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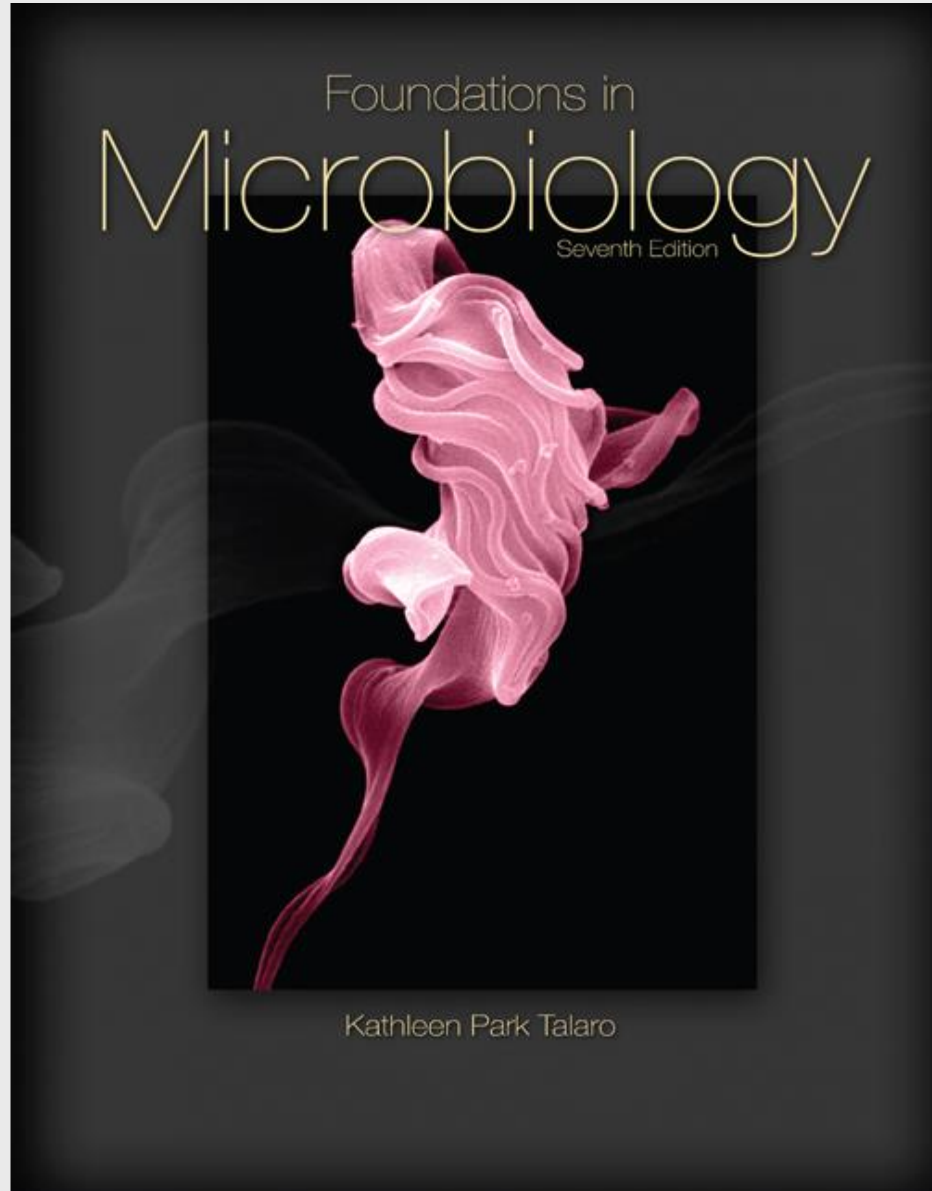
Foundations in Microbiology

Seventh Edition

Talaro

Chapter 7

Elements of Microbial Nutrition, Ecology, and Growth



7.1 Microbial Nutrition

Nutrition – process by which chemical substances (nutrients) are acquired from the environment and used in cellular activities

Essential nutrients – must be provided to an organism

Two categories of essential nutrients:

- **Macronutrients** – required in large quantities; play principal roles in cell structure and metabolism
  - Proteins, carbohydrates
- **Micronutrients or trace elements** – required in small amounts; involved in enzyme function and maintenance of protein structure
  - Manganese, zinc, nickel

Nutrients

- **Organic nutrients** – contain carbon and hydrogen atoms and are usually the products of living things
  - Methane (CH_4), carbohydrates, lipids, proteins, and nucleic acids
- **Inorganic nutrients** – atom or molecule that contains a combination of atoms other than carbon and hydrogen
  - Metals and their salts (magnesium sulfate, ferric nitrate, sodium phosphate), gases (oxygen, carbon dioxide) and water

TABLE 7.1 Principal Inorganic Reservoirs of Elements

Element	Inorganic Environmental Reservoir
Carbon	CO_2 in air; CO_3^{2-} in rocks and sediments
Oxygen	O_2 in air, certain oxides, water
Nitrogen	N_2 in air; NO_3^-, NO_2^-, NH_4^+ in soil and water
Hydrogen	Water, H_2 gas, mineral deposits
Phosphorus	Mineral deposits (PO_4^{3-}, H_3PO_4)
Sulfur	Mineral deposits, volcanic sediments (SO_4^{2-}, H_2S, S^0)
Potassium	Mineral deposits, the ocean (KCl, K_3PO_4)
Sodium	Mineral deposits, the ocean (NaCl, $NaSiO_4$)
Calcium	Mineral deposits, the ocean ($CaCO_3$, $CaCl_2$)
Magnesium	Mineral deposits, geologic sediments ($MgSO_4$)
Chloride	The ocean (NaCl, NH_4Cl)
Iron	Mineral deposits, geologic sediments ($FeSO_4$)
Manganese, molybdenum, cobalt, nickel, zinc, copper, other micronutrients	Various geologic sediments

TABLE 7.2 Nutrient Requirements and Sources for Two Ecologically Different Bacterial Species

	Acidithiobacillus thioxidans	*Mycobacterium tuberculosis*
Habitat	Sulfur springs	Human respiratory tract
Nutritional Type	Chemoautotroph (sulfur-oxidizing bacteria)	Chemoheterotroph (parasite; cause of tuberculosis in humans)
Essential Element	**Provided Principally By**	
Carbon	Carbon dioxide (CO_2) in air	Minimum of L-glutamic acid (an amino acid), glucose (a simple sugar), albumin (blood protein), oleic acid (a fatty acid), and citrate
Hydrogen	H_2O, H^+ ions	Nutrients listed in the previous line, H_2O
Nitrogen	Ammonium (NH_4^+)	Ammonium, L-glutamic acid
Oxygen	Phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), O_2	Oxygen gas (O_2) in atmosphere
Phosphorus	PO_4^{3-}	PO_4^{3-}
Sulfur	Elemental sulfur (S), SO_4^{2-}	SO_4^{2-}
Miscellaneous Nutrients		
Vitamins	None required	Pyridoxine, biotin
Inorganic salts	Potassium, calcium, iron, chloride	Sodium, chloride, iron, zinc, calcium, copper, magnesium
Principal energy source	Oxidation of sulfur (inorganic)	Oxidation of glucose (organic)

Chemical Analysis of Microbial Cytoplasm

- 70% water
- Proteins
- 96% of cell is composed of 6 elements:
  - Carbon
  - Hydrogen
  - Oxygen
  - Phosphorous
  - Sulfur
  - Nitrogen

TABLE 7.3 Analysis of the Chemical Composition of an *Escherichia coli* Cell

	% Total Weight	% Dry Weight		% Dry Weight
Organic Compounds			**Elements**	
Proteins	15	50	Carbon (C)	50
Nucleic acids			Oxygen (O)	20
RNA	6	20	Nitrogen (N)	14
DNA	1	3	Hydrogen (H)	8
Carbohydrates	3	10	Phosphorus (P)	3
Lipids	2	10	Sulfur (S)	1
Miscellaneous	2	4	Potassium (K)	1
Inorganic Compounds			Sodium (Na)	1
Water	70		Calcium (Ca)	0.5
All others	1	3	Magnesium (Mg)	0.5
			Chlorine (Cl)	0.5
			Iron (Fe)	0.2
			Trace metals	0.3

Sources of Essential Nutrients

- Carbon sources
- **Heterotroph** – must obtain carbon in an organic form made by other living organisms such as proteins, carbohydrates, lipids, and nucleic acids
- **Autotroph** – an organism that uses CO_2, an inorganic gas as its carbon source
  - Not nutritionally dependent on other living things

Sources of Essential Nutrients

Nitrogen Sources

- Main reservoir is nitrogen gas (N_2); 79% of earth's atmosphere is N_2
- Nitrogen is part of the structure of proteins, DNA, RNA and ATP – these are the primary source of N for heterotrophs
- Some bacteria and algae use inorganic N sources (NO_3^-, NO_2^-, or NH_3)
- Some bacteria can fix N_2.
- Regardless of how N enters the cell, it must be converted to NH_3, the only form that can be combined with carbon to synthesize amino acids, etc.

Sources of Essential Nutrients

Oxygen Sources

- Major component of carbohydrates, lipids, nucleic acids, and proteins
- Plays an important role in structural and enzymatic functions of cell
- Component of inorganic salts (sulfates, phosphates, nitrates) and water
- O_2 makes up 20% of atmosphere
- Essential to metabolism of many organisms

Sources of Essential Nutrients

Hydrogen Sources

- Major element in all organic compounds and several inorganic ones (water, salts, and gases)
- Gases are produced and used by microbes
- Roles of hydrogen:
  - Maintaining pH
  - Acceptor of oxygen during cell respiration

Sources of Essential Nutrients

Phosphorous (Phosphate Sources)

- Main inorganic source is phosphate (PO_4^{-3}) derived from phosphoric acid (H_3PO_4) found in rocks and oceanic mineral deposits
- Key component of nucleic acids, essential to genetics
- Serves in energy transfers (ATP)

Sources of Essential Nutrients

Sulfur Sources

- Widely distributed in environment, rocks; sediments contain sulfate, sulfides, hydrogen sulfide gas and sulfur
- Essential component of some vitamins and the amino acids: methionine and cysteine
- Contributes to stability of proteins by forming disulfide bonds

Other Nutrients Important in Microbial Metabolism

- Potassium – essential to protein synthesis and membrane function
- Sodium – important to some types of cell transport
- Calcium – cell wall and endospore stabilizer
- Magnesium – component of chlorophyll; membrane and ribosome stabilizer
- Iron – component of proteins of cell respiration
- Zinc, copper, nickel, manganese, etc.

