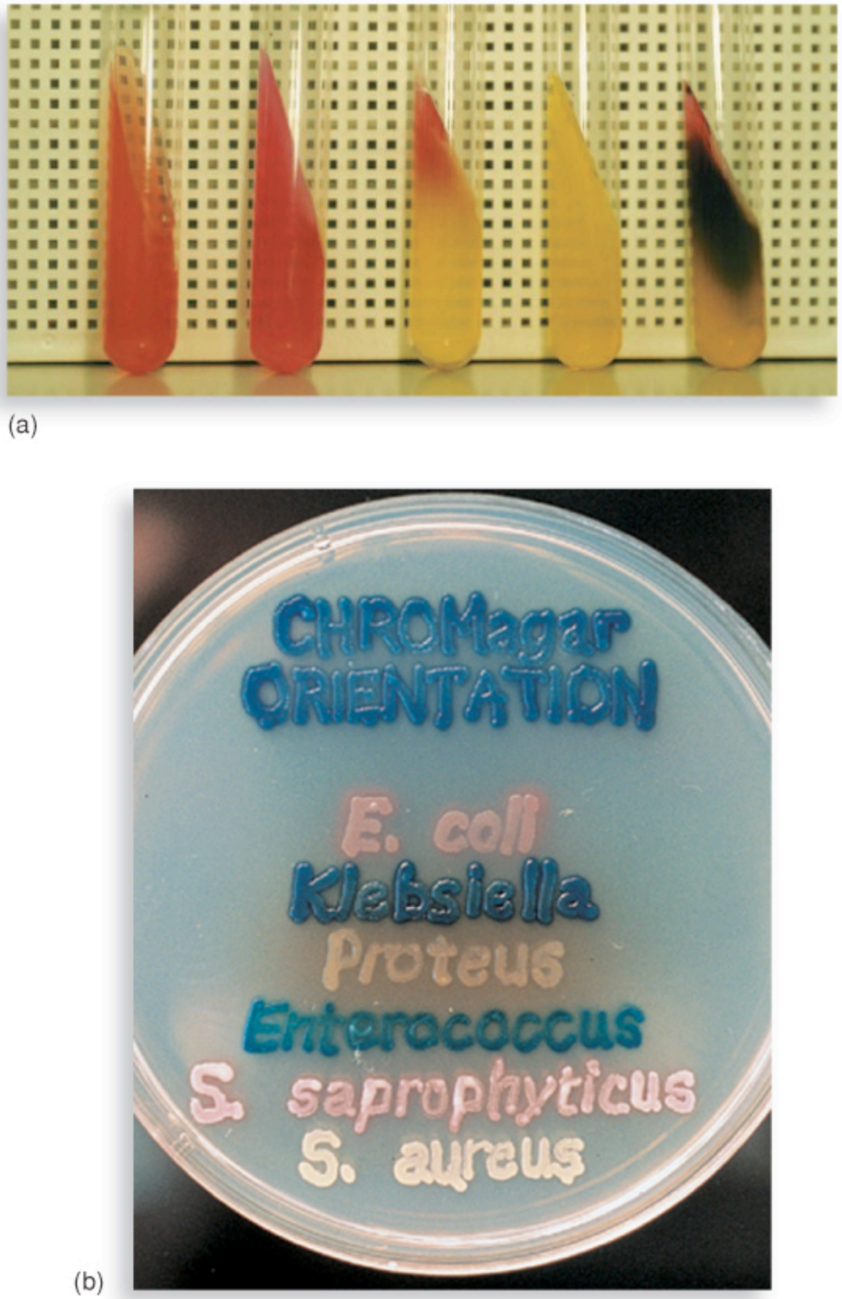


Fig. 3.9 Examples of media that are both selective and differential

Miscellaneous Media

- **Reducing media-** absorbs oxygen or slows its penetration in the medium; used for growing anaerobes or for determining oxygen requirements
- **Carbohydrate fermentation media-** contain sugars that can be fermented and a pH indicator; useful for identification of microorganisms
- **Transport media-** used to maintain and preserve specimens that need to be held for a period of time
- **Assay media-** used to test the effectiveness of antibiotics, disinfectants, antiseptics, etc.
- **Enumeration media-** used to count the numbers of organisms in a sample.

