Chapter 5

Microbial Nutrition

The Common Nutrient Requirements

- **macroelements** (macronutrients)
  - C, O, H, N, S, P, K, Ca, Mg, and Fe
  - required in relatively large amounts
- **micronutrients** (**trace elements**)
  - Mn, Zn, Co, Mo, Ni, and Cu
  - required in trace amounts
  - often supplied in water or in media components

Requirements for Carbon, Hydrogen, and Oxygen

- often satisfied together
  - carbon source often provides H, O and electrons
- autotrophs
  - use carbon dioxide as their sole or principal carbon source
- heterotrophs
  - use organic molecules as carbon sources

Nutritional Types of Microorganisms

Table 5.1
Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (*pp. 202–3*)[a]
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (*pp. 190–96*)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (*pp. 188–90*)
Organotrophs	Organic molecules (*chapter 9*)

[a] For each category, the location of material describing the participating metabolic pathways is given within the parentheses.

Table 5.2 Major Nutritional Types of Microorganisms

Major Nutritional Types[a]	Sources of Energy, Hydrogen/Electrons, and Carbon	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy Inorganic hydrogen/electron (H/e^-) donor CO_2 carbon source	Algae Purple and green sulfur bacteria Cyanobacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy)	Light energy Organic H/e^- donor Organic carbon source (CO_2 may also be used)	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic) Inorganic H/e^- donor CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic) Organic H/e^- donor Organic carbon source	Protozoa Fungi Most nonphotosynthetic bacteria (including most pathogens)

[a]Bacteria in other nutritional categories have been found. The categories are defined in terms of energy, electron, and carbon sources. Condensed versions of these names are given in parentheses.

mixotrophy

- chemical energy source (inorganic)
- inorganic H/e^- donor
- organic carbon source

Requirements for Nitrogen, Phosphorus, and Sulfur

- needed for synthesis of important molecules (e.g., amino acids, nucleic acids)
- nitrogen supplied in numerous ways
- phosphorus usually supplied as inorganic phosphate
- sulfur usually supplied as sulfate via assimilatory sulfate reduction

Sources of nitrogen

- organic molecules
- ammonia
- nitrate via assimilatory nitrate reduction
- nitrogen gas via nitrogen fixation

Growth Factors

- organic compounds
- essential cell components (or their precursors) that the cell cannot synthesize
- must be supplied by environment if cell is to survive and reproduce

Classes of growth factors

- amino acids
  - needed for protein synthesis
- purines and pyrimidines
  - needed for nucleic acid synthesis
- vitamins
  - function as enzyme cofactors

Table 5.3 Functions of Some Common Vitamins in Microorganisms

Vitamin	Functions	Examples of Microorganisms Requiring Vitamin[a]
Biotin	Carboxylation (CO_2 fixation) One-carbon metabolism	*Leuconostoc mesenteroides* (B) *Saccharomyces cerevisiae* (F) *Ochromonas malhamensis* (A) *Acanthamoeba castellanii* (P)
Cyanocobalamin (B_{12})	Molecular rearrangements One-carbon metabolism—carries methyl groups	*Lactobacillus* spp. (B) *Euglena gracilis* (A) Diatoms and many other algae (A) *Acanthamoeba castellanii* (P)
Folic acid	One-carbon metabolism	*Enterococcus faecalis* (B) *Tetrahymena pyriformis* (P)
Lipoic acid	Transfer of acyl groups	*Lactobacillus casei* (B) *Tetrahymena* spp. (P)
Pantothenic acid	Precursor of coenzyme A—carries acyl groups (pyruvate oxidation, fatty acid metabolism)	*Proteus morganii* (B) *Hanseniaspora* spp. (F) *Paramecium* spp. (P)
Pyridoxine (B_6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus* spp. (B) *Tetrahymena pyriformis* (P)
Niacin (nicotinic acid)	Precursor of NAD and NADP—carry electrons and hydrogen atoms	*Brucella abortus*, *Haemophilus influenzae* (B) *Blastocladia pringsheimii* (F) *Crithidia fasciculata* (P)
Riboflavin (B_2)	Precursor of FAD and FMN—carry electrons or hydrogen atoms	*Caulobacter vibrioides* (B) *Dictyostelium* spp. (F) *Tetrahymena pyriformis* (P)
Thiamine (B_1)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	*Bacillus anthracis* (B) *Phycomyces blakesleeanus* (F) *Ochromonas malhamensis* (A) *Colpidium campylum* (P)

[a]The representative microorganisms are members of the following groups: bacteria (B), fungi (F), algae (A), and protozoa (P).