Growth Factors: Essential Organic Nutrients

- Organic compounds that cannot be synthesized by an organism because they lack the genetic and metabolic mechanisms to synthesize them
- **Growth factors** must be provided as a nutrient
  - Essential amino acids, vitamins

Nutritional Types

- Main determinants of nutritional type are:
  - Carbon source – heterotroph, autotroph
  - Energy source
    - **Chemotroph** – gain energy from chemical compounds
    - **Phototrophs** – gain energy through photosynthesis

TABLE 7.4 Nutritional Categories of Microbes by Energy and Carbon Source

Category/ Carbon Source	Energy Source	Example
Autotroph/CO_2	**Nonliving Environment**	
Photoautotroph	Sunlight	Photosynthetic organisms, such as algae, plants, cyanobacteria
Chemoautotroph	Simple inorganic chemicals	Only certain bacteria, such as methanogens, deep-sea vent bacteria
Heterotroph/ Organic	**Other Organisms or Sunlight**	
Chemoheterotroph	Metabolic conversion of the nutrients from other organisms	Protozoa, fungi, many bacteria, animals
Saprobe	Metabolizing the organic matter of dead organisms	Fungi, bacteria (decomposers)
Parasite	Utilizing the tissues, fluids of a live host	Various parasites and pathogens; can be bacteria, fungi, protozoa, animals
Photoheterotroph	Sunlight	Purple and green photosynthetic bacteria

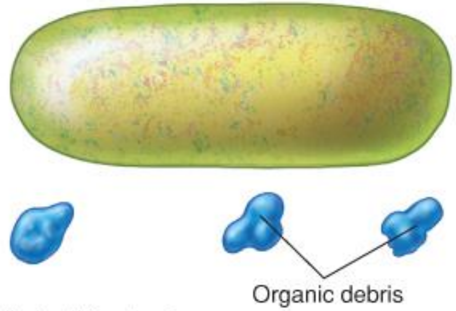
Autotrophs and Their Energy Sources

- Photoautotrophs
  - Oxygenic photosynthesis
  - Anoxygenic photosynthesis
- Chemoautotrophs (lithoautotrophs) survive totally on inorganic substances
- Methanogens, a kind of chemoautotroph, produce methane gas under anaerobic conditions

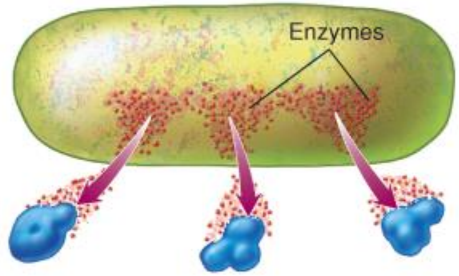
Heterotrophs and Their Energy Sources

- Majority are chemoheterotrophs
  - Aerobic respiration
- Two categories
  - Saprobes: free-living microorganisms that feed on organic detritus from dead organisms
    - Opportunistic pathogen
    - Facultative parasite
  - Parasites: derive nutrients from host
    - Pathogens
    - Some are obligate parasites

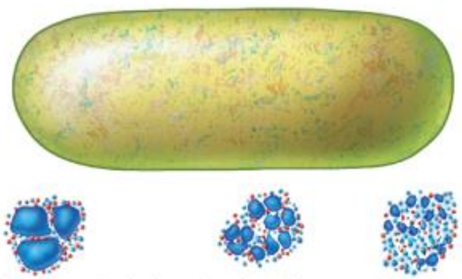
Digestion in Bacteria and Fungi



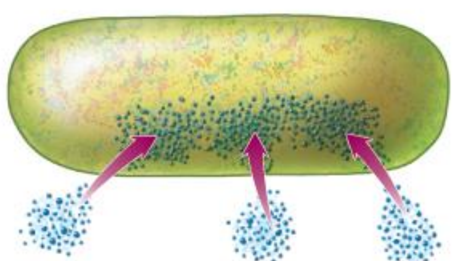
(a) Walled cell is a barrier.



(b) Enzymes are transported outside the wall.



(c) Enzymes hydrolyze the bonds on nutrients.



(d) Smaller molecules are transported across the wall and cell membrane into the cytoplasm.

Transport: Movement of Chemicals Across the Cell Membrane

- **Passive transport** – does not require energy; substances exist in a gradient and move from areas of higher concentration toward areas of lower concentration
  - Diffusion
  - Osmosis – diffusion of water
  - Facilitated diffusion – requires a carrier
- **Active transport** – requires energy and carrier proteins; gradient independent
  - Active transport
  - Group translocation – transported molecule chemically altered
  - Bulk transport – endocytosis, exocytosis, pinocytosis

Diffusion – Net Movement of Molecules Down Their Concentration Gradient

How Molecules Diffuse in Aqueous Solutions

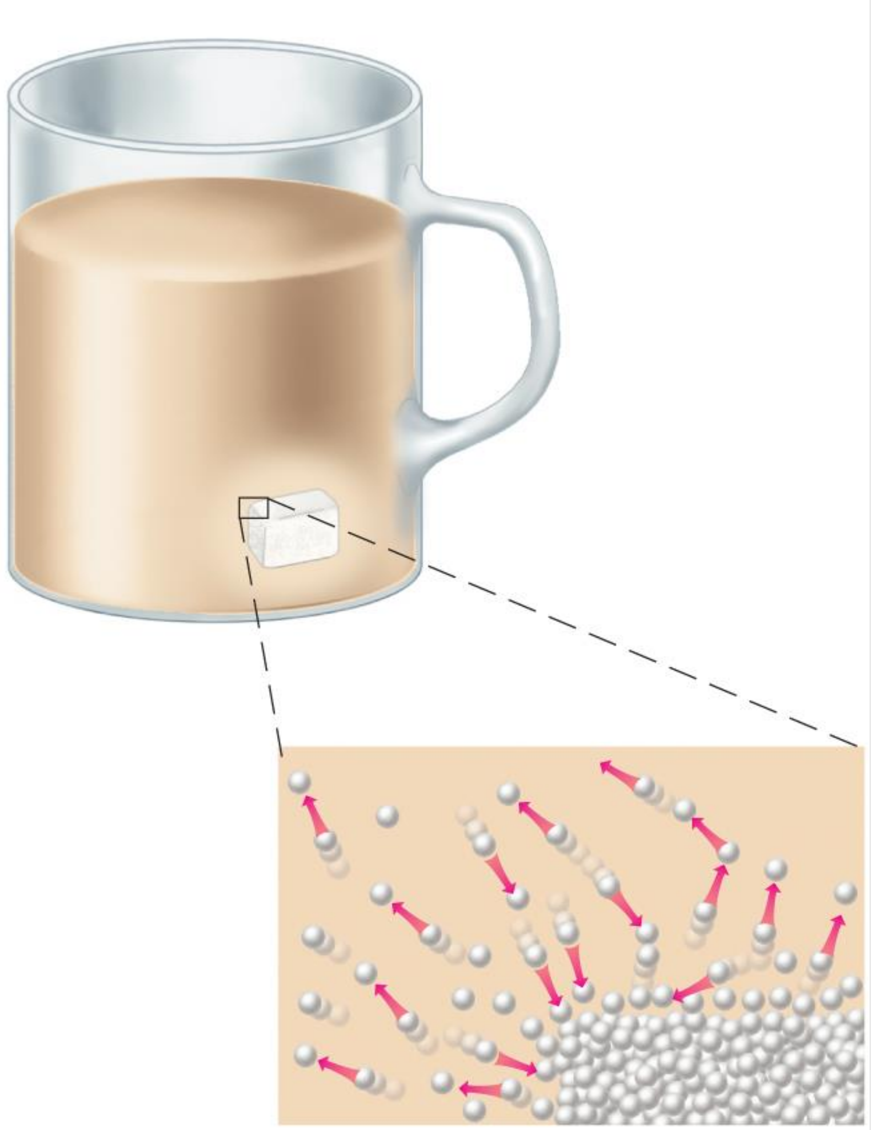
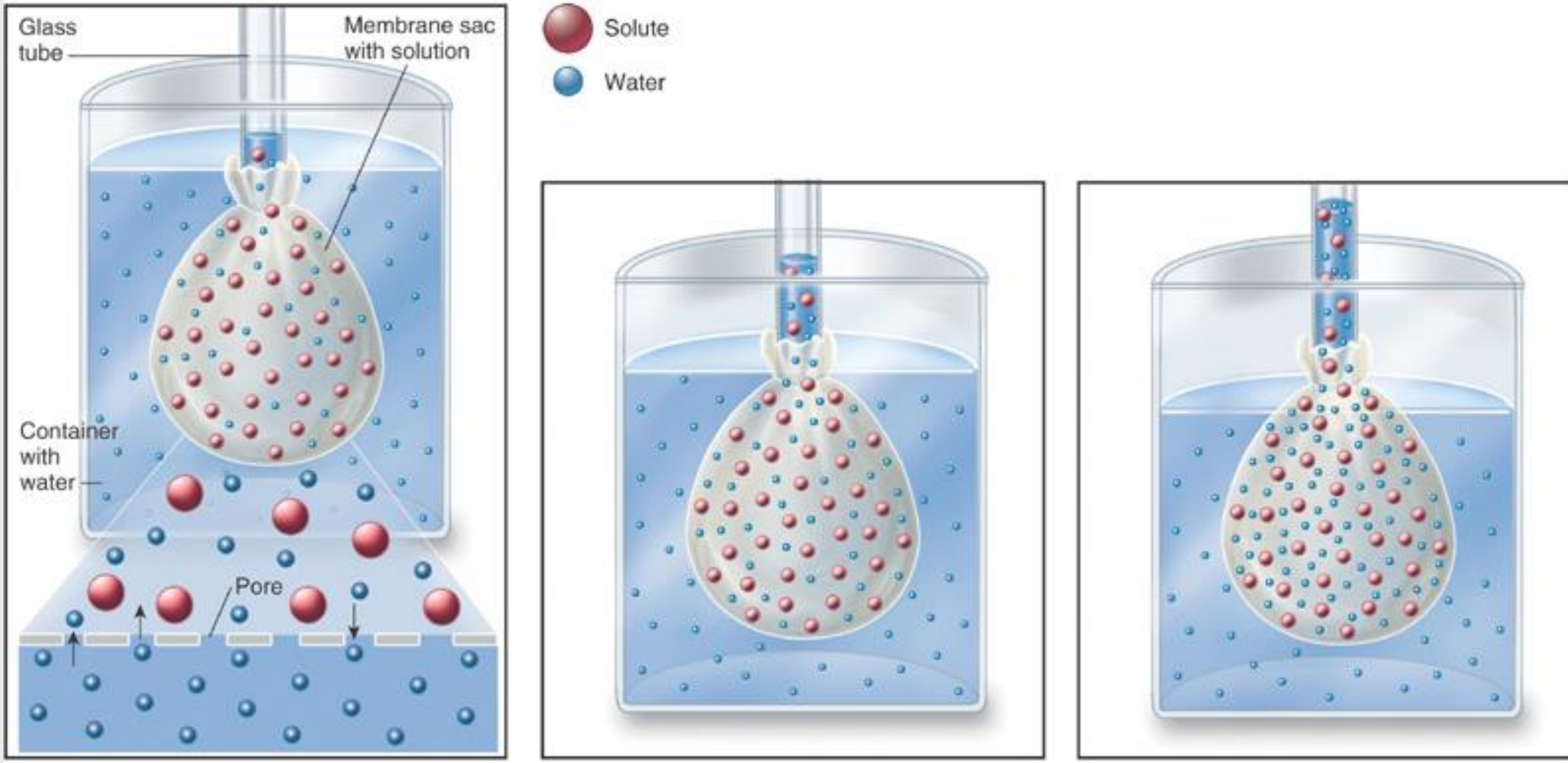