Examples of miscellaneous media are reducing, fermentation and transportation media.

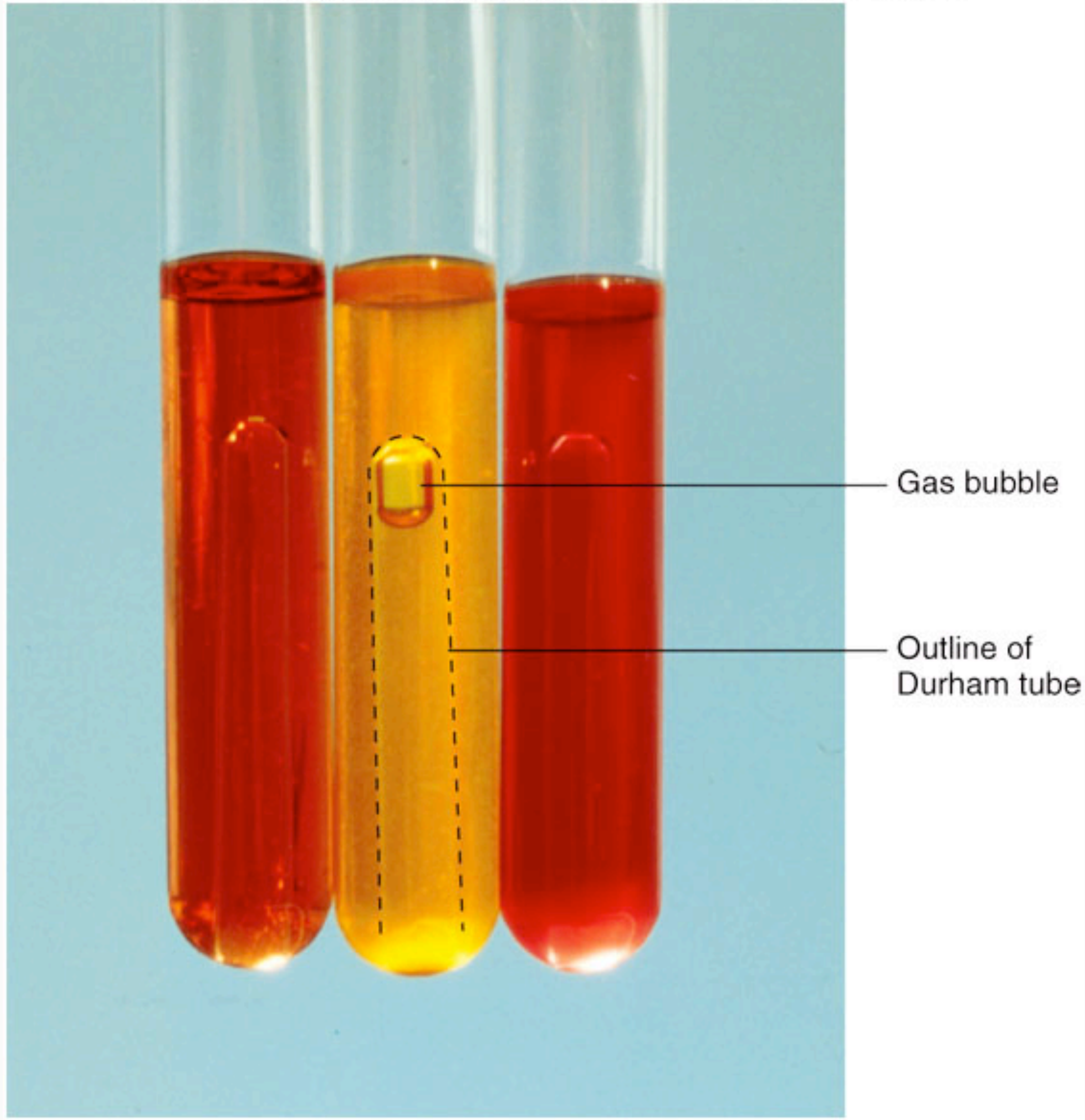


Fig. 3.11 Carbohydrate fermentation broth

Microbial growth

- Incubation
  - Varied temperatures, atmospheric states
- Inspection
  - Mixed culture
  - Pure culture
- Identification
  - Microscopic appearance
- Maintenance and disposal
  - Stock cultures
  - sterilization

Microscope

- Magnification
- Resolution
- Optical microscopes
- Electron microscopes
- Stains

3.2 The Microscope: Window on an Invisible Realm

- Two key characteristics of microscopes: **magnification and resolving power**

A compound microscope is typically used in teaching and research laboratories.