Practical importance of growth factors

- development of quantitative growth-response assays for measuring concentrations of growth factors in a preparation
- industrial production of growth factors by microorganisms

Uptake of Nutrients by the Cell

- Some nutrients enter by **passive diffusion**
- Most nutrients enter by:
  - facilitated diffusion
  - active transport
  - group translocation

Passive Diffusion

- molecules move from region of higher concentration to one of lower concentration because of random thermal agitation
- H_2O, O_2 and CO_2 often move across membranes this way

Facilitated Diffusion

- similar to passive diffusion
  - movement of molecules is not energy dependent
  - direction of movement is from high concentration to low concentration
  - size of concentration gradient impacts rate of uptake

Facilitated diffusion…

- differs from passive diffusion
  - uses carrier molecules (permeases)
  - smaller concentration gradient is required for significant uptake of molecules
  - effectively transports glycerol, sugars, and amino acids
- more prominent in eucaryotic cells than in procaryotic cells

- rate of facilitated diffusion increases more rapidly and at a lower concentration

- diffusion rate reaches a plateau when carrier becomes saturated

carrier saturation effect

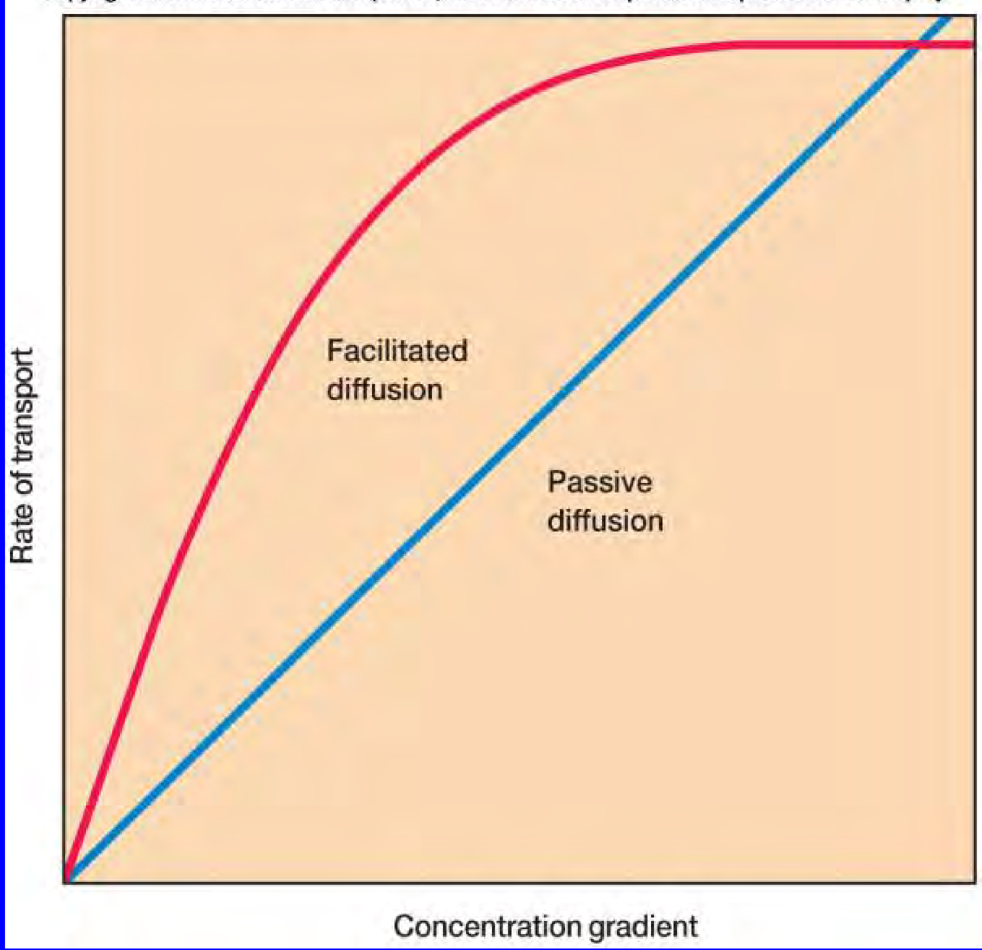


Figure 5.1

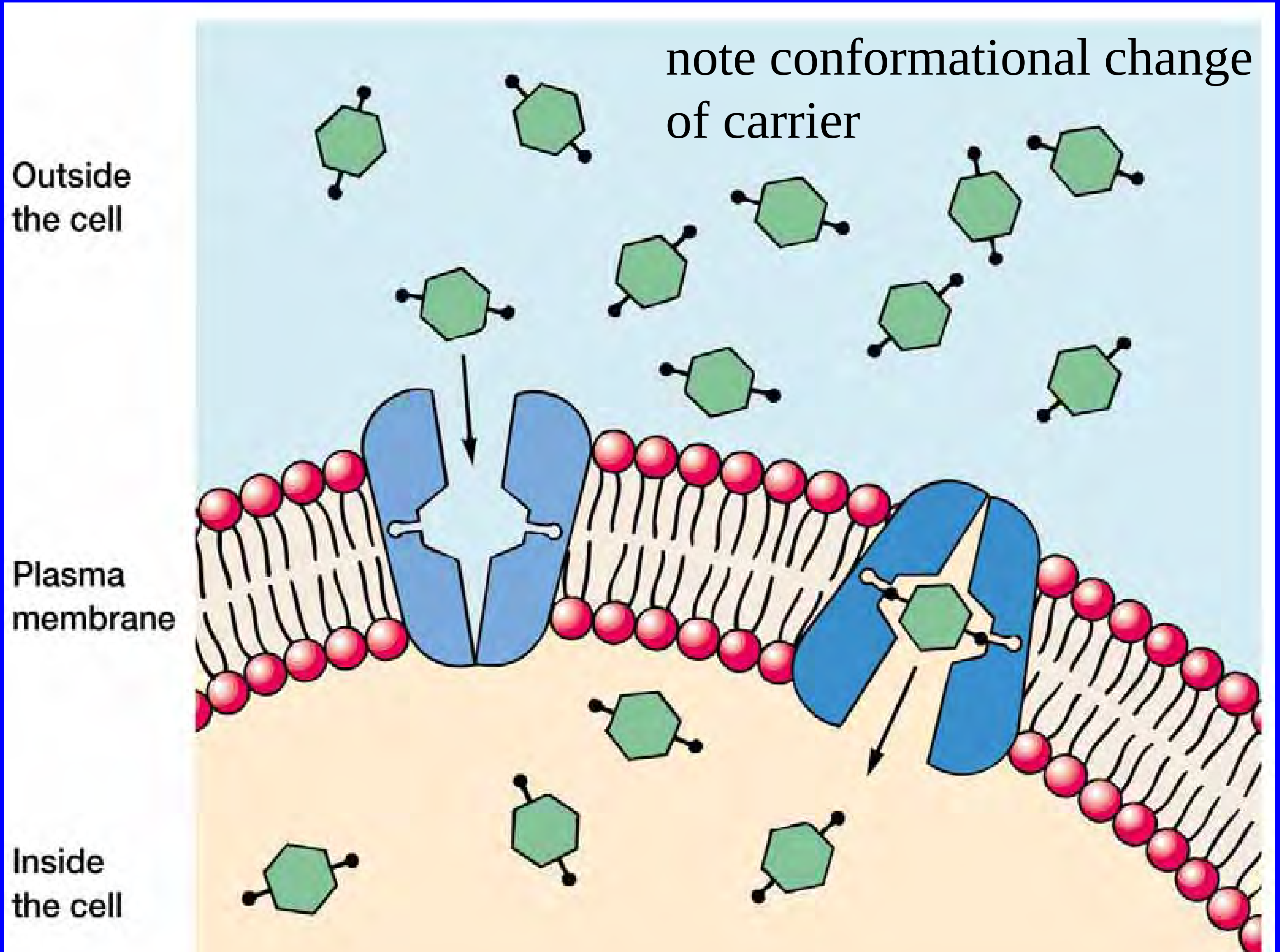

Outside
the cell
Plasma
membrane
Inside
the cell
note conformational change
of carrier

Figure 5.2

Active Transport

- energy-dependent process
  - ATP or proton motive force used
- moves molecules against the gradient
- concentrates molecules inside cell
- involves carrier proteins (permeases)
  - carrier saturation effect is observed

ABC transporters

- ATP-binding cassette transporters
- observed in bacteria, archaea, and eucaryotes