Figure 7.4 Osmosis



a. Inset shows a close-up of the osmotic process. The gradient goes from the outer container (higher concentration of H_2O) to the sac (lower concentration of H_2O). Some water will diffuse the opposite direction but the net gradient favors osmosis into the sac.

b. As the H_2O diffuses into the sac, the volume increases and forces the excess solution into the tube, which will rise continually.

c. Even as the solution becomes diluted, there will still be osmosis into the sac. Equilibrium will not occur because the solutions can never become equal. (Why?)

Figure 7.5 Response to solutions of different osmotic content

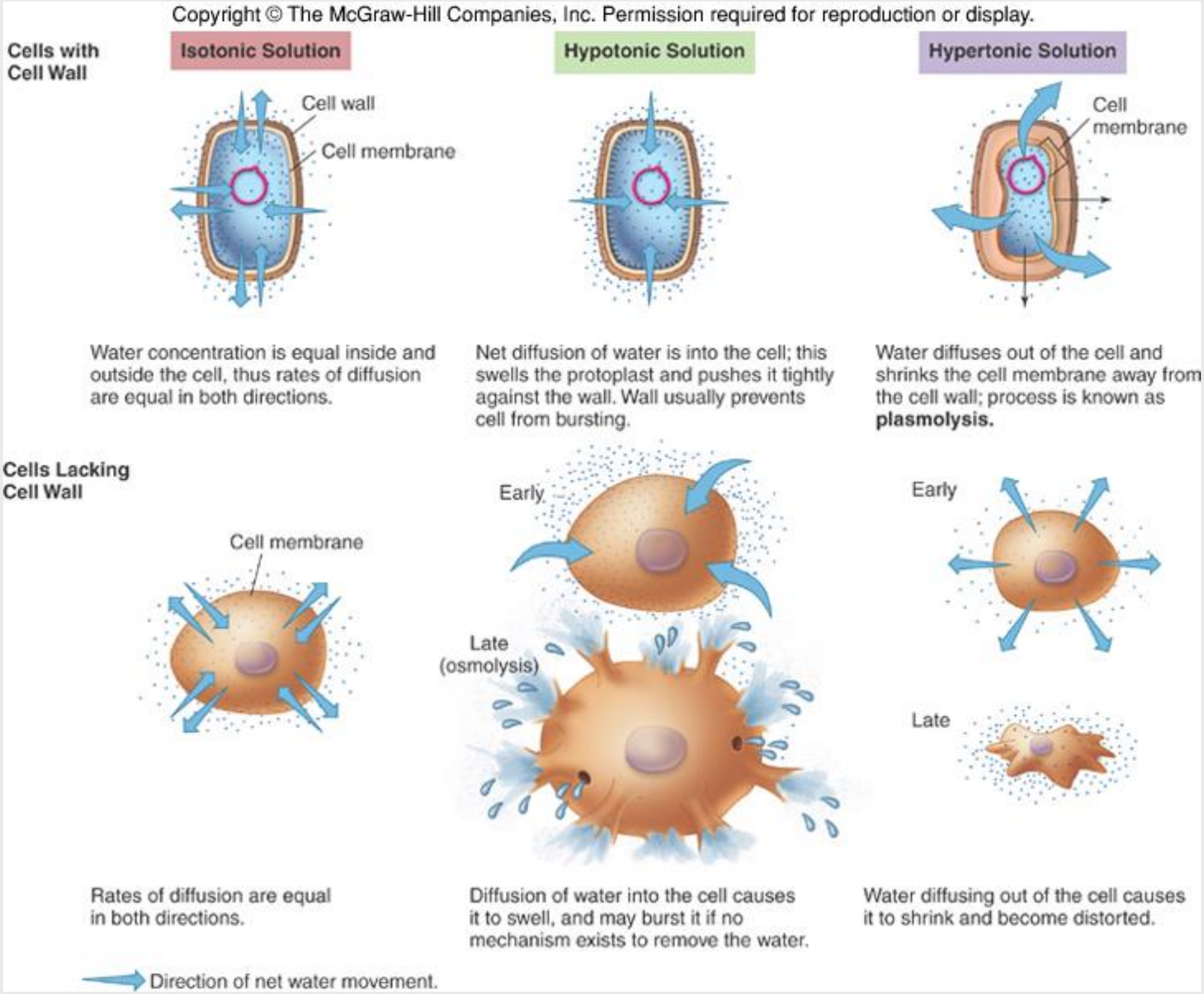


Figure 7.6 Facilitated diffusion

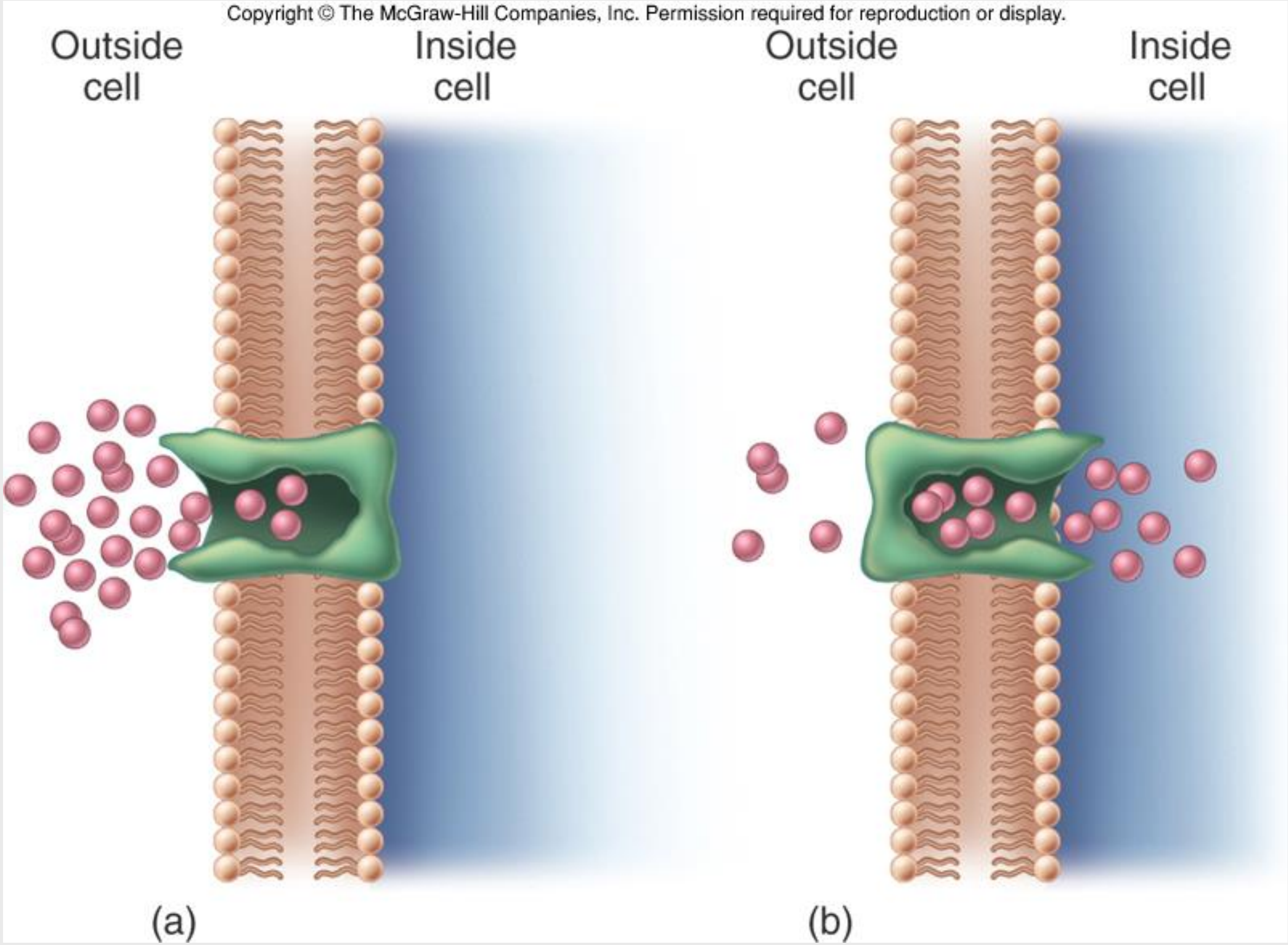


Figure 7.7a Carrier mediated active transport

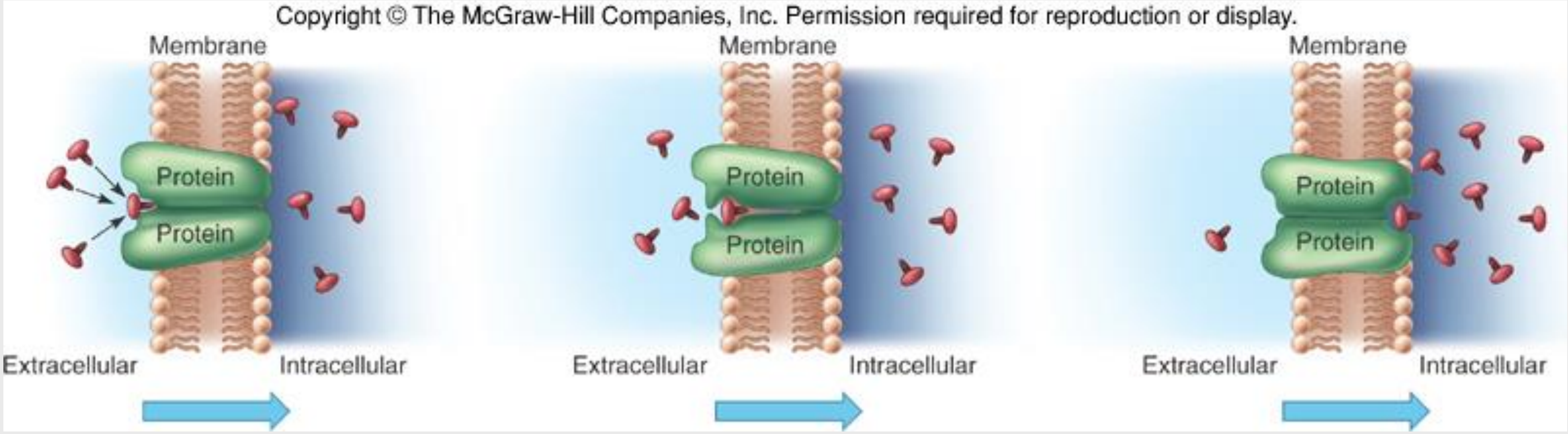


Figure 7.7b Group translocation

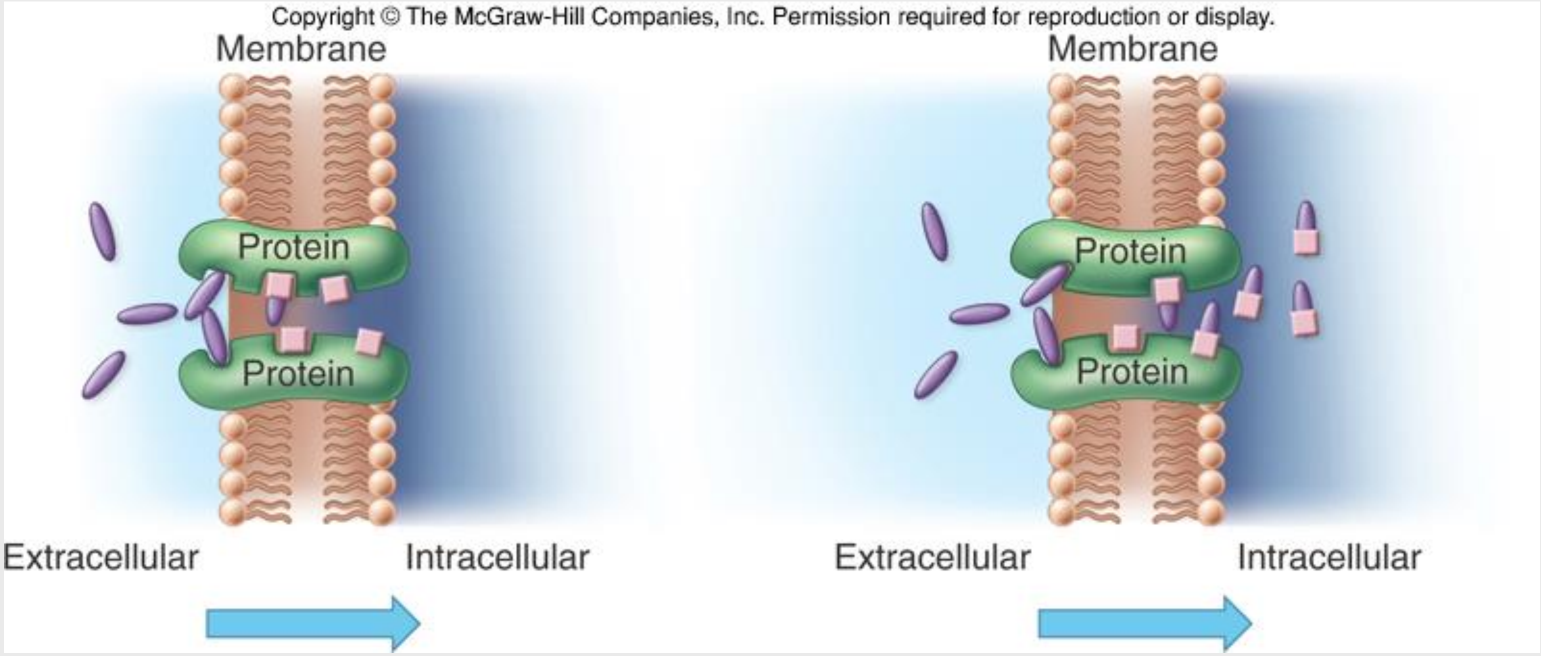


Figure 7.7c Endocytosis

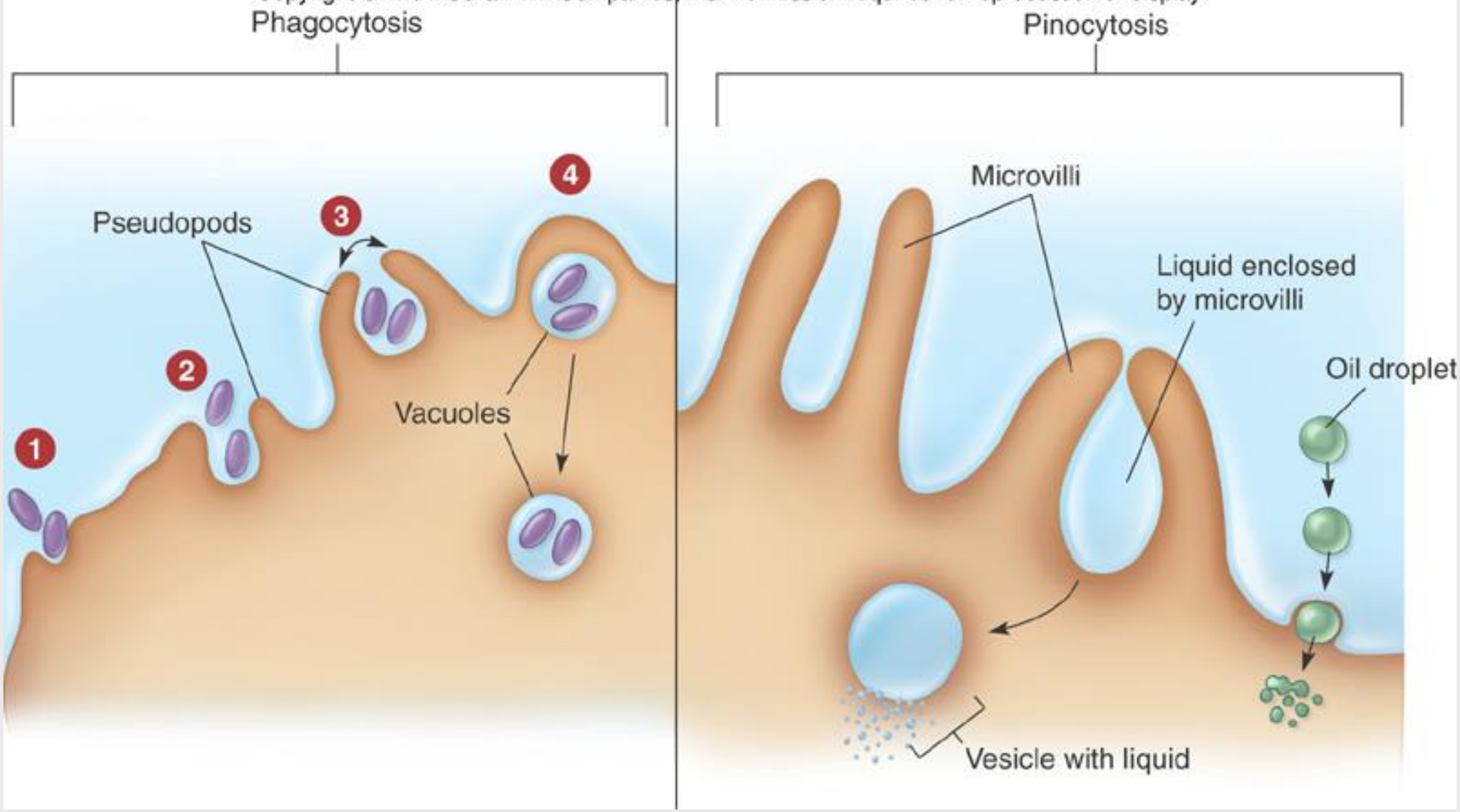


TABLE 7.5 Summary of Transport Processes in Cells

General Process	Nature of Transport	Examples	Description	Qualities
Passive	Energy expenditure by the cell is not required. Substances exist in a gradient and move from areas of higher concentration toward areas of lower concentration in the gradient.	Diffusion	A fundamental property of atoms and molecules that exist in a state of random motion	Nonspecific Brownian movement Movement of small uncharged molecules across membranes