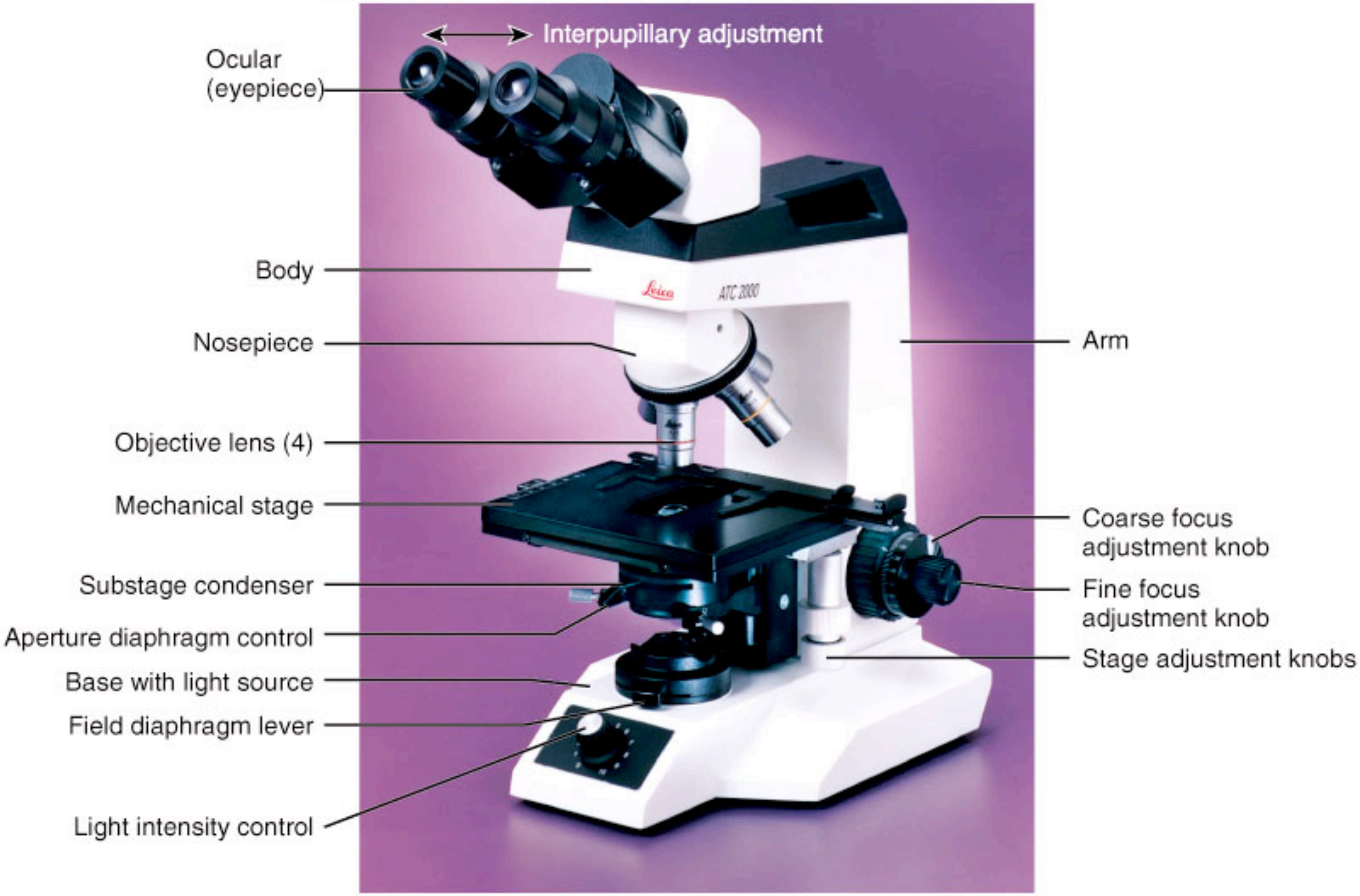


Fig. 3.14 The parts of a student laboratory microscope

Principles of Light Microscopy

- **Magnification**
  - Results when visible light waves pass through a curved lens
  - The light experiences **refraction**
  - An image is formed by the refracted light when an object is placed a certain distance from the lens and is illuminated with light
  - The image is enlarged to a particular degree- the power of magnification
- **Magnification-** occurs in two phases
  - Objective lens- forms the **real image**
  - Ocular lens- forms the **virtual image**
  - Total power of magnification- the product of the power of the objective and the power of the ocular

Resolution

- **Resolution-** the ability to distinguish two adjacent objects or points from one another
- Also known as **resolving power**
  - Resolving power (RP) = $\dfrac{\text{Wavelength of light in nm}}{2 \times \text{Numerical aperture of objective lens}}$
  - Shorter wavelengths provide a better resolution
  - **Numerical aperture**- describes the relative efficiency of a lens in bending light rays
  - Oil immersion lenses increase the numerical aperture

A specimen is magnified as light passes through the objective and ocular lens.