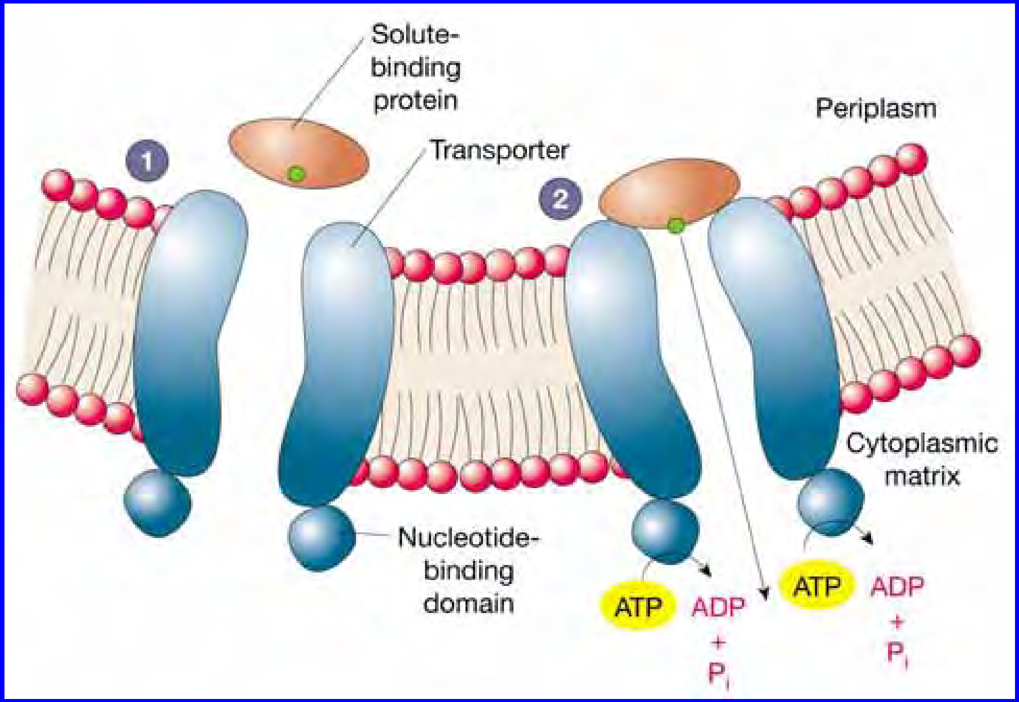
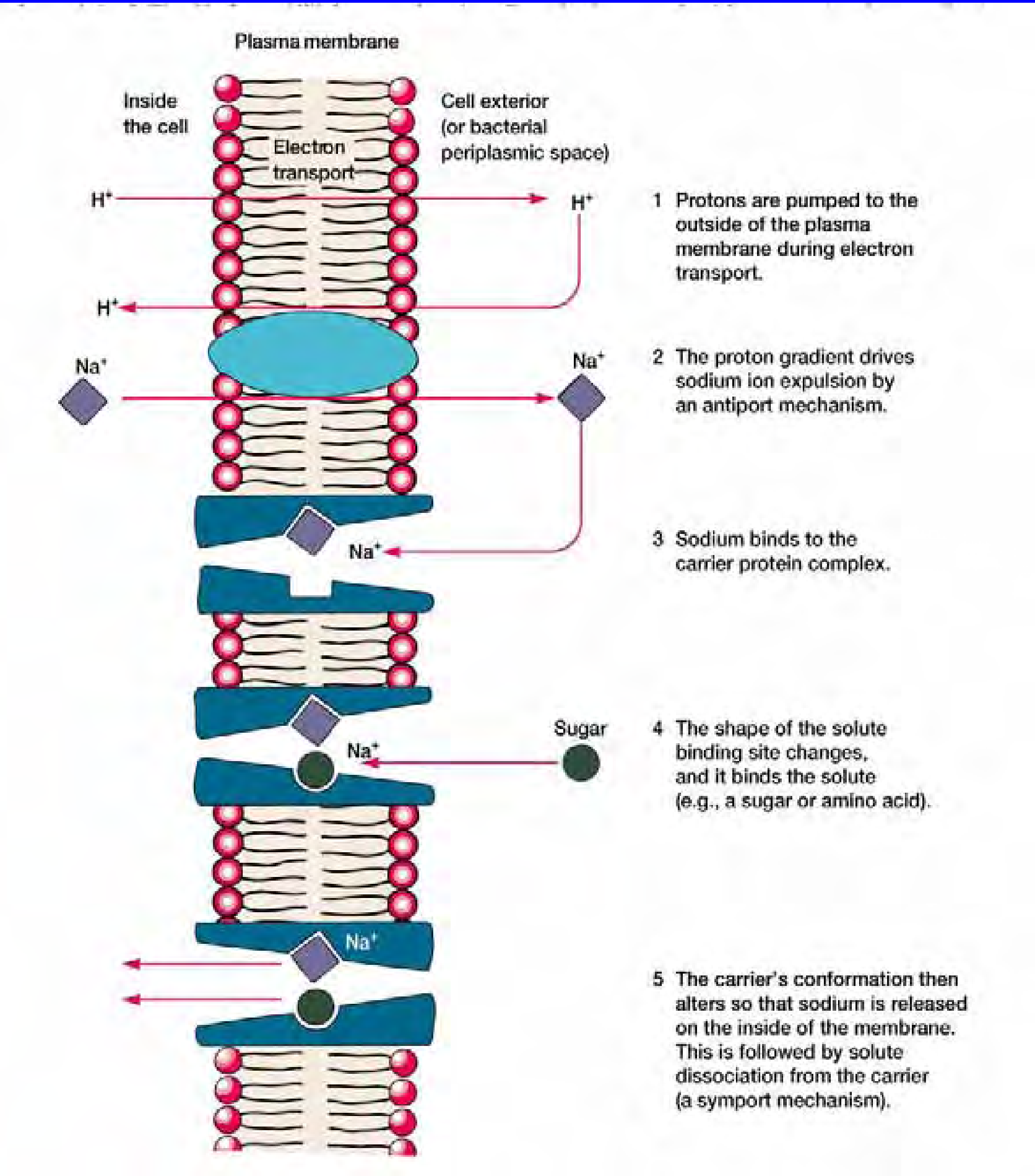


Figure 5.3



antiport

symport

Figure 5.4

Group Translocation

- molecules are modified as they are transported across the membrane
- energy-dependent process