		Facilitated diffusion	Molecule binds to a carrier protein in membrane and is carried across to other side	Molecule specific; transports both ways Transports sugars, amino acids, water
Active	Energy expenditure is required. Molecules need not exist in a gradient. Rate of transport is increased. Transport may occur against a concentration gradient.	Carrier-mediated active transport	Atoms or molecules are pumped into or out of the cell by specialized receptors. Driven by ATP or the proton motive force	Transports simple sugars, amino acids, inorganic ions (Na^+, K^+)
		Group translocation	Molecule is moved across membrane and simultaneously converted to a metabolically useful substance.	Alternate system for transporting nutrients (sugars, amino acids)
		Bulk transport	Mass transport of large particles, cells, and liquids by engulfment and vesicle formation	Includes endocytosis, phagocytosis, pinocytosis

7.2 Environmental Factors That Influence Microbes

- Niche: totality of adaptations organisms make to their habitat
- Environmental factors affect the function of metabolic enzymes
- Factors include:
  - Temperature
  - Oxygen requirements
  - pH
  - Osmotic pressure
  - Barometric pressure

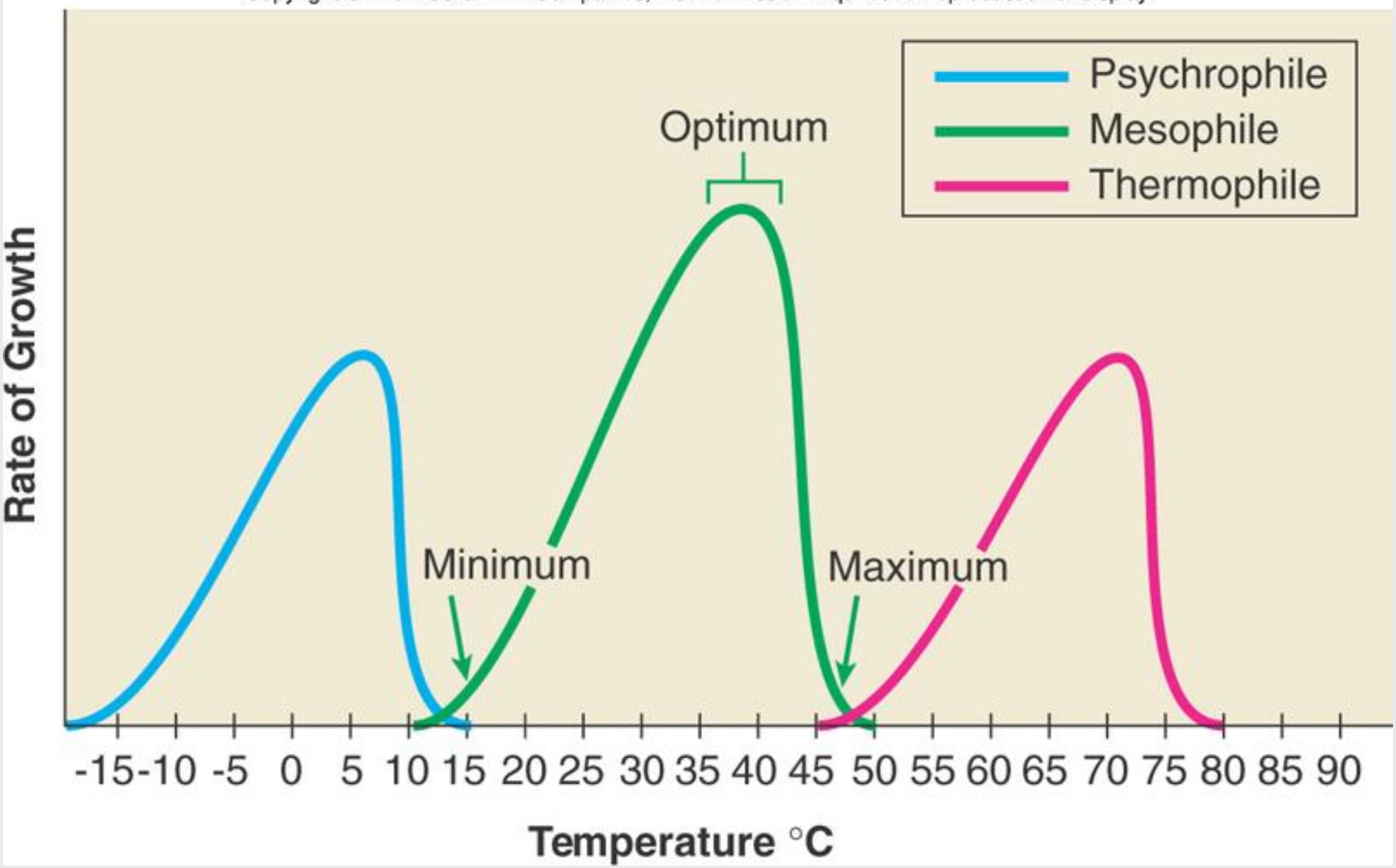
3 Cardinal Temperatures

- **Minimum temperature** – lowest temperature that permits a microbe's growth and metabolism
- **Maximum temperature** – highest temperature that permits a microbe's growth and metabolism
- **Optimum temperature** – promotes the fastest rate of growth and metabolism