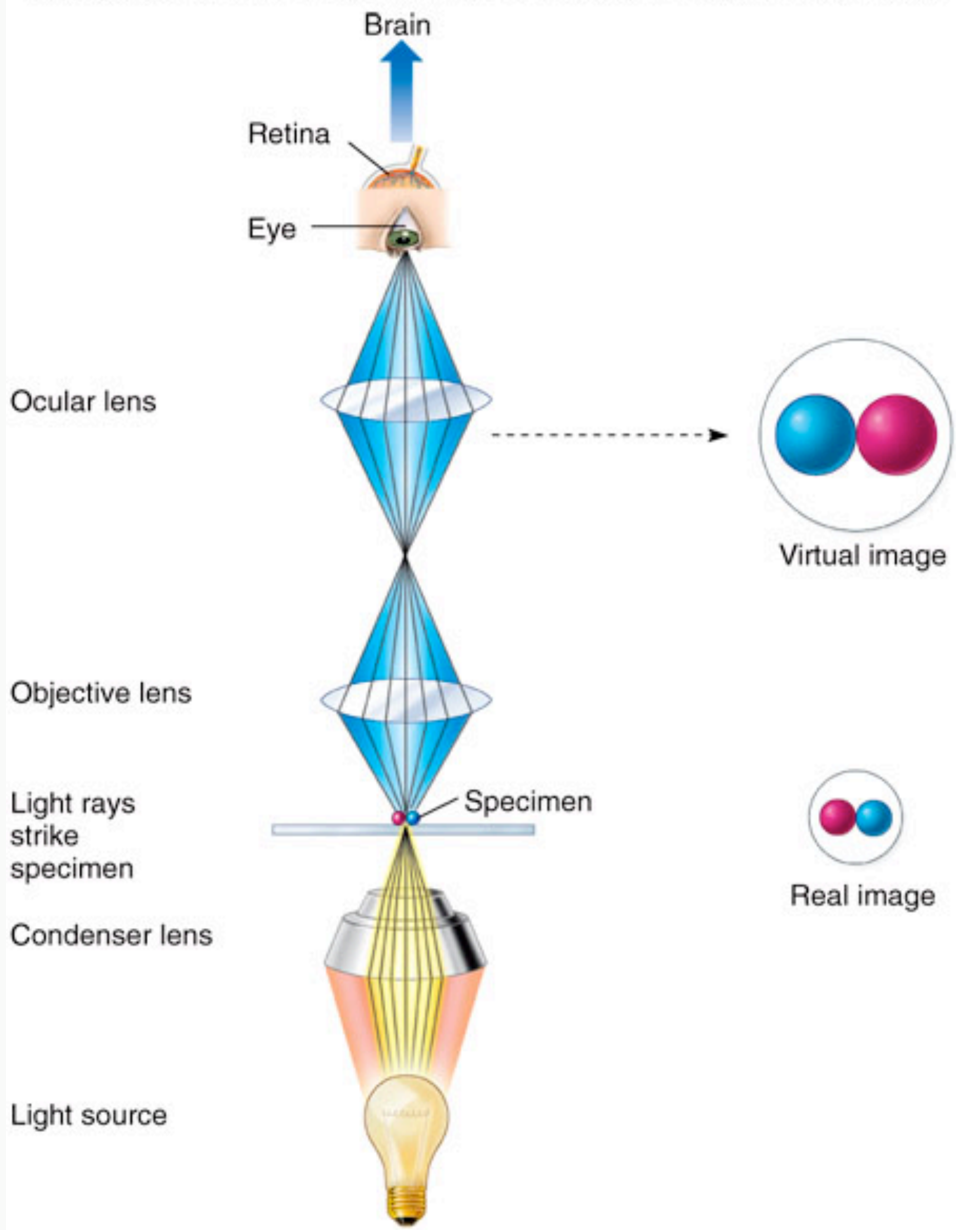
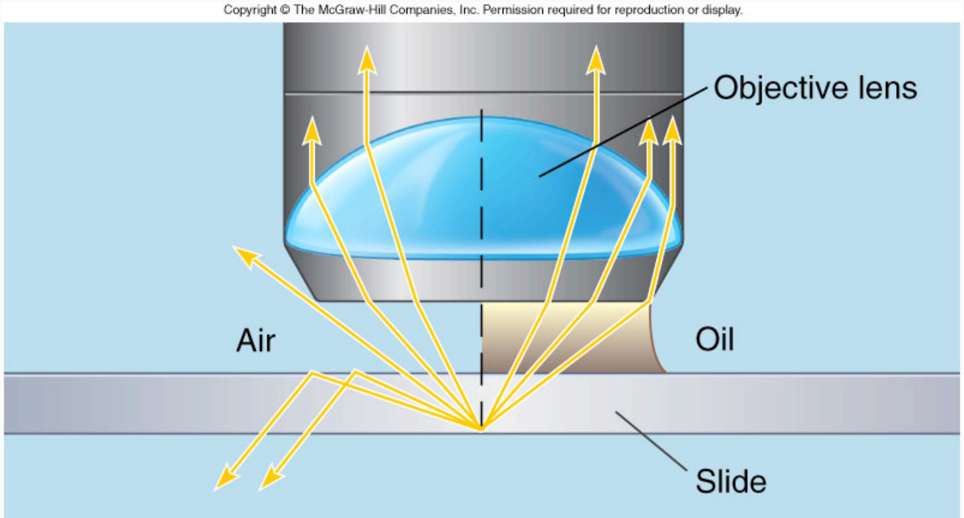