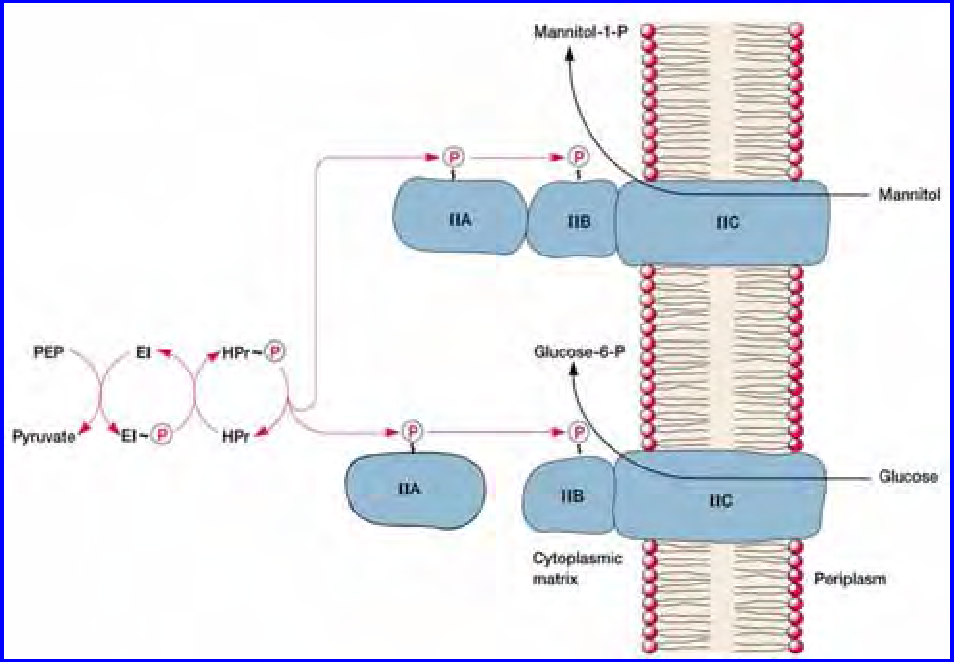


Figure 5.5

Iron Uptake

- ferric iron is very insoluble so uptake is difficult
- microorganisms use siderophores to aid uptake
- siderophore complexes with ferric ion
- complex is then transported into cell

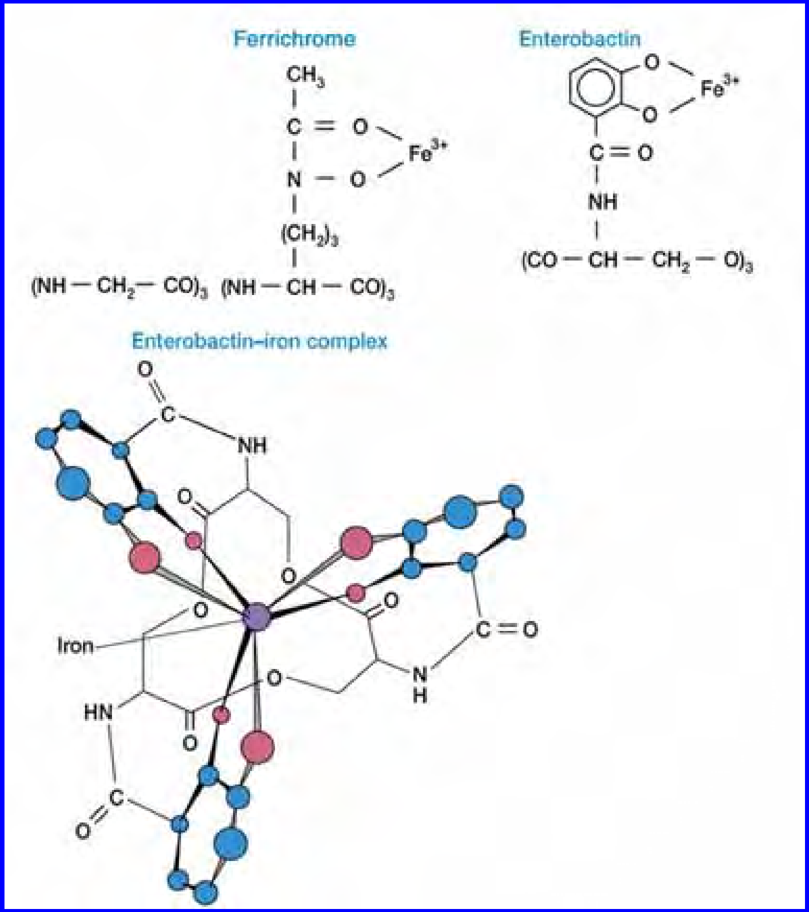


Figure 5.6

Culture Media

- preparations devised to support the growth (reproduction) of microorganisms
- can be liquid or solid
  - solid media are usually solidified with agar
- important to study of microorganisms

Synthetic or Defined Media

- all components and their concentrations are known

Table 5.4 Examples of Defined Media

BG–11 Medium for Cyanobacteria	**Amount (g/liter)**
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na2Mg salt)	0.001
Na_2CO_3	0.02
Trace metal solution[a]	1.0 ml/liter
Final pH 7.4	
Medium for *Escherichia coli*	**Amount (g/liter)**
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Sources: Data from Rippka, et al. *Journal of General Microbiology,* 111:1–61, 1979; and S.S. Cohen, and R. Arbogast, *Journal of Experimental Medicine,* 91:619, 1950.

[a]The trace metal solution contains H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2Mo_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$.

Complex Media

- contain some ingredients of unknown composition and/or concentration

Table 5.5 Some Common Complex Media

Nutrient Broth	**Amount (g/liter)**
Peptone (gelatin hydrolysate)	5
Beef extract	3
Tryptic Soy Broth	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
MacConkey Agar	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Some media components

- peptones
  - protein hydrolysates prepared by partial digestion of various protein sources
- extracts
  - aqueous extracts, usually of beef or yeast
- agar
  - sulfated polysaccharide used to solidify liquid media

Types of Media

- **general purpose media**
  - **support the growth of many microorganisms**
  - **e.g., tryptic soy agar**
- **enriched media**
  - **general purpose media supplemented by blood or other special nutrients**
  - **e.g., blood agar**

Types of media…

- **selective media**
  - **favor the growth of some microorganisms and inhibit growth of others**
  - **e.g., MacConkey agar**
    - **selects for gram-negative bacteria**