3 Temperature Adaptation Groups

1. **Psychrophiles** – optimum temperature below 15^{o}C; capable of growth at 0^{o}C
2. **Mesophiles** – optimum temperature 20^{o}-40^{o}C; most human pathogens
3. **Thermophiles** – optimum temperature greater than 45^{o}C

Figure 7.8 Ecological groups by temperature of adaptation



Gas Requirements

Oxygen

- As oxygen is utilized it is transformed into several toxic products:
  - Singlet oxygen ($^1O_2$), superoxide ion (O_2^-), peroxide (H_2O_2), and hydroxyl radicals (OH^-)
- Most cells have developed enzymes that neutralize these chemicals:
  - Superoxide dismutase, catalase
- If a microbe is not capable of dealing with toxic oxygen, it is forced to live in oxygen free habitats

Categories of Oxygen Requirement

- **Aerobe** – utilizes oxygen and can detoxify it
- **Obligate aerobe** – cannot grow without oxygen
- **Facultative anaerobe** – utilizes oxygen but can also grow in its absence
- **Microaerophilic** – requires only a small amount of oxygen

Categories of Oxygen Requirement

- **Anaerobe** – does not utilize oxygen
- **Obligate anaerobe** – lacks the enzymes to detoxify oxygen so cannot survive in an oxygen environment
- **Aerotolerant anaerobes** – do not utilize oxygen but can survive and grow in its presence

Carbon Dioxide Requirement

All microbes require some carbon dioxide in their metabolism

- **Capnophile** – grows best at higher CO_2 tensions than normally present in the atmosphere