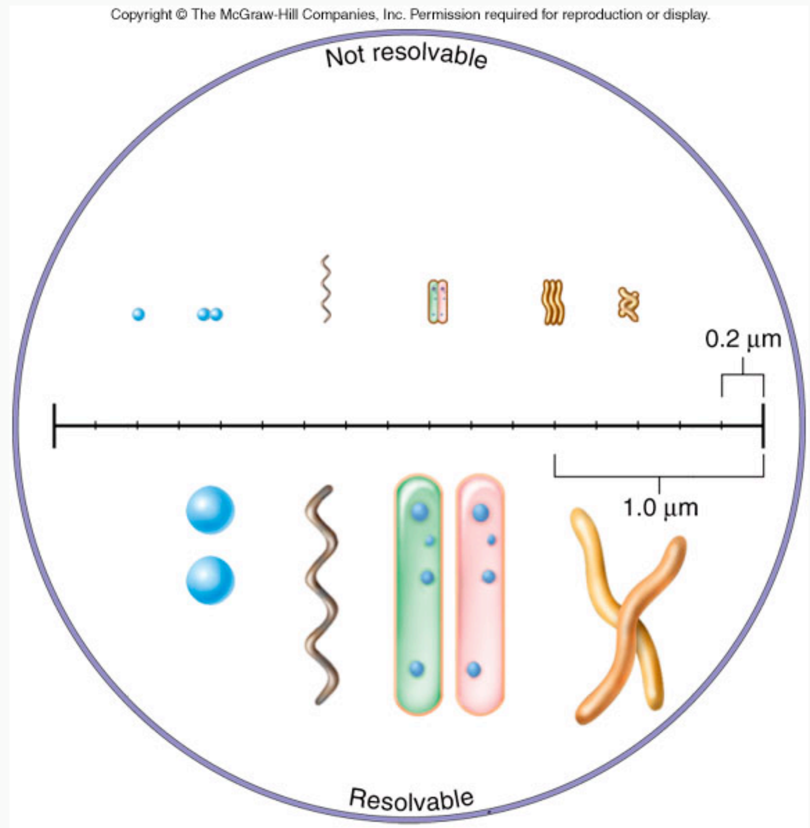