Types of media…

- **differential media**
  - distinguish between different groups of microorganisms based on their biological characteristics
  - e.g., blood agar
    - hemolytic versus nonhemolytic bacteria
  - e.g., MacConkey agar
    - lactose fermenters versus nonfermenters

Isolation of Pure Cultures

- **pure culture**
  - **population of cells arising from a single cell**
- **spread plate, streak plate, and pour plate are techniques used to isolate pure cultures**

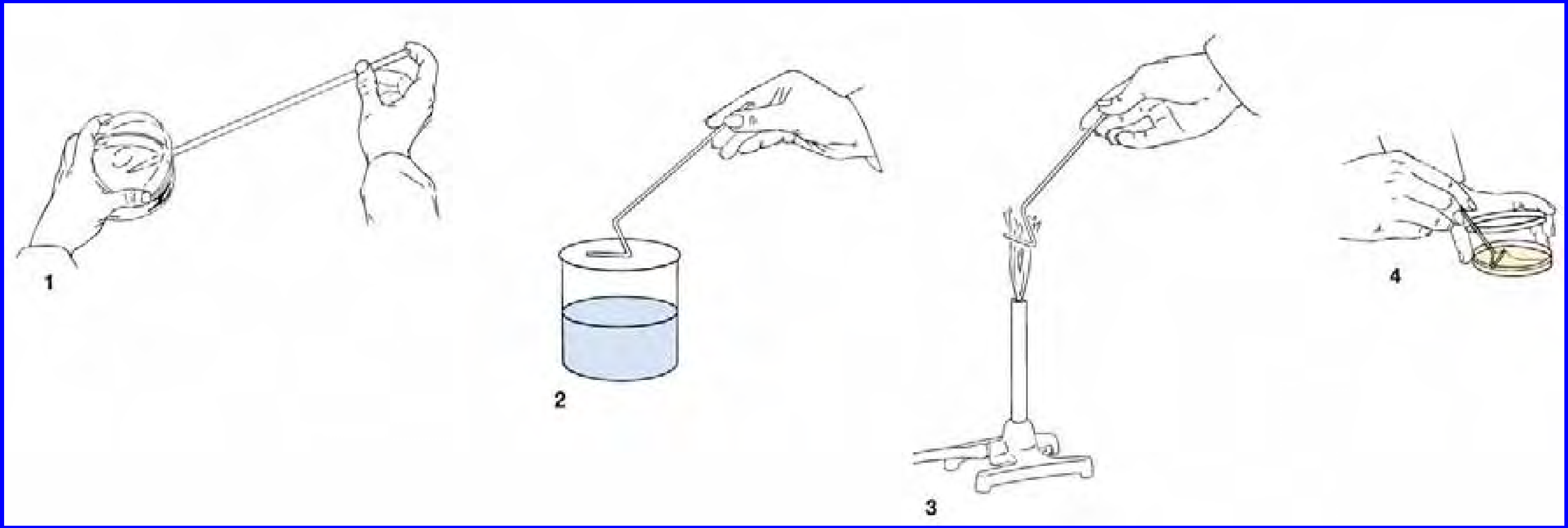
The Spread Plate and Streak Plate

- involve spreading a mixture of cells on an agar surface so that individual cells are well separated from each other
- each cell can reproduce to form a separate colony (visible growth or cluster of microorganisms)