Figure 7.10

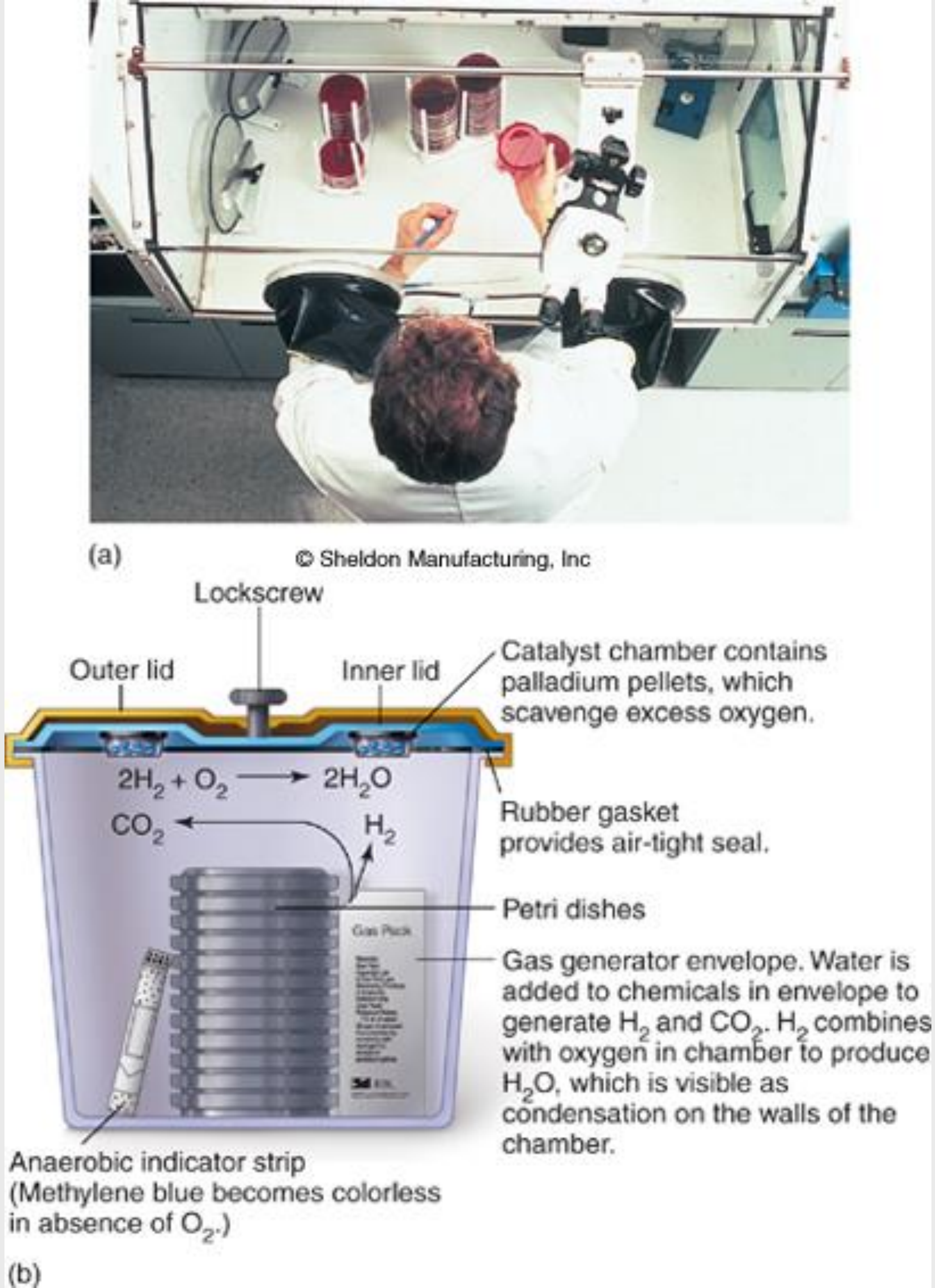

(a)
© Sheldon Manufacturing, Inc
Lockscrew
Outer lid
Inner lid
Catalyst chamber contains palladium pellets, which scavenge excess oxygen.
$2H_2 + O_2 \longrightarrow 2H_2O$
CO_2
H_2
Rubber gasket provides air-tight seal.
Petri dishes
Gas Pack
Gas generator envelope. Water is added to chemicals in envelope to generate H_2 and CO_2. H_2 combines with oxygen in chamber to produce H_2O, which is visible as condensation on the walls of the chamber.
Anaerobic indicator strip (Methylene blue becomes colorless in absence of O_2.)
(b)

Figure 7.11

Effects of pH

- Majority of microorganisms grow at a pH between 6 and 8
- **Obligate acidophiles** – grow at extreme acid pH
- **Alkalinophiles** – grow at extreme alkaline pH

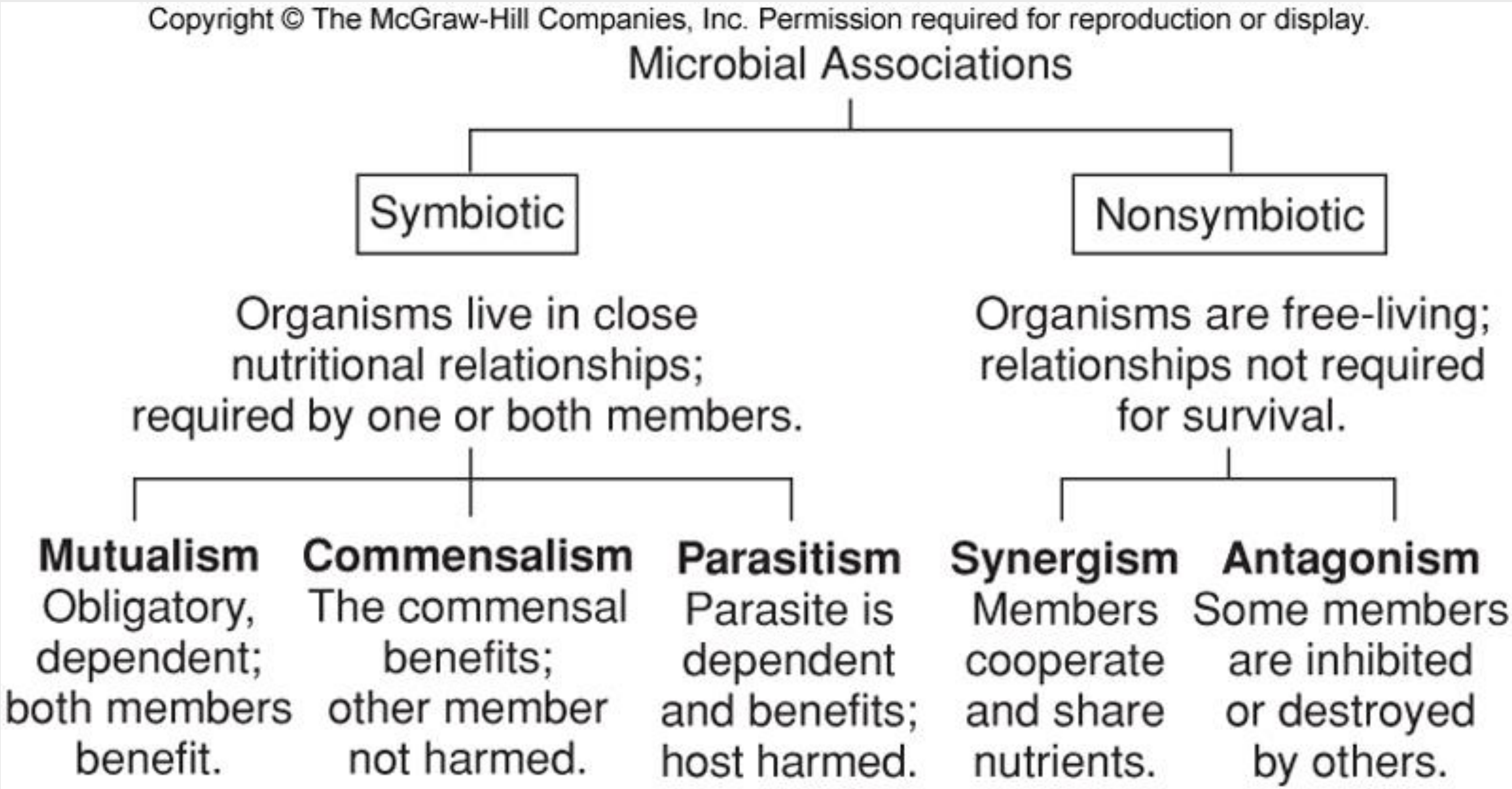
Osmotic Pressure

- Most microbes exist under hypotonic or isotonic conditions
- **Halophiles** – require a high concentration of salt
- **Osmotolerant** – do not require high concentration of solute but can tolerate it when it occurs

Other Environmental Factors

- **Barophiles** – can survive under extreme pressure and will rupture if exposed to normal atmospheric pressure

Ecological Associations Among Microorganisms



Ecological Associations Among Microorganisms

- Symbiotic – two organisms live together in a close partnership
  - **Mutualism** – obligatory, dependent; both members benefit
  - **Commensalism** – commensal member benefits, other member neither harmed nor benefited
  - **Parasitism** – parasite is dependent and benefits; host is harmed

Ecological Associations Among Microorganisms

- Non-symbiotic – organisms are free-living; relationships not required for survival
  - **Synergism** – members cooperate to produce a result that none of them could do alone
  - **Antagonism** – actions of one organism affect the success or survival of others in the same community (competition)
    - Antibiosis

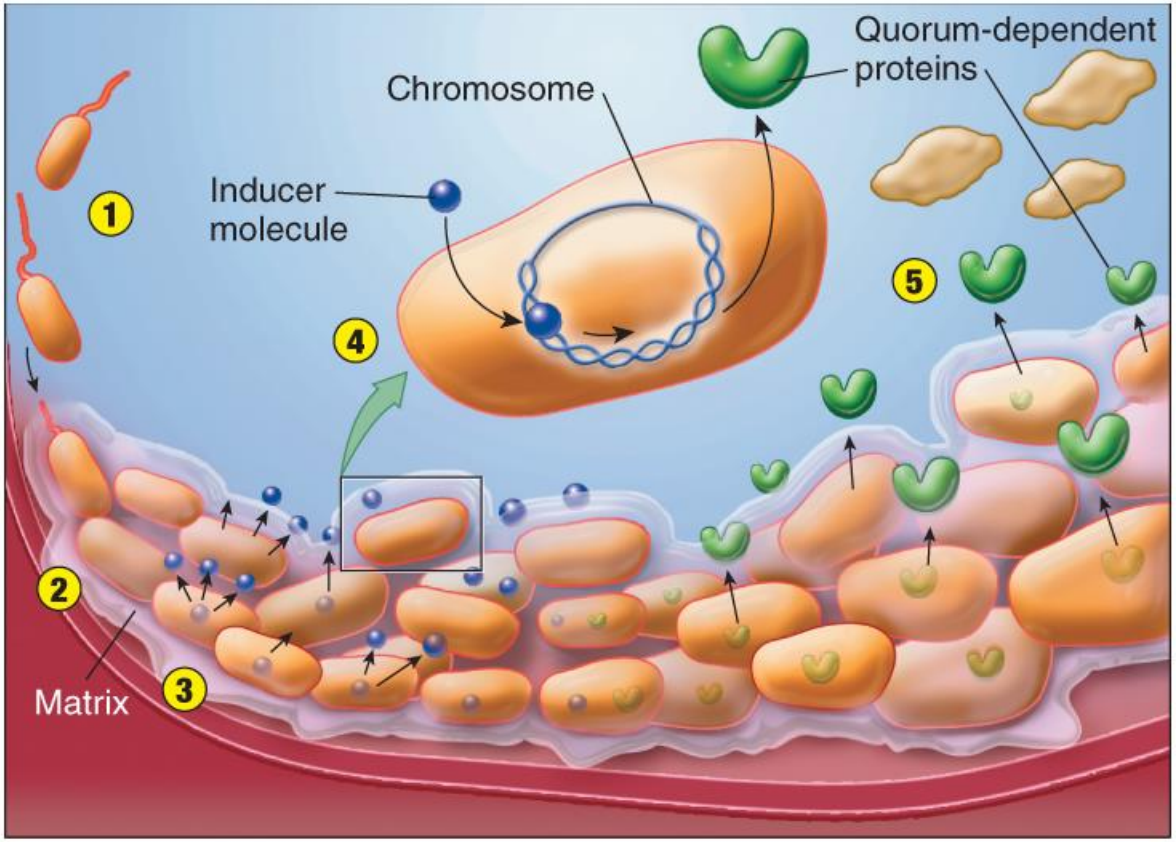
Interrelationships Between Microbes and Humans