Fig. 3.15 The pathway of light and the two Stages in magnification of a compound microscope.

Resolution can be increased by using immersion oil.





Figs. 3.17 and 3.18 Workings of an oil immersion lens,and effect of magnification.

Comparison of optical and electron microscopes.

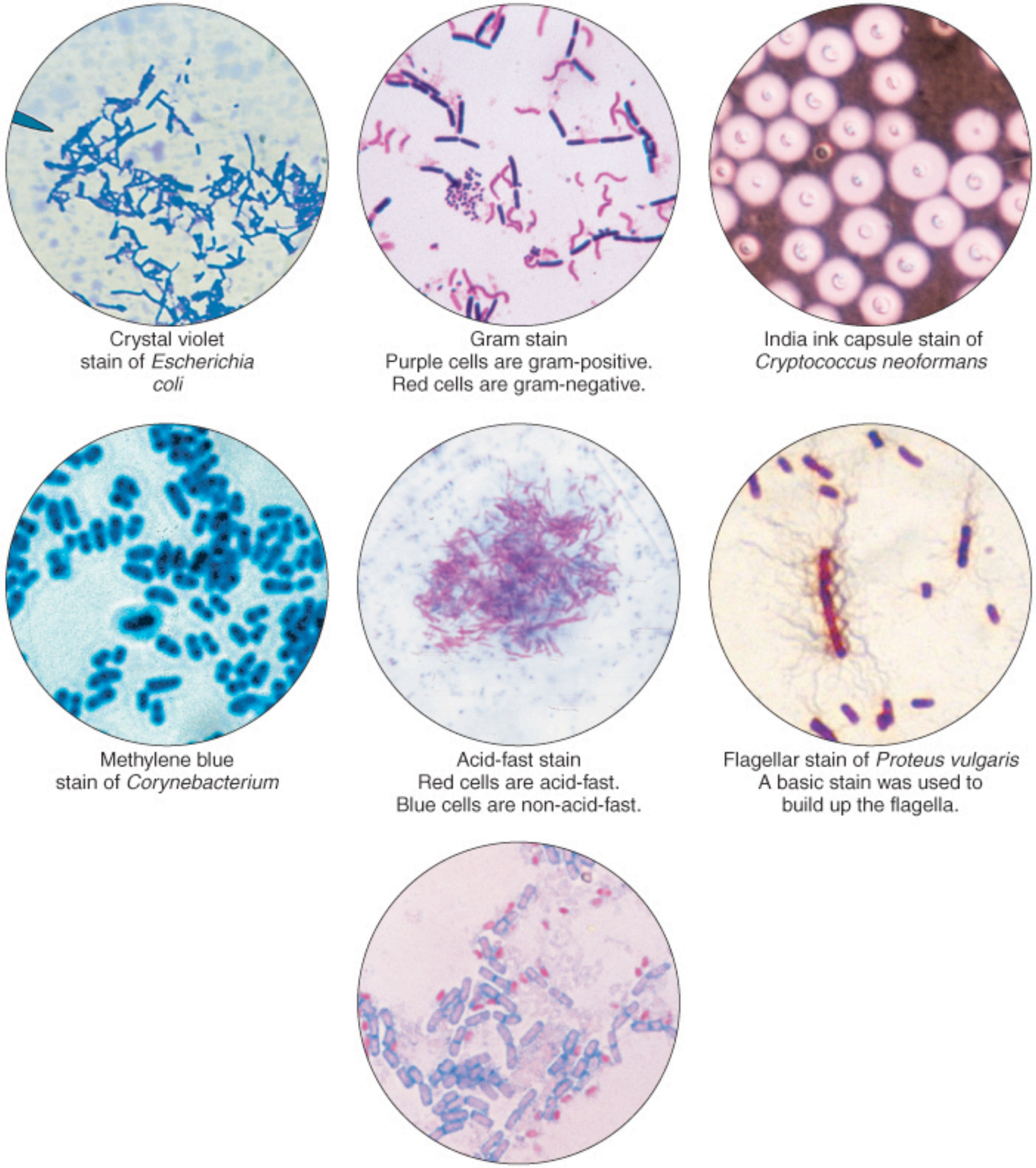
TABLE 3.6 Comparison of Light Microscopes and Electron Microscopes