Spread-plate technique

1. dispense cells onto medium in petri dish

4. spread cells across surface



2. - 3. sterilize spreader

Figure 5.7

Streak plate technique

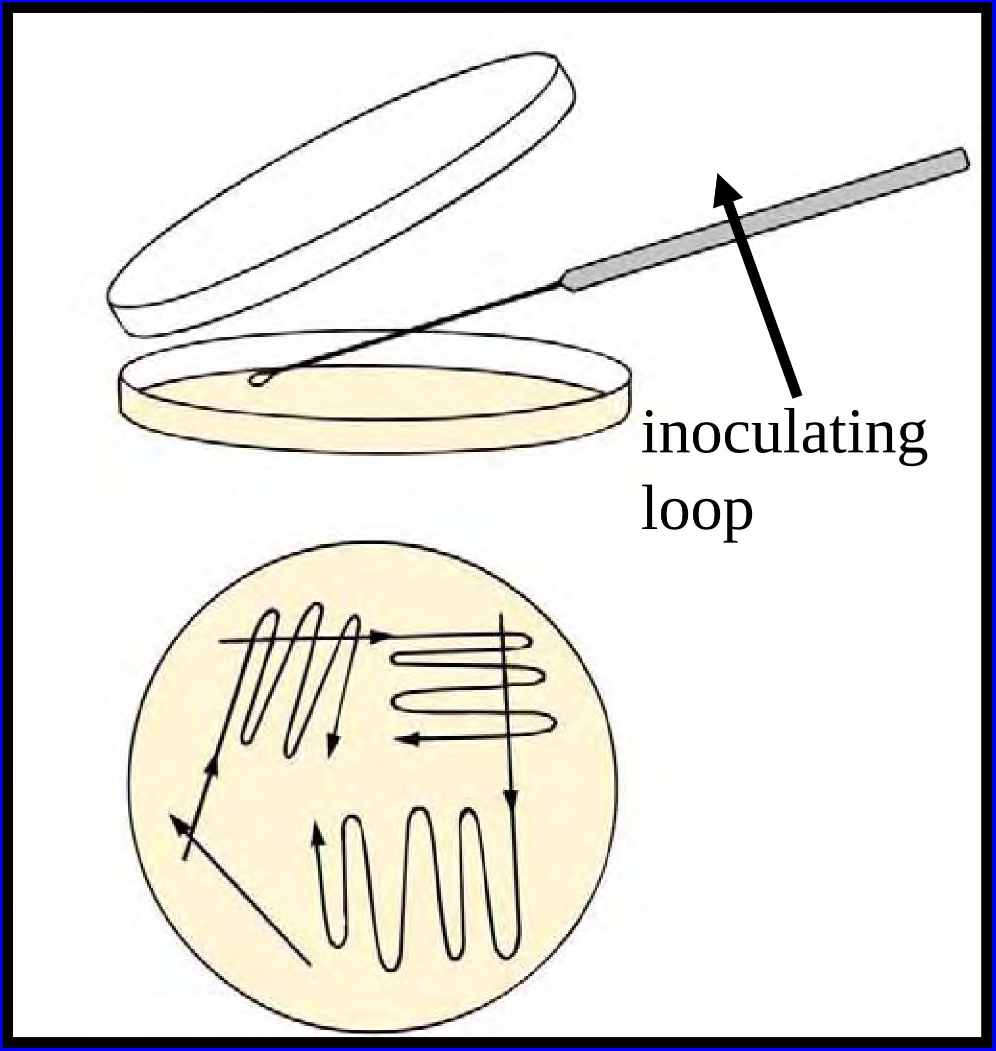


Figure 5.8

The Pour Plate

- sample is diluted several times
- diluted samples are mixed with liquid agar
- mixture of cells and agar are poured into sterile culture dishes