- Human body is a rich habitat for symbiotic bacteria, fungi, and a few protozoa **- normal microbial flora**
- Commensal, parasitic, and synergistic relationships

Microbial Biofilms

- **Biofilms** result when organisms attach to a substrate by some form of extracellular matrix that binds them together in complex organized layers
- Dominate the structure of most natural environments on earth
- Communicate and cooperate in the formation and function of biofilms – **quorum sensing**

Figure 7.13



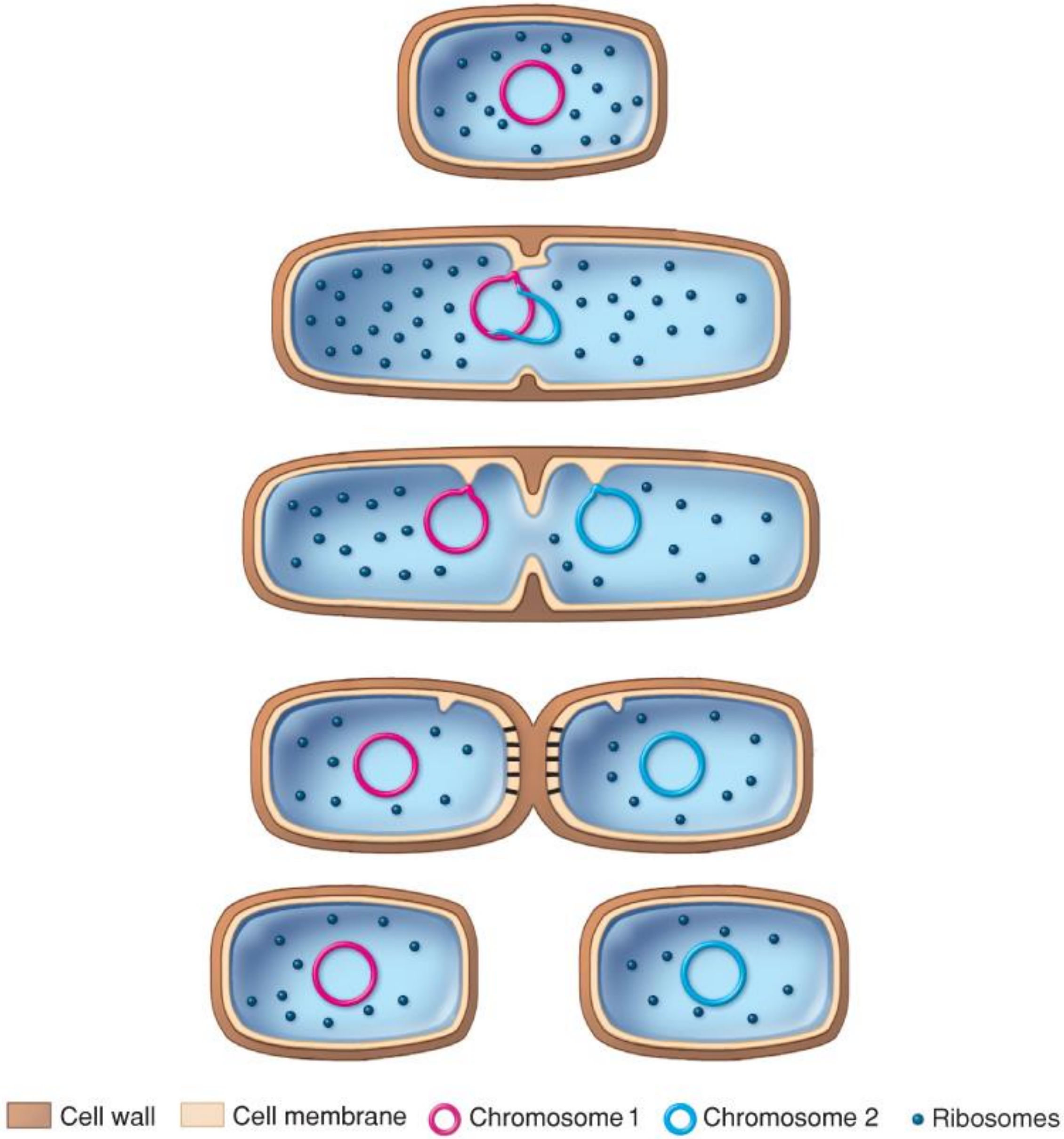
1. Free-swimming cells settle on a surface and remain there.
2. Cells synthesize a sticky matrix that holds them tightly to the substrate.
3. When biofilm grows to a certain density (quorum), the cells release inducer molecules that can coordinate a response.
4. Enlargement of one cell to show genetic induction. Inducer molecule stimulates expression of a particular gene and synthesis of a protein product, such as an enzyme.
5. Cells secrete their enzymes in unison to digest food particles.

7.3 The Study of Microbial Growth

- Microbial growth occurs at two levels: growth at a cellular level with increase in size, and increase in population
- Division of bacterial cells occurs mainly through **binary fission** (transverse)
  - Parent cell enlarges, duplicates its chromosome, and forms a central transverse septum dividing the cell into two daughter cells

Figure 7.14

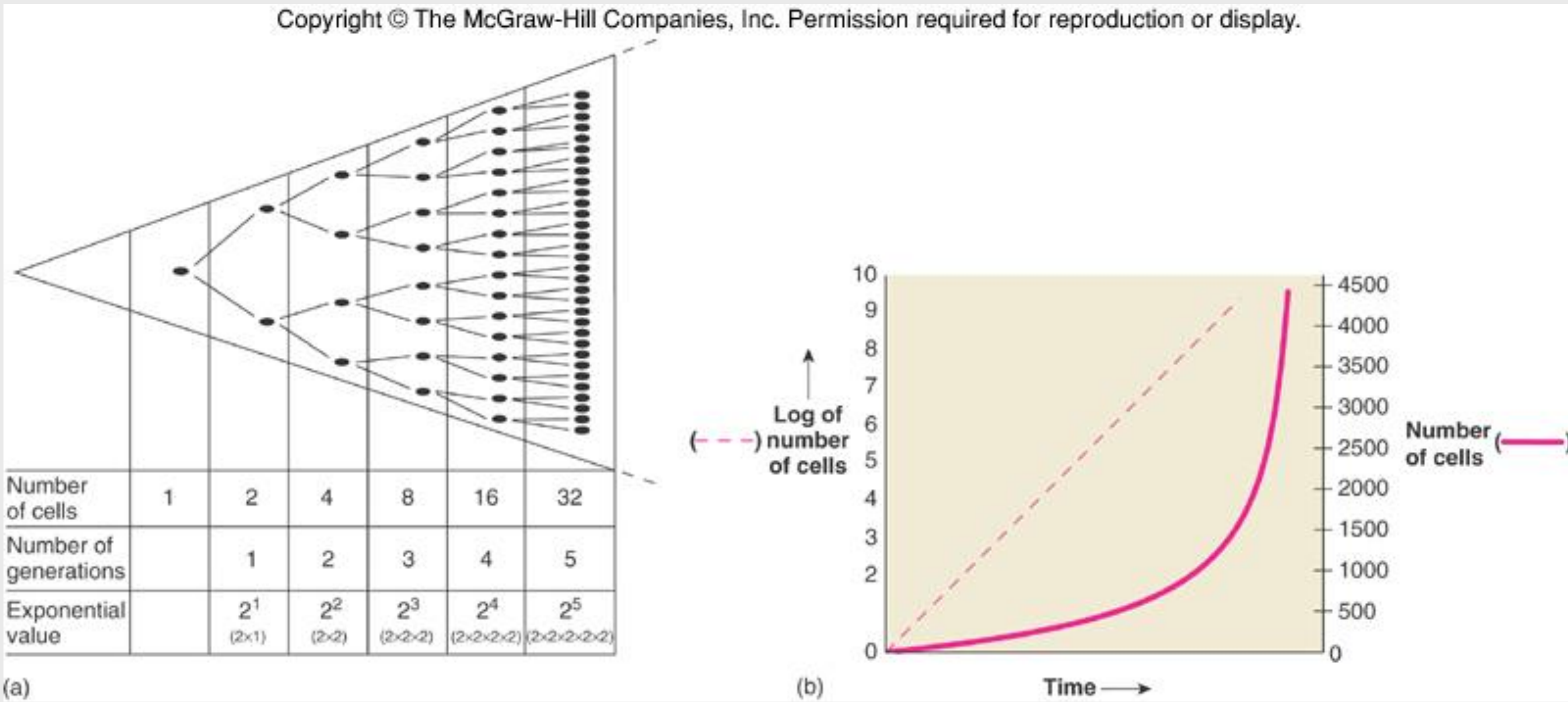
1. A young cell at early phase of cycle
2. A parent cell prepares for division by enlarging its cell wall, cell membrane,and overall volume. Midway in the cell,the wall develops notches that will eventually form the transverse septum, and the duplicated chromosome becomes affixed to a special membrane site.
3. The septum wall grows inward, and the chromosomes are pulled toward opposite cell ends as the membrane enlarges. Other cytoplasmic components are distributed (randomly) to the two developing cells.
4. The septum is synthesized completely through the cell center, and the cell membrane patches itself so that there are two separate cell chambers.
5. At this point, the daughter cells are divided. Some species will separate completely as shown here, while others will remain attached, forming chains or doublets, for example.