Characteristic	Light or Optical	Electron (Transmission)
Useful magnification	2,000×	1,000,000× or more
Maximum resolution	200 nm	0.5 nm
Image produced by	Light rays	Electron beam
Image focused by	Glass objective lens	Electromagnetic objective lenses
Image viewed through	Glass ocular lens	Fluorescent screen
Specimen placed on	Glass slide	Copper mesh
Specimen may be alive	Yes	No
Specimen requires special stains or treatment	Not always	Yes
Colored images possible	Yes	No

Table 3.6 Comparison of light microscopes and Electron microscopes

Stains

- Positive stains
  - Dye binds to the specimen
- Negative stains
  - Dye does not bind to the specimen, but rather around the specimen.



(a) **Simple Stains** (b) **Differential Stains** (c) **Special Stains**

Crystal violet stain of *Escherichia coli*

Gram stain
Purple cells are gram-positive.
Red cells are gram-negative.

India ink capsule stain of *Cryptococcus neoformans*

Methylene blue stain of *Corynebacterium*

Acid-fast stain
Red cells are acid-fast.
Blue cells are non-acid-fast.

Flagellar stain of *Proteus vulgaris*
A basic stain was used to build up the flagella.

Spore stain, showing spores (red) and vegetative cells (blue)

Figure 3.25

Optical microscopes

- All have a maximum magnification of 2000X
  - Bright-field
  - Dark-field
  - Phase-contrast
  - Differential interference
  - Fluorescent
  - Confocal

Summary of optical and electron microscopes.

TABLE 3.5 Comparisons of Types of Microscopy

Microscope	Maximum Practical Magnification	Resolution	Important Features
Visible light as source of illumination			
Bright-field	2,000×	0.2 µm (200 nm)	Common multipurpose microscope for live and preserved stained specimens; specimen is dark, field is white; provides fair cellular detail
Dark-field	2,000×	0.2 µm	Best for observing live, unstained specimens; specimen is bright, field is black; provides outline of specimen with reduced internal cellular detail
Phase-contrast	2,000×	0.2 µm	Used for live specimens; specimen is contrasted against gray background; excellent for internal cellular detail
Differential interference	2,000×	0.2 µm	Provides brightly colored, highly contrasting, three-dimensional images of live specimens
Ultraviolet rays as source of illumination			
Fluorescent	2,000×	0.2 µm	Specimens stained with fluorescent dyes or combined with fluorescent antibodies emit visible light; specificity makes this microscope an excellent diagnostic tool
Confocal	2,000×	0.2 µm	Specimens stained with fluorescent dyes are scanned by laser beam, multiple images (optical sections) are combined into three-dimensional image by a computer; unstained specimens can be viewed using light reflected from specimen
Electron beam forms image of specimen			
Transmission electron microscope (TEM)	100,000×	0.5 nm	Sections of specimen are viewed under very high magnification; finest detailed structure of cells and viruses is shown; used only on preserved material
Scanning electron microscope (SEM)	650,000×	10 nm	Scans and magnifies external surface of specimen; produces striking three-dimensional image

Table 3.5 Comparison of types of microscopy