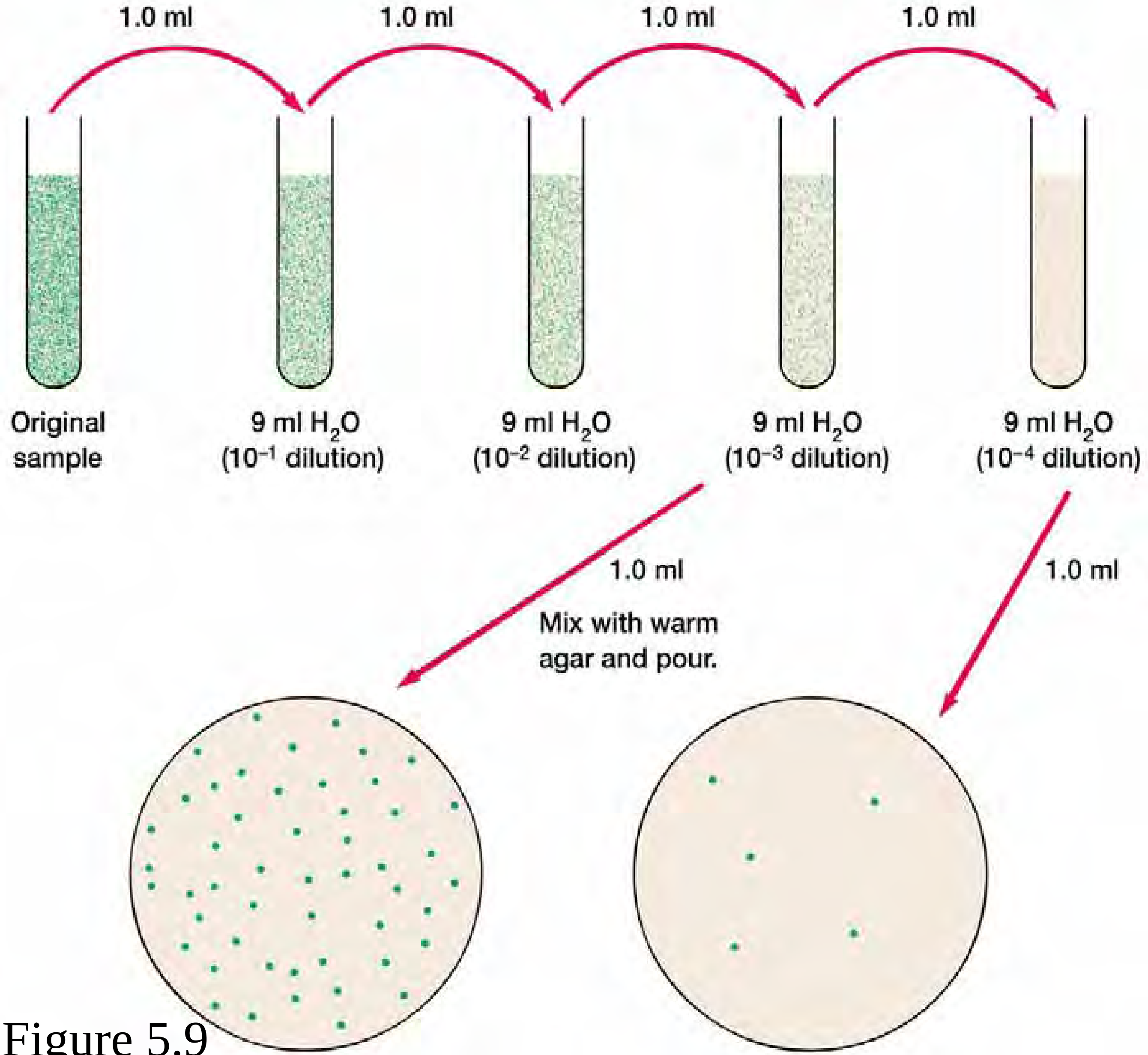

1.0 ml
1.0 ml
1.0 ml
1.0 ml
Original sample
9 ml H_2O (10^{-1} dilution)
9 ml H_2O (10^{-2} dilution)
9 ml H_2O (10^{-3} dilution)
9 ml H_2O (10^{-4} dilution)
1.0 ml
Mix with warm agar and pour.
1.0 ml

Figure 5.9

Colony Morphology and Growth

- **individual species form characteristic colonies**

Figure 5.10b

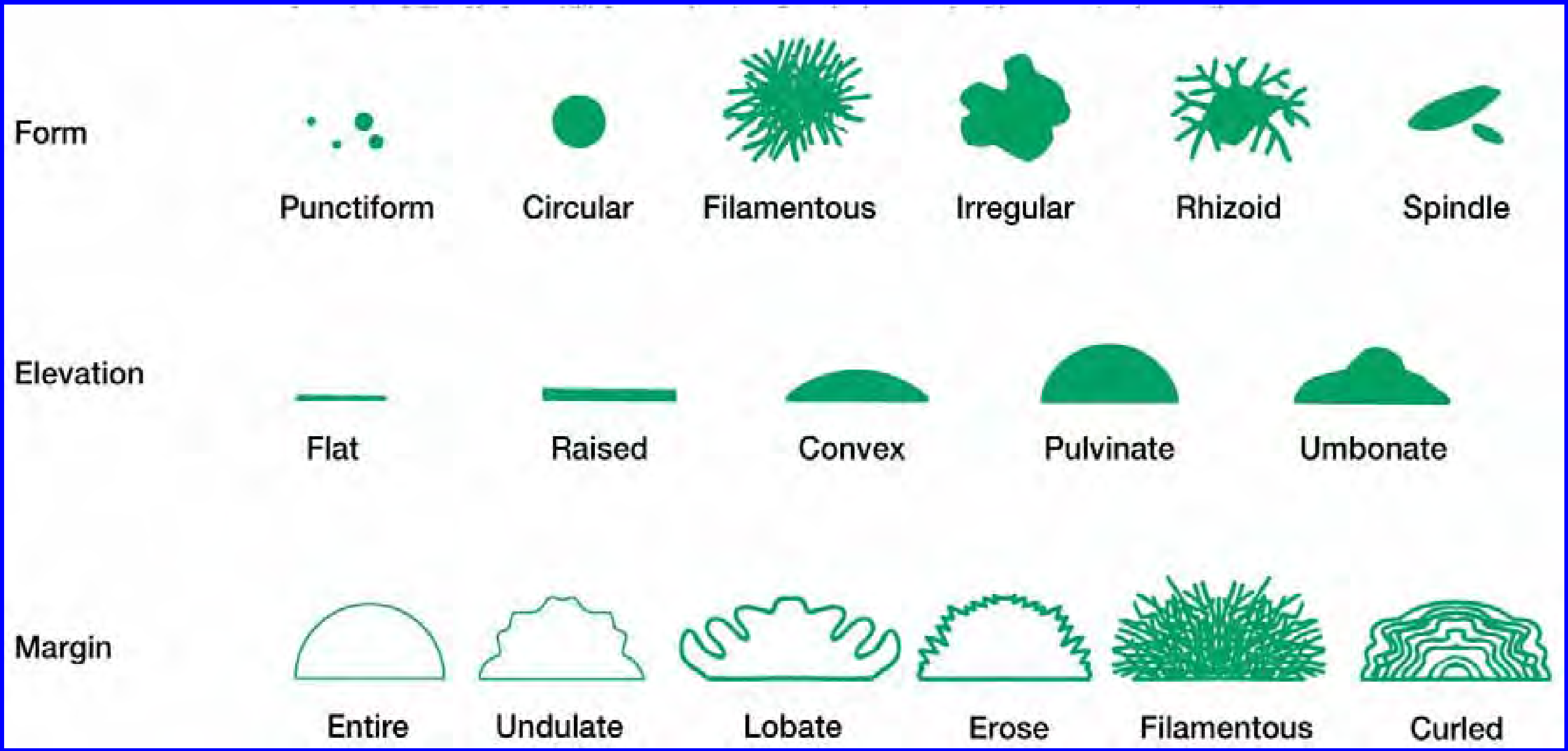

Form
Punctiform
Circular
Filamentous
Irregular
Rhizoid
Spindle
Elevation
Flat
Raised
Convex
Pulvinate
Umbonate
Margin
Entire
Undulate
Lobate
Erose
Filamentous
Curled

Figure 5.10a

Colony growth

- most rapid at edge of colony
  - oxygen and nutrients are more available at edge
- slowest at center of colony
- in nature, many microorganisms form biofilms on surfaces