Rate of Population Growth

- Time required for a complete fission cycle is called the **generation,** or **doubling** time
- Each new fission cycle increases the population by a factor of 2 – **exponential** growth
- Generation times vary from minutes to days

Figure 7.15 Mathematics of population growth



Rate of Population Growth

- Equation for calculating population size over time:

$$N_f = (N_i)2^n$$

N_f is total number of cells in the population

N_i is starting number of cells

Exponent n denotes generation time

2^n number of cells in that generation

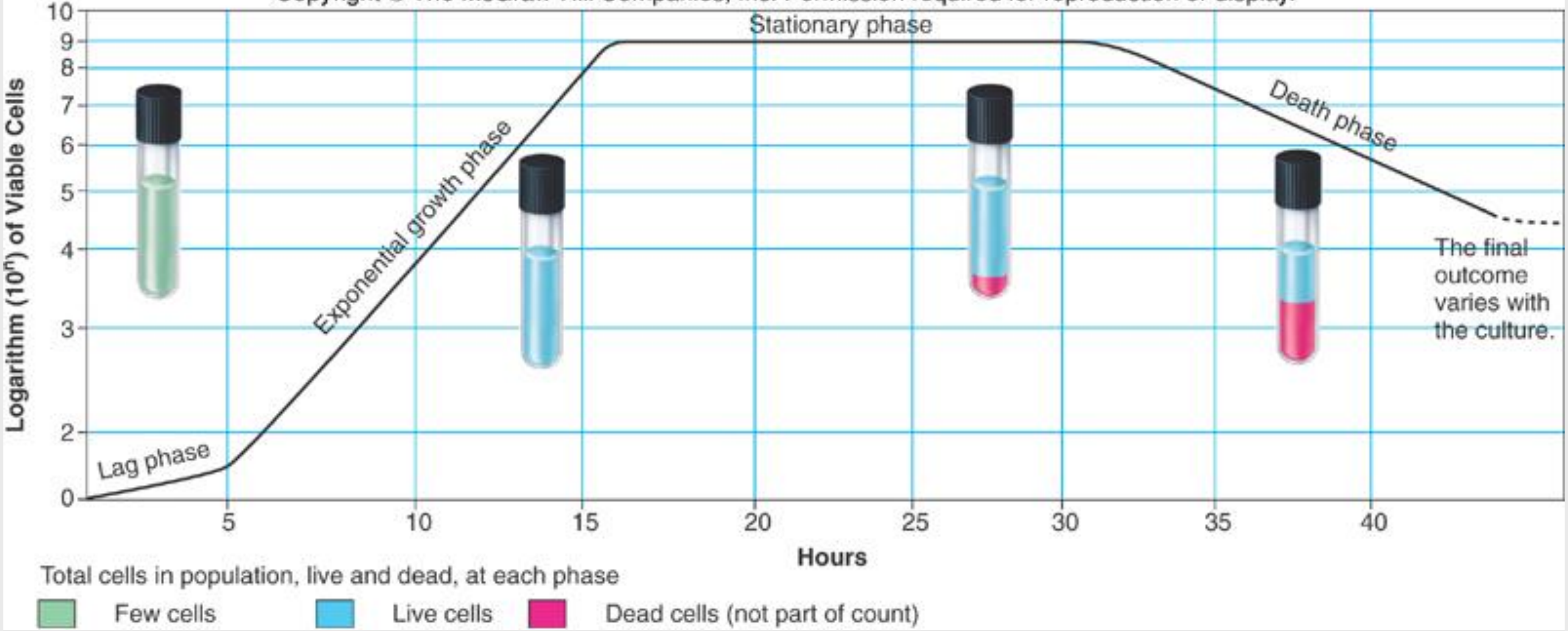
The Population Growth Curve

In laboratory studies, populations typically display a predictable pattern over time – **growth curve**

Stages in the normal growth curve:

1. **Lag phase** – "flat" period of adjustment, enlargement; little growth
2. **Exponential growth phase** – a period of maximum growth will continue as long as cells have adequate nutrients and a favorable environment
3. **Stationary phase** – rate of cell growth equals rate of cell death caused by depleted nutrients and O_2, excretion of organic acids and pollutants
4. **Death phase** – as limiting factors intensify, cells die exponentially

Figure 7.16 Growth curve in a bacterial culture



Methods of Analyzing Population Growth

- Turbidometry – most simple
- Degree of cloudiness, turbidity, reflects the relative population size
- Enumeration of bacteria:
  - Viable colony count
  - Direct cell count – count all cells present; automated or manual

Figure 7.17 Turbidity measurements

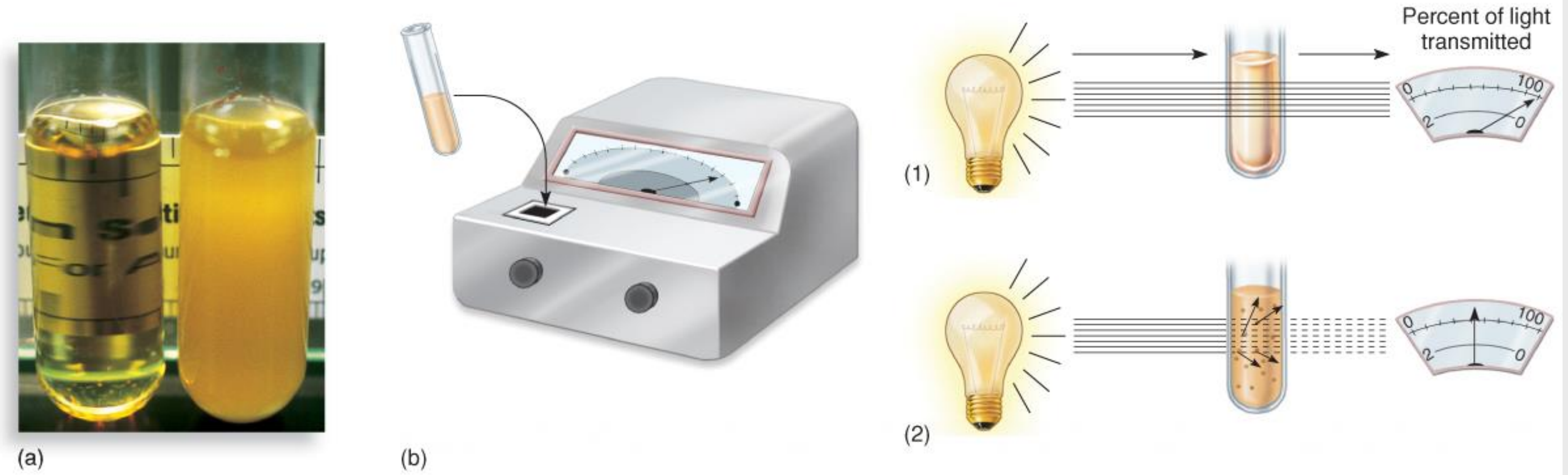


Figure 7.18 Direct microscopic count of bacteria

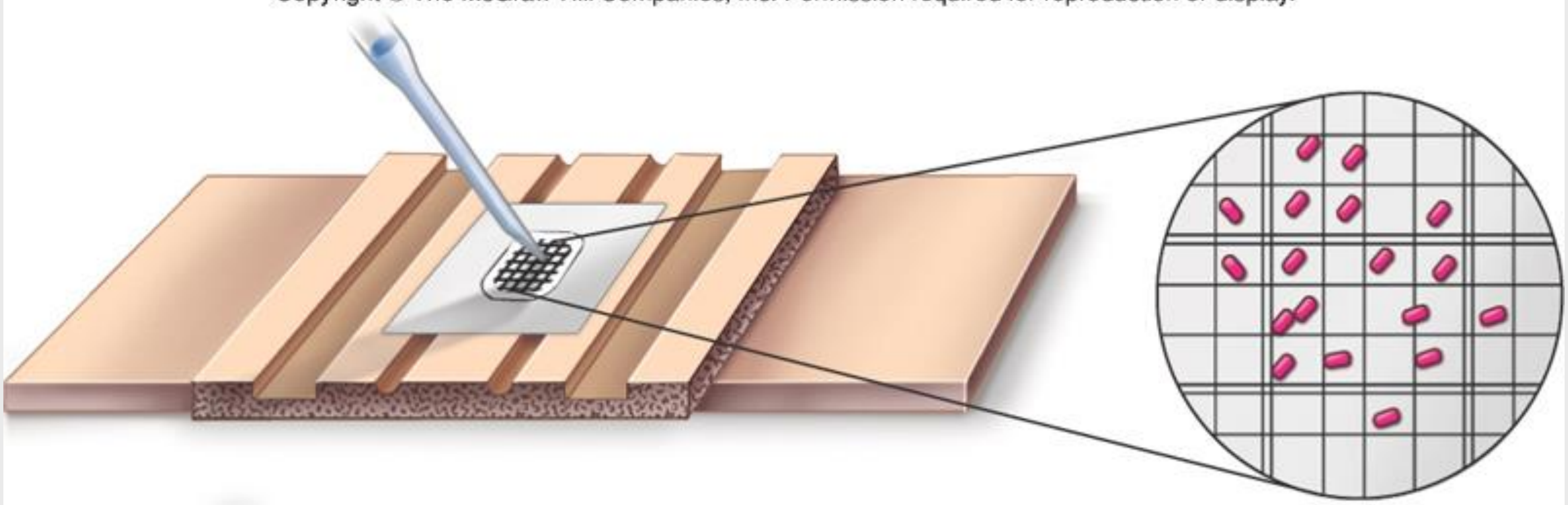


Figure 7.19 Coulter Counter